博 士 学 位 論 文

フェノタイプ及びターゲットベースドスクリーニングによる
新規ヘモグロビン症治療薬の創製

片 山 勝 史

北海道大学大学院生命科学院

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略語表

本博士論文中では便宜上、以下の略語を用いた。

Ac	acetyl
ADMET	absorption, distribution, metabolism, excretion and toxicity
All	allyl
APCI	atmospheric-pressure chemical ionization
aq.	aqueous
Arg	arginine
Asp	aspartic acid
AUC	area under the curve
BA	bioavailability
bid	*bis in die* (twice a day)
BM	bone marrow
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bu	butyl
Cbz	benzyloxycarbonyl
CL	clearance
C_{max}	maximum concentration
COMU	1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate
CYP	cytochrome P450
DAC	decitabine
Dba	dibenzylideneacetone
DIPEA	*N*,*N*-diisopropylethylamine
DMA	*N*,*N*-dimethylacetoamide
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNMT	DNA (cytosine-5)-methyltransferase
dppf	1,1'-bis(diphenylphosphino)ferrocene
EC_{50}	50% effective concentration
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EHMT	euchromatic histone-lysine *N*-methyltransferase
ESI	electrospray ionization
Et	ethyl
E_{max}	maximum efficacy
FCM	flow cytometry
FDA	Food and Drug Administration

GLP	G9a-like protein
Glu	glutamic acid
h	hour(s)
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HAT	histone acetyltransferases
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HDAC	histone deacetylase
hERG	human ether-à-go-go related gene
Hex	hexyl
HPFH	hereditary persistence of fetal hemoglobin
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HTS	high-throughput screening
HU	hydroxyurea
HUDEP	human umbilical cord blood-derived erythroid progenitor
IC_{50}	50% inhibitory concentration
Ile	isoleiucine
^{i}Pr	isopropyl
IV	intravenous
IVtMN	in vitro micronucleus
KMT	lysine methyltransferase
LC/MS	liquid chromatography/mass spectrometry
LCR	locus control region
Leu	leucine
LiHMDS	lithium bis(trimethylsilyl)amide
Log D	logarithm of ocatanol–water distribution coefficient
Log P	logarithm of ocatanol–water partition coefficient
Lys	lysine
MBI	mechanism-based inhibition
MC	methylcellulose
MDCK	Madin-Darby Canine Kidney
Me	methyl
min	minute(s)
mRNA	messenger ribonucleic acid
Ms	methanesulfonyl
MS	metabolic stability
MW	molecular weight

μW	microwave
n, *n*-	normal
NaHMDS	sodium bis(trimethylsilyl)amide
Nf	nonafluorobutanesulfonyl
NMR	nuclear magnetic resonance
PD	pharamcodynamics
PDB	protein data bank
PEG	polyethylene glycol
Pen	pentyl
Ph	phenyl
Pin	pinacolato
PK	pharmacokinetics
pK_a	logarithm of acidity constant
po	*per os* (oral administration)
Py	pyridine
qd	*quaque die* (once a day)
qPCR	quantitative polymerase chain reaction
quant.	quantitative yield
RCM	ring closing metathesis
rt	room temperature
SAH	*S*-adenosylhomocysteine
SAM	*S*-adenosylmethionine
SAR	structure–activity relationship
sat.	saturated
SCD	sickle cell disease
SD	standard deviation
SEM	standard error of the mean
t, *t*-, *tert*-	tertiary
$t_{1/2}$	elimination half-life
TBAI	tetrabutylammonium Iodide
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofolate or tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin-layer chromatography
Ts	toluenesulfonyl
Val	valine
VOC	vaso-occlusive crisis
Xphos Pd G2	chloro(2-dicyclohexylphosphino-2', 4', 6'-tri-i-propyl-1, 1'-biphenyl)(2'-amino-1, 1'-biphenyl-2-yl) palladium (II)

序論

成人ヘモグロビン（HbA; $\alpha_2\beta_2$）は 2α 鎖と 2β 鎖からなる四量体タンパク質であり、2 つの対称性 αβ 二量体で配置し、成人血液中の酸素運搬を担っている。一方、新生児の赤血球では β-グロビン鎖の代わりとして γ-グロビン鎖が優勢な α-グロビンの対応物として機能し、胎児性グロビン（HbF; $\alpha_2\gamma_2$）の形成をもたらす。そして出生直後、γ-グロビンはグロビンスイッチングと呼ばれるプロセスを介して β-グロビンに置き換えられる [1,2]（Figure 1）。

鎌状赤血球症（SCD）と β-サラセミアは一般的に知られている単遺伝子性異常ヘモグロビン症であり、ヘモグロビンの β-グロビン鎖の構造と産生に影響を与え、重度の溶血性貧血を引き起こす [3]。β-サラセミアは、成人ヘモグロビンの主成分の 1 つである β-グロビン鎖の合成の低下または欠如を特徴とする単一遺伝子障害である [4]。SCD では、成人ヘモグロビンの β 鎖上の単一の点突然変異（β6 Glu→Val）によって鎌状赤血球ヘモグロビン（HbS; $\alpha_2\beta^s_2$）が生じ、脱酸素状態になることで溶血と血管閉塞が引き起こされる [5]。これらのヘモグロビン症は、アフリカ、中東、インド亜大陸、東南アジア、及び地中海を含む多くの地域で広く発生し、世界中の疾病負荷を着実に増加させていることが知られている [5]。

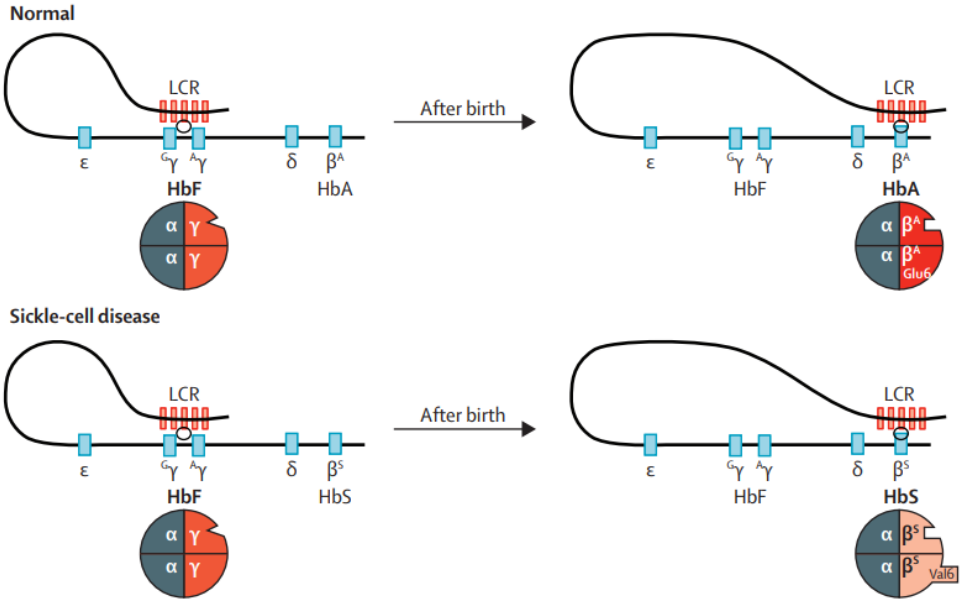


Figure 1. Fetal hemoglobin and sickle cell disease. The predominant hemoglobin before birth is fetal hemoglobin (HbF; $\alpha_2\gamma_2$) with a small contribution of adult hemoglobin (HbA; $\alpha_2\beta_2$). After birth, only a small residual production of HbF and HbA predominates in the total amount of hemoglobin. Sickle cell disease, due to its β^S mutation of β-globin, only manifests after birth once the contribution of HbF wanes.[6]

臨床研究では、赤血球での HbF 産生の増加が、β-サラセミアと SCD の疾患進行の改善につながることが示されており、赤血球あたり約 30%の HbF の閾値濃度が臨床的効果を示すことが知られている。これは、HbF が成人期まで持続する良性の無症候性遺伝性疾患である遺伝性高胎児血色素症（HPFH）において、その患者の HbF のレベルが約 30%であり、

症状が SCD や β-サラセミアの患者に比べて軽く通常の日常生活を送ることより設定されている[7]。したがって、HbF 産生を増加させる治療薬は、ヘモグロビン症患者の臨床的罹患率と死亡率を低下させるための重要な治療戦略となっている。 SCD の治療薬として承認されたヒドロキシ尿素（HU）は、HbF 産生を誘発し、SCD 患者の苦痛を軽減することができる。しかしながら、その有効性の低さや副作用、及びすべての患者で薬理反応を引き出すことができないため、その臨床的有用性には重要な制限がある[8]。

現在の SCD の標準治療は、主にヒドロキシ尿素による治療や、水分補給、輸血、疼痛管理を含む支持療法である。重度の異常ヘモグロビン症の患者は、生存するために日常的な輸血が必要であり、生命を脅かす合併症である鉄過剰症を来すことがある[9]。近年、血管閉塞発作（VOC）の発生を減少させる L -グルタミン[10]及び抗 P セレクチン抗体であるクリザンリズマブ[11]をはじめ、ヘモグロビンに結合し HbS の酸素化状態をアロステリックに調節するボキセロートル（GBT-440）[12]が FDA によって承認されている。

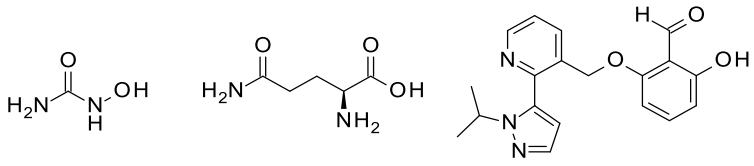


Figure 2. Chemical structures of FDA approval drugs for treating SCD.

また現在では、SCD を治療するための他のいくつかの化学療法剤が開発されている。例えば、DNA メチル化酵素阻害剤（5-アザシチジンやデシタビンなど）、ヒストン脱アセチル化酵素阻害剤（酪酸やパノビノスタットなど）、及び抗糖尿病薬（メトホルミンなど）が、前臨床研究でさまざまなメカニズムを介して HbF 産生を誘導するために開発されている[13]。しかしながら、これらの薬剤の多くは上記の疾患に対する治療効果についての概念実証をすでに示しているものの、安全性及び有効性に関しては未だ改善の余地がある。他の臨床的選択肢には、β-グロビン突然変異を是正するための遺伝子または細胞治療などの技術が挙げられるが、これらは依然として形成期にある[14]。したがって、様々なヘモグロビン症の治療のために、より効率的かつ低い毒性で HbF の産生を誘導することができる新しいタイプの薬剤を創出することが緊急に必要とされている。

筆者は、フェノタイプ及びターゲットベースドスクリーニングによる 2 つのアプローチから新規 β-サラセミア及び鎌状赤血球症治療薬の獲得を目指し、研究に着手した。フェノタイプスクリーニングは、細胞や動物モデル全体での表現型の変化を観察することで薬効を評価する手法であり、疾患に深く関連した細胞などのモデルを使って化合物のスクリーニングを行うため、初期の段階でヒットしてくる化合物が臨床試験に近い薬の候補になりうるという利点がある（Figure 3）。一方、ターゲットベースドスクリーニングは医薬標的（タンパク質など）と薬物の結合強度を構造最適化によって強めていく手法であり、標的の反応を利用したシンプルな評価系であるため簡便で誤差が小さい点や、標的のタンパク質の立体構造を見ながら化合物のデザインができる点が利点として挙げられる。筆者はこ

れら 2 つのアプローチによって創薬研究を行うことで、それぞれの利点を活かした合成展開が可能となると考えた。

第 1 章では、HTS ヒット化合物からフェノタイプスクリーニングにより合成展開を実施し、より剛性が高く、ユニークな構造を持つ 2-アザスピロ[3.3]ヘプタン誘導体の取得について述べる。第 2 章では、HbF 産生を誘導できる強力な G9a/GLP 阻害剤の取得を目指し、側鎖部の最適化による hERG 阻害活性と PK プロファイルを改善した化合物の取得について述べる。第 3 章では、さらなる構造最適化研究による DS79932728 の獲得とその薬理評価について述べる。

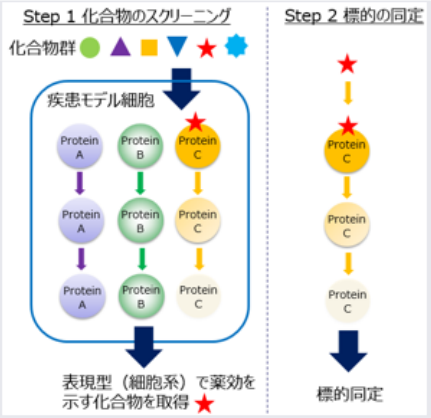
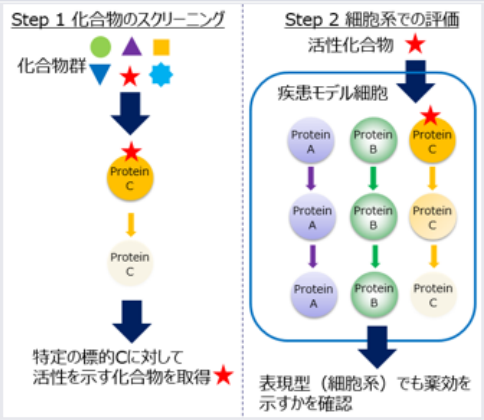
Category	表現型スクリーニング Phenotypic Screening	標的ベースのスクリーニング Target-based Screening
手法	細胞や動物モデル全体での表現型変化を観察することで、薬効評価する手法	医薬標的（タンパク質など）と薬物の結合強度を構造最適化によって強めていく手法
スクリーニングの流れ		
	Step1: 化合物が上の図のタンパク質A~Cのいずれかに作用することで、その疾患モデルに対して効果を出す化合物を取得 Step2: step1でヒットしてきた化合物がなぜ効果を出したのか、そのメカニズムを検証	Step1: 特定の疾患の1つの標的タンパク質に対して高活性を示す化合物を取得する Step2: step1でヒットしてきた化合物が非常に多くのタンパク質反応系が複雑に存在する細胞の中で、疾患に関連した現象(表現型)を示すかを検証
利点	・疾患に深く関連した細胞などのモデルを使って化合物のスクリーニングを行うため、初期の段階でヒットしてくる化合物が臨床試験に近い薬の候補になりうる	・特定の標的の反応を利用したシンプルな系を利用するので、評価は容易 ・標的のタンパク質立体構造を見ながら化合物のデザインができる（SBDDができる）

Figure 3. 表現型スクリーニング（Phenotypic Screening）と標的ベースのスクリーニング（Target-based Screening）の概要と利点

本論

第 1 章　フェノタイプスクリーニングによる *In vivo* で HbF 誘導効果を示すリード化合物の獲得

1-1　HTS ヒットの獲得

まず、血中曝露が良好な望ましい薬物動態学的（PK）プロファイル及び薬力学的（PD）特性を備えた HbF の新規誘導物質を取得するために、ヒトの赤芽球細胞株（HUDEP-2 細胞）[15] を用いたフェノタイプスクリーニングを社内化合物ライブラリー17,958 化合物に対して実施することとした。ヒト γ-グロビン特異的な抗体によるサンドイッチ αLISA を用いた評価系において、陽性対照であるデシタビン（0.5 μM）の活性を 1 とすると 0.25 以上の HbF 誘導活性を示す化合物（2.5 μM）をヒットと定義した。本スクリーニングにより 18 のヒット化合物を獲得し、各ヒット化合物を構造ごとに次の 7 つのカテゴリーに分類した（Figure 1-1）。

（1）ヌクレオシドアナログ（idoxuridine）
（2）既知のヒストンメチルトランスフェラーゼ阻害剤（UNC0638）
（3）9,10-ジオキソ-1 アントラキノン誘導体
（4）4-キノリルピロリジン誘導体
（5）キナゾリン誘導体
（6）2,3-ジヒドロピロロキノロン誘導体
（7）3-フェニル-イソキサゾール誘導体

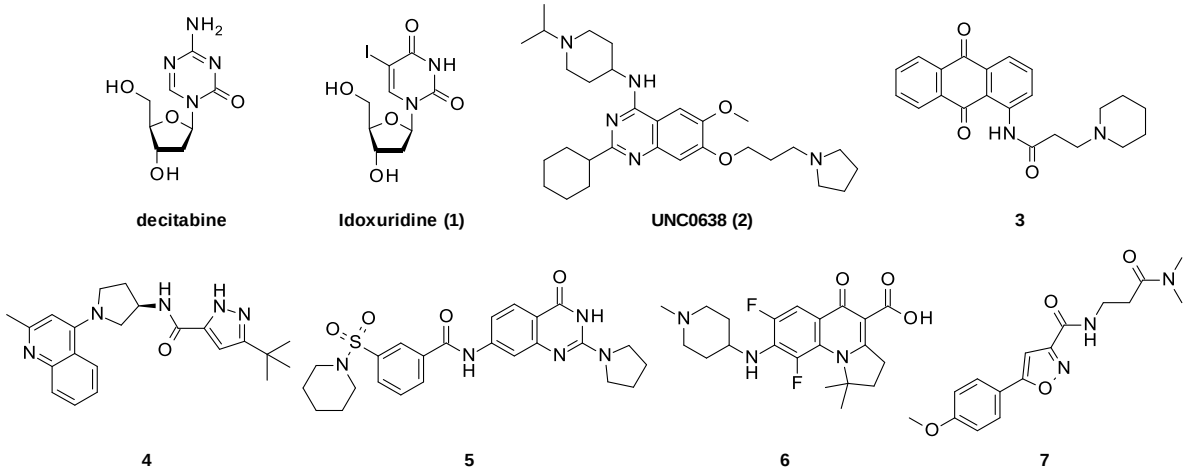
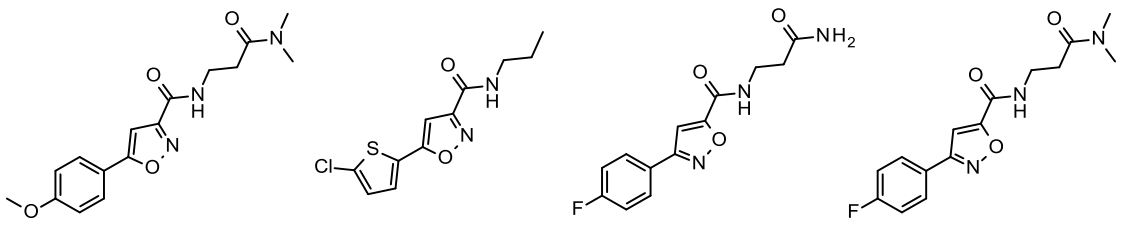


Figure 1-1. Chemical structures of hit compounds.

これら 7 つのカテゴリーの中より、合成展開を実施する誘導体を選抜することとした。（1）のヌクレオシドアナログ（idoxyuridine）は、陽性対照のデシタビンとは異なるウリジンタイプのヌクレオシドアナログである。その構造活性相関からデオキシタイプのヌクレオシドのみ活性を示すことがわかり、DNA への取り込みが作用機構と考え、遺伝毒性の観点から合成展開は実施しないこととした。（2）は、ヒストンメチルトランスフェラーゼ阻害剤（UNC0638）である（2、3 章においてこのターゲットの阻害剤開発について述べる）。

（3）は細胞毒性と HbF 誘導活性を解離できず、さらにアントラキノン構造は遺伝毒性の懸念を持つため、合成展開を実施しなかった。(4)、(5)、(6）は合成展開を実施したものの明確な構造活性相関の取得が困難であった（data not shown)。Figure 1-2 に示す（7）及びその周辺のヒット化合物 **8–10** は、HUDEP2 における HbF 誘導活性は弱いものの（E_{max} : 最も薬効が確認された濃度における陽性対照化合物デシタビンとの薬効比)、後に示すサル骨髄、ヒト CD34+細胞を用いた高次評価系で安定した HbF 誘導活性が確認されたため、合成展開を実施することとした。



7
EC_{50} >10 μM
E_{max} = 20%@10 μM

8
EC_{50} >10 μM
E_{max} = 15%@10 μM

9
EC_{50} >10 μM
E_{max} = 18%@2.0 μM

10
EC_{50} >10 μM
E_{max} = 24%@2.5 μM

Figure 1-2. Chemical structures of hit compounds **7**–**10**.

1-2　3-フェニル-イソキサゾール誘導体の合成展開

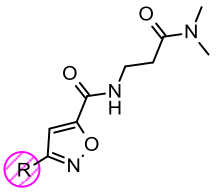
3-フェニル-イソキサゾール誘導体の合成展開を実施するにあたって評価系の見直しをしたところ、先述したヒット化合物 **7–10** は HUDEP2 に対する HbF 誘導活性がいずれも 10 μM より弱く、化合物の構造活性相関を取得するための評価系としては適さないと判断した。したがって、新たにヒト末梢血由来 CD34 赤芽球細胞（BM-CD34）[*1] を用いた評価系を構築し [16]、各化合物の HbF 誘導活性を評価することにした。

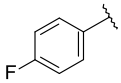
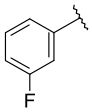
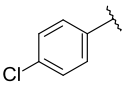
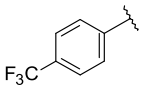
まず、ヒット化合物 **10** の左末端のフルオロフェニル部の変換を行った（Table 1-1)。無置換体 **13** は活性が向上したものの、*o*-フルオロ体 **11** 及び *m*-フルオロ体 **12** は活性が消失した。このことより、F 基の位置が活性に重要であることがわかった。また、化合物 **10** の F 基を Cl 基や CF_3 基、シクロヘキシル基に変換した化合物 **14–16** も同様に活性が消失した。これより、左部は sp^2 タイプであるフェニル基が活性を示すのに重要であり、さらに *p* 位の置換基の大きさも活性に影響を与えることがわかった。

*1

ヒト骨髄由来 CD34 陽性細胞を 1×10^4 cells/mL に調製後、37 °C、5% CO_2 インキュベータ内で 7 日間培養することにより前赤芽球へ誘導し、被験化合物及びデシタビンを 10 μL/well にて添加した。化合物添加培養 5 日後、細胞を回収し、FITC 標識 Fetal Hemoglobin Monoclonal Antibody (HBF-1)で染色して γ-globin 陽性率をフローサイトメーター（MACSQuant）にて解析した。陽性対照化合物であるデシタビンは EC_{50}=0.073 μM を示し、SN 比も高い値を示したため、化合物の HbF 誘導活性を確認する評価系が構築できたと判断した。

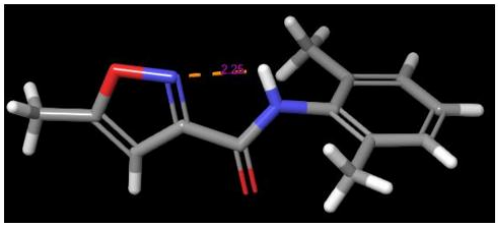
Table 1-1. SAR of substituents at the 3-position of the isoxazole ring.

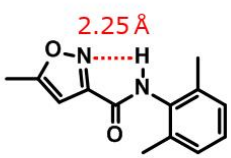


Compound	R	BM-CD34 EC_{50} (μM)[a]	Emax (%)[b]	Metabolic stability[c] human/monkey (%)
10		2.8	56	87/10
11		>5	-	>100/4
12		>5	-	>100/55
13		1.7	51	81/4.7
14		>5	-	83/3.8
15		>5	-	95/16
16		>5	-	>100/34

[a] Compound concentration that induces HbF by 50% if the maximum induction of HbF by decitabine is taken as 100%.
[b] Maximum effect of the compound as a percentage of that of decitabine. Human BM-CD34$^+$ cells were used in the assay.
[c] Remaining percentage of test compounds after 0.5 h incubation with human/monkey liver microsomes (0.5 mg/mL).

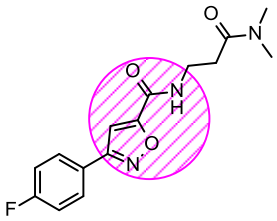
*2

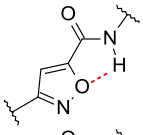
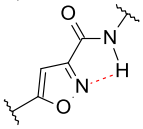
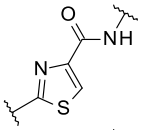
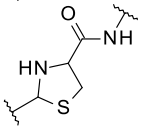
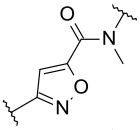
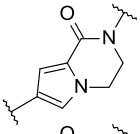
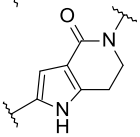
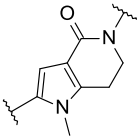




化合物 **10** 及び **17** の類縁体である上記のアシルイソキサゾール体の単結晶構造（CCDC: 1171030）[17] において、アミドの N-H とイソキサゾールの窒素原子間の距離は 2.25 Å となり、分子内水素結合を形成している可能性が示唆された。

Table 1-2. SAR of acyl isoxazole moieties.



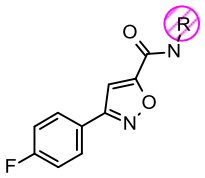
Compound	R	BM-CD34 EC_{50} (μM)[a]	Emax (%)[b]	Metabolic stability[c] human/monkey (%)
10		2.8	56	87/10
17		2.6	53	77/17
18		>5	-	53/0.1
19[d]		>5	-	90/32
20		>5	-	98/36
21		>5	-	94/41
22		>5	-	99/39
23		>5	-	>100/42

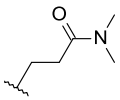
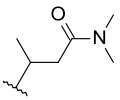
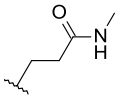
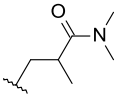
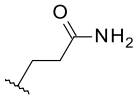
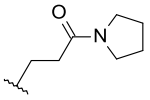
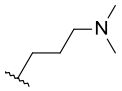
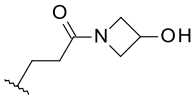
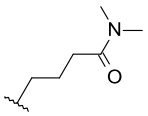
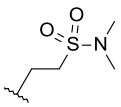
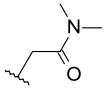
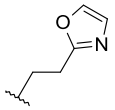
[a] Compound concentration that induces HbF by 50% if the maximum induction of HbF by decitabine is taken as 100%.
[b] Maximum effect of the compound as a percentage of that of decitabine. Human BM-CD34$^+$ cells were used in the assay.
[c] Remaining percentage of test compounds after 0.5 h incubation with human/monkey liver microsomes (0.5 mg/mL).
[d] Diastereomer mixture.

次に、ヒット化合物 **10** の中心部（アシルイソキサゾール部）の変換を行った（Table 1-2）。まず、イソキサゾールの位置異性体 **7**、**8**（Figure 1-1）が活性を示した結果を踏まえて、イソキサゾールの位置置換体 **17** を合成・評価したところ、活性は化合物 **10** よりわずかに向上したが、E_{max} は減弱した。続いて、イソキサゾール環をチアゾール環に変換した化合物 **18**、チアゾリジン環に変換した化合物 **19** は、活性が消失した。また、化合物 **10** のアミ

ド部を N-メチル化（**20**）したところ、活性は消失した。これらの結果より、化合物 **10** 及び **17** は中心部のアミドの N-H とイソキサゾールとの間に分子内水素結合を形成し（Table 1-2 参照）[*2]、コンフォメーションを固定化している可能性が示唆された。これらの結果に基づいて、縮合環誘導体 **21–23** を設計・合成したが、これらの化合物はいずれも活性を示さなかった。

Table 1-3. SAR of dimethylamide and linker moieties.



Compound	R	BM-CD34 EC_{50} (μM)[a]	Emax (%)[b]	Metabolic stability[c] human/mon (%)	Compound	R	BM-CD34 EC_{50} (μM)[a]	Emax (%)[b]	Metabolic stability[c] human/mon (%)
10		2.8	56	87/10	**29**[d]		>5	-	91/35
24		2.6	89	>100/59	**30**[d]		>5	-	61/52
25		N.D.[e]	-	88/60	**31**		1.7	50	80/1.6
26		>5	-	N.T.[f]	**32**		1.7	69	>100/49
27		4.1	55	N.T.[f]	**33**		4.2	65	91/47
28		6.1	41	84/11	**34**		>5	39	87/25

[a] Compound concentration that induces HbF by 50% if the maximum induction of HbF by decitabine is taken as 100%.
[b] Maximum effect of the compound as a percentage of that of decitabine. Human BM-CD34$^+$ cells were used in the assay.
[c] Remaining percentage of test compounds after 0.5 h incubation with human/monkey liver microsomes (0.5 mg/mL).
[d] Racemate.
[e] Not ditected.
[f] Not tested.

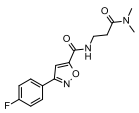
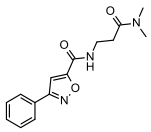
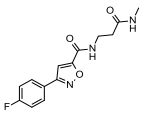
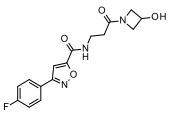
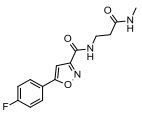
続いて、ヒット化合物 **10** のメチレン鎖及びジメチルアミド基の変換を行った（Table 1-3）。化合物 **10** のジメチルアミド基をモノメチルアミド基に変換した化合物 **24** はわずかに活性が向上した。一方、カルバモイル誘導体 **25** は低い溶解度を示し、活性を検出することができなかった。また、ジメチルアミド基をジメチルアミノ基 **26** に変換すると活性が低下し、アミド部分が活性に重要であることが示唆された。アルキル鎖の一炭素原子延長した化合物 **27** 及び短縮した化合物 **28** は、化合物 **10** より活性は低下したものの、なおも IC_{50} 値は 1 桁の μM の活性を示した。イソキサゾール部分とジメチルアミド部分の間のリンカ

ーにメチル基を導入した化合物 **29**、**30** は、活性が低下した。この結果により、メチレン鎖部周辺は活性を示すスペースが狭く、あまり大きい置換基は許容されないことが明らかとなった。次に、ジメチルアミドを環化させたシクロペンチル体 **31** は活性が向上したが、サル肝ミクロソームにおける代謝安定性が悪化した。そこで代謝部位と考えられる環状部に水酸基を導入したアゼチジン体 **32** は代謝安定性が回復し、活性も保持した。ジメチルアミド部をジメチルスルホンアミド **33** 及びオキサゾール **34** に変換したところ、活性が減弱した。以上、*in vitro* 活性及び代謝安定性の結果より、化合物 **10**、**13**、**24**、**32** そして化合物 **24** の位置異性体である **35** をリード化合物として選抜し、ADMET の評価及びサルの PK 試験を実施することとした。

1-3　代表化合物の初期 ADMET と PK プロファイル

先に選抜した化合物の ADMET プロファイル及び PK プロファイルを Table 1-4 に示す。

Table 1-4. Chemical structures, activity, ADMET profiles, and PK profiles of isoxazole derivatives.

Compound	**10**	**13**	**24**	**32**	**35**
Structure					
BM-CD34 EC_{50}	2.8 μM	1.7 μM	2.6 μM	1.7 μM	4.4 μM
E_{max}	56%	51%	89%	69%	51%
MW (g/mol)	305	287	291	333	291
LCLog P	3.7	3.3	3.1	2.5	3.2
CYP(1A2/2C9/2D6/3A4)[a]	6/6/5/<0	N.T.[e]	<0/5/0/<0	N.T.[e]	N.T.[e]
Solubility[b](μg/mL) (JP1/JP2)	>100/>100	>100/>100	44/44	>100/>100	26/22
hERG inhibition (1/10 μM)	1.3%/7.1%	N.T.[e]	2.6%/8.7%	2.4%/7.2%	N.T.[e]
Plasma protein binding (monkey)	58%	55%	61%	56%	59%
PK parameters (monkey: 1 mg/kg, p.o.)					
C_{max} (ng/mL)	1362[c]	245	3845[d]	626	460
AUC_{last} (ng*h/mL)	4365[c]	365	30026[d]	2037	2354

[a] % Direct inhibition of CYP at 10 μM.

[b] JP1/JP2: Japanese Pharmacopoeia first/second test fluid (pH = 1.2/6.8)

[c] 10 mg/kg, p.o.

[d] 5 mg/kg, p.o.

[e] Not tested.

いずれの化合物も分子量が 300 g/mol 前後、Log P は 2.5–3.7、HBD は 5 以下、HBA は 10 以下となり、経口バイオアベイラビリティの優れた薬物を予測するための経験則（Lipinski の法則）である「Rule of five」[18] を満たした。溶解度は、ジメチル体 **10**、**13** やヒドロキシアゼチジン体 **32** は良好であったが、モノメチル体 **17**、**35** は中程度であった。hERG 阻害活性 [19] 及びシトクロム P450（CYP）阻害活性については、評価した化合物は 10 μM においてほとんど示さなかった。これは、化合物の脂溶性は低く、塩基性置換基を持たないためだと考えられる。また、これらの化合物は、サル血漿タンパク質に中程度に結合した（55–61%）。

PK 試験では、まず化合物 **10**、**13** は血中曝露量（AUC_{last}）が小さく、これは代謝安定性が悪いためだと考えられる。化合物 **32** も血中曝露量が中程度となったが、これは水酸基を導入したことで脂溶性が下がり、膜透過性が低下したことにより血中に吸収されにくくなってしまったと考えられる。化合物 **24** と位置異性体 **35** を投与量ベースで比較すると、化合物 **24** の方が良好な血中曝露量を示した。以上より、化合物 **24** を代表化合物として選抜し、mRNA レベルでの HbF 誘導評価、標的探索、毒性 *in vitro* 細胞毒性試験や *in vivo* 試験を実施することとした。

1-4　mRNA レベルの *in vitro* 評価

HUDEP2 細胞を用いて mRNA レベルでの γ-グロビン及び β-グロビンの発現変動を測定することにより、HbF 誘導活性のある化合物 **24** が遺伝子レベルでグロビンスイッチングが起きているかを確認した（Figure 1-3）。

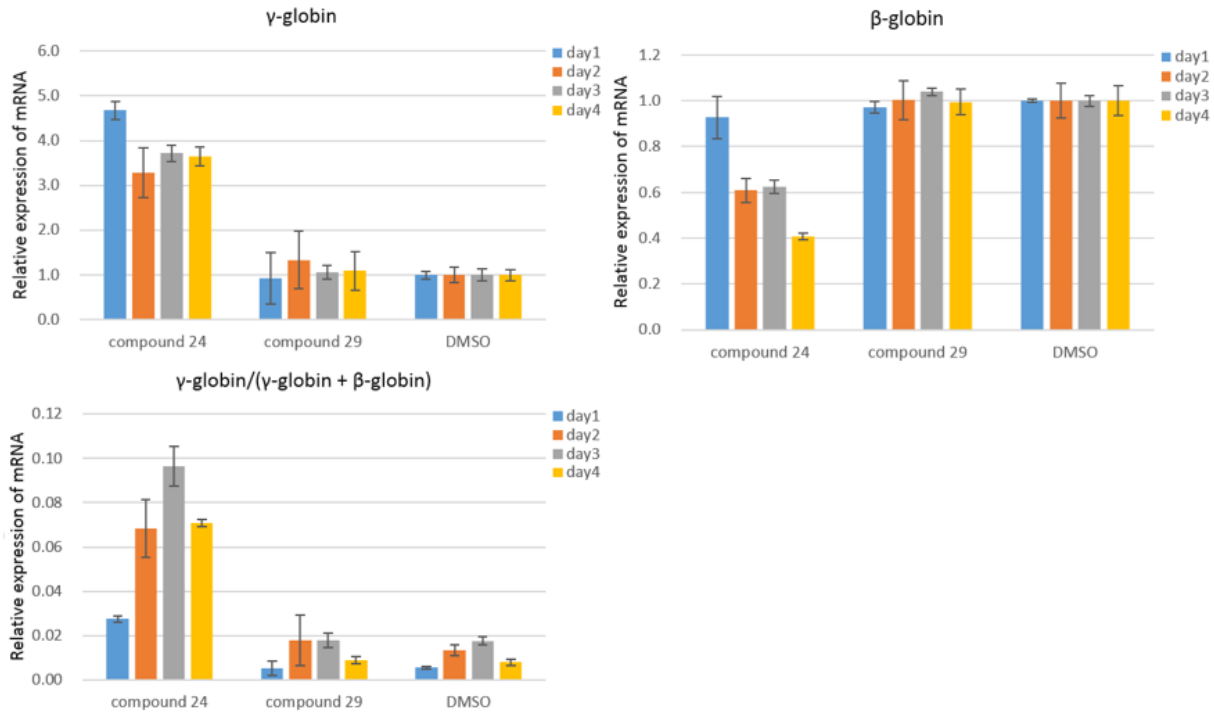


Figure 1-3. Relative expression of γ-globin mRNA induced by compounds **24** and **29** in HUDEP2 cell. Cells were harvested 1, 2, 3, and 4 d after exposure to compounds and subjected to analysis. Data, obtained independently and in triplicate, are shown as mean ± standard deviation.

HbF 誘導活性のある化合物 **24** は、γ-グロビンの mRNA の発現の増加と β-グロビンの減少が確認されたが、HbF 誘導活性のない化合物 **29** では γ-グロビン、β-グロビンともにコントロールである DMSO 添加群との差が見られなかった。以上より、タンパク質レベルで HbF 誘導活性が確認されている化合物 **24** において、mRNA レベルでも γ-グロビン発現へのグロビンスイッチングが確認された。

次に、HUDEP2 細胞で見られた mRNA レベルでグロビンスイッチングが高次の細胞であるヒト CD34+細胞でも見られるかを確認した（Figure 1-4）。ヒト CD34+細胞を赤芽球誘導培地で 1 週間培養後に、化合物をそれぞれ終濃度 5 μM で添加した。化合物 **24** において、グロビンにおける γ-グロビンの割合（γ/(γ+β)）は増加し、HbF 誘導活性のない化合物 **29** との差が明確に見られた。一方、γ-グロビン単独及び β-グロビン単独の変動は、ばらつきが大きかった。CD34+細胞におけるこれら mRNA 発現量比のばらつきは、化合物添加の影響等により細胞の分化速度や細胞数などに差異が生じ、株化細胞に比べ細胞の均一性が低くなったことによると考えられた。例数の増加やタイムコースなどより詳細な検討を行うことにより、これらのばらつきは軽減されると期待される。

以上より、γ-グロビン、β-グロビンの遺伝子発現変動を測定した結果、化合物 **24** はヒト赤芽球前駆細胞（CD34+細胞、HUDEP2）において γ-グロビン mRNA の増加と β-グロビン mRNA の減少を誘導し、mRNA レベルで γ-グロビン発現へのグロビンスイッチングを確認することができた。

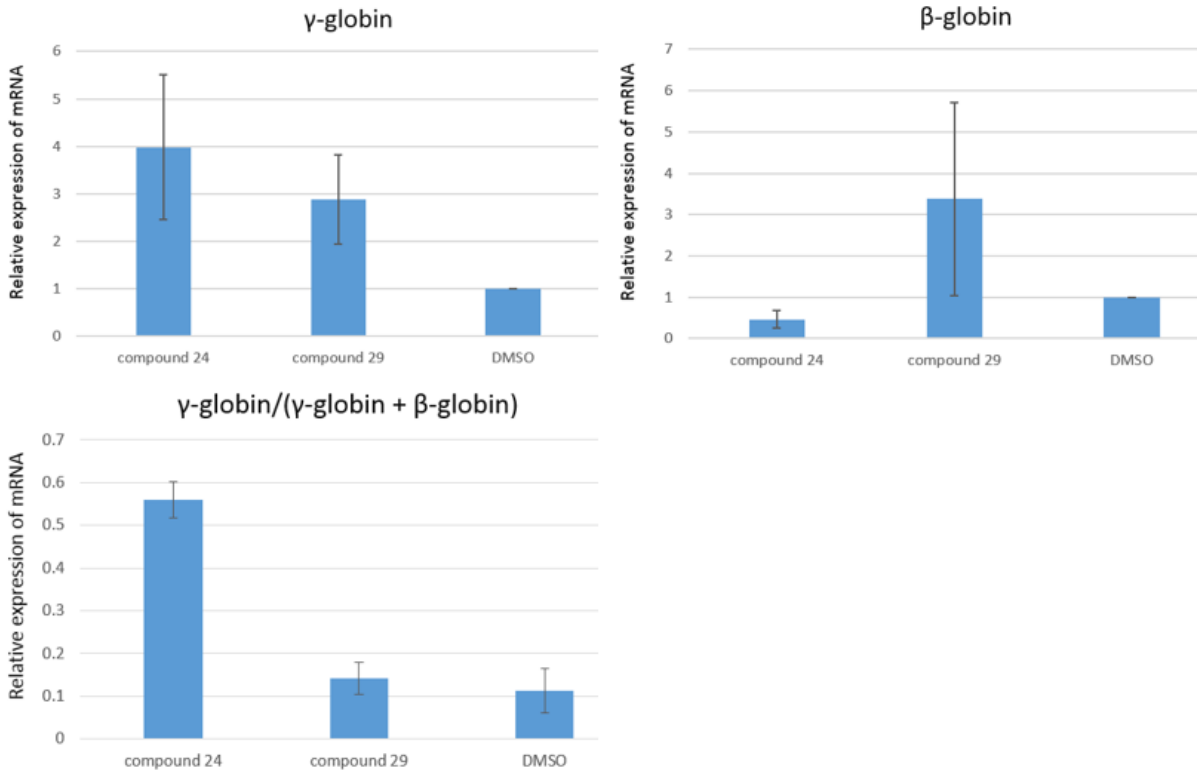


Figure 1-4. Relative mRNA expression analysis of γ- or β-globin gene in human CD34+ cell.

1-5　化合物 24 を用いた標的探索①（エピジェネティクスな作用）

グロビン遺伝子発現の制御は主として 2 つの機序があり、エピジェネティクス（クロマチン調節因子）な作用と DNA 結合転写因子の作用があると考えられている [13]。本節では、まず化合物 **24** のエピジェネティクス関連酵素に対する阻害作用を確認することとした。

一般に、HDAC、LSD-1、ヒストンメチルトランスフェラーゼ及び DNA メチルトランスフェラーゼ阻害剤などのエピジェネティクス酵素阻害剤は、*in vitro* 及び *in vivo* で HbF を誘導することが知られている [20]。そこでまず、3-フェニル-イソキサゾール誘導体の HbF 発現誘導に関する作用機序を探るために、エピジェネティクス酵素阻害パネルアッセイを実施した。HbF 誘導活性体 **24** 及び不活性体 **29** の 2 化合物について n = 2, 10 μM で以下のパネルに対する阻害活性をセルフリーの系で評価した（各パネルの詳細は実験項を参照）。

DMT demethylase	14 種
DNMT DNA Methyl transferase	4 種
HAT Histone acethyltrasferase	9 種
HDAC Histone Deacetylase	16 種
HMT Histone methyltransferase	28 種
Histone 関連 kinase	10 種
DNA repair 関連	3 種
Ubiquitin 修飾酵素	6 種
SMMT　small molecule methyltransferase	3 種（カウンターとして）

合計 93 パネル

上記の一連のパネルアッセイで、化合物 **24** が 20%以上の阻害活性を示す酵素はなかった。したがって、本パネルアッセイの結果からは、本系統化合物のエピジェネティクス関連酵素に対する阻害作用は確認されず、本系統化合物は DNA とヒストン（クロマチン構造）の直接修飾には関与していないことが示唆された。

1-6　化合物 24 を用いた標的探索②（DNA 結合転写因子の作用）

次に、グロビン遺伝子発現の制御のもう一つの機序である、DNA 結合転写因子の作用を確認することとした。

β-サラセミア及び SCD におけるゲノムワイド関連解析及び遺伝子解析からの数多くの知見により、転写因子 BCL11a、KLF、LRF は独立して γ-グロビンの発現を抑制し [21]、LIN28b はその発現を増加させるとする論文が多数報告されている（Figure 1-5A）[22]。例えば、*bcl11a* 及び *lrf* の発現を調整またはノックダウンすることにより、マウスモデルにおいて *in vitro* と同様に赤芽球細胞株においても HbF の発現が再活性化されたことが最近明らかとなっている [23]。また、シンバスタチンなどのあるスタチン系薬剤は、KLF1 及び BCL11a を抑制することによって、ヒト赤血球細胞の HbF をアップレギュレートすることが報告されている [24]。現在までに同定された HbF 産生に関与するこれら転写因子の中で、BCL11a が最も強力なリプレッサーとして知られており、BCL11a の研究が盛んに行われている。例えばごく最近、BCL11a の γ-グロビンの発現を抑制の詳細なメカニズムが報告されており [25]、成

人の赤血球細胞では BCL11a は立体障害を介して NF-Y の結合を妨げ、γ-グロビンの発現を抑制するが、胎児期の赤血球細胞では NF-Y が γ-グロビンプロモーターに結合して発現を活性化していることが明らかとされている。

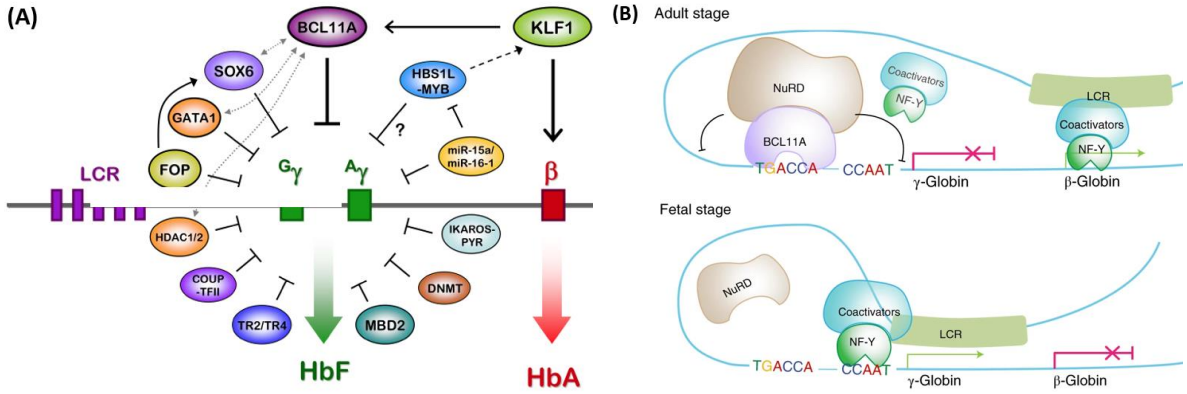


Figure 1-5. (A) Transcription factors and epigenetic factors involved in the suppression of γ-globin expression.[26] (B) Competitive binding between NF-Y and BCL11A controls hemoglobin switching.[25]

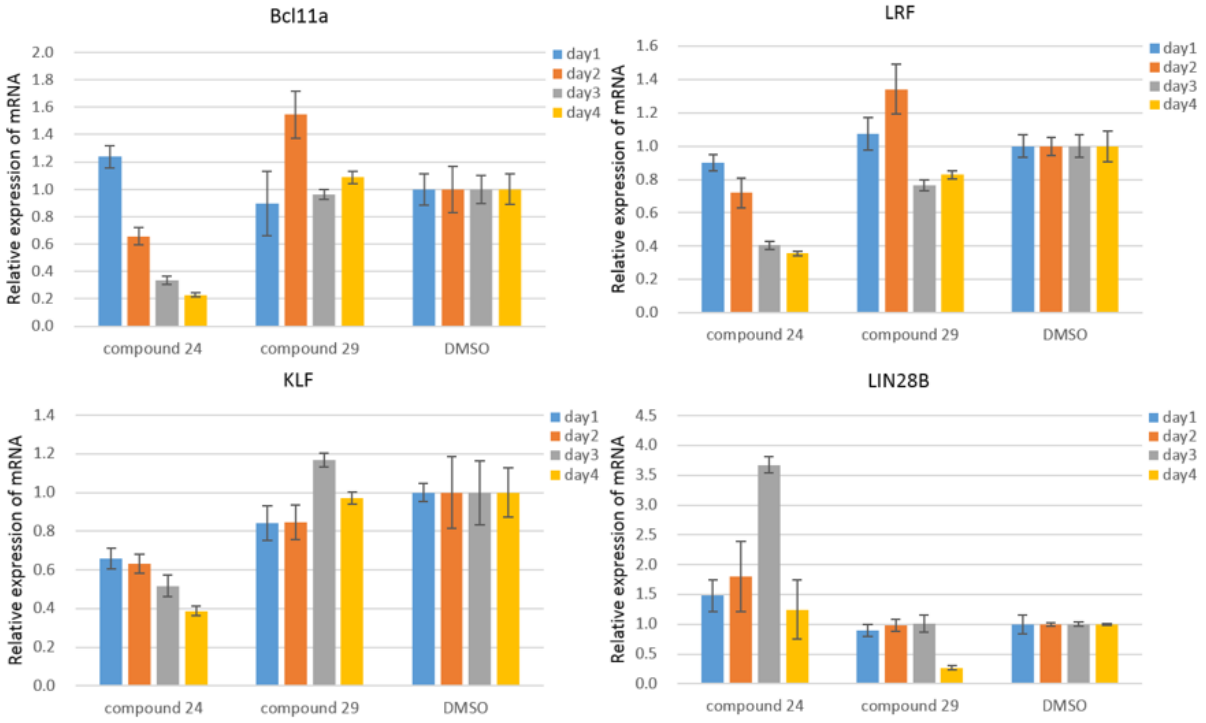


Figure 1-6. Relative mRNA expression analysis of transcriptional factors related to HbF induction in HUDEP2 cell.

そこで、化合物 **24** が上記の転写因子を調節することによって HbF を誘導するのか否かを調べた（Figure 1-6）。HUDEP-2 を化合物 **24**（終濃度 5 μM）で処理した後、1、2、3、及び 4 日目に遠心により細胞を回収し、RNeasy マイクロキットを用いて RNA 抽出を行い、

NanoDrop を用いて RNA 濃度を測定した。SuperScript VILO を用いて、42 °C で 1 時間、逆転写反応を行った。得られた cDNA に対して 7900HT Fast Real-Time PCR System（Applied Biosystems）を用いて qPCR を行った。発現量は⊿⊿Ct 法により、ACTB を内部標準遺伝子として補正を行い、各培養日における DMSO 添加群に対する相対的発現量を求めた。化合物 **24** は、*bcl11a*、*klf* 及び *lrf* の発現をそれぞれ減少させたが、HbF 誘導活性を有さないネガティブコントロールの化合物 **29** は、それらの発現に影響しなかった。また、HbF 発現の正の調節因子である *lin28b* は、化合物 **24** によってアップレギュレートされたが、化合物 **29** はアップレギュレートしなかった。これらの結果より、化合物 **24** のグロビンスイッチングは、これら転写因子の発現量の制御を介して調節していることが示唆された。

1-7　化合物 24 の *in vitro* 細胞毒性評価

骨髄抑制とは、骨髄の機能が低下して血液細胞の生産能力が減退する現象である。骨髄における造血はコロニー形成能を有する未熟な造血細胞によって担われているが、骨髄が抑制されるとコロニー形成能が低下する。したがって、化合物のコロニー形成能への影響を測定することで骨髄を抑制するか否かを評価できると考えた。未熟な造血細胞のほとんどは CD34 抗原を発現していることから、骨髄由来 CD34+細胞を使用してそのコロニー形成能に及ぼす化合物 **24** の影響を検討した（Figure 1-7）。

化合物 **24** は、15 μM までほとんど増殖抑制が認められなかったが、30 μM においてコントロールと比較して約 20%の増殖抑制が認められた。一方、ポジティブコントロールのデシタビンでは、濃度依存的な骨髄抑制作用が認められ、20%抑制率での濃度は 0.016 μM であり、自社化合物に比べ顕著な骨髄抑制作用を示した。

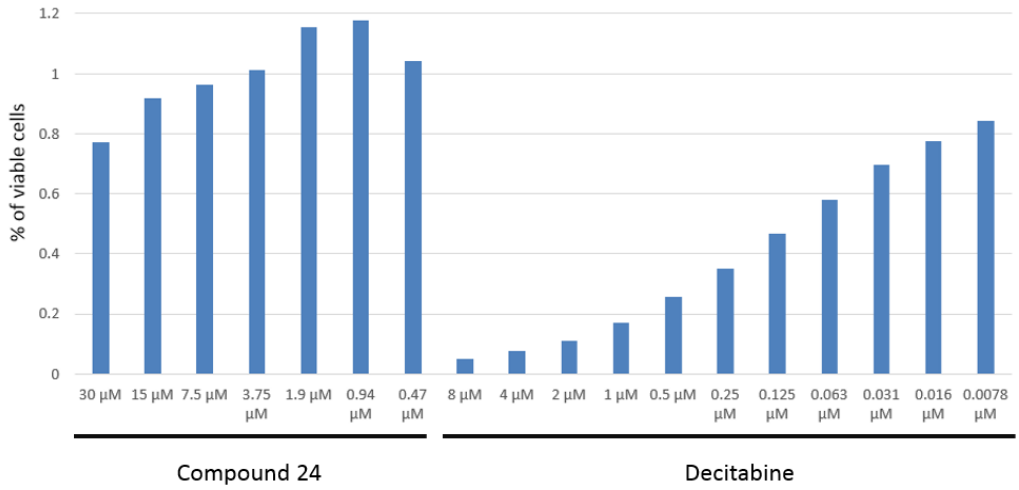


Figure 1-7. Evaluation of myelosuppressive effects of compound **24** and decitabine.

また、デシタビン及び化合物 **24** の HbF 誘導活性と CD34+細胞増殖抑制作用のプロットした図を Figure 1-8 に示す。デシタビンの臨床有効血中濃度は C_{max} = 285–338 nM と知られており [27]、その濃度における HbF 誘導活性は 70–75%を示したが同時に骨髄毒性も 40%程度確認された。一方、化合物 **24** は、HbF 誘導活性を 70–75%示す濃度はおおよそ 1–2 μM

であり、その濃度において骨髄毒性は示さなかった。また、HbF 誘導活性を 70–75%示す濃度の 10 倍の濃度である 10–20 μM においても骨髄毒性は 10%程度と依然低い値を示した。以上より、化合物 **24** の活性発現濃度と細胞抑制発現濃度のマージンはデシタビンに比べて非常に広かったことがわかった。

次に、化合物 **24** を用いて Ames 試験及び *in vitro* 小核（IVtMN）試験を実施したところ、どちらも陰性だった（data not shown）。したがって、化合物 **24** は遺伝毒性の懸念が小さく、ヒドロキシ尿素やデシタビンよりも安全性が高いことが期待できる [28]。

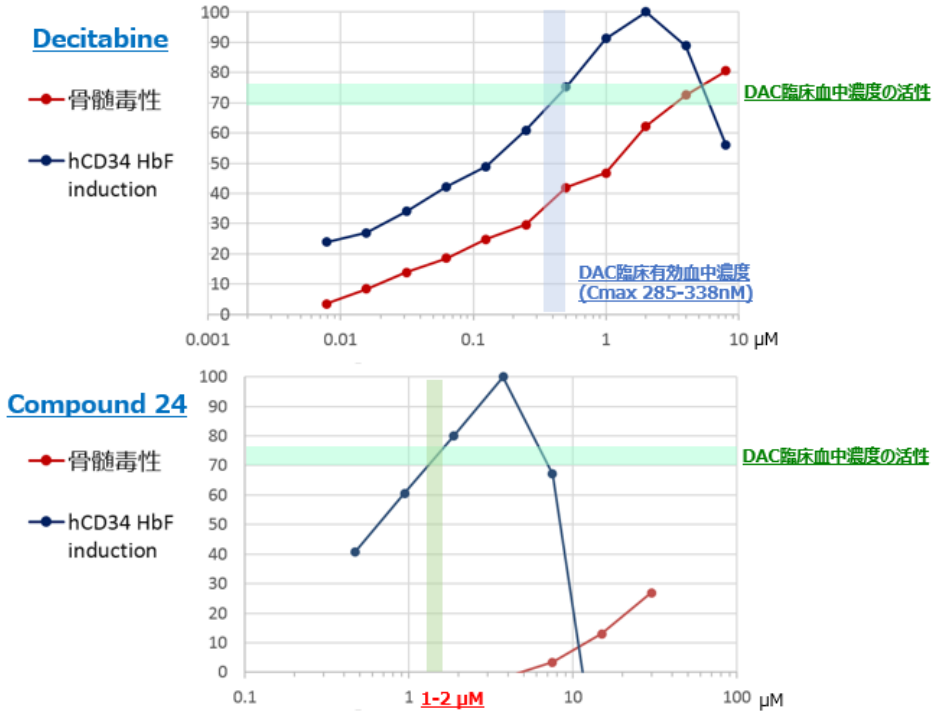


Figure 1-8. Relationship between the active expression concentration of decitabine and compound **24** and the myelosuppressive expression concentration.

1-8　化合物 24 の *in vivo* 評価

カニクイザルモデルを用いて、化合物 **24** を経口投与した場合に骨髄に蓄積された赤芽球において HbF 発現を誘導できるか否かを確認することとした。

デシタビンは、非ヒト霊長類において経口投与によって HbF を増強することが報告されているため [29]、化合物 **24** の評価に先立ってデシタビンを投与した実験群を陽性対照として評価系の構築を検証した（Figure 1-9）。骨髄全血細胞（Bone Marrow Cell：BMC）より抽出した RNA を用いて qPCR し、Hb の γ-グロビンと β-グロビンの mRNA 発現量の比率（γ/β）を活性の指標として解析した。1 試験目では、デシタビンを 5 mg/kg、10 mg/kg（いずれも 1 日 1 回投与）で実施したところ、用量依存的に γ/β の増加を確認した。また、1 試験目（Figure 1-9a）及び 3 試験目（Figure 1-9c）では、vehicle 群と比較して有意差は認められなかったが、2 試験目（Figure 1-9b）では vehicle 群と比較して有意差が認められた。なお、γ-グロビン mRNA 及び β-グロビン mRNA の基礎発現は各個体で異なるため、vehicle 群平均値で補正した数値を算出し、Means ± SD で表記した（Table 1-5：1 試験目は n = 2 のため

SD 表記なし)。デシタビンは、投与量の増加に対応して HbF 誘導活性が亢進する傾向が認められた。以上より、デシタビンの用量依存的な作用が認められたため、次に本実験条件で化合物 **24** を評価することとした。

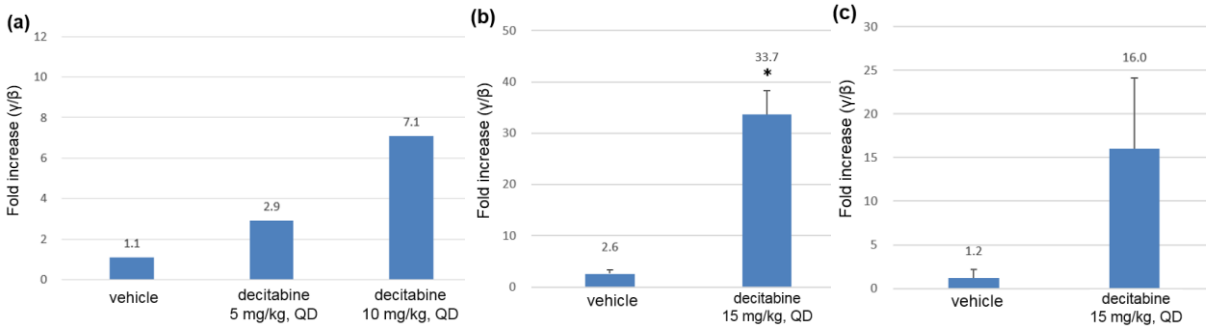


Figure 1-9. Induction of HbF by decitabine in normal cynomolgus monkeys. (a) Cynomolgus monkeys were administered decitabine (5 or 10 mg/kg, once a day) or vehicle orally from Day 0 to Day 6. (n = 2) (b), (c) Cynomolgus monkeys were administered decitabine (15 mg/kg, once a day) or vehicle orally from Day 0 to Day 6. Data show the mean (+SD) fold increase in the ratio of Hb γ-/β-subunit mRNA expression (n = 3; *p < 0.05, Dunnett's test).

Table 1-5. Fold change value of γ-globin against β-globin gene relative to vehicle control.

decitabine	vehicle	5 mg/kg QD	10 mg/kg QD	15 mg/kd QD
(a) 1試験目	1.0	2.6	6.4	
(b) 2試験目	1.0 ± 0.8			12.8 ± 4.6
(c) 3試験目	1.0 ± 1.0			13.2 ± 8.1

Gene expression of γ-globin and β-globin were determined in erythroblasts from cynomolgus monkey bone marrow. Each unit is calculated by using following formula and compared with vehicle control; γ-globin /β-globin = (γ-globin gene expression at day 6/γ-globin gene expression at day 0)/(β-globin gene expression at day 6/β-globin gene expression at day 0).

化合物 **24** については、まず 7.5、15 mg/kg（いずれも 1 日 1 回投与）で経口投与による HbF 誘導活性を評価した（Figure 1-10a)。化合物 **24** は、期待通り用量依存的に γ-グロビン発現を誘導し、15 mg/kg 投与群で有意な γ/β 比の上昇が認められた（*p < 0.05, Dunnett 試験)。また、試験期間中いずれのサルにおいても体重変化は認められなかった。血液学的検査では、15 mg/kg 群において網状赤血球数の減少が認められたものの、赤血球数、平均赤血球容積、平均赤血球ヘモグロビン量、平均赤血球ヘモグロビン濃度、血中ヘモグロビン濃度、ヘマトクリット値、血小板数、白血球数には大きな変化は認められなかった。

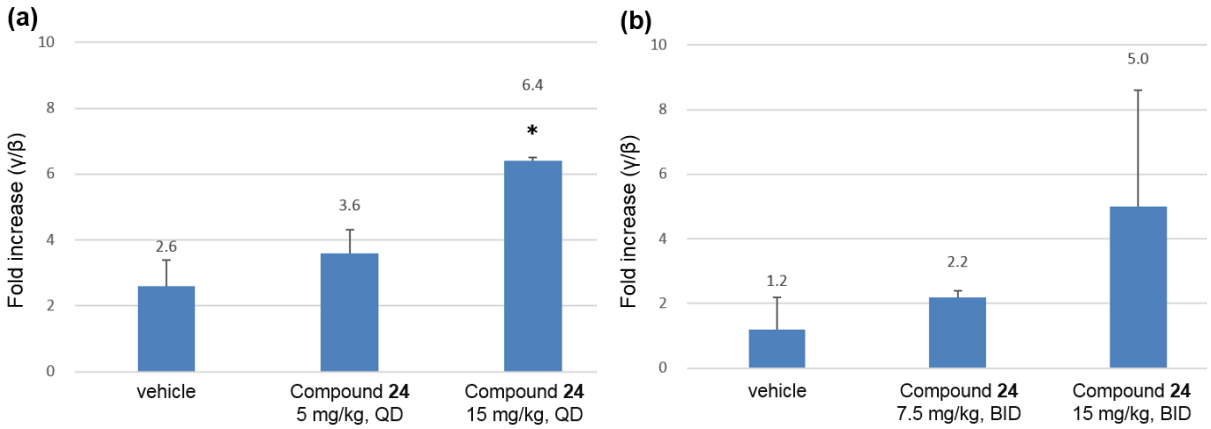


Figure 1-10. Induction of HbF by compound **24** in normal cynomolgus monkeys. (a) Cynomolgus monkeys were administered compound **24** (5 or 15 mg/kg, once a day) or vehicle orally from Day 0 to Day 6. (b) Cynomolgus monkeys were administered compound **24** (7.5 or 15 mg/kg, twice a day) or vehicle orally from Day 0 to Day 6. Data show the mean (+SD) fold increase in the ratio of Hb γ-/β-subunit mRNA expression (n = 3; *p < 0.05, Dunnett's test).

Table 1-6. Fold change value of γ-globin against β-globin gene relative to vehicle control.

compound 24	vehicle	5 mg/kg QD	10 mg/kg QD	7.5 mg/kd BID	15 mg/kg BID
(a) 1試験目	1.0 ± 0.8	1.4 ± 0.7	2.4 ± 0.1		
(b) 2試験目	1.0 ± 1.0			1.8 ± 0.2	4.2 ± 3.6

次に、先の *in vivo* 評価結果を化合物 **24** の PK データを用いて PK/PD の考察を行うこととした（Figure 1-11）。化合物 **24** の血漿中タンパク結合率（61%）を考慮すると、フリー体の EC_{50} は 6.6 μM となり、5 mg/kg の場合では C_{max} でも EC_{50} に届かないことがわかった。したがって、5 mg/kg では十分な血中曝露量を示さなかったために有意差を持って薬効を示さなかったと考えられる。一方、15 mg/kg では、フリー体の EC_{50} = 6.6 μM 以上の血中化合物濃度を 8 時間近く持続することがわかる。したがって、15 mg/kg では薬効に有意差が認められたと考えられる。以上より、次に明確な薬効と用量依存性を確認すべく、7.5 mg/kg、15 mg/kg の 2 用量にて、1 日 2 回投与で *in vivo* 試験を実施することとした。

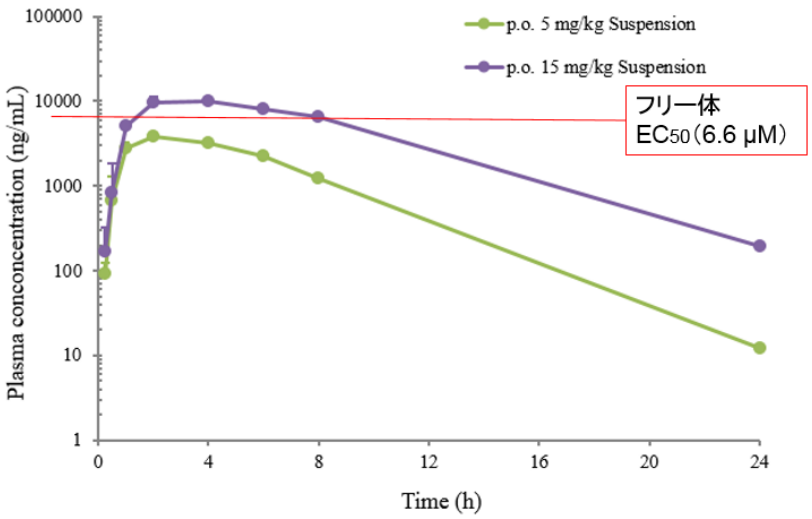


Figure 1-11. PK data of compound **24** and EC_{50} value in free form.

1 日当たりの投与回数を 1 回から 2 回に増加させた第 2 の実験では、用量依存的に γ-グロビン mRNA 発現の増加が観察され、15 mg/kg、1 日 2 回投与群が平均値としては高値であった（Figure 1-10b）。しかしながら、サルの 3 匹中 1 匹で化合物の薬理反応が認められなかったため（それぞれ個体の γ/β は 9.6, 4.6, 0.9）、有意差は認められなかった。なお、本試験では、先と同様に毒性面では全ての群で血液パラメーターの有意な変動は見られなかった。

解析結果の検証を行ったところ、化合物 **24** の 15 mg/kg、1 日 2 回投与群において最終日の最終投与後 12 時間での血漿中濃度が単回の PK データより算出したシミュレーションの計算値より約 1/10 低いことが判明した（Figure 1-12）。どのタイミングからシミュレーション通りの血中曝露ができていないかは不明であるが、HbF 誘導活性の強度が試験前の想定よりも低かった要因は、化合物曝露が十分でなかったためだと考えられる。この原因としては、①連投による代謝酵素誘導が起こり、代謝速度が速くなり血漿中濃度の低下につながった可能性、②8 時間と 24 時間の血漿中濃度推移の見積りが適切でなかった（その間の濃度データがより正確な PK 把握に必要だった）可能性、③化合物 **24** の PK データと曝露データロットが異なるので、ロット差の可能性が考えられる。

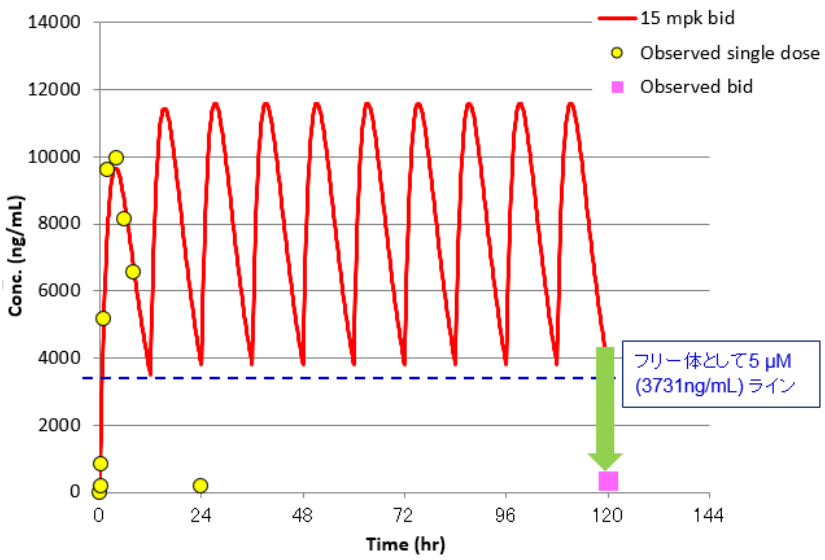


Figure 1-12. Simulation of 15 mg/kg (bid: red line) of compound **24** and measured value 12 hours after final administration (pink)

1-9　2-アザスピロ[3.3]ヘプタン誘導体の合成展開

次に、先の化合物 **24** の *in vivo* 試験の結果を踏まえて、活性を保持したまま PK 試験で持続的な曝露を示す化合物の取得を目指すこととした。

本系統の ADME 及び PK プロファイルを確認すると、サルにおける代謝安定性が悪く、PK においても半減期が短いという課題があった。この原因として、ジメチル部が CYP による代謝により脱メチル化されてしまった点や、このアミド部がアミラーゼやエステラーゼによって加水分解されてしまった可能性が示唆された。また、先の SAR 情報より、リンカー部周辺は活性を示すスペースが狭いことがわかっているので、2-アザスピロ[3.3]ヘプタン誘導体を設計及び合成することとした（Figure 1-13）。

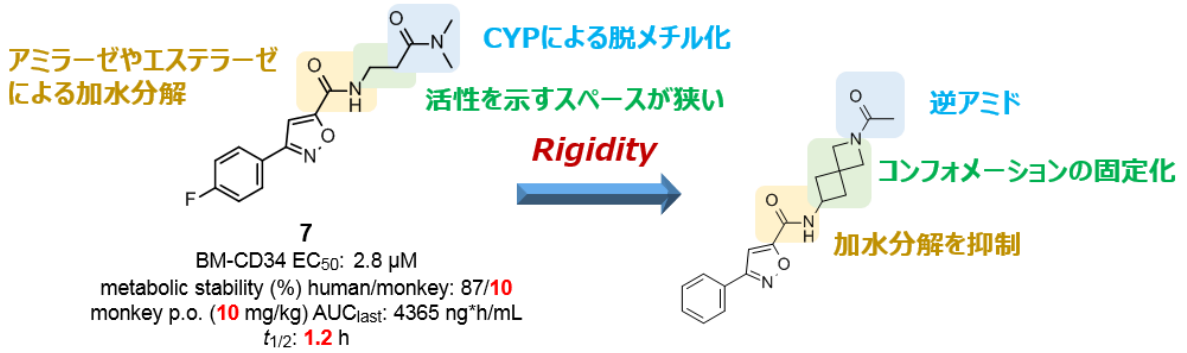
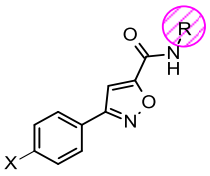


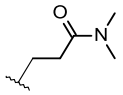
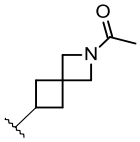
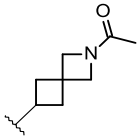
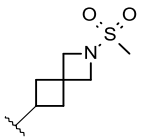
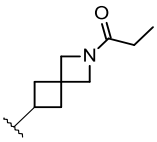
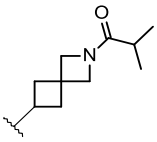
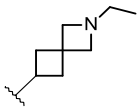
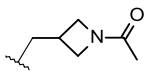
Figure 1-13. Compound design and strategies for improving PK profiles.

剛直性が高く独特の構造を持つ 2-アザスピロ[3.3]ヘプタンは、4-アミノピペリジンの生物学的等価体として認識され [30,31]、さらに代謝安定性を改善することが知られている [32–35]。このスピロ部はコンフォメーションを固定化しつつ立体障害の小さい細長い置換基となり、

活性を保持するのに最適だと考えた。また、ジメチル部は逆アミドにすることでメチル基の除去を抑制した。また、アミド部周辺は立体障害を少し大きくすることでエステラーゼ等による加水分解を抑制できると考えた。

Table 1-7. SAR of 2-azaspiro[3.3]heptane derivatives.



Compound	X	R	BM-CD34 EC_{50} (μM)[a]	Emax (%)[b]	Metablic stability[c] human/monkey (%)
24	F		2.6	89	>100/59
36	F		2.9	64	84/79
37	H		2.8	54	>100/64
38	H		3.9	45	76/87
39	H		>5	47	68/69
40	H		>5	27	94/41
41	H		5.0	50	100/87
42	H		>5	-	50/17

[a] Compound concentration that induces HbF by 50% if the maximum induction of HbF by decitabine is taken as 100%.
[b] Maximum effect of the compound as a percentage of that of decitabine. Human BM-CD34$^+$ cells were used in the assay.
[c] Remaining percentage of test compounds after 0.5 h incubation with human/monkey liver microsomes (0.5 mg/mL).

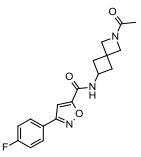
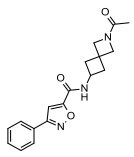
上記の考察を踏まえて設計・合成した化合物 **36** は、期待通り EC_{50} 値 2.9 μM の活性を示

し、サル肝ミクロソームにおける代謝安定性も改善した（Table 1-7）。同様に、左部のフルオロ基を除去した化合物 **37** も活性を保持した。化合物 **36** 及び **37** は化合物 **24** と比較して分子量及び LCLog P が増加しているにもかかわらず代謝安定性が改善された。続いて、化合物 **37** のアセチル基をメタンスルホニル基 **38**、プロピオニル基 **39**、2-メチルプロピオニル基 **40**、またはエチル基 **41** で置き換えると、活性が低下した。また、比較のために非剛直構造のアシルアゼチジン誘導体 **42** を合成・評価したところ、活性及び代謝安定性は低下し、スピロ構造の優位性が確認された。以上より、これらの結果に基づいて、良好な *in vitro* 活性及び代謝安定性を示した化合物 **36** 及び **37** を用いて、初期 ADMET 及び PK 試験を実施することとした。

1-10　化合物 36、37 の初期 ADMET と PK プロファイル

先に選抜した化合物 **36**、**37** の ADMET プロファイル及び PK プロファイルを Table 1-8 に示す。これら化合物は、化合物 **24** と比較して溶解性はおおむね良好で、CYP 阻害活性や hERG 阻害活性は非常に弱いことが明らかとなり、有望な骨格であることが示唆された。PK 試験では、化合物 **36**、**37** は良好な血中曝露量を示し、特に化合物 **37** は AUC_{last} が 4212 ng·h/mL となり、化合物 **36** の 2 倍の血中曝露量を示した。また、化合物 **37** の高用量での PK 試験を実施したところ、15 mg/kg において C_{max} は 11310 ng/mL、AUC_{last} は 65877 ng·h/mL となり、投与量と血中曝露量に線形性を示すことがわかった。以上より、化合物 **37** を用いて *in vivo* 試験を実施することとした。

Table 1-8. Chemical structures, activity, ADMET profiles, and PK profiles of compound **36**, **37**.

Compound	**36**	**37**
Structure		
BM-CD34 EC_{50} (E_{max})	2.9 μM (64%)	2.8 μM (54%)
MW (g/mol)	343	325
LCLog P	3.3	3.2
CYP(1A2/2C9/2D6/3A4)[a]	1/<0/1/<0	3/<0/2/<0
Solubility[b](μg/mL) (JP1/JP2)	>100/>100	>100/>100
hERG inhibition (10 μM)	N.T[c]	18%
Plasma protein binding (monkey)	72%	59%
PK parameters (monkey: 1 mg/kg, p.o.)		
C_{max} (ng/mL)	460	545
AUC_{last} (ng*h/mL)	2354	4212

[a] % Direct inhibition of CYP at 10 μM.

[b] JP1/JP2: Japanese Pharmacopoeia first/second test fluid (pH = 1.2/6.8)

[c] Not tested.

1-11　化合物 37 の *in vivo* 評価

化合物 **37** を用いて、15 mg/kg 及び 30 mg/kg の 1 日 2 回投与にて *in vivo* 試験を実施した（Figure 1-14）。その結果、用量依存性をもって HbF 誘導活性を示し、特に 30 mg/kg 投与群では有意な HbF 誘導活性が認められた。一方、安全性の面では課題を残した。高用量群で嘔吐物が確認され、5 日目以降で用量依存的な体重減少が認められた。また、血液学的検査においては、血小板数、白血球数などに用量依存的な低値が認められた。そして、試験中化合物 **37** の 30 mg/kg 投与群における 1 匹のサルにおいて、経口投与後に一過性の低血糖が観察され、このサルは実験から除外された。

実験間の比較をするために vehicle 群平均値で補正した数値を算出し、Means ± SD で Table 1-9 に表記した。試験間の比較となるが、化合物 **37** の 15 mg/kg、1 日 2 回投与群において、デシタビン 15 mg/kg、1 日 1 回投与群（約 13 倍）と同等以上の高い薬効が示唆された。

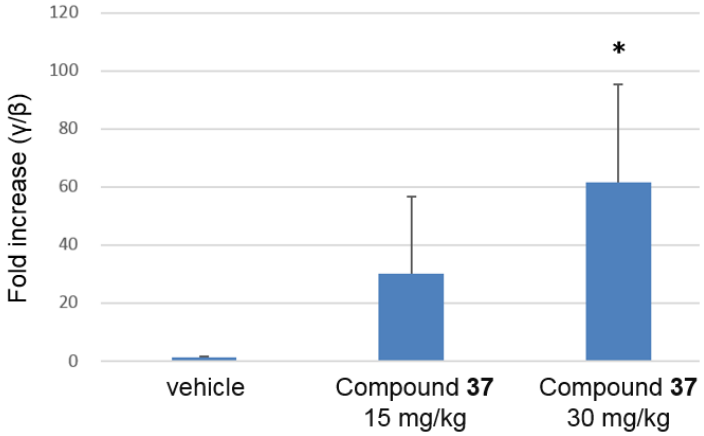


Figure 1-14. Induction of HbF by compound **37** in normal cynomolgus monkeys. Cynomolgus monkeys were administered compound **37** (15 or 30 mg/kg, twice a day) or vehicle orally from Day 0 to Day 6. Data show the mean (+SD) fold increase in the ratio of Hb γ-/β-subunit mRNA expression (n = 3 or 4; *p < 0.05, Dunnett's test).

Table 1-9. Fold change value of γ-globin against β-globin gene relative to vehicle control.

compound 37	vehicle	15 mg/kg BID	30 mg/kg BID
1試験目	1.0 ± 0.3	24.6 ± 21.6	49.9 ± 27.6

また、化合物 **37** の 30 mg/kg、1 日 2 回投与群において最終日の最終投与後 12 時間での血漿中濃度の実測値は、単回の PK 試験より算出したシミュレーションの計算値とほぼ一致した（Figure 1-15）。したがって、化合物 **37** は想定通りの曝露ができていたことが示唆され、化合物 **24** よりも高曝露であったために高い薬効を示したと考えられた。

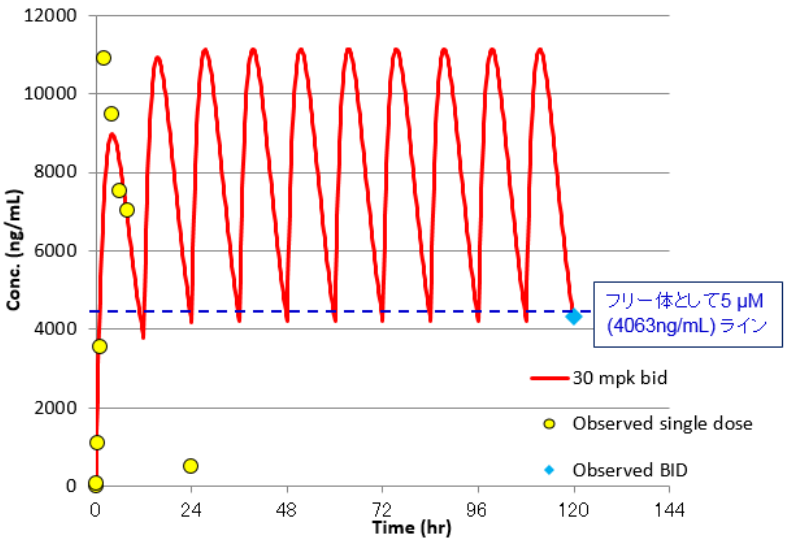
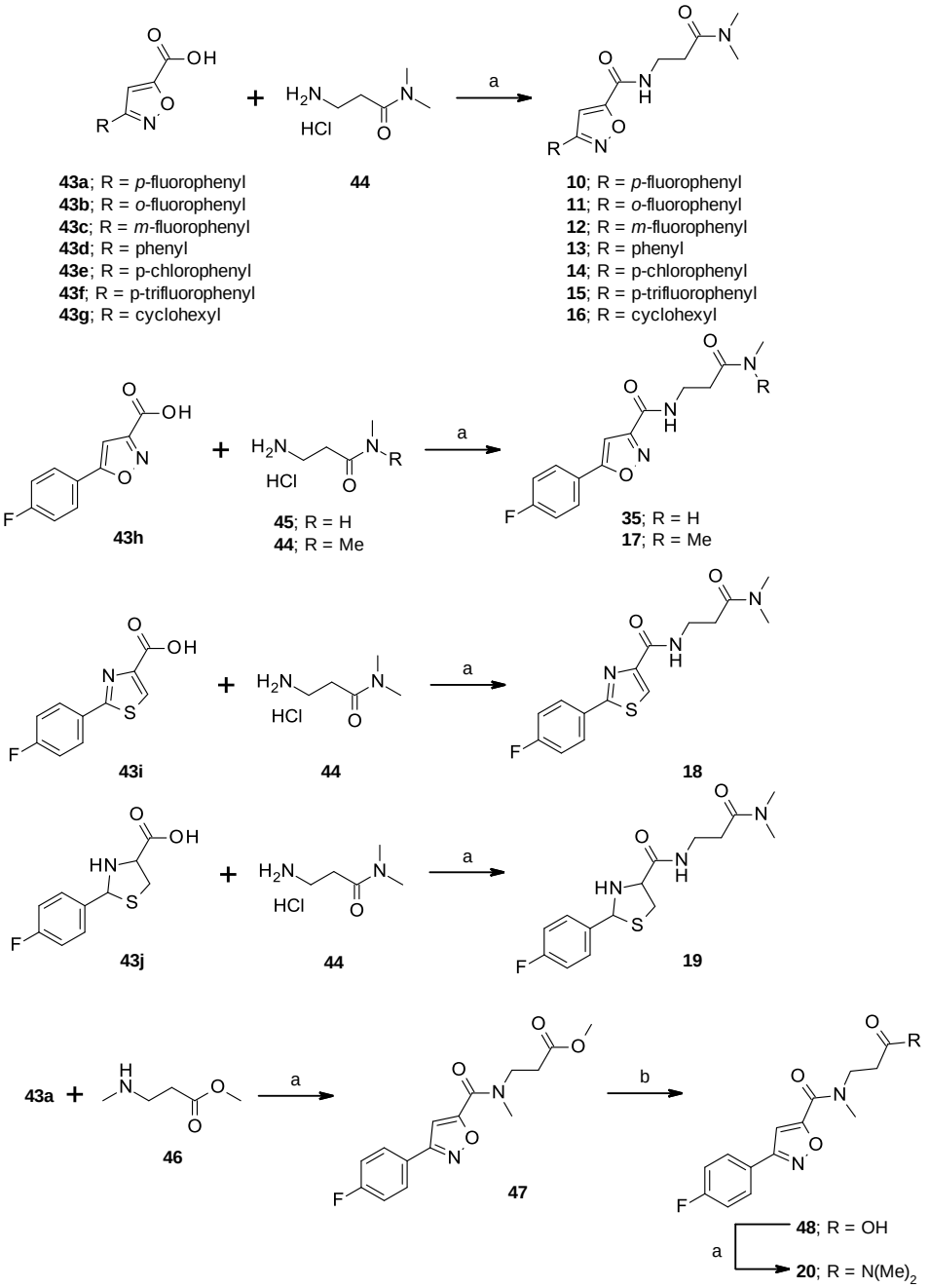


Figure 1-15. Simulation of 35 mg/kg (bid: red line) of compound **37** and measured value 12 hours after final administration (light blue).

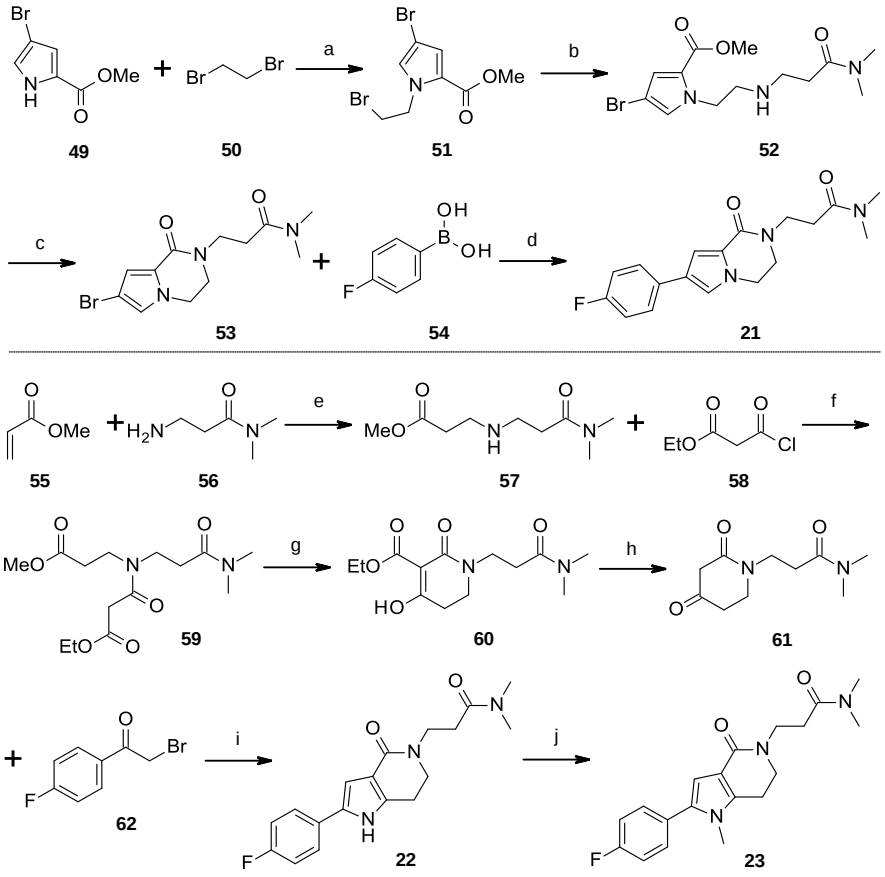
1-12　3-フェニル-イソキサゾール誘導体の合成

化合物 **10–20** 及び **35** の合成法を Scheme 1-1 に示す。市販のカルボン酸 **43a–43j** とアミン塩酸塩 **44** の縮合反応は、HABU または EDCI を縮合剤として用いることで、目的とする化合物 **10–19** を得た。化合物 **35** は、カルボン酸 **43h** とアミン塩酸塩 **45** を縮合することで得た。化合物 **20** は、まずカルボン酸 **43a** とアミン **46** を縮合し、続いて加水分解を行った後に生じたカルボン酸 **48** とジメチルアミンを縮合させることで得た。

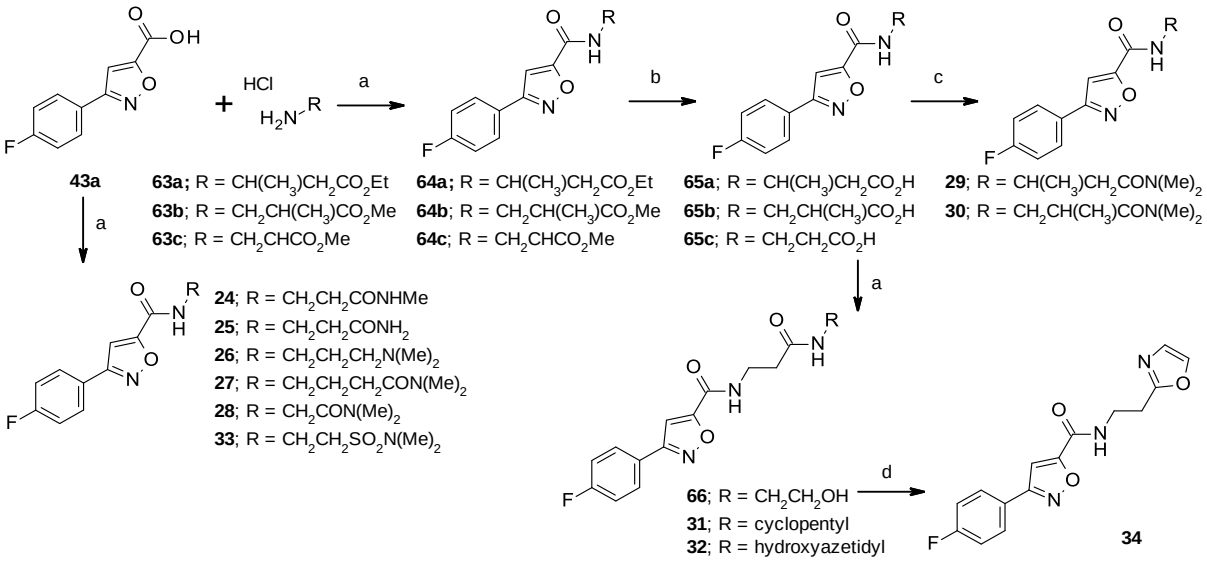


Scheme 1-1. Synthesis of compounds **10**–**20**, **35**. *Reagents and conditions*: a) HABU or EDCI, DIPEA or Et_3N, DMF, rt, 1 h, 25%–62%; b) LiOH-H_2O, THF, MeOH, H_2O, rt, 2 h, 52%.

化合物 **21–23** の合成法を Scheme 1-2 に示す。市販のピロール-2-メチルエステル **49** をアルキル化し、さらに N-アルキル化してアミン **52** を得た。続いて、DMSO 中 150 °C で加熱することでラクタム体 **53** を得た後、ボラン酸 **54** と鈴木-宮浦カップリングを行うことで化合物 **21** を得た。化合物 **22**、**23** の合成は、まずアクリル酸メチル **55** とアミン **56** を用いて aza-Michael 反応により化合物 **57** を得た。その後、N-アシル化、続くラクタム化を行うことで化合物 **60** を得た。続いて、化合物 **60** を脱炭酸後、ブロモケトン **62** を用いて Hantzsch ピロール反応によって化合物 **22** を得た。化合物 **23** は、化合物 **22** の N-メチル化により得た。



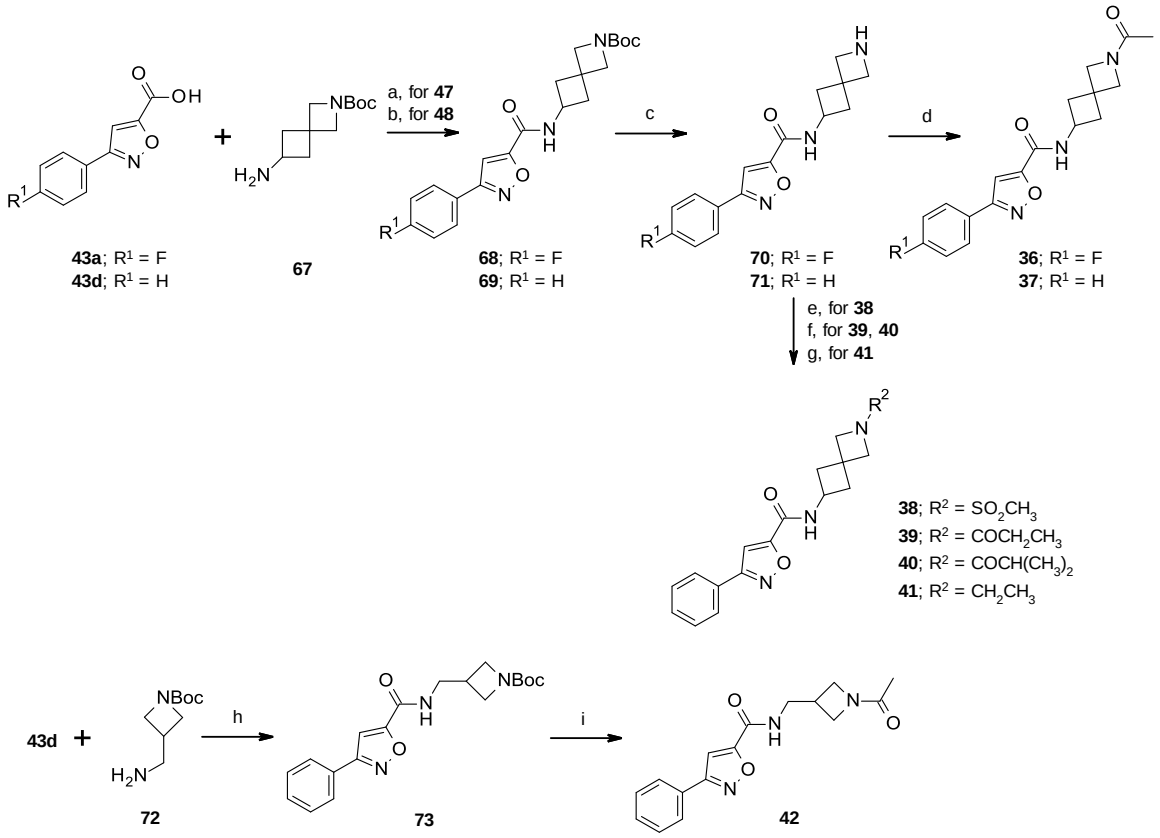
Scheme 1-2. Synthesis of compounds **21**–**23**. *Reagents and conditions*: a) NaH, DMF, rt to 80 °C, 1 h, 77%; b) 3-amino-*N*,*N*-diethylpropanamide, K_2CO_3, DMF, rt to 80 °C, 2 h, 12%; c) DMSO, 150 °C, 4 h, 87%; d) $Pd(PPh_3)_2Cl_2$ (10 mol%), K_2CO_3, dioxane, H_2O, rt to 120 °C, 1 h, μW, 33%; e) MeOH, rt to 50 °C, 16 h, 33%; f) DIPEA, CH_2Cl_2, 0 °C, 1 h, 45%; g) NaH, EtOH, 0 °C to rt, 17 h, 89%; h) AcOH, H_2O, rt to 90 °C, 2 h, 100%; i) NH_4OAc, EtOH, rt, 17 h, 17%; j) NaH, MeI, DMF, 0 °C to rt, 2 h, 76%.



Scheme 1-3. Synthesis of compounds **24**–**34**. *Reagents and conditions*: (a) corresponding amine, HATU or HBTU, DIPEA, DMF, 0 °C to rt, 1 h, 15%–80%; (b) LiOH-H_2O, THF, H_2O, rt, 1 h–15 h; (c) Me_2NH-HCl, HATU, DIPEA, DMF, 0 °C to rt, 1 h, 30%–93%; (d) PPh_3, hexachloroethane, Et_3N, CH_2Cl_2 rt, 16 h, 32%.

化合物 **24**–**34** の合成法を Scheme 1-3 に示す。化合物 **24**–**28** 及び **33** の合成は、カルボン酸 **43a** と対応するアミンを用いて縮合反応を行うことで得た。化合物 **29**、**30** の合成は、まずカルボン酸 **43a** と種々のエステル体 **63a**、**63b** をそれぞれ縮合し、続いて加水分解を行い、さらにジメチルアミンと縮合することで得た。化合物 **31**、**32** は、カルボン酸 **65c** と対応するアミンを用いて縮合反応を行うことでそれぞれ得た。化合物 **34** は、カルボン酸 **65c** とヒドロキシエチルアミンを縮合した後、トリフェニルホスフィンを作用させることでオキサゾール環を構築し、目的とする化合物 **34** を得た。

化合物 **36**–**42** の合成法を Scheme 1-4 に示す。まず、市販の化合物 **43a**、**43d** と市販のアミン **67** を縮合し、化合物 **68**、**69** をそれぞれ得た。続いて、酸性条件下で Boc 基を除去した後、得られたアミン **70**、**71** をアセチル化することで目的とする化合物 **36**、**37** を得た。化合物 **38** は、化合物 **71** の N-スルホニル化を行うことで得た。同様に、化合物 **39** 及び **40** は、対応する酸塩化物を用いてアシル化を行うことで得た。化合物 **41** は、アセトアルデヒドを用いた還元的アミノ化によって得た。化合物 **42** は、化合物 **36** と同様の方法で、化合物 **43d** 及び **72** から合成した。



Scheme 1-4. Synthesis of compounds **36**–**42**. *Reagents and conditions*: a) **43a**, COMU, Et_3N, DMF, 0 °C to rt, 3 h, 56%; b) (i) **43d**, $SOCl_2$, Et_3N, DMF, CH_2Cl_2, rt to 50 °C, 4 h; (ii) **67**, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h, 91% over 2 steps; c) TFA, CH_2Cl_2, 0 °C, 3 h, 54%–85%; d) AcCl, Py., CH_2Cl_2, 0 °C to rt, 1 h, 80%–88%; e) MsCl, Py, CH_2Cl_2, 0 °C to rt, 3 h, 71%; f) carbonyl chloride, Py., CH_2Cl_2, DMF, 0 °C to rt, 3 h, 90%–96%; g) acetaldehyde, $NaBH(OAc)_3$, THF, 0 °C to rt, 1 h, 61%; h) HATU, DIPEA, DMF, 0 °C to rt, 1 h, 41%; i) (i) TFA, CH_2Cl_2, 0 °C, 3 h, 61%; (ii) AcCl, Py., CH_2Cl_2, 0 °C to rt, 1 h, 86%.

1-13　小括

近年の医薬品パイプラインならびに新規標的及びメカニズムの減少は、多くの治療分野に影響を与え、創薬研究の実質的な課題となっている。そこで現在、表現型に基づくスクリーニングが新規薬剤型の生産性を改善し、新薬発見の鍵となるアプローチのひとつとして期待されている。実際、1999–2008 年において FDA に承認された新薬を解析すると、ファーストインクラスの 62%はフェノタイプスクリーニングによって見出されたと報告されており [36]、さらに現在も依然として増加している [37,38]。

この知見を踏まえて、筆者はフェノタイプスクリーニングによって新規 HbF 誘導剤の取得を目指した。まず、ハイスループットのフェノタイプスクリーニングで約 18,000 の化合物を評価し、18 の化合物がヒットした。そのうち、ヒット化合物の 1 つである 3-フェニル-イソキサゾール誘導体から合成展開を実施し、*in vitro* における HbF 誘導活性を保持しつつサルの代謝安定性が改善した化合物 **24** を取得した。化合物 **24** は、既知のエピジェネティクスのモジュレーターに影響を与えることなく、Bcl11a や LRF などの HbF の主たる転

写調節因子をダウンレギュレートすることを明らかにした。また、化合物 **24** の活性発現濃度と細胞抑制発現濃度のマージンはデシタビンに比べて非常に広く、Ames 試験及び *in vitro* 小核（IVtMN）試験で陰性であったことより、化合物 **24** は毒性の懸念が小さく、ヒドロキシ尿素やデシタビンよりも安全性が高いことが期待できる。

化合物 **24** は、サルを用いた *in vivo* 試験において 7.5、15 mg/kg（いずれも 1 日 1 回投与）の経口投与で評価したところ、期待通り用量依存的に γ-グロビンの mRNA 発現を誘導し、15 mg/kg 投与群で有意な γ/β 比の上昇が認められた。一方、1 日当たりの投与回数を 1 回から 2 回に増加させた第 2 の実験では、用量依存的に γ-グロビン発現のより大きな増加が観察されたものの、有意差は認められなかった。解析結果の検証を行ったところ、化合物 **24** の 15 mg/kg、1 日 2 回投与群において最終日の最終投与後 12 時間での血漿中濃度がシミュレーションの計算値より約 1/10 低いことが判明した。

次に、活性を保持したまま PK 試験で持続的な曝露を示す化合物の取得を目指し、コンフォメーションの固定化した 2-アザスピロ[3.3]ヘプタン骨格に着目した。期待通り、2-アザスピロ[3.3]ヘプタン誘導体 **37** は活性を保持しつつ、サル肝ミクロソームにおける代謝安定性も改善した。化合物 **37** を用いて、15 mg/kg、及び 30 mg/kg の 1 日 2 回投与群にて *in vivo* 試験を実施した結果、用量依存的に HbF 誘導活性を示し、特に 30 mg/kg 投与群では有意な HbF 誘導活性が認められた。また、化合物 **37** の 30 mg/kg 投与群において最終日の最終投与後 12 時間での血漿中濃度の実測値は、シミュレーションの計算値とほぼ一致した。したがって、化合物 **37** は想定通りの曝露ができていたことが示唆され、化合物 **24** よりも高曝露であったために高い薬効を示したと考えられた。また、試験間の比較となるが 15 mg/kg、1 日 2 回投与群（vehicle 補正後、約 25 倍）でも、デシタビンの 15 mg/kg、1 日 1 回投与群（約 13 倍）よりも薬効が強い可能性が推測された。

以上より、ヒット化合物 **10** からフェノタイプスクリーニングにより合成展開を実施し、より剛性が高く、ユニークな構造を持つ 2-アザスピロ[3.3]ヘプタン誘導体 **37** を取得した。臨床現場では、ヒドロキシ尿素の細胞毒性などの問題を解決した新しく安全で効果的な HbF 誘導剤が求められており、化合物 **37** は β-サラセミア及び SCD の治療薬の開発に役立つと期待される。

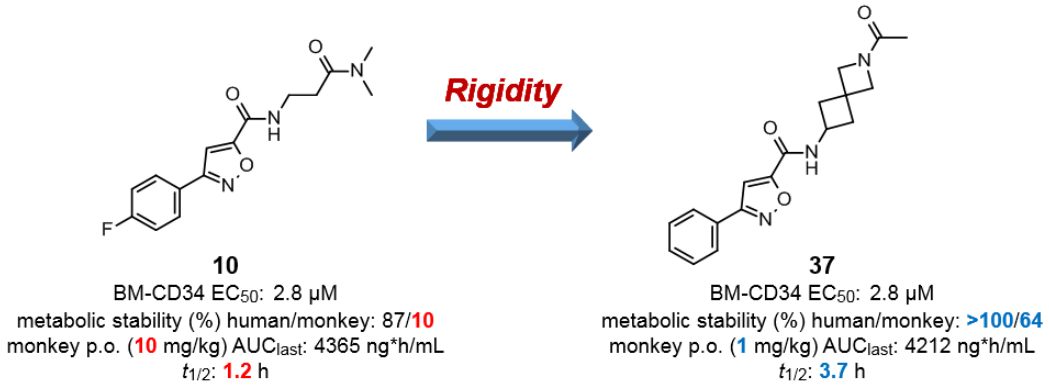


Figure 1-16. Summary of section 1.

第 2 章　G9a/GLP 阻害作用を示すリード化合物の獲得

2-1　背景

エピジェネティクス制御機構とは、DNA やヒストンへの後天的な化学修飾を通して、DNA の塩基配列の変化によらない遺伝子発現を制御・伝達するシステムのことである [39]。エピジェネティックスの異常は、癌などの深刻な病気につながる可能性があり、現在創薬標的として注目されている。エピジェネティクスによる遺伝子発現調節において重要な役割を果たしているのは、アセチル基、メチル基等の化学修飾を導入する「書き込み酵素」、それらを取り除く「消去酵素」、及び、アセチル基やメチル基を認識し相互作用する「読み取りタンパク質」である（Figure 2-1）[40]。このうち、ヒストンのメチル化は、リシン、アルギニン残基側鎖上で起こり、遺伝子発現の促進・抑制に関与している。例えば、ヒストンタンパク質 H3 の 4 番目のリシン残基（H3K4）、H3K36、H3K79 などのメチル化は転写活性化が起こっている遺伝子のプロモーター領域に確認され、H3K9、H3K27、H4K20 などのメチル化は転写抑制が起こっている領域に見出されている。こうしたメチル化を行う“writer”の役割を担う酵素ファミリーは、ヒストンメチル化酵素（Histone methyltransferase：HMT）と呼ばれている。

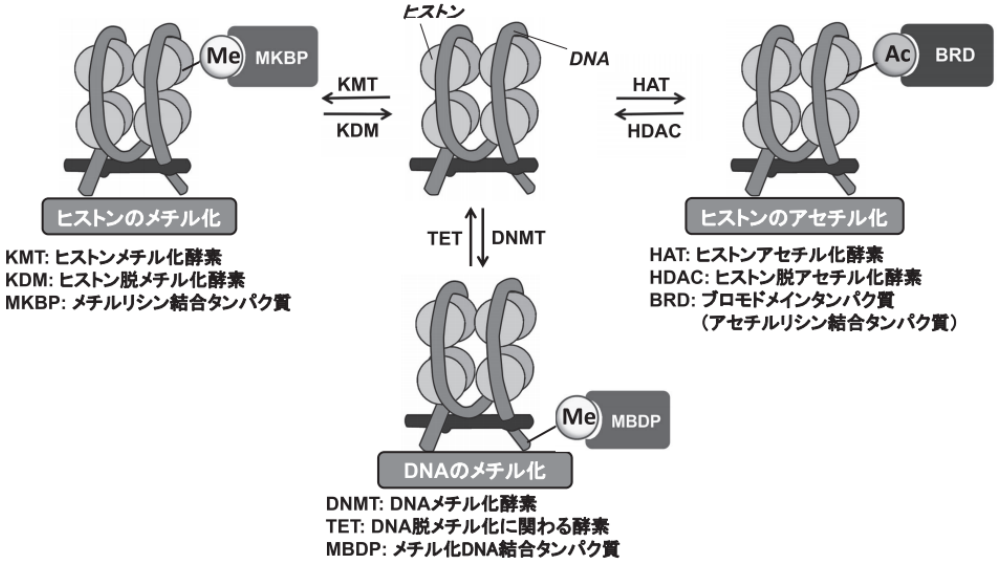


Figure 2-1. Epigenetics in DNA methylation and histone acetylation and methylation.[40]

前章で述べたように、グロビン遺伝子発現の制御は主として 2 つの機序があり、クロマチン調節因子の作用としては、グロビンスイッチングがグロビン遺伝子クラスターにおいてエピジェネティックに変化し、DNA とヒストン（クロマチン構造）の直接修飾を伴う。例えば、成人赤血球細胞の γ-グロビン遺伝子は、胎児赤血球細胞と比較して、シトシンメチル化の増加及び活性ヒストン修飾（H3K36m3、H3K9ac、H3K27ac）の損失と関連している [41]。これらのエピジェネティックな変化は、DNA メチル化酵素 1（DNMT1）のノックダウンによって HbF の抑制が解除されるという知見と一致し、実際に DNMT1 阻害剤のデシタビンは、臨床試験中である [42]。また、ヒストン脱アセチル化酵素（HDAC）阻害剤が胎児様ヒストンアセチル化を回復させ、HbF を増加させるという報告もある [43]。その他、ヒス

トン脱メチル化酵素（LSD1）やアルギニンメチル化酵素（PRMT5）などがグロビン遺伝子発現と関与していることが知られている。その中で、筆者はリシンメチル化酵素であるG9a/GLPに着目した。

G9a（EHMT2、KMT1C）は、メチル供与体である*S*-アデノシルメチオニン（SAM）によってヒストンタンパク質H3上の9番目のリシン残基（H3K9）のモノメチル化、ジメチル化を触媒する。GLP（EHMT1、KMT1D）は、G9aとそれぞれのSETドメインにおいて80%の配列相同性を有するG9a様タンパク質であり、主にH3K9のトリメチル化を触媒する。これらはヒストンのメチル化を調節することにより、DNA複製、損傷と修復、及び遺伝子発現において重要な役割を果たす[44]。さらに、G9aは多くの腫瘍細胞で過剰発現しており、腫瘍の発生等の多くの病原性プロセスに関連していることが明らかとなっている[45-47]。このように、G9a及びGLPは抗癌剤の優れた新しい治療標的と注目されており、癌治療の新しい選択肢として新規G9a/GLP阻害剤の開発が積極的に行われている。

一方、癌以外の適応症を狙った研究も報告されている。2015年にKrivegaらはG9a/GLP阻害剤であるUNC0638（Figure 2-4）がヒトCD34+赤芽球前駆細胞において総ヘモグロビンの最大30%のHbFを上昇させることを報告した[48]。また、メカニズム解析の結果、HbFの上昇は胎児のγ-グロビンの有意な活性化と成人のβ-グロビン転写の抑制に関連していた。すなわち、β-グロビン遺伝子の転写はLDB1/GATA-1/TAL1/LMO2複合体と遠隔遺伝子座制御領域（LCR）によって調節されるが、G9aを阻害するとγ-グロビン遺伝子プロモーターからH3K9me2が排除されて先のLDB1複合体を占有し、γ-グロビン遺伝子プロモーターとLCRの相互作用が促進されることでγ-グロビンの転写が活性化される（Figure 2-2）。

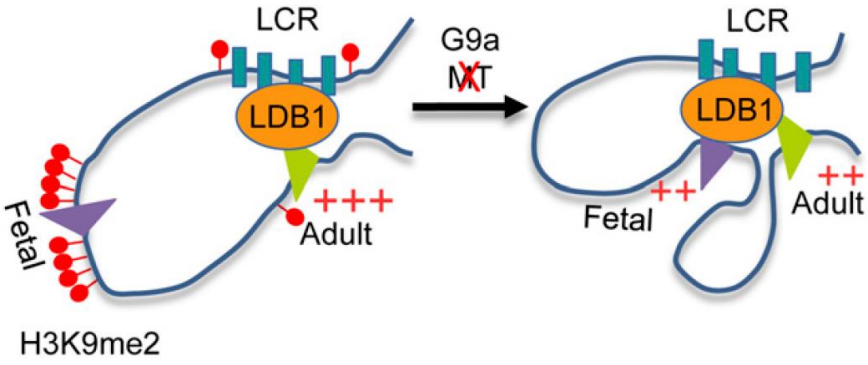


Figure 2-2. Model describing the role of G9a established H3K9me2 in regulation of fetal and adult β-globin genes expression in adult erythrocytes.[48]

また、同年、Rennevilleらは G9a及びGLPの個々の遺伝子ノックダウンがヒト赤血球細胞においてγ-グロビン遺伝子発現とHbF産生を誘導し、G9a及びGLPがγ-グロビン抑制に関与するエピジェネティックな調節因子であることを報告している[49]。さらに、G9a及びGLPの生体内の発現部位を The Human Protein Atlas[*3, 50]で調査したところ、G9a及びGLPのRNAやタンパク質が標的部位である骨髄に高発現していることがわかった。したがって、HbF産生を誘導できる強力なデュアルG9a/GLP阻害剤の開発は、β-サラセミア及びSCDを治療するための標的アプローチとして妥当であると考えられた。実際、Epizyme

社は SCD の治療薬として経口投与可能な G9a 阻害剤である EZM8266（構造は非公開）を開発した[51]。以上より、2 章において筆者は、β-サラセミア及び SCD を治療薬の創製として新規 G9a/GLP 阻害剤の取得を目指すこととした。

2-2　既知 G9a/GLP 阻害剤の総括

過去 10 年間で、数多くの G9a/GLP 阻害剤が報告されている[52]。現在の G9a/GLP 阻害剤は、結合モードに基づいて 1）SAM 補因子競合阻害剤、2）基質（ペプチド）競合阻害剤、の 2 つのグループに分類できる（Figure 2-3）。以下にそれぞれの阻害剤について紹介する。

SAM 競合阻害剤は、G9a 及び GLP の SAM 結合部位に結合し、SAM と競合することによって G9a の基質のメチル化が抑制されて G9a 活性が阻害される。BIX01338（Figure 2-4）は、基質競合阻害剤である BIX01294 とともに HTS によって見つかり、G9a に対して 4.7 μM の IC_{50} 値を示した[53]。また、BRD9539 は、BIX01338 の構造に基づいて設計及び合成され、IC_{50} 値は G9a に対して 6.3 μM を示した[54]。しかし、BRD9539 は細胞ベースのアッセイでは活性を示さず、これは細胞透過性が低いことが原因として考えられた。また、Chaetomium から抽出された真菌マイコトキシンであるチャエトシンは、2005 年にリシン特異的ヒストンメチル化酵素阻害剤として最初に報告された[55]。チャエトシンは、IC_{50} = 2.5 μM で G9a を選択的に阻害し、細胞内の H3K9me2 及び H3K9me3 レベルを選択的に低下させる。シネフンギンは、抗真菌性抗生物質として Eli Lilly によって Streptomycesgriseoleus から分離された[56]。構造は SAM に類似しており、IC_{50} = 30 μM で G9a 活性を阻害する。

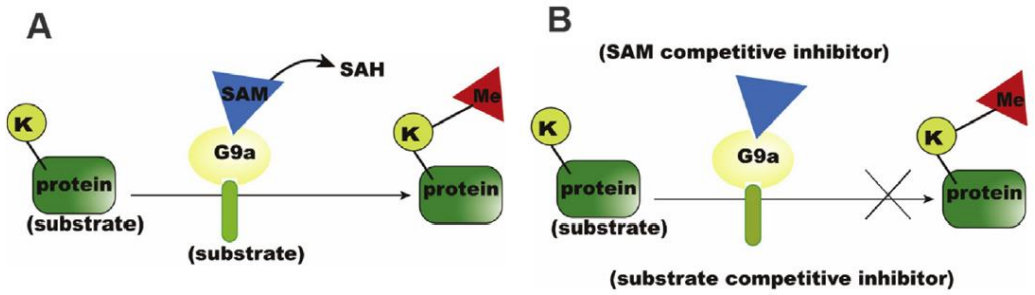
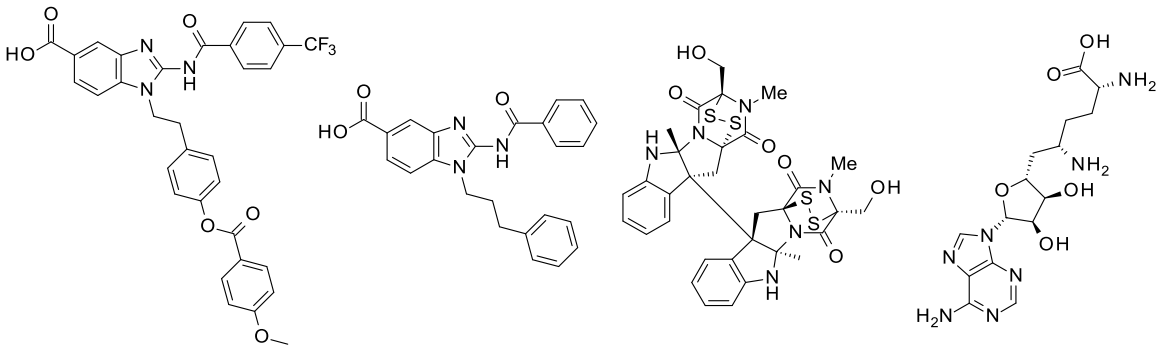


Figure 2-3. The mechanism of action of G9a and G9a inhibitors. (A) G9a catalyzes the methylation of histone3 protein with *S*-adenosylmethionine (SAM) as a methyl donor. (B) The mechanism of action of G9a inhibitors. Substrate competitive inhibitors bind the substrate site in G9a and block the methylation of substrates. SAM competitive inhibitors compete with endogenous SAM, thus inhibit the G9a activity. (K: Lysine; SAM: *S*-adenosyl methionine; SAH: *S*-adenosylhomocysteine; Me: Methyl group).[52]

*3

The Human Protein Atlas（HPA）は、スウェーデンの研究グループが提供しているヒトのタンパク質の発現情報データベースである。様々なヒトの組織やガン細胞、細胞株におけるタンパク質発現情報・遺伝子（RNA）発現情報及びタンパク質局在を調べることができる。



BIX01338
G9a IC_{50}= 4.7 μM

BRD9539
G9a IC_{50}= 6.3 μM

Chaetocin
G9a IC_{50}= 2.5 μM

Sinefungin
G9a/GLP IC_{50}= 30/28 μM

Figure 2-4. Chemical structures of SAM competitive G9a inhibitors.

基質（ペプチド）競合阻害剤は、ヒストンの G9a への結合部位を直接占有し、SAM と競合するのではなく、G9a の基質部位に特異的に結合する。一般に、基質競合阻害剤は SAM 競合阻害剤よりも G9a に対して優れた選択性を示し、阻害活性も強いことから精力的に研究が行われている。BIX01294（**74**）は、HTS によって得られた最初の G9a/GLP 基質競合阻害剤である [53]（Figure 2-5）。そして、このキナゾリン誘導体のさらなる SAR 研究によって以下のことが明らかとなった。(1) BIX01294 のベンジル基は G9a 阻害活性には不要である、(2)リシンをミミックした側鎖のアルコキシアミノ基が阻害活性に非常に重要である、(3) 2 位に疎水性基を導入することによって細胞透過性が改善し、細胞アッセイにおいても阻害活性を示す。この考察に基づき、G9a/GLP に対してそれぞれ IC_{50} = 15/19 nM を示す強力な G9a/GLP 阻害剤 UNC0638（**75**）が報告された [57]。しかしながら、PK プロファイルが不十分であり、動物実験での使用は制限されていた。UNC0631 は、脂溶性がより高く（Log P =5.5 vs Log P =4.8 for **75**）膜透過性が改善し、様々な細胞株で高い G9a 阻害活性を示した [58]。また、UNC0638 の SAR 研究によって、2 位のシクロヘキシル基が *in vivo* における代謝安定性の低下の原因であることが示唆され、UNC0638 の 2 位のシクロヘキシル基を 4,4-ジフルオロピペリジンに変換した強力な G9a/GLP 阻害剤 UNC0642（**77**）が得られた [59]。UNC0642 は、UNC0638 と比較して酵素アッセイ及び細胞アッセイにおいて活性が向上し、さらに優れた *in vivo* PK プロファイルを持つ。7 位にメチルフリル基を導入したキノリン誘導体 CM-272 は、G9a と DNMT の二重阻害剤であり、血液腫瘍の増殖を効果的に阻害し、そのアポトーシスを促進することが報告されている [60]。さらに、アミノインドール A-366（**79**）[61] や EPZ035544（**80**）[62-64]、2-アミノベンズイミダゾール体 **81** [65] など、完全に異なる骨格の G9a/GLP 阻害剤が報告されている。しかしながら、いずれの骨格においても hERG 阻害活性示す問題点が挙げられる（社内データ：Figure 2-5 参照）。また、UNC0638（**75**）や UNC0642（**77**）などのキナゾリン誘導体は、最近創薬のために積極的に研究されているが [66,67]、このケモタイプは膜透過性が非常に悪いという問題がある（例：UNC0638（**75**）、UNC0642（**77**）：MDCK P_{app} = 0.22、0.21×10^{-6} cm/s）。さらに、非キナゾリン構造に基づく強力な G9a/GLP 阻害剤に関する文献報告はごくわずかであり競合は少ないと考えられる。以上より、筆者は Epizyme 社から報告された EPZ035544（**80**）を出発化合物として強力な新規 G9a/GLP 阻害剤の創製を目指すこととした。

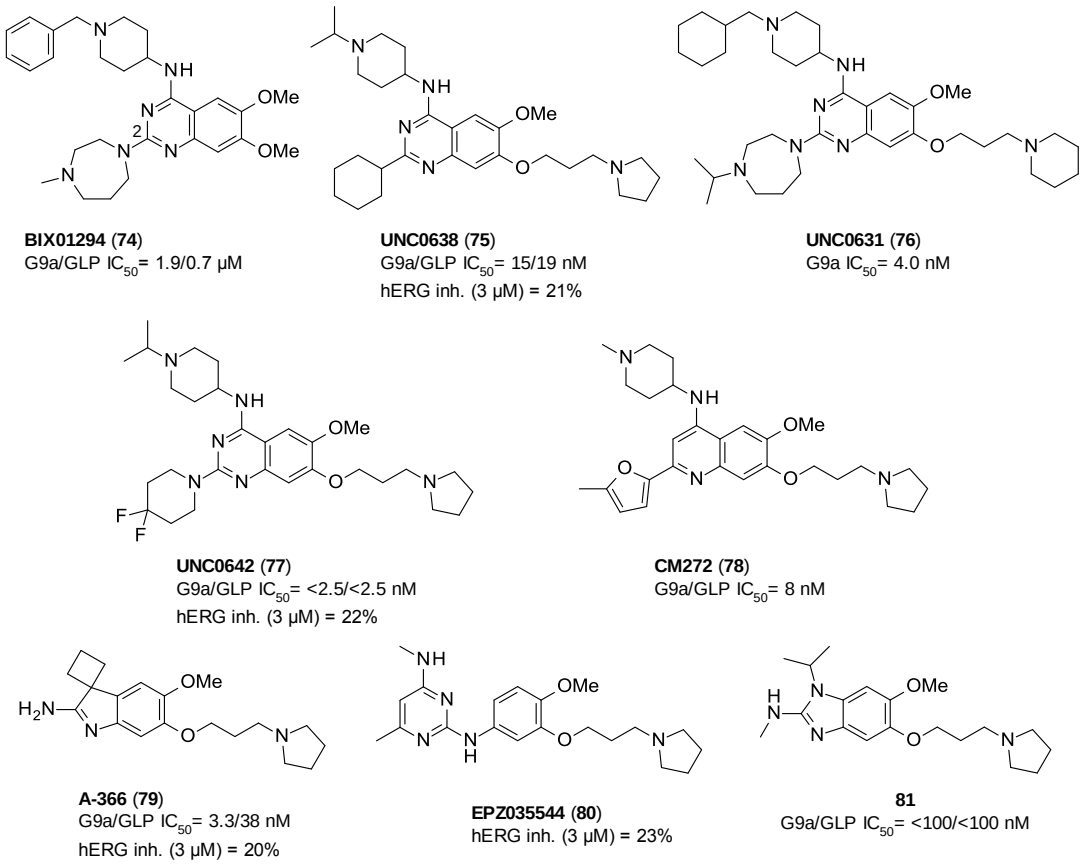
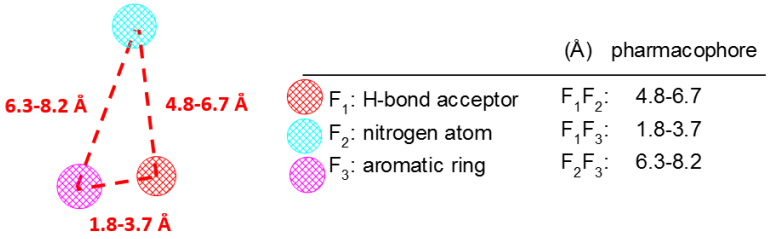


Figure 2-5. Chemical structures of substrate competitive G9a/GLP inhibitors.

2-3　EPZ035544 からの合成展開

EPZ035544（**80**）は、社内の *in vitro* アッセイを使用して評価したところ、G9a/GLP に対してそれぞれ 16.9/185 nM の IC_{50} 値で阻害活性を示した。一方で、化合物 **80** のマウス PK プロファイルは、バイオアベイラビリティ（BA）が 17%と良好ではなく、さらに hERG 阻害活性（42% at 10 μM）を示した。一般に、hERG を阻害することによって心電図の QT 間隔が延長し、致死的心室不整脈を誘発する懸念があるため、hERG は創薬展開において初期段階から抑えるべきオフターゲットだと考えられている。化合物 **80** が hERG 阻害活性を示した原因を考察したところ、側鎖部において芳香環と塩基性置換基が適切な距離にあり典型的な hERG 阻害活性を示す構造であるからだと考えられた。したがって、筆者はまず、hERG 阻害活性の改善に向けた取り組みとして hERG 阻害活性を示す化合物のファーマコフォアモデルについて着目した。Aronov によって提案された hERG 阻害活性を示すファーマコフォアモデルを Table 2-1 に示す [68,69]。

Table 2-1. Pharmacophore hypotheses by Aronov et al.[68]



水素結合アクセプター（F_1）と窒素原子（F_2）は 4.8–6.7 Å、水素結合アクセプター（F_1）と芳香族環（F_3）は 1.8–3.7 Å、窒素原子（F_2）と芳香族環（F_3）は 6.3–8.2 Å の位置関係にある化合物は、hERG 阻害活性を示す傾向があると考察されている。続いて、hERG 阻害活性を示す EPZ035544 の最安定配座を分子モデリングソフトウェア Maestro （version 11.4）を用いて計算し、それぞれの距離を算出した（Figure 2-6）。EPZ035544 は、最安定配座において F_1F_2 = 6.02Å、F_1F_3 = 2.80Å、F_2F_3 = 7.26Å となり、Aronov によって提案された hERG ファーマコフォアモデルに全て含まれていることがわかった。

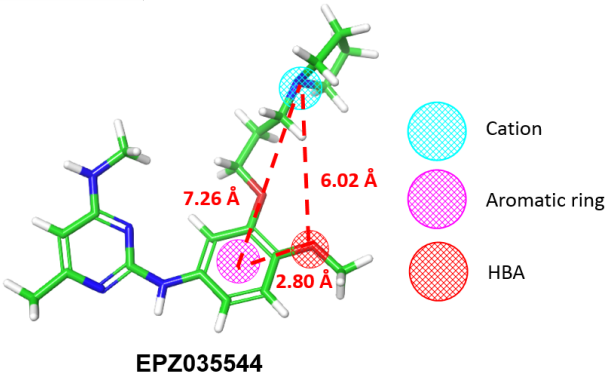


Figure 2-6. Aronov hERG pharmacophore overlaid on calculated molecular model structures of compound **80** for the most stable conformer. Pharmacophore points are shown as color-coded mesh spheres according to the legend, with distances (Å) between features marked by dashed red lines.

次に、Figure 2-5の既知のG9a/GLP阻害剤の化学構造に注目すると、これら阻害剤の右側の側鎖の多くが3-（ピロリジン-1-イル）プロポキシ基を有することがわかる。この側鎖は、基質結合部位のリシン残基を模倣しており、活性を示すためには適切な位置に塩基性官能基を導入する必要があることが示唆された。また、この側鎖の共結晶構造（化合物**75**のPDB code：3RJW[59]及び化合物**79**のPDB code：4NVQ[61]）を注意深く観察すると、プロポキシ基は真っ直ぐに伸びるのではなく丸く歪んでいることがわかった（Figure 2-7）。

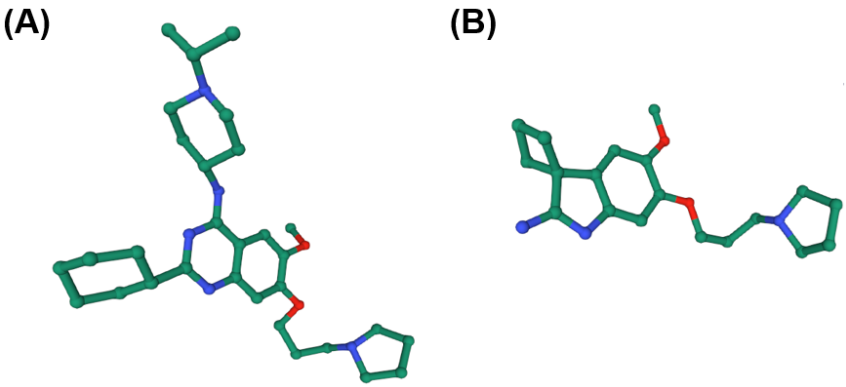


Figure 2-7. Active conformations of (A) compounds **75**[59] and (B) **79**[61] obtained from co-crystal structures with G9a.

EPZ035544の活性コンフォメーションを確認すべく、G9aとの共結晶を取得したところ、化合物**75**や化合物**79**と同様に側鎖のプロポキシ基は丸く歪んでいることがわかった。

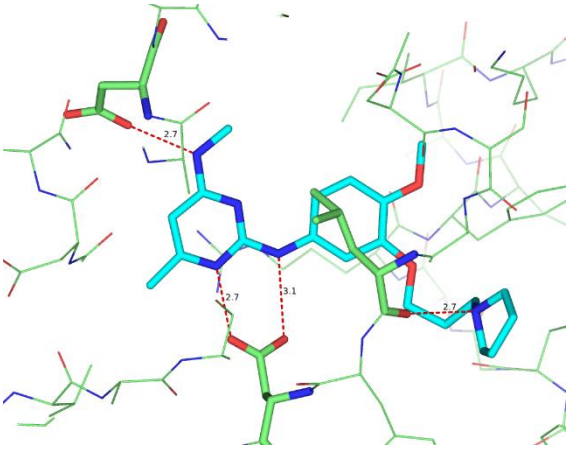


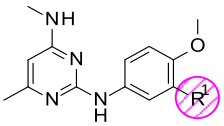
Figure 2-8. X-ray crystal structure of EPZ035544 (**80**) bound to G9a (PDB code: 7BTV).

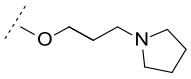
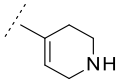
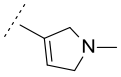
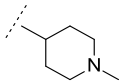
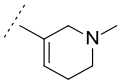
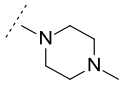
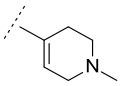
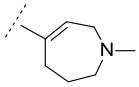
以上のことから、側鎖部を活性コンフォメーションのように曲がったまま環化して固定化することで、G9a/GLP活性を保持しつつhERG阻害ファーマコフォアモデルから外れ、hERG阻害が改善するのではないかと考え、本仮説の検証を実施することとした。一般に、コンフォメーションの固定化は、化合物の結合親和性、PKプロファイル、及びオフターゲットプロファイル（hERG阻害及びCYP阻害）の改善に役立つため、ドラッグデザインの最も効果的な方法の1つと考えられている[70,71]。なお、G9aとGLPの活性値には正の相関関係があるため（詳細は3-3参照）、今後はG9a阻害活性について言及することとする。

まず、右側構造を 5 員環で環化したジヒドロピロリル基 **82** は活性が大きく低下したが、6 員環で環化した N-メチル-3-テトラヒドロピリジル基 **83** は、IC_{50} 値が 451 nM を示した（Table 2-2）。続いて、窒素原子をずらした N-メチル-4-テトラヒドロピリジル基 **84** は、活性が 10 倍以内の低下に留まった（IC_{50} = 92.9 nM for G9a）。一方、化合物 **84** のデスメチル誘導体 **85**、ピペリジン誘導体 **86**、及びピペラジン誘導体 **87** は、活性が大きく低下した。次に、7 員環のテトラヒドロアゼピニル基 **88** は、最も強力な G9a/GLP 阻害活性を示した

（IC_{50} = 15.1 nM for G9a、IC_{50} = 74.1 nM for GLP）。このことより、環サイズが G9a/GLP の活性に大きな影響を与えることがわかった。

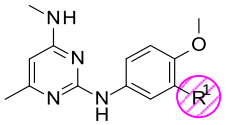
Table 2-2. SAR of side chain.

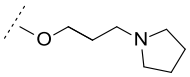
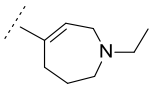
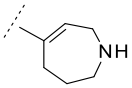
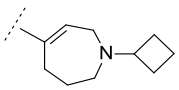
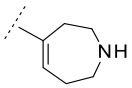
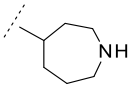
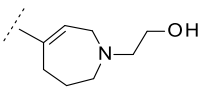


Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]	Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]
80		16.9 ± 0.322	185 ± 14.8	**85**		225 ± 9.09	1156 ± 60.3
82		1494 ± 68.6	2772 ± 111	**86**		588 ± 34.4	N. T.[b]
83		451 ± 23.3	864 ± 47.4	**87**		> 5000	N. T.[b]
84		92.9 ± 5.21	277 ± 12.3	**88**		15.1 ± 0.449	74.1 ± 2.98

[a] The IC_{50} values are presented as the average of four technical replicates ± the standard error of the mean (SEM).
[b] Not tested.

Table 2-3. SAR of tetrahydroazepine group.



Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]	Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]
80		16.9 ± 0.322	185 ± 14.8	**92**		10.5 ± 0.208	44.1 ± 1.41
89		4.31 ± 0.179	32.3 ± 1.10	**93**		145 ± 7.55	1066 ± 51.8
90		83.3 ± 2.69	479 ± 17.5				
91[b]		28.2 ± 1.10	342 ± 14.5	**94**		213 ± 14.4	1586 ± 60.1

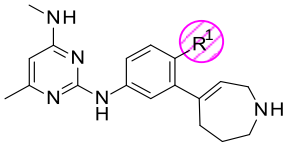
[a] The IC_{50} values are presented as the average of four technical replicates ± the standard error of the mean (SEM).
[b] Racemate.

次に、テトラヒドロアゼピン側鎖の SAR を調べた（Table 2-3）。デスメチル誘導体 **89** は、

メチル誘導体 **88** と比較して G9a/GLP 阻害活性が向上し、化合物 **80** と比較して G9a に対して 4 倍、GLP に対して 6 倍強くなった。この置換基変換は、G9a 阻害活性に非常に好ましい効果をもたらし、化合物 **89** は IC_{50} = 4.31 nM という強力な G9a 阻害活性を示した。オレフィン部分の位置異性体 **90** または飽和のアゼパン誘導体 **91** は活性が低下し、適切な位置のオレフィン部分が活性を示すために重要であることが示唆された。次に、テトラヒドロアゼピン環内窒素の置換基の最適化を検討した。エチル基 **92** は、メチル基 **88** と比較して活性が向上したが、無置換体 **89** と比較して活性が低下した。同様に、置換基をより大きくしたシクロブチル基 **93** や極性基を導入したヒドロキシエチル基 **94** は活性が低下した。化合物 **89** と G9a の複合体の共結晶構造からも、テトラヒドロアゼピン体のアミノ基の先にはあまりスペースがないことや、側鎖の周辺は Tyr1067、Tyr1154、Phe1158 等の疎水性置換基が多いため極性基が許容されないことが示唆された（2-4, Figure 2-9 参照）。

次に、化合物**89**のフェニル環のメトキシ基のSARを調査した（Table 2-4）。 このメトキシ基をクロロ基**95**、メチル基**96**、またはシアノ基**97**に置き換えると、活性がわずかに低下した。 また、ジフルオロメトキシ基**98**は活性を保持したが、トリフルオロメトキシ基**99**はG9aとGLPの両方に対する活性を完全に低下させることもわかった。 化合物**89**とG9aの複合体の共結晶構造（2-4, Figure 2-9参照）から、メトキシ基は浅い結合溝を占め、トリフルオロメトキシ基などのより大きな基は結合溝と衝突する可能性が高いため、活性が低下することが示唆された。なお、他の部位、特にピリミジン部の4位のメチルアミノ基と6位のメチル基のSAR研究を実施したが（>30化合物）、化合物**89**より活性が向上する化合物を得ることができなかった（data not shown）。

Table 2-4. SAR of methoxy group.



Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]	Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]
89		4.31 ± 0.179	32.2 ± 1.10	**97**		15.2 ± 0.503	43.2 ± 2.29
95		6.32 ± 0.221	27.1 ± 0.921	**98**		3.25 ± 0.114	14.7 ± 0.449
96		14.2 ± 0.554	109 ± 4.75	**99**		853 ± 36.2	3801 ± 145

[a] The IC_{50} values are presented as the average of four technical replicates ± the standard error of the mean (SEM).

2-4　化合物 80 及び化合物 89 と G9a の共結晶構造解析

一般に、基質競合阻害剤はメチル基のドナーであるSAMと競合するのではなく、G9aへのヒストンの結合部位を直接占有し、G9aの基質部位に特異的に結合する。先に合成・評価したテトラヒドロアゼピン誘導体のG9aへの結合様式を調べるために、化合物**89**とG9aの共

結晶構造を決定した（Figure 2-9）。予想通り、化合物**89**は他の基質競合阻害剤と同様にヒストンペプチド結合溝に占有し、テトラヒドロアゼピン部分はリシン結合部位及びSAMの硫黄原子と相互作用することが明らかとなった。また、テトラヒドロアゼピンのアミノ基は、Leu1086のカルボニル基の酸素に水素結合を提供し、G9aと重要な相互作用を形成している。化合物**89**のアミノピリミジン部は、Asp1088及びAsp1078の側鎖と水素結合を形成する。化合物**89**のメトキシ基は、Phe1158、Ile1161、及びLys1162の側鎖によって形成された疎水性ポケットにはまっている。

続いて、化合物**80**と化合物**89**のそれぞれのG9aとの共結晶構造より、重ね合わせを実施した（Figure 2-10）。両複合体で、タンパク質側に顕著な構造の差異は無く、Arg1157の側鎖による相互作用も両化合物で確認され、側鎖のアミノ基の位置もほぼ重なった。また、Arg1157の側鎖は、Leu1086の反対側の結合リガンドの面にリガンドと平行にパッキングすることもわかった。

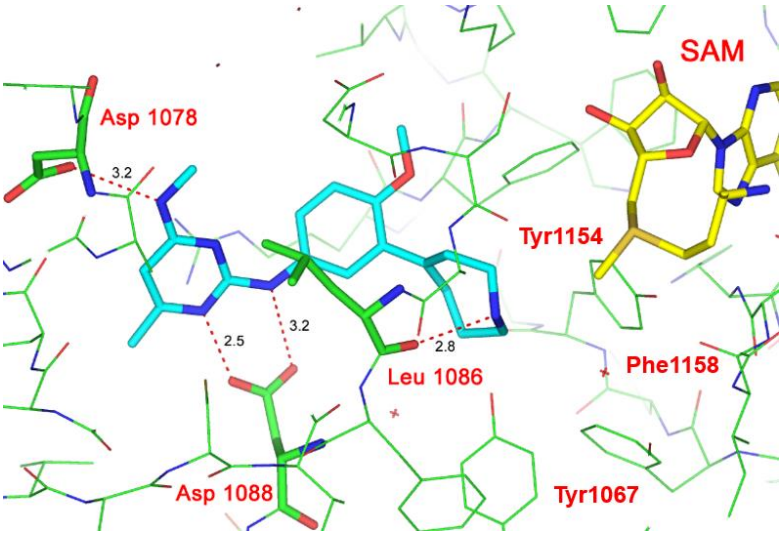


Figure 2-9. X-ray crystal structure of compound **89** bound to G9a (PDB code: 7BUC).

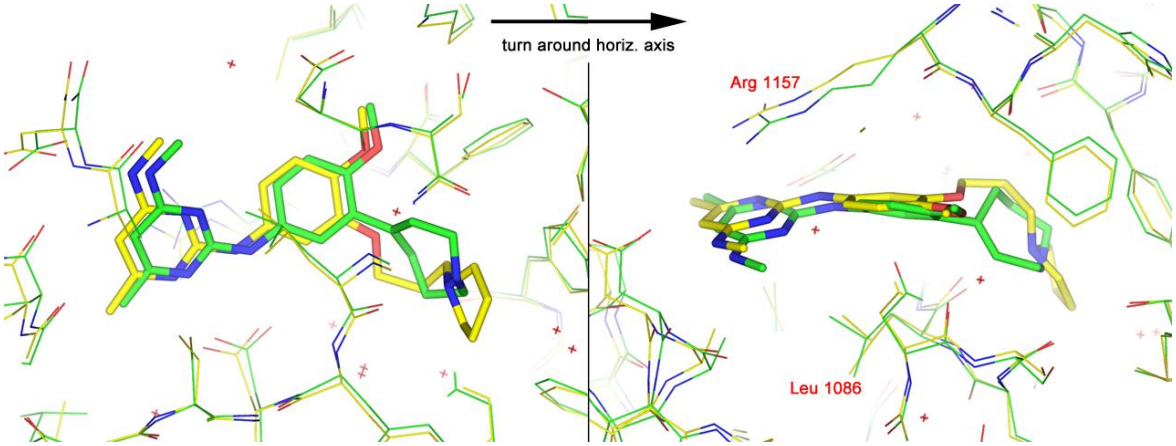


Figure 2-10. Overlay of X-ray crystal structure of compounds **80** (yellow; PDB code: 7BTV) and **89** (green; PDB code: 7BUC) bound to G9a.

2-5　化合物 89 のヒト培養骨髄 CD34 +細胞を用いた *in vitro* 評価

ヒト培養骨髄CD34+細胞を用いて、成人型β-グロビンから胎児型γ-グロビンへのグロビンmRNA発現の切り替え（グロビンスイッチング）を計測することによって代表的な化合物（化合物**79**、**80**、及び**89**）の細胞活性を評価した（Figure 2-11）[72]。 化合物**80**の細胞グロビンスイッチング活性は、化合物**79**のそれとほぼ同じであり（Figure 2-11A）、化合物**89**の活性は、化合物**79**の活性よりわずかに高かった（Figure 2-11B）。

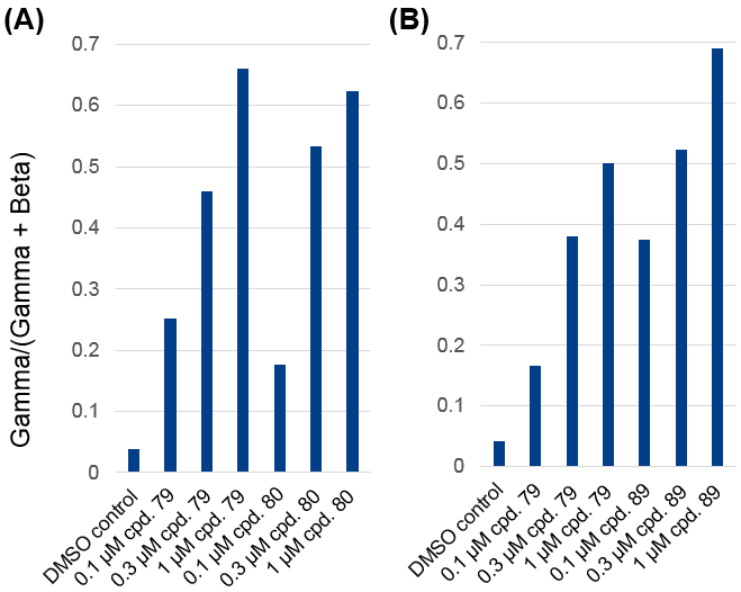


Figure 2-11. Effect of compounds **79**, **80** (A), and **89** (B) on the cellular globin switching activity (γ/γ+β globin mRNA ratio) using human bone marrow-derived hematopoietic progenitor CD34+ cells.

2-6　代表化合物の初期ADMET及びPKプロファイル

Table 2-5. In vitro ADME parameters of compounds **80**, **88** and **89**.

Compound	Log D	MDCK P_{app} (10^{-6} cm/s)	Solubility[a] (μg/mL) (JP1/JP2)	Metabolic stability[b] (m/mon/h)	CYP inhibition (1A2/2C8/2C9/2C19/2D6/3A4)	MBI[c] (% remaining, 3A4)	hERG inhibition[d]
80	0.8	3.1	740/590	48/8/99	5/2/0/3/8/8	93.6	42%
88	1.3	8.4	710/360	23/2/76	N.T.[e]	87.3	17%
89	0.5	1.4	680/640	34/24/96	0/0/0/9/3/0	80.5	1%

[a] JP1/JP2: Japanese Pharmacopoeia first/second test fluid (pH 1.2/6.8).

[b] The percentage (%) of the tested compounds remaining after 0.5 h of incubation with mouse/monkey/human liver microsomes (0.5 mg/mL).

[c] The % remaining at a concentration of 100 μM of compounds reacted with CYP3A4 probe substrates after 30 min of preincubation in human liver microsomes.

[d] Inhibition at 10 μM.

[e] Not tested.

化合物**80**及び2-3において高活性を示した化合物**88**、**89**のADMETプロファイルをTable 2-5に示す。化合物**88**及び**89**は、脂溶性が低いため（Log D = 0.5–1.3）、MDCK細胞における膜透過性試験では中程度の膜透過性（P_{app} = 1.4–8.4 × 10^{-6} cm/s）を示した。また、これらの化合物は酸性pHと中性pHにおける溶解度やヒト肝ミクロソームにおける代謝安定性は良好であった。一方、これらの化合物はマウスの肝ミクロソームでは代謝安定性は中程度であり、サルの肝ミクロソームでは不安定であった。10 μMの濃度におけるシトクロムP450（CYP）酵素、すなわちCYP1A2、CYP2C8、CYP2C9、CYP2C19、CYP2D6、及びCYP3A4の阻害については、化合物**80**及び**89**はほとんど阻害を示さなかった。 CYP3A4に対するメカニズムベースの阻害（MBI）の阻害効果は、これらの化合物は示さなかった[73]。さらに、化合物**88**及び**89**は、化合物**80**（42% at 10 μM）と比較して、hERG阻害活性が改善した（化合物**88**、**89**それぞれ17%, 1% at 10 μM）。なお、テトラヒドロアゼピン誘導体でhERG阻害活性が改善した理由は、化合物**89**の塩基性は（pK_a = 9.35 : ADMET predictor 9.5による計算値、以下同様）化合物**80**（pK_a = 9.21）と大きく変わらなかったことから、コンフォメーションの固定化によってhERGに対する親和性が低下したと考えられる。そこで、先と同様に、化合物**89**の最安定配座を分子モデリングソフトウェアMaestro （version 11.4）を用いて計算し、それぞれの距離を算出した（Figure 2-12B）。

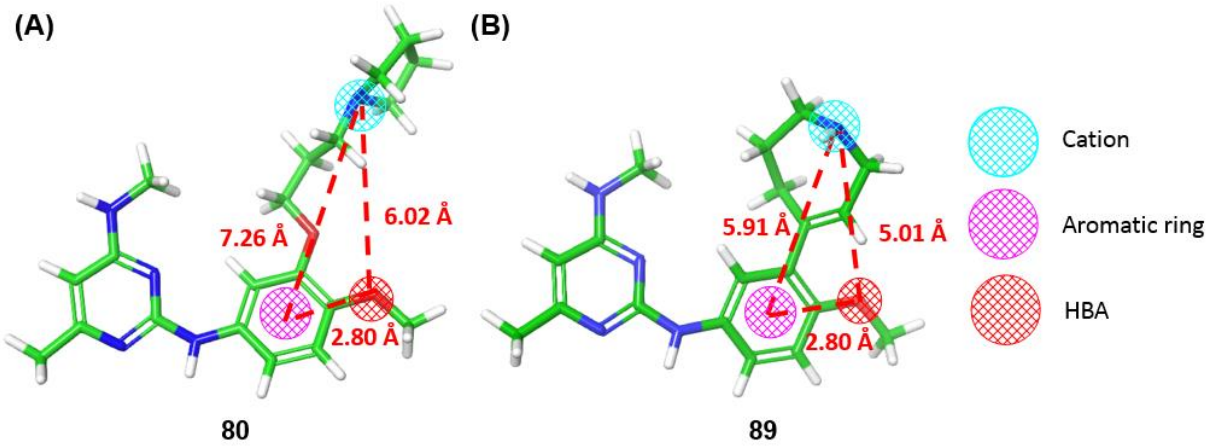


Figure 2-12. Aronov hERG pharmacophore overlaid on calculated molecular model structures of compounds **80** (A) and **89** (B) for the most stable conformer. Pharmacophore points are shown as color-coded mesh spheres according to the legend, with distances (Å) between features marked by dashed red lines.

化合物**89**はF_2F_3 = 5.91Åとなり、hERGファーマコフォアモデル（F_2F_3 = 6.3–8.2 Å）から外れた。すなわち、化合物**89**のhERG阻害活性が改善されたのは、側鎖部の環化によってコンフォメーションが固定化し、Aronovによって提案されたhERGファーマコフォアモデルから外れたことによってhERGに対する親和性が低下したためだと考えられる。

Table 2-6. Mouse PK profiles of compounds **80**, **88** and **89**.

Compound	PO[a] (10 mg/kg; n=2)				IV[b] (1 mg/kg; n=2)				
	C_{max} (μM)	AUC_{inf} (μM·h)	T_{max} (h)	BA (%)	C_0 (μM)	AUC_{inf} (μM·h)	$T_{1/2}$ (h)	Vd_{ss} (L/kg)	CL_{inf} (mL/min/kg)
80	0.22	2.86	5.50	17	0.26	1.71	4.0	8.3	27.4
88	0.21	3.14	0.63	26	0.22	1.2	5.6	14.9	38.7
89	0.45	3.15	2.00	40	0.40	0.79	7.0	24.6	62.9

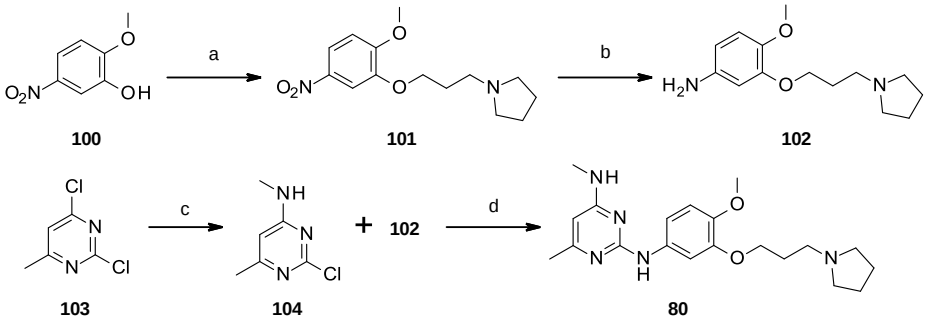
[a] Dosing vehicle: 0.5% MC.

[b] Dosing vehicle: DMSO/Tween80/saline= 1/1/8.

次に、化合物**80**及びテトラヒドロアゼピン誘導体**88**、**89**のPKプロファイルをTable 2-6に示す。マウスにおいて1 mg/kgの用量でIV投与した場合、化合物**80**、**88**、及び**89**は、クリアランスは中程度の値（それぞれ、27.4、38.7、及び62.9 mL/min/kg）を示し、半減期はそれぞれ4.0、5.6、7.0時間と好ましい値を示した。また、経口投与（10 mg/kg）において、テトラヒドロアゼピン誘導体**88**、**89**の方が化合物**80**よりわずかに血中曝露量（AUC_{inf}）が向上し（**88**：AUC_{inf} = 3.14 μM･h、**89**：AUC_{inf} = 3.15 μM･h）、さらに良好なバイオアベイラビリティ（**88**：BA = 26%、**89**：BA = 40%）を示した。なお、化合物**89**は化合物**88**と比較して高いC_{max}（0.45 μM）、バイオアベイラビリティ及び長い半減期を示したが、それはマウスにおける代謝安定性が化合物**88**より良好であるためだと考えられた（**88**: 2%、**89**: 24%）。

2-7　2,4-ジアミノ-6-メチルピリミジン誘導体の合成

化合物**80**の合成法をScheme 2-1に示す。

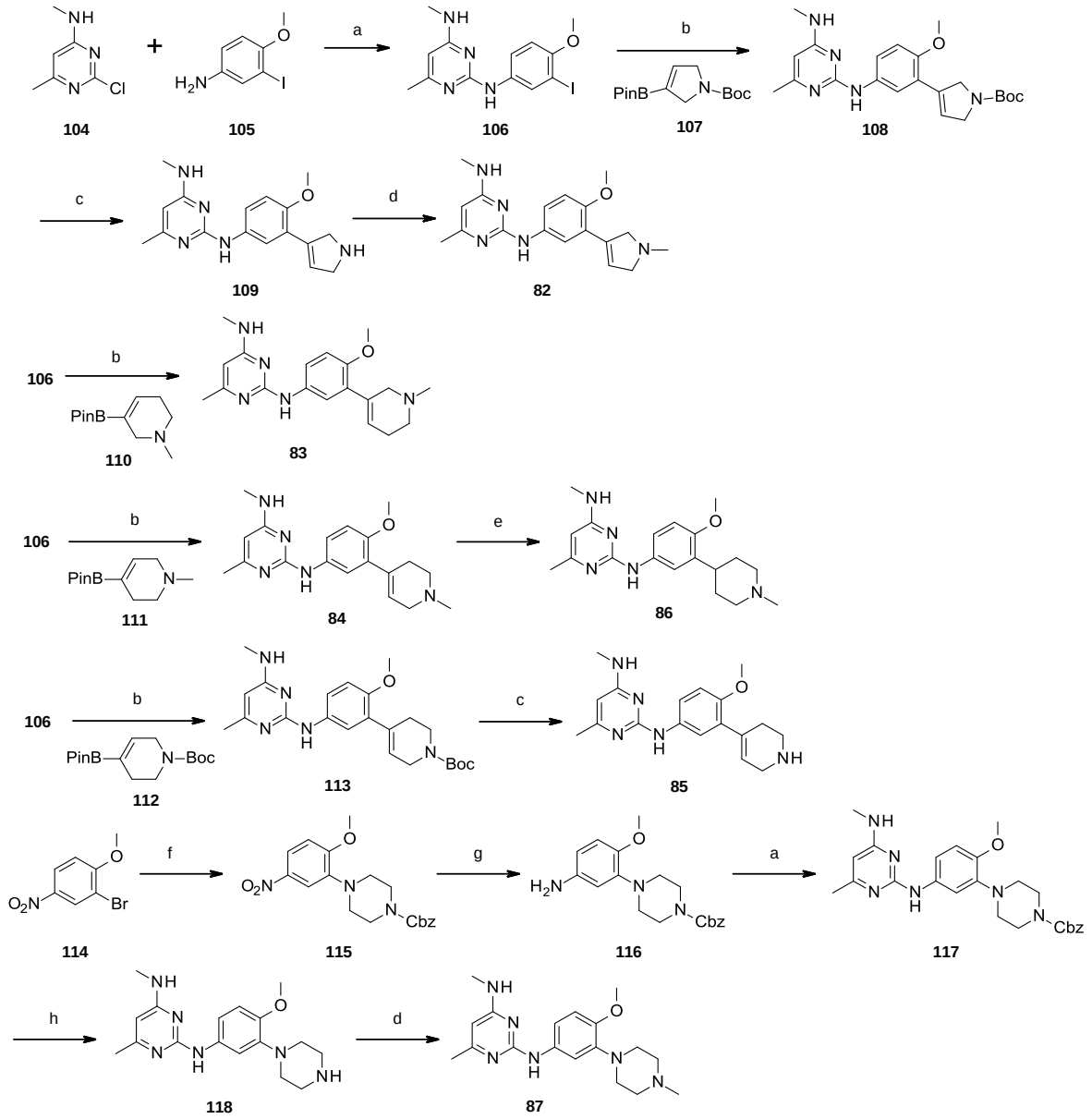


Scheme 2-1. Synthesis of compound **80**. *Reagents and conditions*: (a) 1-chloro-3-iodopropane, K_2CO_3, MeCN, rt to 80 ºC, 2 h; pyrrolidine, NaI, TBAI, K_2CO_3, MeCN, rt to 80 ºC, 3 h, 68% over 2 steps; (b) H_2, Pd/C, EtOH, rt, 3 h, 74%; (c) MeNH-HCl, K_2CO_3, DMF, rt, 5 h, 56%; (d) TFA, ^{i}PrOH, μW, 140 ºC, 1 h, 95%.

市販の化合物**100**を出発物質とし、1-クロロ-3-ヨードプロパンを用いてO-アルキル化を行った後、ピロリジンを作用させて化合物**101**を得た。化合物**101**に対してニトロ基の還元

を行い、アニリン体**102**を得た。一方、ジクロロピリミジン**103**に対して位置選択的にメチルアミノ基を導入し、化合物**104**を得た後、先の化合物**102**とS_NAr反応を行うことで目的とする化合物**80**を得た。

次に、化合物**82**–**85**の合成法をScheme 2-2に示す。



Scheme 2-2. Synthesis of compounds **82**–**87**. *Reagents and conditions*: (a) TFA, ^{i}PrOH, rt to 140 °C, μW, 100%; (b) Pd(dppf)Cl_2 (20 mol%), K_2CO_3, dioxane, H_2O, μW, 120 ºC, 1 h, 89%–98%; (c) TFA, CH_2Cl_2, rt, 0.5 h, 87%–89%; (d) formaldehyde, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 1 h, 82%–86%; (e) H_2, $Pd(OH)_2$/C, MeOH, rt, 12 h, 44%; (f) $Pd_2(dba)_3$ (10 mol%), Xantphos, ^{t}BuONa, toluene, rt to 100 ºC, 2 h, 66%; (g) Fe, NH_4Cl, MeOH, H_2O, rt to 80 ºC, 3 h, 73%; (h) H_2, $Pd(OH)_2$/C, AcOH, MeOH, rt, 3 h, 93%.

先に得た化合物**104**と化合物**105**を用いてS_NAr反応を行うことでヨード体**106**を得た後、

ボロン酸エステル**107**と鈴木-宮浦カップリングを行うことで化合物**108**を得た。続いて、Boc基を除去し、さらに生じたアミンに対して還元的アミノ化反応を行うことで目的とする化合物**82**を得た。同様に、ヨード体**106**とボロン酸エステル**110**及び**111**をそれぞれ用いて鈴木-宮浦カップリングを行うことで、化合物**83**、**84**をそれぞれ得た。化合物**86**は、化合物**84**より加水素反応によってアルケン部を還元することで得た。化合物**85**は、ヨード体**106**とボロン酸エステル**112**を用いて鈴木-宮浦カップリングを行った後、TFAによってBoc基を除去することで得た。続いて、化合物**87**の合成法はまず、市販の化合物**111**に対してBuchwald-Hartwigアミノ化反応によってピペラジル基を導入し、化合物**115**を得た。続いて、鉄を用いてニトロ基を還元後、S_NAr反応を行うことで化合物**117**を得た。次に、Cbz基を除去後、還元的アミノ化反応によってN-メチル化し、目的とする化合物**87**を得た。

次に、テトラヒドロアゼピン体**88**–**99**の合成を目指すこととした。近年のFDA承認薬の構造的分析を行った論文によると[74]、最も一般的な7員環複素環のトップ5に多数の縮環に加えて単環アゼパン及びテトラヒドロアゼピンが含まれている。これらの7員環複素環は、例としてFigure 2-13Aに挙げるように医薬品や天然物にも良く含まれる官能基だが、それらの合成法は6員環のピペリジンや5員環のピロリジンと比較して報告例が少ない。

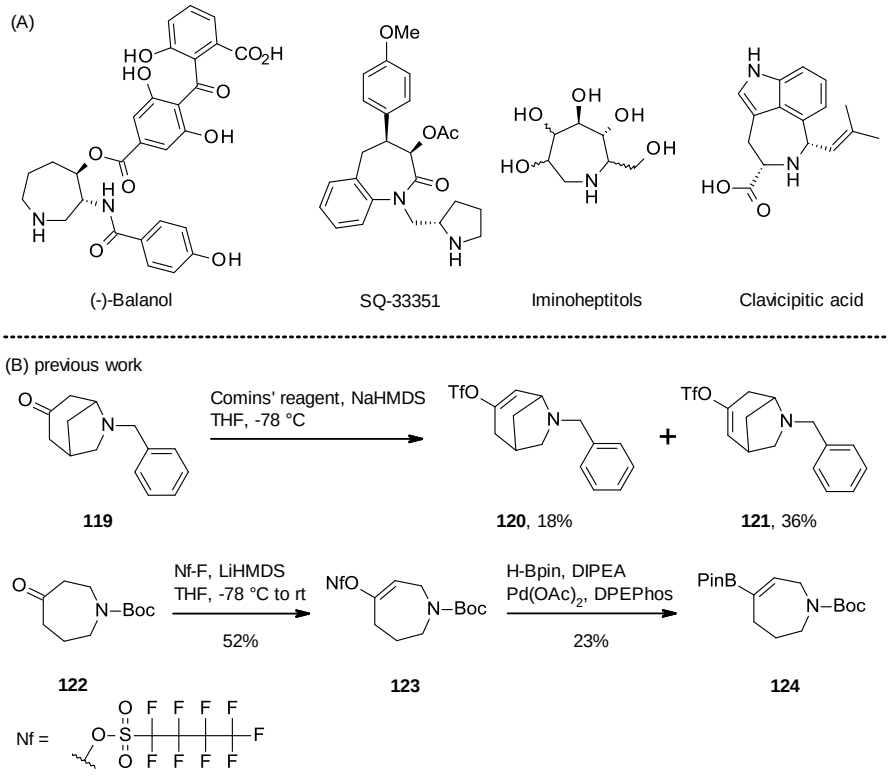


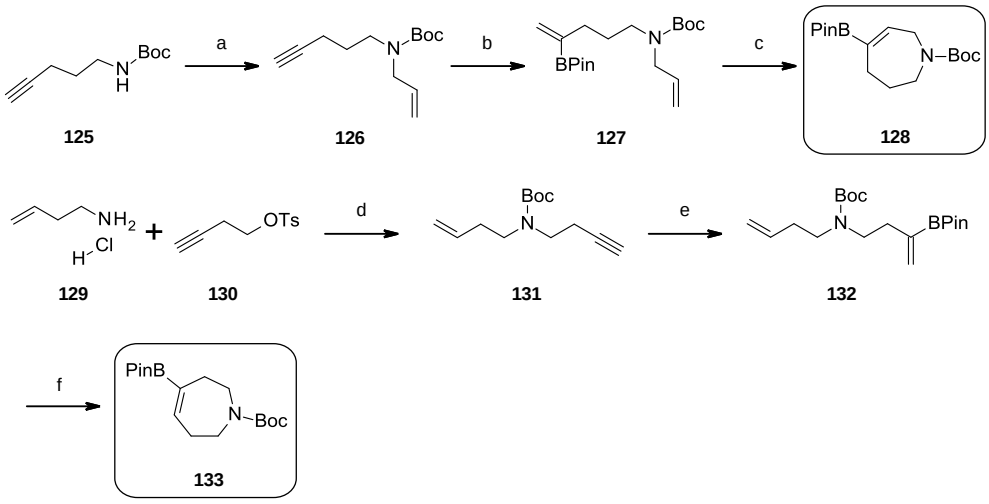
Figure 2-13. (A) Example of azepane and tetrahydroazepine containing natural products and drugs. (B) Previous reports on the synthesis of tetrahydroazepine.

テトラヒドロアゼピンカルボキシラートの合成は、いくつか報告例がある（Figure 2-13B）。Zaveriらは、6-ベンジル-6-アザビシクロ[3.2.1]オクタノン**119**に対してComin's試薬を作用させることでトリフラート体**120**及び**121**をそれぞれ低収率ながら得ている[75]。また、Clement

らは、*N*-Bocアゼピノン**122**に対して–78 ºC中LiHMDSを用いることで位置選択的に脱プロトン化を行い、ノナフレート体**123**を収率52%で得ている[76]。なお、位置選択性はアリルカルバメート窒素の遠距離誘導効果に起因すると推察されている。その後、彼らはノナフレート体**123**をボロン酸ピナコールエステルに変換しているが、収率が23%と低収率であった。

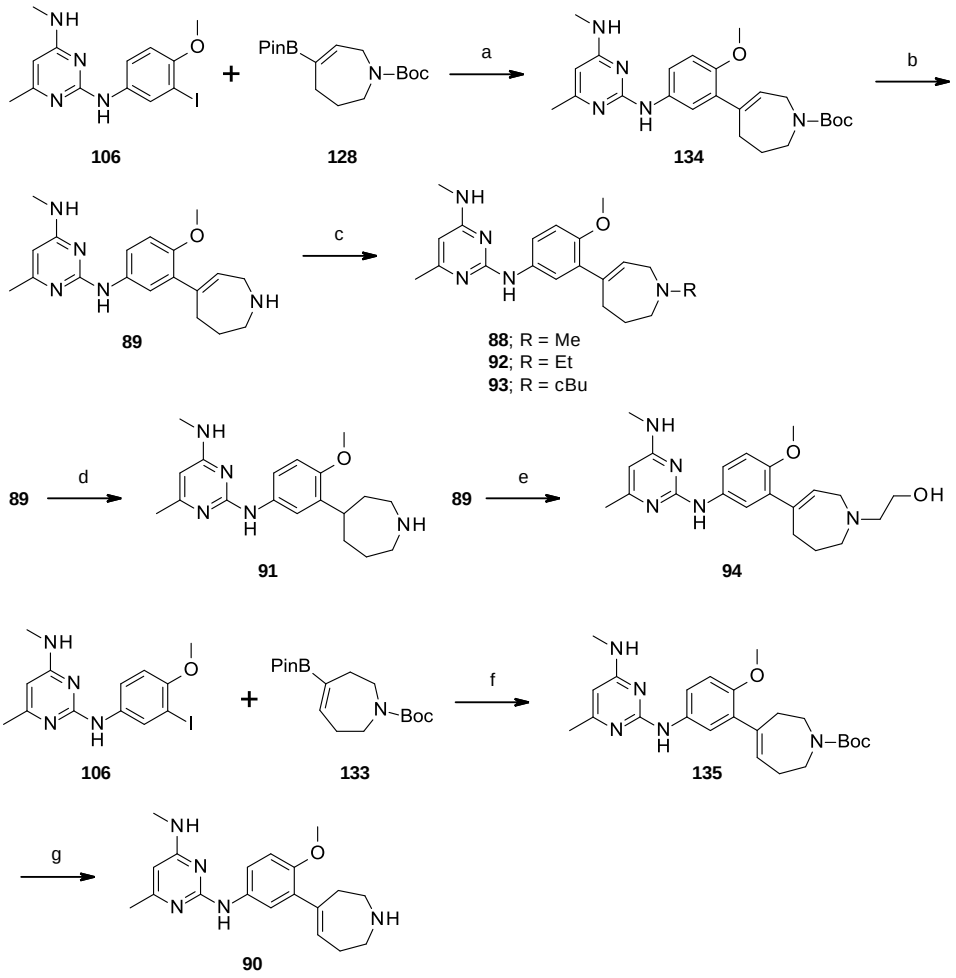
以上のように、*N*-Bocアゼピノンからピナコールボラン体を得るのは、位置選択性の問題や変換効率（収率）の低下の問題があり、より効率的な合成法が求められる。そこで筆者は、Grubbs触媒を用いた閉環メタセシス（RCM）反応を用いることで、テトラヒドロアゼピンのピナコールボラン体を合成することとした（Scheme 2-3）。

化合物 **125** をアリル化した後、アルキン体 **126** に対して位置選択的なヒドロホウ素化によって化合物 **127** を得た[77]。続いて、化合物 **127** に対して、Grubbs 触媒を用いて閉環メタセシス（RCM）反応を用いることで目的とするテトラヒドロアゼピン体 **128** を得た。化合物 **128** の位置異性体 **133** の合成も同様に、化合物 **129** に対して N-アルキル化を行った後、位置選択的なヒドロホウ素化を行うことで化合物 **132** を得た。そして、化合物 **132** に対して Grubbs 触媒を用いて閉環メタセシス（RCM）反応を用いることで、目的とするテトラヒドロアゼピン体 **133** を得た。



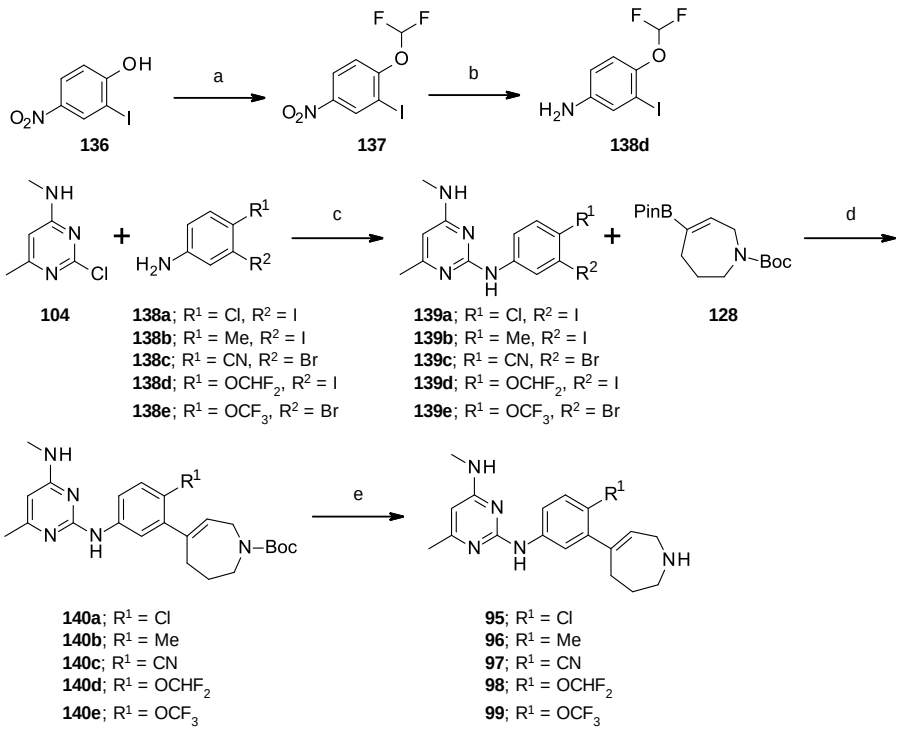
Scheme 2-3. Synthesis of compounds **128**, **133**. *Reagents and conditions*: (a) allyl iodide, NaH, DMF, rt, 3 days, 77%; (b) $(Pin)_2B_2$, CuCl, LiCl, AcOK, DMF, rt, 2 h, 63%; (c) 2nd Grubbs (5 mol%), CH_2Cl_2, rt, 15 h, 84%; (d) K_2CO_3, MeCN, rt to 85 ºC, 1 day; Boc_2O, rt, 1 h, 68%; (e) $(Pin)_2B_2$, CuCl, LiCl, AcOK, DMF, rt, 2 days, 58%; (f) 2nd Grubbs (5 mol%), CH_2Cl_2, rt, 3 h, 92%.

次に、化合物 **88–94** の合成法を Scheme 2-4 に示す。化合物 **106** と先に得た化合物 **128** を用いて鈴木-宮浦カップリングを行った後、Boc 基を除去することで化合物 **89** を得た。続いて、対応するアルデヒドまたはシクロブタノンを用いて還元的アミノ化反応を行うことで、目的とする化合物 **88**、**92** 及び **93** を得た。また、化合物 **89** に対して接触還元することで還元体 **91** を得た。また、化合物 **89** に対してブロモエタノールを用いて N-アルキル化を行うことで化合物 **94** を得た。化合物 **90** は、先と同様に、化合物 **106** と先に得た化合物 **133** を用いて鈴木-宮浦カップリングを行った後、Boc 基を除去することで得た。



Scheme 2-4. Synthesis of compounds **88**–**94**. *Reagents and conditions*: (a) Pd(dppf)Cl_2 (20 mol%), K_2CO_3, dioxane, H_2O, rt to 120 °C, 1 h, μW, 98%; (b) TFA, CH_2Cl_2, rt, 0.5 h, 87%; (c) aldehyde or cyclobutanone, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 1 h, 74%–83%; (d) H_2, $Pd(OH)_2$/C, MeOH, rt, 12 h, 80%; (e) 2-bromoethanol, NaI, Cs_2CO_3, MeCN, rt to 80 ºC, 2 h, 69%; (f) Pd(dppf)Cl_2 (20 mol%), K_2CO_3, dioxane, H_2O, rt to 120 °C, 1 h, μW, 93%; (g) TFA, CH_2Cl_2, rt, 0.5 h, 82%.

次に、化合物**95**–**99**の合成法をScheme 2-5に示す。市販のフェノール体**136**に対してジフルオロ化した後、鉄によってニトロ基を還元することでアニリン体**138d**を得た。化合物**104**と対応するアニリン**138a**–**138e**を用いてS_NAr反応を行うことで、化合物**139a**–**139e**をそれぞれ得た。続いて、化合物**128**と鈴木-宮浦カップリングを行った後、Boc基を除去することで目的とする化合物**95**–**99**をそれぞれ得た。



Scheme 2-5. Synthesis of compounds **95**–**99**. *Reagents and conditions*: (a) diethyl (bromodifluoromethyl)phosphonate, KOH, MeCN, H_2O, 0 °C, 3 h, 79%; (b) Fe, NH_4Cl, MeOH, H_2O, 80 ºC, 3 h, 92%; (c) **104**, TFA, ^{i}PrOH, μW, 140 ºC, 1 h, 52%–96%; (d) Pd(dppf)Cl_2 (20 mol%), K_2CO_3, dioxane, H_2O, rt to 120 °C, 1 h, μW, 86%–98%; (e) TFA, CH_2Cl_2, rt, 0.5 h, 65%–89%.

2-8　小括

2015 年、Renneville らはヒストンメチルトランスフェラーゼ（HMT）である G9a または GLP の個々の遺伝子ノックダウンが HbF 産生を誘導することを明らかとした。したがって、HbF 産生を誘導できる強力な G9a/GLP 阻害剤の開発は、β-サラセミア及び SCD を治療するための標的アプローチとなる可能性があると考えられ、筆者は Epizyme 社から報告された EPZ035544（**80**）を出発化合物として強力な新規 G9a/GLP 阻害剤の創製を目指すこととした。化合物 **80** は、社内の *in vitro* アッセイを用いて評価したところ、G9a/GLP に対してそれぞれ IC_{50} 値 16.9/185 nM の阻害活性を示し、hERG 阻害活性を示した（42% at 10 μM）。さらに、この化合物のマウス PK プロファイルは良好ではなかった（BA: 17%）。本章では、G9a/GLP 阻害活性を保持しながら、hERG 阻害活性と PK プロファイルを改善した化合物の獲得を目指した。

まず、合成展開を開始する前に、hERG阻害活性の改善に向けた取り組みとしてhERG阻害活性を示すファーマコフォアモデルについて着目した。EPZ035544は、最安定配座においてAronovによって提案されたhERGファーマコフォアモデルに全て含まれていることがわかった。

一方、EPZ035544のG9aとの共結晶を取得し、右側の側鎖の3-（ピロリジン-1-イル）プロポキシ基の活性コンフォメーションを確認したところ、真っ直ぐに伸びるのではなく丸く歪んでいることがわかった。

以上のことから、側鎖部を活性コンフォメーションのように曲がったまま環化して固定化することで、G9a/GLP活性を保持したままhERG阻害が改善するのではないかと考え、本仮説の検証を実施することとした。コンフォメーションの固定化は、化合物の結合親和性、PKプロファイル、及びオフターゲットプロファイル（hERG阻害及びCYP阻害）の改善に役立つため、一般にドラッグデザインの最も効果的な方法の1つと考えられている。期待通り、テトラヒドロアゼピン体**89**は、化合物**80**と比較してG9aに対して4倍、GLPに対して6倍活性が向上した。また、化合物**89**とG9aのX線結晶構造を決定したが（PDB code: 7BUC）、化合物**80**と同様の結合モードが観察され、テトラヒドロアゼピンのアミノ基はLeu1086のカルボニル酸素と水素結合を形成し、G9aと重要な相互作用を示すことがわかった。さらに、化合物**89**のhERG阻害活性は10 μMにおいて1%となり、化合物**80**より改善した。化合物**89**のhERG阻害活性が改善した理由は、側鎖部の環化によってコンフォメーションが固定化し、Aronovによって提案されたhERGファーマコフォアモデルから外れたためだと考察した。

次に、マウス PK 試験を実施したところ、化合物 **89** は、化合物 **88** と比較して優れた PK プロファイルを示した（AUC_{inf} = 3.15 μM*h、BA = 40%、C_{max} = 0.45 μM）。

合成の点では、側鎖のテトラヒドロアゼピン体の誘導体合成を志向した合成を目指し、位置選択的なヒドロホウ素化及び Grubbs 触媒を用いた閉環メタセシス（RCM）反応を用いることで、効率的なテトラヒドロアゼピン体の合成法を確立した。

以上より、側鎖の設計、合成、活性評価、及び構造最適化により、化合物 **89** が同定され、化合物 **80** と比較して G9a/GLP 阻害活性が増加した。さらに、化合物 **89** は、化合物 **80** よりも hERG 阻害活性が改善し、マウスにおける PK プロファイルも改善した（BA: 17%→40%）。

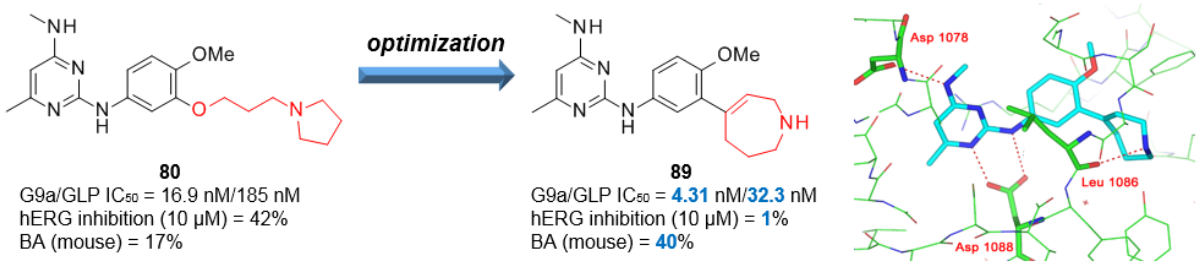


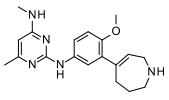
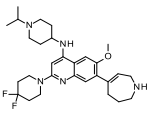
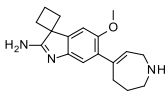
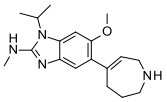
Figure 2-14. Summary of section 2.

第 3 章　HbF 誘導効果を示す新規 G9a/GLP 阻害剤の獲得

3-1　テトラヒドロアゼピン側鎖の他骨格への応用

前章では、既知のG9a/GLP阻害剤の側鎖としてよく用いられている3-（ピロリジン-1-イル）プロポキシ基の変換の検討を行い、テトラヒドロアゼピン基が活性やhERG阻害活性及びPKプロファイルの点で有用であることを述べた。本章ではまず、側鎖のテトラヒドロアゼピン基の汎用性を調べるために、既知のG9a/GLP阻害剤の他の中心骨格に導入し、活性やADMETプロファイル、PKプロファイルを取得した（Table 3-1）。

Table 3-1. Chemical structures, activity, ADMET profiles, and PK profiles of tetrahydroazepine derivatives.

Compound	**89**	**141**	**142**	**143**
Structure				
G9a IC_{50}[a] (nM)	4.31 ± 0.179	2.00 ± 0.0496	4.50 ± 0.155	22.8 ± 1.71
GLP IC_{50}[a] (nM)	32.3 ± 1.10	6.15 ± 0.366	33.9 ± 2.57	390 ± 34.4
Log D (pH = 7.4)	0.5	0.3	-0.8	-0.3
MDCK Papp (10^{-6} cm/s)	1.4	0.23	2.7	0.6
Metabolic stability % (human/monkey/mouse)	96/24/34	87/72/88	99/47/95	98/92/63
hERG inhibition (1/3/10/30 μM)	2/-1/1/23	N.T.[b]	5/6/5/5	8/2/3/8
PK parameters (mouse: 10 mg/kg, p.o.)				
C_{max} (μM)	0.45	0.02	0.04	0.03
AUC_{inf} (μM*h)	3.15	0.02	0.64	0.16
BA %	40	N.T.[b]	4.5	1.6

[a] The IC_{50} values are presented as the mean of four technical replicates ± the standard error of the mean (SEM).

[b] Not tested.

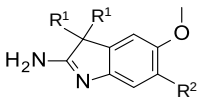
まず、Figure 2-5に示すG9a/GLP阻害剤**77**及び**78**の活性結果に基づいて、キノリン誘導体**141**を合成したところ、期待通り化合物**89**よりも強力な活性を示した（IC_{50} = 2.00 nM for G9a、6.15 nM for GLP）。次に、インドール誘導体A-366（**79**）にテトラヒドロアゼピル基を導入した化合物**142**は、活性が保持された。最後に、既知のベンズイミダゾール**81**にテトラヒドロアゼピル基を導入したベンズイミダゾール化合物**143**は、化合物**89**と比較して活性が5倍程度減少したが、なおもG9aに対してIC_{50} = 22.8 nMを示した。また、既知のG9a/GLP阻害剤の課題であったhERG阻害活性がいずれの骨格においても改善傾向を示した（<10% at 10 μM）。以上より、テトラヒドロアゼピン側鎖が様々な中心骨格に対してもG9a/GLP活性を示し、汎用性を有することを明らかとした。

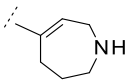
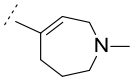
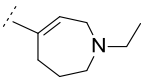
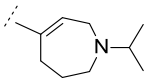
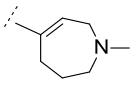
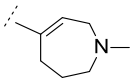
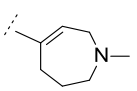
キノリン誘導体**141**は、ADMETプロファイルの結果、代謝安定性は良好だが膜透過性が悪く、PKプロファイルもC_{max}、AUC_{inf}と共に低い値を示した。分子量も500 g/molを超えており、合成展開の優先度は低いと考えた。また、ベンズイミダゾール化合物**143**は、PKプロファイルにおいてAUC_{inf}は化合物**141**よりは良いが、C_{max}は低い値を示した。そして何より活性が他の骨格に比べて低いことより、合成展開の優先度は低いと考えた。以上より、膜透過性と代謝安定性が良好で、PKプロファイルにおいても血中曝露量（AUC_{inf}）が中程度を示したインドール誘導体に焦点を当て、G9a/GLP阻害活性を保持しながらPKプロファイルが改善された新規化合物の取得を目指すこととした。

3-2　インドール誘導体の合成展開

まず、インドール誘導体のテトラヒドロアゼピン環の窒素置換基の変換及びシクロブチル基の変換を行った（Table 3-2）。

Table 3-2. SAR of Indole derivatives.



Compound	R^1	R^2	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]
142			4.50 ± 0.155	33.9 ± 2.57
144 (DS79932728)			12.6 ± 0.228	75.7 ± 2.21
145			3.13 ± 0.178	20.3 ± 0.765
146			8.57 ± 0.426	61.6 ± 2.51
147			32.0 ± 1.45	155 ± 4.80
148			172 ± 11.2	1057 ± 67.8
149			719 ± 54.4	3491 ± 143

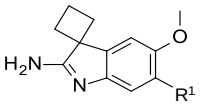
[a] The IC_{50} values are presented as the average of four technical replicates ± the standard error of the mean (SEM).

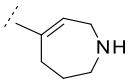
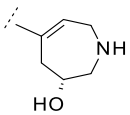
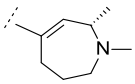
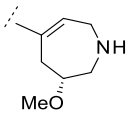
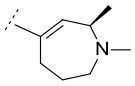
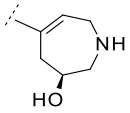
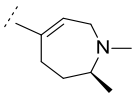
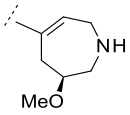
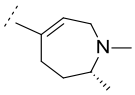
エチル基**145**は活性を保持したが、メチル基**144**は活性が1/3に低下し、イソプロピル基**146**は活性が1/2に低下した。続いて、化合物**144**のシクロブチル基をシクロペンチル基**147**及びシクロヘキシル基**148**で置き換えると、活性は減弱した（それぞれIC_{50} = 32, 172 nM）。これらの活性結果は、A-366誘導体の結果と一致し[61]、これら誘導体がA-366と同じG9a/GLPタン

パク質部位に結合することが示唆された。同様に、テトラヒドロピラニル体**149**は、活性が大きく減弱し、この部位において極性基の導入は困難であることがわかった。

次に、SARをさらに取得して活性を向上させるために、テトラヒドロアゼピン環上の置換基導入を行った（Table 3-3）。テトラヒドロアゼピン環のα位にメチル基を導入した化合物**150–153**においては、化合物**152**のみ活性が保持傾向を示したものの、他の化合物は残念ながら活性が低下した。また、テトラヒドロアゼピン環のβ位にヒドロキシ基またはメトキシ基を導入した化合物**154–157**は、化合物**156**のみ活性が保持傾向を示したものの、軒並み活性が低下した。これらの活性結果について共結晶構造に基づく考察は、3-4で説明する。

Table 3-3. SAR of Indole derivatives.



Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]	Compound	R^1	G9a IC_{50} (nM)[a]	GLP IC_{50} (nM)[a]
142		4.50 ± 0.155	33.9 ± 2.57	**154**		155 ± 16.5	1424 ± 78.4
150		2148 ± 93.3	>5000	**155**		2331 ±99.8	>5000
151		353 ± 18.7	2174 ± 126	**156**		34.6 ± 2.70	385 ± 21.6
152		20.7 ± 0.559	85.5 ± 2.19	**157**		3151 ±142	>5000
153		102 ± 3.83	205 ± 7.05				

[a] The IC_{50} values are presented as the average of four technical replicates ± the standard error of the mean (SEM).

3-3　G9a阻害活性値とGLP阻害活性値の相関

これまで合成してきた132個のG9a/GLP阻害剤を用いてG9a阻害活性値とGLP阻害活性値の相関関係を調べた（Figure 3-1）。R^2値は0.87となり、高い正の相関関係があることがわかった（なお、活性比はG9a/GLP =約3.1でG9aに対する活性の方が強かった）。これは、G9aとGLPの相同性は80%と高く、G9a/GLP阻害剤と相互作用するアミノ酸残基もほぼ同じであるためだと考えられる。

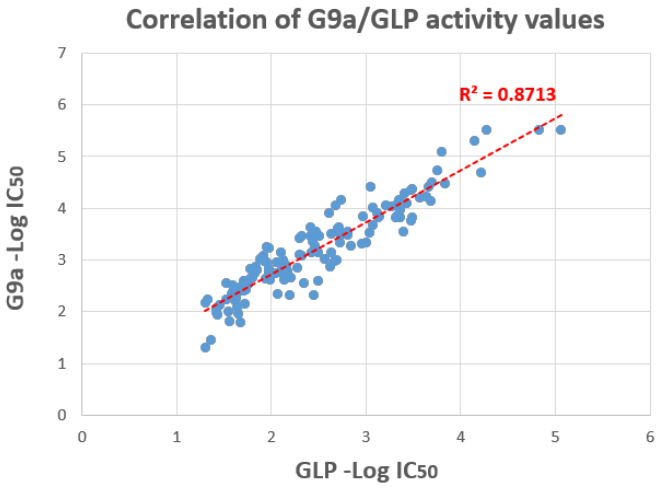


Figure 3-1. The R^2 value was calculated from the G9a/GLP inhibitory activity values of the 132 compounds synthesized in-house.

3-4　化合物144とG9aの共結晶構造解析

新たに見出された新規インドール誘導体の結合様式を確認するために、化合物**144**とG9aのX線共結晶構造を決定した（Figure 3-2）。化合物**144**とG9aの共結晶構造は、以前報告されたA-366（**79**）[61]と同じ結合部位であり、SAMに対して非競合的阻害様式を示すことが明らかとなった。水素結合パターンは、Leu1086の側鎖とテトラヒドロアゼピンのアミノ基、及びAsp1078、Asp1088、Asp1074の側鎖とアミジン基の間において相互作用をすることがわかった。これらの結合様式もA-366と類似していた。

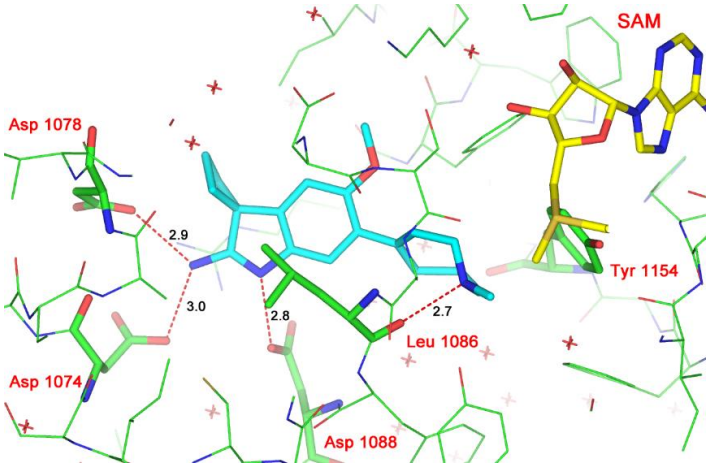


Figure 3-2. X-ray crystal structure of compound **144** bound to G9a (PDB code: 7DCF).

この共結晶構造に基づいて、化合物**150**–**157**の活性結果を考察した。化合物**150**、**151**及び**153**は、Y1154による立体反発のために活性が低下した。そして、化合物**152**のみが低い立体反発のために活性が保持傾向を示したと推察した。さらに、メトキシ誘導体**155**及び**157**の活性は、同様にY1154による立体反発のために大幅に減弱したと推察した。一方、ヒドロキシ誘導体**154**及び**156**は、Y1154の主鎖のカルボニル基と水素結合を形成し、特に化合物**156**

の水素結合角は化合物**154**よりも優れているため、活性が保持することになったと考えられる。

続いて、分子モデリングソフトウェアMaestro（version 11.4） を用いて、Figure 3-2の複合体の化合物**144**の構造を、化合物**150–157**へと変換したときのポテンシャルエネルギーを分子力学シミュレーションソフトウェアImpactで計算し、pIC_{50}との関係を調べた（Figure 3-3）。ここでのポテンシャルエネルギーは、変換した置換基とG9aタンパク質のぶつかりの程度を大まかに反映した値である。化合物**152**、**156**等の活性が強い化合物はポテンシャルエネルギーが小さく、活性が弱い化合物**150**、**151**、**155**、及び**157**は、ポテンシャルエネルギーが大きくなっていることがわかった。以上より、テトラヒドロアゼピン誘導体**150–157**の活性は、G9aタンパク質とのぶつかり具合が大きく影響していることが示唆された。

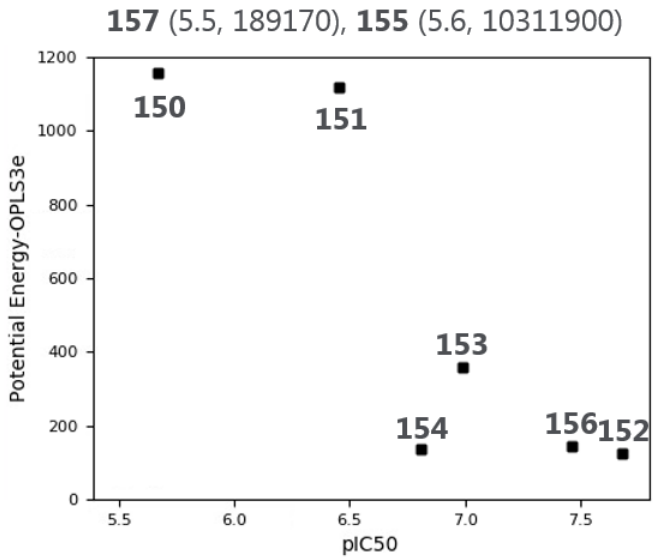


Figure 3-3. Correlation between potential energy and pIC_{50}.

次に、化合物**144**と前章で述べたピリミジン体**89**の共結晶構造の重ね合わせ図を作成し、結合様式を比較することとした（Figure 3-4）。

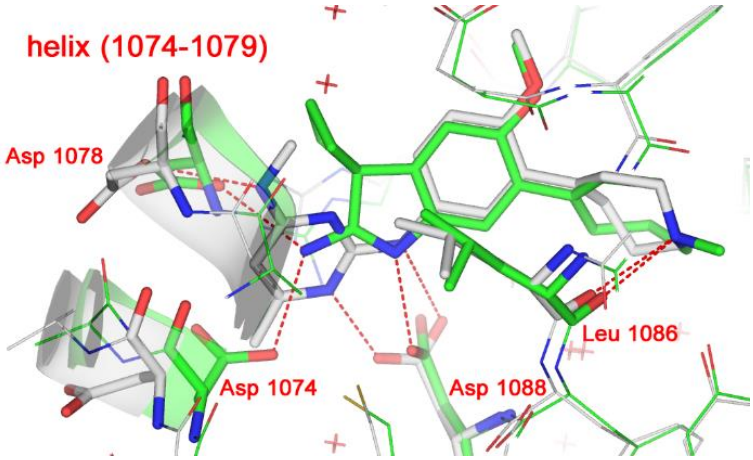
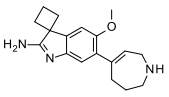
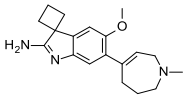
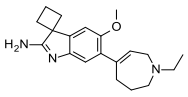


Figure 3-4. Overlay of the X-ray crystal structures of compounds **89** (gray, PDB code: 7BUC) and **144** (green, PDB code: 7DCF) bound to G9a.

各阻害剤のテトラヒドロアゼピン部分はほぼ同じ位置にあり、Leu1086の側鎖に水素結合していることがわかった。一方、G9aタンパク質のヘリックス（1074–1079残基）は各共結晶でずれており、化合物**144**の場合は阻害剤により近づいていた。各阻害剤の分子サイズの違いを考慮すると、テトラヒドロアゼピン部分とLeu1086の相互作用が保存されることより、この相互作用が活性部位における阻害剤の位置を制御していると考えられる。その結果、ヘリックス（1074-1079残基）との相互作用様式が阻害剤間で異なったと考えられる。それに伴い、化合物**144**の第一級アミノ基は、Asp1078及びAsp1074の側鎖と水素結合を形成するが、化合物**79**のアミノ部分は、Asp1074とではなく、Asp1078の側鎖のみと水素結合を形成する。さらに、Asp1088との相互作用において、化合物**79**はアミノピリミジン部と水素結合及び塩橋を形成するが、化合物**144**のインドール窒素はAsp1088とのみ塩橋を形成する。

3-5　代表化合物の初期ADMETとPKプロファイル

Table 3-4. Physicochemical properties and ADMET profiles of compounds **142**, **144**, and **145**.

Compound	**142**	**144**	**145**
Structure			
Log D (pH = 7.4)	–0.8	–0.1	0.1
Solubility[a] (μg/mL) (JP1/JP2)	590/590	620/600	>650/>650
MDCK Papp (10^{-6} cm/s)	2.7	0.8	0.6
MS % (human/monkey/mouse)	47/95/99	21/79/98	14/86/95
CYP direct inhibition[c]	0/0/0/2/0/1	7/4/8/5/4/10	0/0/0/0/0/1
MBI[d]	82	94	90
hERG inhibition (1/3/10/30 μM)	5/6/5/5	–6/–9/–6/1	4/4/8/16
Ames assays[f]	N.T.[f]	Negative	N.T.[f]
Rat hepatocyte toxicity (IC_{50})	N.T.[f]	>300 μM	N.T.[f]

[a] JP1/JP2: Japanese Pharmacopoeia first/second test fluid (pH = 1.2/6.8).

[b] The percentage (%) of the tested compound remaining after 0.5 h of incubation with mouse/monkey/human liver microsomes (0.5 mg/mL).

[c] The percentage (%) inhibition of 1A2/2C8/2C9/2C19/2D6/3A4 at 10 μM.

[d] The percentage (%) remaining at a concentration of 100 μM of compound reacted with CYP3A4 probe substrates after 30 min of preincubation in human liver microsomes.

[e] Ames assays were performed up to 1000 μg/well with or without S9 using *Salmonella* TA98 and TA100.

[f] Not tested.

代表的な化合物**142**、**144**、及び**145**のADMETプロファイルをTable 3-4に示す。これら化

合物は低いLog D値を示し、脂溶性が低いことがわかった。また、溶解度は酸性pH及び中性pHいずれにおいても高く、サルとヒトの肝臓ミクロソームにおいても両方で高い代謝安定性を示した。CYP阻害やCYP3A4のメカニズムベースの阻害（MBI）においても、3化合物は阻害活性を示さなかった。また、hERG阻害活性は、最大30 μMの濃度でも化合物**142**、**144**は10%以下の阻害率となり、化合物**89**と比較して改善された（23% at 30 μM）。なお、化合物**144**は、Ames試験でも陰性であり、ラット肝細胞毒性試験でもIC_{50} >300 μMとなり安全性の高いG9a/GLP阻害剤と考えられる。

次に、化合物**142**、**144**、及び**145**のマウスPKプロファイルをTable 3-5に示す。化合物**142**は、これらの化合物の静脈内（IV）投与で最も高いC_0値を示したが、経口投与での血中曝露量は低く、バイオアベイラビリティ（BA）も4.5%と最も低かった。一方、化合物**144**は、CL_{inf}がやや高めにもかかわらず、経口投与において3化合物の中で最も高い血中曝露量（AUC_{inf}）とBAを示した。化合物**145**はIV投与でCL_{inf}が低かったが、経口曝露では化合物**144**よりも低かった。以上より、マウスでの血中曝露量（AUC_{inf}）が高く、BAが96%と良好であった化合物**144**を用いて次にサルPK試験を実施した。

Table 3-5. Mouse pharmacokinetics profiles of compounds **142**, **144**, and **145**.

Compound	PO[a] (10 mg/kg; n = 2)				IV[b] (1 mg/kg; n = 2)				
	C_{max} (μM)	AUC_{inf} (μM·h)	T_{max} (h)	BA (%)	C_0 (μM)	AUC_{inf} (μM·h)	$T_{1/2}$ (h)	Vd_{ss} (L/kg)	CL_{inf} (mL/min/kg)
142	0.04	0.64	3.75	4.5	2.4	1.4	21.1	36.5	39.4
144	0.44	12.0	1.5	96	1.5	1.2	11.9	31.1	43.8
145	0.51	3.6	1.00	11	1.3	3.4	13.9	14.7	15.0

[a] Dosing vehicle: 0.5% methylcellulose (MC) solution.

[b] Dosing vehicle: DMA/Tween80/saline = 10/10/80.

Table 3-6 に、化合物 **144** のサル PK プロファイルを示す。化合物 **144** は、AUC_{inf} = 1.36 μM·h と高い血中曝露量を示し、経口投与による *in vivo* サル PD 研究に適したプロファイルを持っていることを確認した。したがって、化合物 **144** を用いて高次評価を実施することとした。

Table 3-6. Monkey pharmacokinetics profile of compound **144**.

Compound	PO[a] (5 mg/kg; n = 2)				IV[b] (0.5 mg/kg; n = 2)				
	C_{max} (μM)	AUC_{inf} (μM·h)	T_{max} (h)	BA (%)	C_0 (μM)	AUC_{inf} (μM·h)	$T_{1/2}$ (h)	Vd_{ss} (L)	CL_{inf} (mL/min/kg)
144	0.11	1.36	3.00	38	0.86	0.35	22.7	109	77.7

[a] Dosing vehicle: 0.5% MC solution.

[b] Dosing vehicle: DMA/Tween80/saline = 10/10/80

次に本系統化合物のADMEとPKプロファイルの結果を基に、ADMEパラメーターと薬物動態の傾向について考察することとした。まず、脂溶性の指標であるLog Dと膜透過性の指標であるMDCK透過性をプロットしたところ、おおよそ相関があることがわかった（Figure 3-5A）。本系統の化合物は、一般的に膜透過性が良いとされる10×10^{-6} cm/s以上の値（薬物の吸収率が約90%以上となる膜透過性の値）[78]を示す化合物は少なく、膜透過性に課題があったことがわかる。また、MDCK 透過性が10×10^{-6} cm/s以上の値を示すには、おおよそLog D = 2以上が好ましいことがわかった。続いて、膜透過性の指標であるMDCK透過性とPK試験において経口投与時のAUC_{inf}とをプロットしたところ、こちらもおおよそ相関があることがわかった（Figure 3-5B）。したがって、本系統のような脂溶性が低く、膜透過性があまり良好でない化合物に対しては、MDCK透過性の増加がAUC_{inf}、すなわち経口吸収性及び血中曝露量の改善につながることが示唆された。

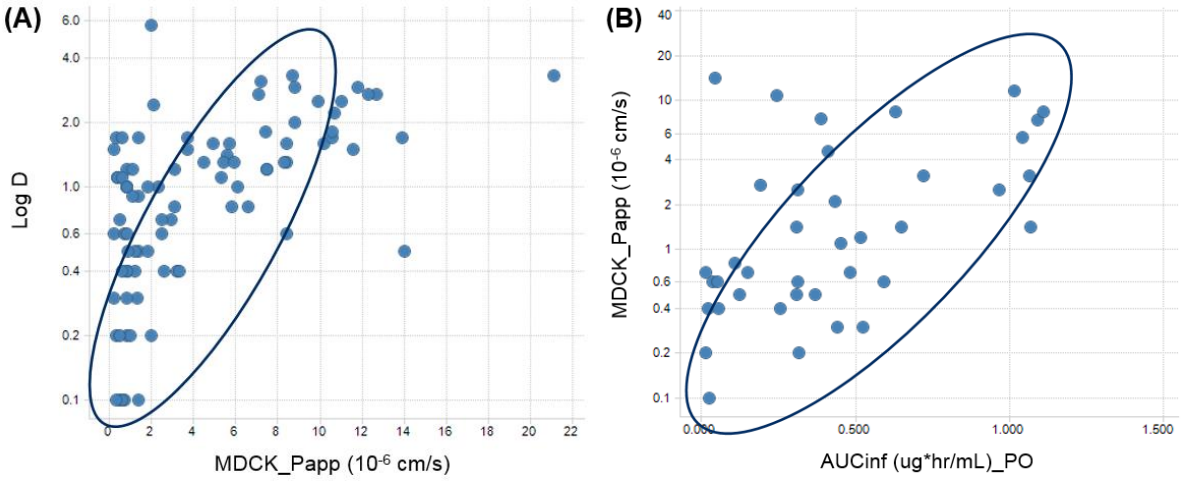


Figure 3-5. (A) Correlation between MDCK permeability and Log D. (B) Correlation between AUC_{inf} and MDCK.

3-6　化合物 144 のサル骨髄単核細胞を用いた *in vitro* 評価

カニクイザル骨髄単核細胞を用いて、化合物**144**、デシタビン及びヒドロキシ尿素のHbF産生誘導活性を評価した（Figure 3-6）。デシタビンは、カニクイザル骨髄単核細胞におけるHbF産生を増強するという報告があるため[79]、陽性対照として用いた。予想通り、γ-グロビン陽性細胞の割合がデシタビンと化合物**144**において濃度依存的に増加し、化合物**144**がカニクイザル骨髄単核細胞においてデシタビンと同様のHbF産生を誘導できることが明らかとなった。一方、ヒドロキシ尿素ではγ-グロビン陽性細胞の増加は観察されなかった。これは、ヒドロキシ尿素の薬理作用のメカニズムがストレス赤血球生成に基づいており、グロビンスイッチングを検出する本モデルではストレス赤血球生成を検出できないため、ヒドロキシ尿素はγ-グロビン発現の増加を示さなかったと考えられる。また、デシタビン及びヒドロキシ尿素と比較して、β-グロビン陽性細胞の減少は化合物**144**の方が比較的穏やかであり、化合物**144**が赤血球の増殖及び分化を劇的に阻害せず、デシタビンやヒドロキシ尿素よりも安全性が高いことが示唆された。

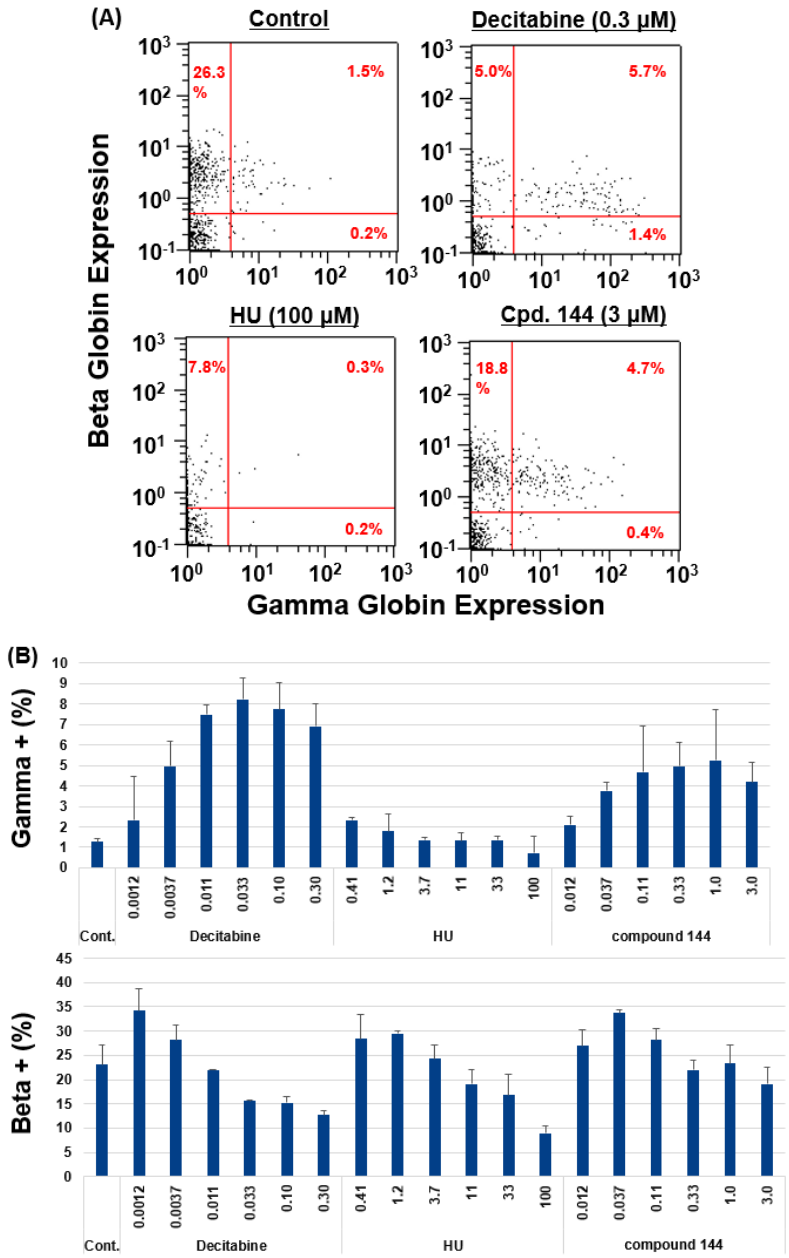


Figure 3-6. Assessment (F-cell; flow cytometry [FCM]) of *in vitro* activity of decitabine, hydroxyurea (HU), and compound **144** using cynomolgus monkey bone marrow mononuclear cells. (A) γ-Globin and β-globin levels in the cells were evaluated by FCM. Representative FCM plots of each compound are shown. (B) The percentage of γ-globin- and β-globin-positive cells at each concentration of the compounds is shown. Data are expressed as the mean ± the standard deviation of the three biological replicates.

3-7　化合物 144 のヒト培養骨髄 CD34+細胞を用いた *in vitro* 評価

化合物**144**を用いて、2-5と同様にヒト培養骨髄CD34+細胞による成人型β-グロビンから胎児型γ-グロビンへのグロビンmRNA発現の切り替え（グロビンスイッチング）を評価した（Figure 3-7）[72]。化合物**144**の細胞グロビンスイッチング活性は、化合物**79**のそれとほぼ同じであることがわかった。以上より、化合物**144**はサル細胞だけでなくヒト細胞においても

HbF活性を示すことがわかった。

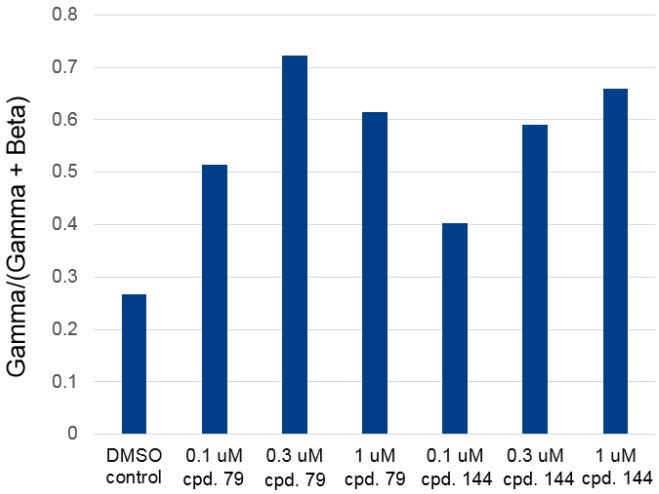


Figure 3-7. Effects of compounds **79** and **144** on the cellular globin switching activity (γ/γ+β-globin mRNA ratio) using human bone marrow-derived hematopoietic progenitor CD34+ cells. Data are expressed as the average response from two biological replicates.

3-8　化合物 144 の静脈切開したカニクイザルモデルを用いた *in vivo* 評価

代表化合物**144**を用いて*in vivo*におけるγ-グロビンのタンパク質発現量の誘導を評価することとした。一般に、関連する胚（ε）、胎児（γ）、及び成体（β）遺伝子からなるヒトβ-グロビン遺伝子座を有するトランスジェニックマウスは、ヒトで起こるような一過性の胎児から成体へのヘモグロビンスイッチングを確認するためのシステムである。 しかし、これらのマウスのヒトγ-グロビンはマウスの胚性グロビン遺伝子（ε-グロビン）として機能することが報告されており[80]、このマウスのモデルでグロビンスイッチングによる薬理学的有効性を確認することには限界があると考えた。 実際、ヒドロキシ尿素はSCDのトランスジェニックマウスモデルでは、HbFの誘導効果を示さないことが報告されている[81]。 一方、ヘモグロビンスイッチングを保持するヒヒまたはカニクイザルモデルでHbFを誘導することが示されている[82]。上記の知見に基づいて、γ-グロビンの発現パターンが保存されている非ヒト霊長類、すなわちカニクイザルを用いて化合物**144**のγ-グロビン産生の誘導効果を確認することとした[83]。

化合物**144**は、ヒドロキシ尿素を対照として静脈切開したカニクイザルモデル（週に3回静脈切開して、血中ヘモグロビンレベルが10.5 g/dL未満の貧血モデルを確立した）を使用して、γ-グロビンを示すF-網状赤血球（F-ret）値を分析し、γ-グロビン産生の誘導を確認した（Figure 3-8）。化合物**144**を1日目から5日目まで15 mg/kgの用量で3匹のサル（化合物144-1、144-2及び114-3）に1日2回経口投与した。ヒドロキシ尿素は、SCD治療において臨床的に使用される最高用量に相当する35 mg/kgの用量で3匹のサル（HU-1、HU-2及びHU-3）に1日目から5日目まで1日1回経口投与した。

結果は、化合物 **144** は 15mg/kg で γ-グロビンのタンパク質発現量の誘導を示し、ヒドロキシ尿素と同等の薬効を示した。また、化合物 **144** の連続投与によっておおよそ 30%の HbF が誘導されており（Figure 3-8B）、序論で述べたように β-サラセミアと SCD の疾患進行の

改善につながるおおよそ 30%の HbF が誘導されているので、臨床でも同様に改善効果を示すことが期待できる。

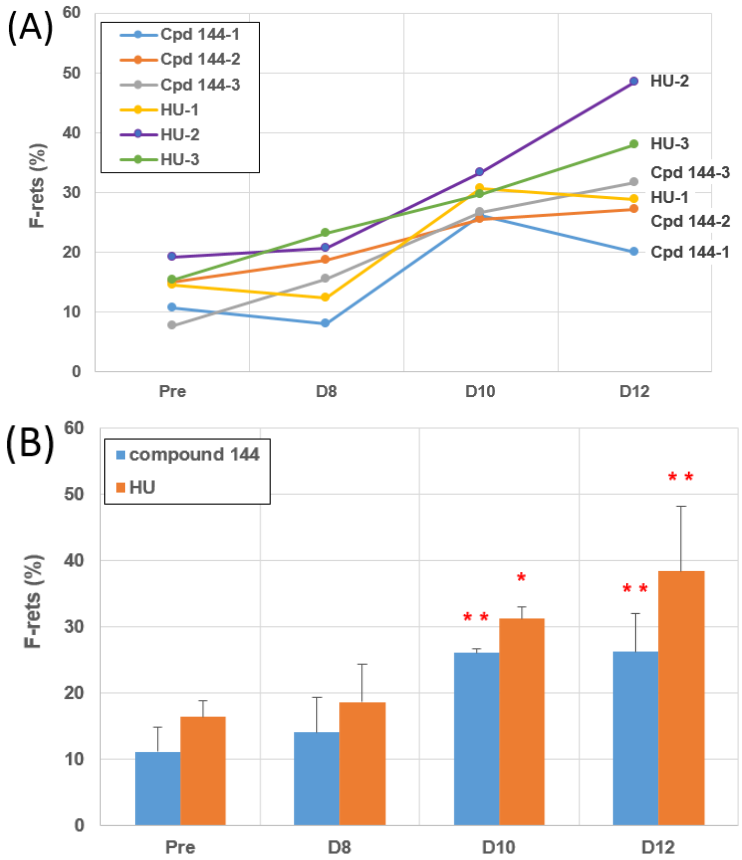


Figure 3-8. Efficacy of compound **144** and HU on F-reticulocytes (F-rets) per flow cytometry. (A) Individual monkey data on the rate of change in F-rets before administration (pre-treatment) and on days 8, 10, and 12. (B) Average rate of change in F-rets in the HU and compound 10 groups. F-ret data for each treatment group are expressed as the mean ± the standard deviation of the three monkeys. *P <0.05 and **P <0.01 indicate statistically significant differences compared with each pre-treatment group.

次に、本試験中の PK データを解析した（Figure 3-9）。単回投与の PK データより算出したシミュレーションを緑線で示し、投与してから 1 時間、2 日、4 日、7 日後の実際の血中薬物濃度を青点で示した。化合物 **144** がいずれの期間でもシミュレーション通りの血中薬物濃度を示しており、連続投与による血中薬物濃度の積み上がりや、血中曝露量が低下することなく薬効を示していたことがわかった。

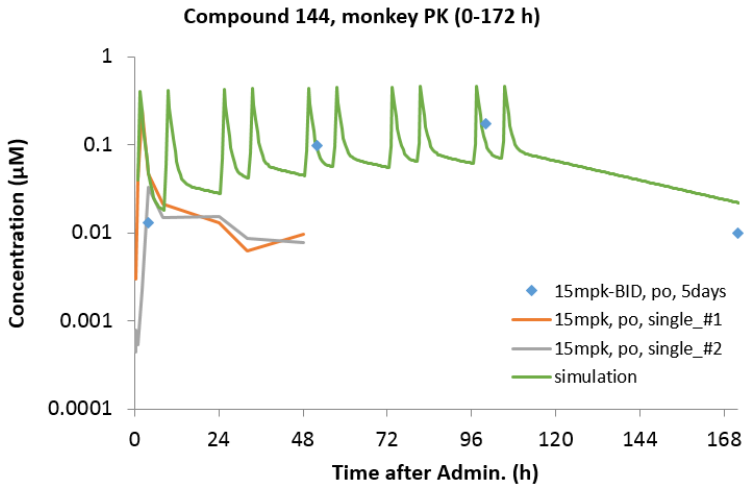


Figure 3-9. Measured values of plasma concentration of compound **144** in an *in vivo* test (blue) and simulated monkey plasma concentration profile of compound **144** (green) at a dose of 15 mg/kg twice daily over 7 days of administration using the average PK parameters at a single dose (orange and gray).

続いて、本試験において化合物の投与前から投与後にわたって好中球及び血小板の濃度を確認した（Figure 3-10）。化合物 **144** 及びヒドロキシ尿素いずれも試験中止の基準値を下回ることなく、*in vivo* 試験を通して骨髄抑制を含む安全性の問題は観察されなかった。筆者の知る限り、化合物 **144** は、カニクイザルモデルで γ-グロビン産生の誘導を示した最初の G9a/GLP 阻害剤となった。

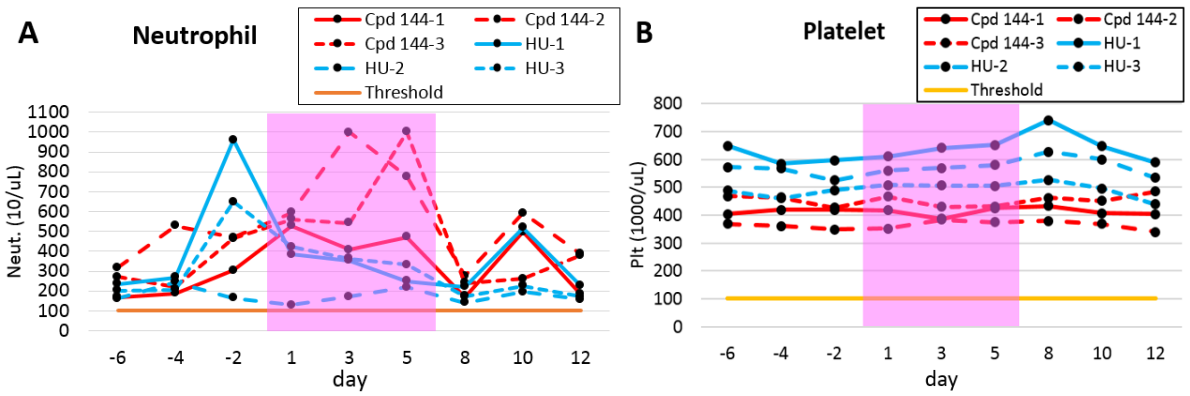
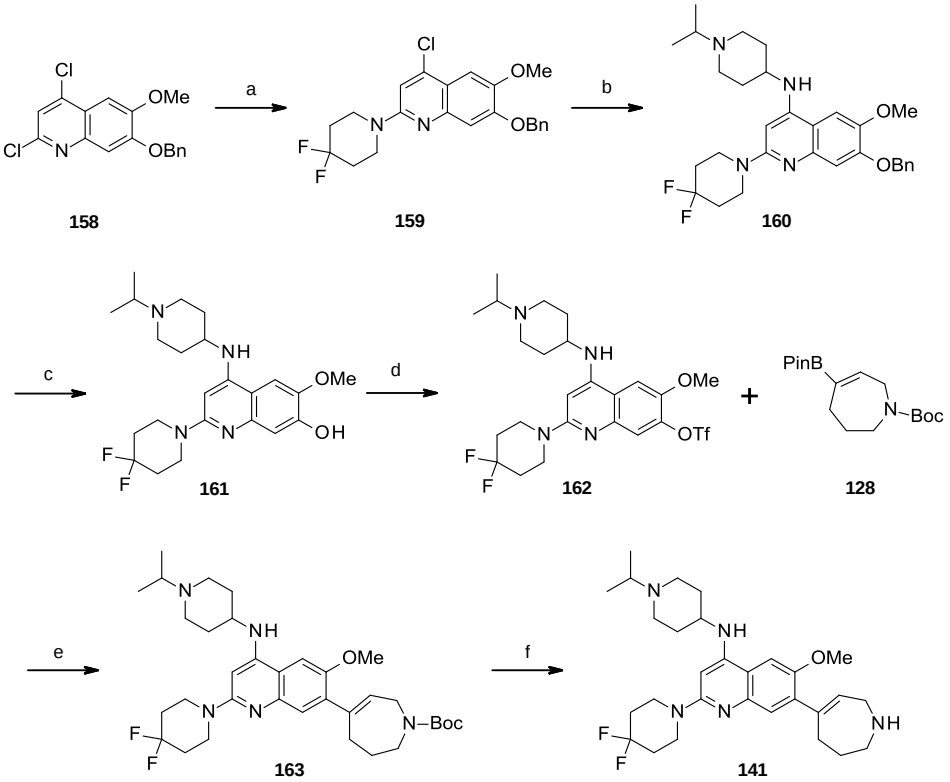


Figure 3-10. Tolerability was monitored by measuring neutrophil (A) and platelet (B) concentrations. Neutrophil and platelet concentrations were evaluated 6, 4, and 2 days before administration and 1, 3, 5, 8, 10, and 12 days after it. The pink range is the dosing period.

3-9　キノリン誘導体 141 及びベンズイミダゾール誘導体 143 の合成

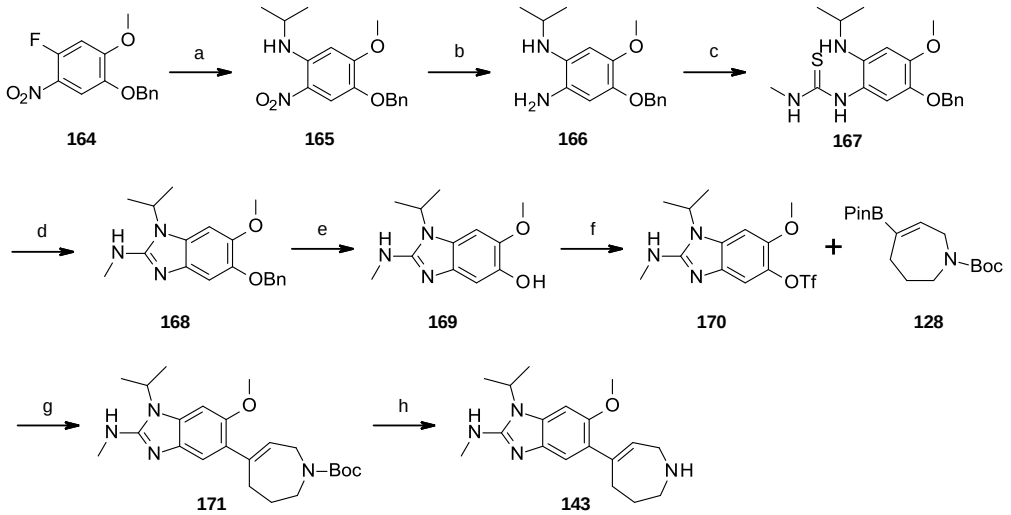
化合物 **141** の合成法を Scheme 3-1 に示す。既知の化合物 **158**[66] を出発物質とし、Buchwald-Hartwig アミノ化反応を 80 度に抑えることで 2 位選択的にジフルオロピペリジル基を導入し、化合物 **159** を得た。続いて、Buchwald-Hartwig アミノ化反応をマイクロウェーブ照射下 120 度で行うことで、目的とするジアミノキノリン体 **160** を得た。次に、ベンジル基を

除去後、生じたアルコールに対してトリフラート化することで化合物 **162** を得た。その後、前章で得たボラン酸ピナコールエステル体 **128** と鈴木-宮浦カップリングを行うことで化合物 **163** を得た後に、Boc 基を除去することで目的とする化合物 **141** を得た。



Scheme 3-1. Synthesis of compound **141**. *Reagents and conditions*: (a) 4,4-difluoropiperidine, $Pd_2(dba)_3$ (5 mol%), Xantphos, NaO^{t}Bu, toluene, 80 °C, 1 h, 68%; (b) 4-amino-1-isopropylpiperidine, $Pd_2(dba)_3$ (5 mol%), Xantphos, NaO^{t}Bu, dioxane, μW, 120 °C, 1.5 h, 44%; (c) H_2, Pd/C, MeOH, rt, 1 h. quant.; (d) $PhNTf_2$, K_2CO_3, DMF, rt, 1 h, 64%; (e) Xphos Pd G2 (10 mol%), K_3PO_4, dioxane, H_2O, μW, 90 °C, 1 h, 92%; (f) TFA, CH_2Cl_2, rt, 1 h, 99%.

次に、化合物 **143** の合成法を Scheme 3-1 に示す。既知の化合物 **164**[84] に対してイソプロピル基を導入し、鉄によってニトロ基を還元することでアニリン体 **166** を得た。続いて、チオウレア化をして化合物 **167** を得た後にメチル化続く環化を経てアミノイミダゾール環を構築し、化合物 **167** を得た。次に、ベンジル基を除去後、トリフラート化して化合物 **170** を得た。その後は Scheme 3-1 と同様の工程を経て目的とする化合物 **143** を得た。

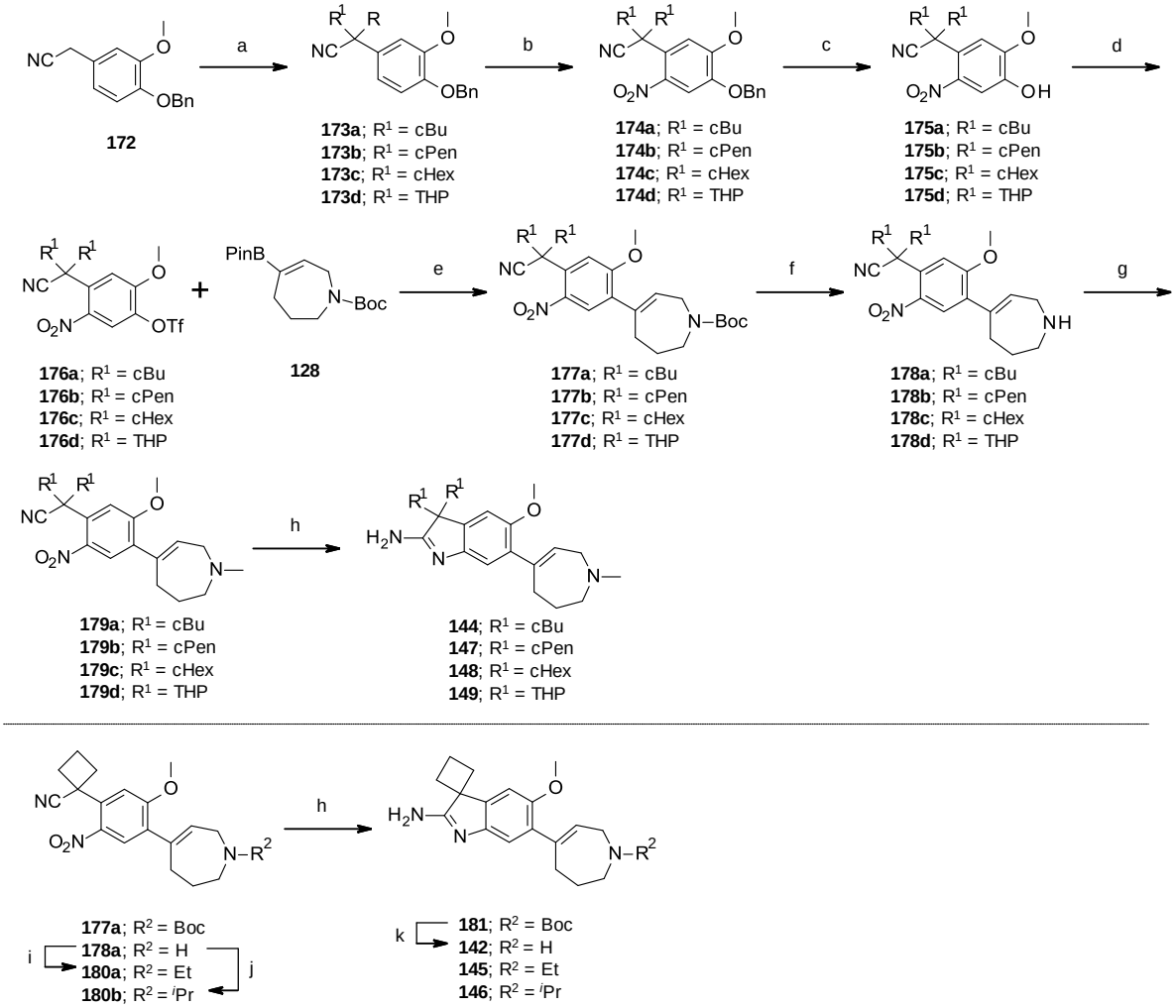


Scheme 3-2. Synthesis of compound **143**. *Reagents and conditions*: (a) isopropylamine, Cs_2CO_3, MeCN, μW, 50 °C, 1 h, 16%; (b) Fe, NH_4Cl, MeOH, H_2O, 80 ºC, 1.5 h, quant. (c) methyl isothiocyanate, THF, rt, 18 h, 67%; (d) MeI, MeCN, CH_2Cl_2, rt, 21 h; MeOH, 55 °C, 5 h, 60%; (e) H_2, Pd/C, EtOH, AcOEt, rt, 2 h, 83%; (f) $PhNTf_2$, Cs_2CO_3, DMF, rt, 1 h, quant.; (g) XPhos Pd G2 (10 mol%), K_3PO_4, 1,4-dioxane, H_2O, rt to 80 °C for 0.5 h, 74%; (h) TFA, CH_2Cl_2, rt, 1 h, 51%.

3-10　インドール誘導体の合成

次に、化合物**142**及び**144**−**149**の合成法をScheme 3-3に示す。市販のフェニルアセトニトリル**172**を出発物質とし、ベンジル位のジアルキル化を行った後、硝酸による位置選択的ニトロ化により化合物**174a**−**174d**を得た。続いて、ベンジル基の除去をSweisらによって報告された条件（H_2; 10%Pd/C; エタノール; 70度）[61]で行ったが、ニトロ基も一部還元されてしまい、高収率かつ再現性良く得ることが困難であった。そこで条件検討を行ったところ、水素源として1,4-シクロヘキサジエン[85]を用いることで再現性良く高収率でベンジル基の除去を行うことができ、目的とする化合物**175a**−**175d**を得た。これは、水素の当量を制御し、過反応を抑制できたため、ベンジル基の除去が選択的に進行したと考えられる。筆者の知る限り、ニトロ基及びシアノ基存在下で1,4-シクロヘキサジエン、Pd/Cを用いてベンジル基を選択的に除去した最初の例である。続いて、生じたアルコールに対してトリフラート化とそれに続くボラン酸ピナコールエステル体**128**との鈴木-宮浦カップリングにより化合物**177a**−**177d**が得られた。酸性条件下でのBoc基の除去とそれに続くN-メチル化により化合物**179a**−**179d**を得た。次のスピロ[シクロアルキル-1,3'-インドール]-2'-アミノ骨格の構築は、最初にSweisらによって報告された条件（H_2; 10%Pd/C; 酢酸、またはZn; 酢酸）[61]で行ったが、変換率が低く、低収率となった。これは、反応途中に生成されるヒドロキシアミン体の溶解性が悪く、析出してしまい、変換率が低くなったと考えられた。したがって、一度ヒドロキシアミンを粗生成物として得た後、THF及び無水酢酸中、水素雰囲気下で還元することにより、系中でヒドロキシアミンがアセチル化されて溶解性が向上し、目的とするアミノインドール誘導体**144**、**147**−**149**を収率良くそれぞれ得た。一方、先に得たBoc基で保護された誘導体**177a**に対して還元的環化を行うことで化合物**181**を得た後に、Boc基を除去することで化合物**142**が得られた。また、先に得た化合物**178a**に対してN-エチル化やN-イソ

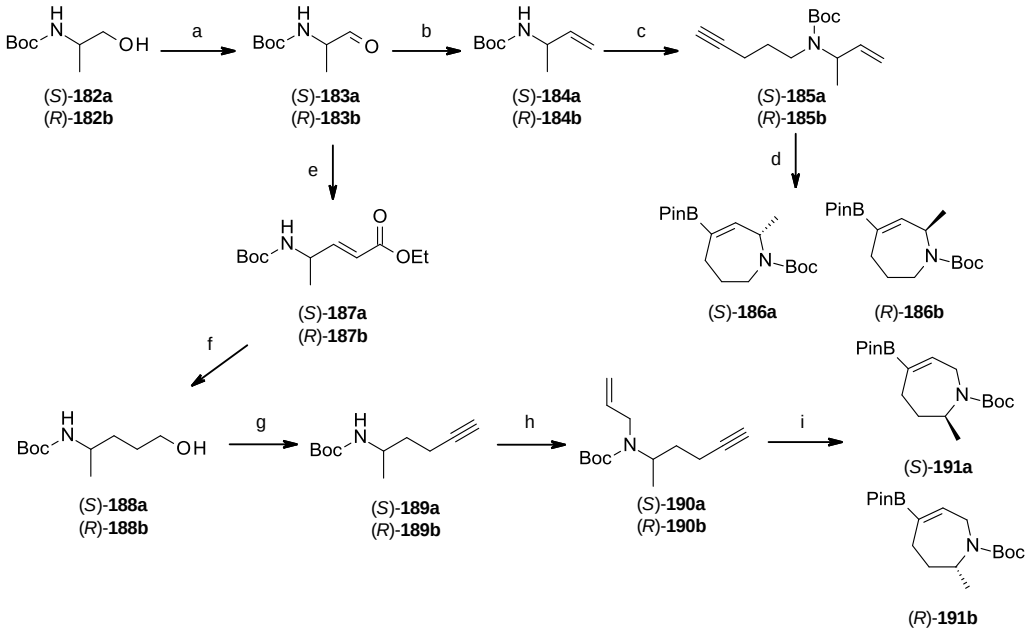
プロピル化を行うことで**180a**、**180b**をそれぞれ得た。最後にこれら化合物に対して還元的環化を行うことで目的とする**145**、**146**をそれぞれ得た。



Scheme 3-3. Synthesis of compounds **142**, **144**−**149**. *Reagents and conditions*: (a) dibromoalkane, NaH, DMF, 0 °C to rt, 1 h, 67%−76%; (b) HNO_3, Ac_2O, AcOH, 0 °C, 30 min, 59%−88%; (c) 10% Pd/C, 1,4-cyclohexadiene, EtOH, AcOEt, rt to 80 °C, 30 min, 72%−100%; (d) Tf_2O, Et_3N, CH_2Cl_2, 0 °C, 30 min, 72%−96%; (e) Pd(dppf)Cl_2 (20 mol%), K_2CO_3, dioxane, H_2O, μW, 120 °C, 1 h, 79%−95%; (f) TFA, CH_2Cl_2, rt, 30 min, 77%−97%; (g) formaldehyde, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 1 h, 84%−94%; (h) Zn, AcOH, THF, 40−50 °C, 5−10 min; H_2, 10% Pd/C, Ac_2O, THF, rt, 10−20 min, 35%−62% over 2 steps; (i) EtI, K_2CO_3, MeCN, rt, 2 h, 75%; (j) acetone, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 30 min, 53%; (k) TFA, CH_2Cl_2, rt, 12 h, 74%.

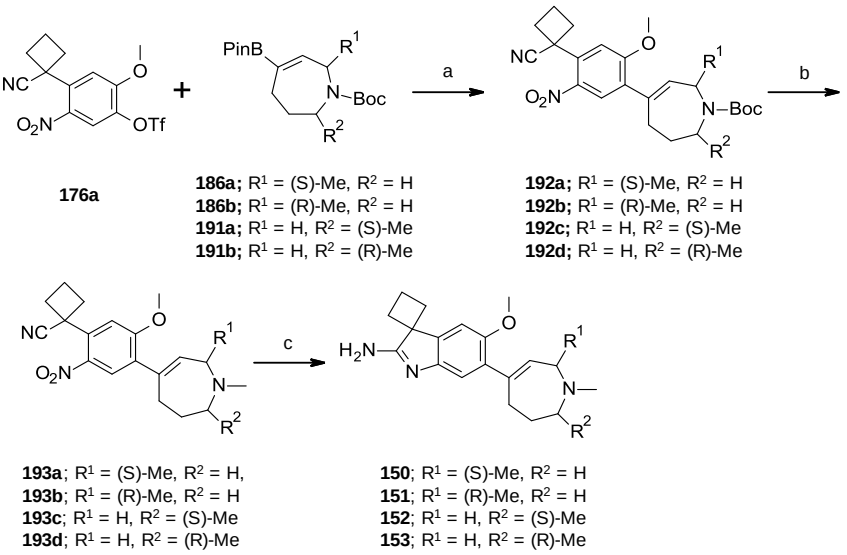
次に、テトラヒドロアゼピン環のアミノ基の α 位にメチル基を導入したボロン酸エステル **186a**、**186b** 及び **191a**、**191b** の合成法を Scheme 3-4 に示す。市販のキラルなアラニノール誘導体 **182a**、**182b** のアルコールを Dess-Martin 酸化し、続く Wittig 反応によってアリルアミン **184a**、**184b** をそれぞれ得た。続いて、5-クロロ-1-ペンチンを用いて N-アルキル化を行うことで **185a**、**185b** を得た後、Scheme 2-4 と同様に位置選択的ホウ素化及び RCM に

よって目的とする化合物 **186a**、**186b** をそれぞれ得た。次に、先に得た **183a**、**183b** に対してトリフェニルカルボエトキシメチレンホスホランを作用させることで、不飽和エステル体 **187a**、**187b** を得た。続いて、水素化反応によってオレフィンを還元した後、水素化ホウ素リチウムによってエステルを還元し、アルコール体 **188a**、**188b** を得た。得られたアルコールを Dess-Martin 酸化し、続く大平-Bestmann アルキン化によって化合物 **189a**、**189b** を得た。その後、ヨウ化アリルを用いて N-アルキル化を行うことで化合物 **190a**、**190b** を得た。その後は先と同様の工程を経て目的とする化合物 **191a**、**191b** をそれぞれ得た。

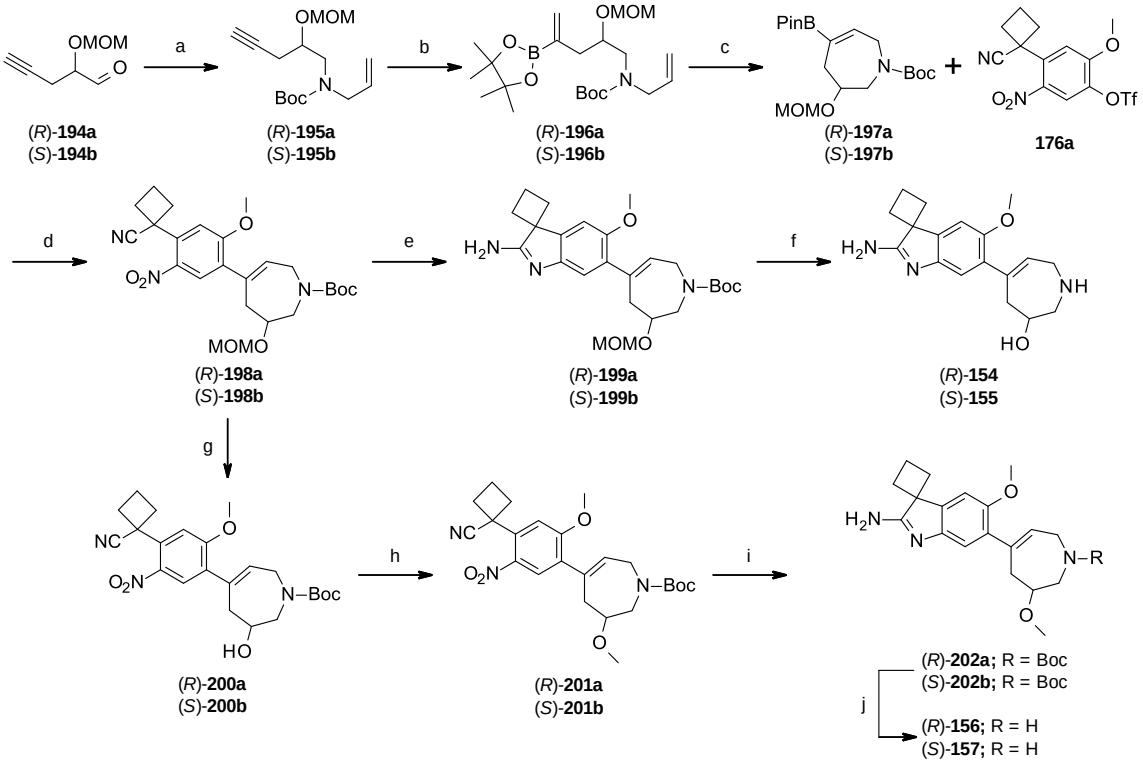


Scheme 3-4. Synthesis of tetrahydroazepines **186a**, **186b**, **191a** and **191b**. *Reagents and conditions*: (a) Dess-Martin periodinane, CH_2Cl_2, rt, 0.5 h, 82%−84%; (b) Ph_3PMeBr, ^{t}BuOK, THF, 0 °C, 0.5 h; aldehyde, THF, 0 °C to rt, 1 h, 77%−82%; (c) 5-chloro-1-pentyne, ^{t}BuOK, DMF, 70 °C, 5 h; 17%−39%; (d) 1) $(Pin)_2B_2$, CuCl, LiCl, AcOK, DMF, rt, 5 h, 28%−89%; 2) 2nd Grubbs (5 mol%), CH_2Cl_2, rt, 16 h, 88%−96%; (e) $Ph_3P{=}CHCO_2Et$, THF, rt, 1 h, 77%−85%; (f) 10% Pd/C, MeOH, rt, 1 h; $LiBH_4$, THF, 50 °C, 3 h, 66%−96% over 2 steps; (g) 1) Dess-Martin periodinane, CH_2Cl_2, rt, 0.5 h, 67%−79%; 2) Ohira-Bestmann Reagent, K_2CO_3, MeOH, rt, 15 h, 25%−51%; (h) allyl iodide, NaH, DMF, rt, 2 h, 72%−86%; (i) 1) $(Pin)_2B_2$, CuCl, LiCl, AcOK, DMF, rt, 5−15 h, 60%−63%; 2) 2nd Grubbs (5 mol%), CH_2Cl_2, rt, 2 h, 50%−63%.

次に、化合物 **150**−**153** の合成法を Scheme 3-5 に示す。先に合成した化合物 **176a** とボラン酸エステル体 **186a**、**186b**、**191a**、**191b** を用いて鈴木-宮浦カップリングを行うことで、化合物 **192a**−**192d** を得た後、Boc 基の除去、続く N-メチル化によって **193a**−**193d** を得た。その後は、Scheme 3-3 と同様の条件で還元的環化を行い目的とする化合物 **150**−**153** をそれぞれ得た。



Scheme 3-5. Synthesis of compounds **150**−**153**. *Reagents and conditions*: (a) $Pd(dppf)Cl_2$ (20 mol%), K_2CO_3, dioxane, H_2O, μW, 120 °C, 1 h, 65%−99%; (b) 1) TFA, CH_2Cl_2, rt, 30 min, 86%−100%; 2) formaldehyde, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 1 h, 65%−91%; (c) Zn, AcOH, THF, 50 °C, 20 min; H_2, 10% Pd/C, Ac_2O, THF, rt, 20 min, 54%−81% over 2 steps.



Scheme 3-6. Synthesis of compounds **154**−**157**. *Reagents and conditions*: (a) $AllNH_2$-HCl, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 30 min, 49%−72%; Boc_2O, Et_3N, THF, rt, 2 h, 65%−86%; (b) $(Pin)_2B_2$, CuCl, LiCl, AcOK, DMF, rt, 5 h, 78%−95%; (c) 2nd Grubbs (5 mol%), CH_2Cl_2, rt, 2 d, 31%−59%; (d) $Pd(dppf)Cl_2$ (20 mol%), K_2CO_3, dioxane, H_2O, μW, 120 °C, 1 h, 93%−97%; (e) Zn, AcOH, THF, 50 °C, 10 min; H_2, 10% Pd/C, Ac_2O, THF, rt, 20 min, 43%−53% over 2 steps; (f) 4 M

HCl/dioxane, MeOH, rt, 2 h, 73%−87%; (g) 4 M HCl/dioxane, MeOH, rt, 2 h; Boc_2O, Et_3N, THF, rt, 2 h, 79%−81% over 2 steps; (h) MeI, NaH, DMF, rt, 15 h, 78%−92%; (i) Zn, AcOH, THF, 50 °C, 10 min; H_2, 10% Pd/C, Ac_2O, THF, rt, 20 min, 30%−37% over 2 steps; (j) TFA, CH_2Cl_2, rt, 1 h, 52%−100%.

次に、化合物**154**−**157**の合成法をScheme 3-6に示す。既知の化合物**194a**、**194b**とアリルアミンを用いて還元的アミノ化反応を行い、さらにBoc化することで化合物**195a**、**195b**を得た。続いて、位置選択的ホウ素化及びRCMを行い化合物**197a**、**197b**を得た後、化合物**176a**と鈴木-宮浦カップリングを行うことで、化合物**198a**、**198b**をそれぞれ得た。その後、還元的環化を行い、さらに酸性条件下にてMOM基及びBoc基を除去することで目的とする化合物**154**、**155**をそれぞれ得た。一方、先に得た化合物**198a**、**198b**に対してMOM基及びBoc基を除去した後に、N-Boc化することで化合物**200a**、**200b**をそれぞれ得た。続いて、O-メチル化した後に還元的環化を行い、さらにBoc基を除去することで、目的とする化合物**156**、**157**をそれぞれ得た。

3-11　小括

前章では、通常G9a/GLP阻害剤の側鎖に用いられていた3-（ピロリジン-1-イル）プロポキシ基の代替基としてコンフォメーションを固定化したテトラヒドロアゼピン基の発見について述べた。この章ではまず、テトラヒドロアゼピン基の汎用性を調べるために、この側鎖を既知のG9a/GLP阻害剤の種々中心コアに導入した。すなわち、テトラヒドロアゼピン基をキノリン誘導体､インドール誘導体､ベンズイミダゾール誘導体に導入したところ、いずれもG9a/GLP阻害活性を示し、テトラヒドロアゼピン側鎖が様々な中心コアに適用可能であることを明らかとした。活性とADMETプロファイルの結果（特に膜透過性と代謝安定性）に基づき、筆者はインドール誘導体に焦点を当て、G9a/GLP阻害活性を保持しながらPKプロファイルが改善された新規化合物の獲得を目指した。

インドール誘導体の SAR を取得し、強い G9a/GLP 阻害活性を示す化合物 **144**（DS79932728）を得た。化合物 **144** のマウスでの AUC_{inf} が高く経口 BA が良好であるため（BA = 96%）、サル PK 試験に化合物 **144** を選抜した。化合物 **144** は、サルの PK 試験においても良好な PK プロファイルを示し、経口投与による *in vivo* サル薬効試験に適したプロファイルを持つことを確認した。

静脈切開されたカニクイザルモデルを用いて、*in vivo* にて化合物 **144** の γ-グロビンの遺伝子発現の誘導を評価したところ、化合物 **144**（15 mg/kg, 1 日 2 回投与）は、ヒドロキシ尿素（35 mg/kg, 1 日 1 回投与: 臨床的に使用される最高用量に相当）と同等の薬効を示した。また、*in vivo* 試験を通して、骨髄抑制を含む安全性の問題は観察されなかった。筆者の知る限り、化合物 **144** はカニクイザルモデルで γ-グロビン産生の誘導を示した最初の G9a/GLP 阻害剤である。また、サルモデルにおける γ-グロビン産生の誘導を示す低分子の報告も現時点でわずかであるため [86-89]、DS79932728（**144**）は今後の薬剤開発にとって有用な情報であると考えられる。そして本研究で得られた知見は、β-サラセミア及び SCD の治療の研究に役立つと期待される。

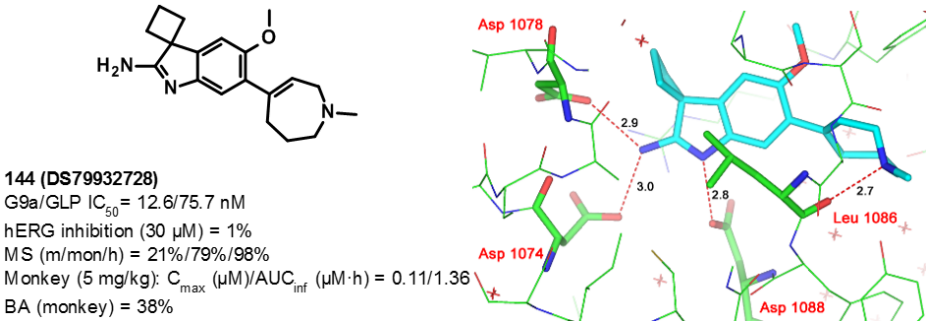


Figure 3-11. Summary of section 3.

3-12　フェノタイプ及びターゲットベースドスクリーニングの総括

本研究は、新規ヘモグロビン症治療薬の創製を目指し、第 1 章ではフェノタイプスクリーニングによる HTS ヒット化合物からの合成展開を実施し、第 2、3 章では G9a/GLP というターゲットベースでの合成展開を実施した。本研究を通して、それぞれの利点、課題を比較しながら述べていきたい。

まず、合成展開の開始時に鍵となる化合物は、フェノタイプスクリーニングの場合は PK プロファイルが良く、安全性の高いドラッグライクな化合物を選ぶことができる。本研究においても第 1 章では、rule of five に含まれるヒット化合物 **10** から合成展開を開始したため、PK プロファイルや安全性が良好な化合物 **37** を取得できたと考えられる。一方、ターゲットベースの場合は標的が確定しているので、ファーマコフォアがある程度固まっており、例えば今回の場合は基質競合剤であるのでリシン残基をミミックする塩基性化合物である必要があった。塩基性化合物は hERG 阻害活性を持つという懸念があるものの、ファーマコフォアが決まっているためにスキャホールドホッピングなど大胆な構造変換が困難であった。

次に、合成展開における SAR の取得についてだが、フェノタイプスクリーニングの場合は活性値の強弱が小さく、合成展開の方針等を探るのが困難な傾向がある。本研究（第 1 章）においても、活性が 1–5 μM の中で SAR 取得をする必要があり、化合物選抜等にも苦労した。一方、ターゲットベースの場合は、合成展開における評価系が細胞系である必要はなく、さらに酵素阻害活性値の強弱が大きく誤差も小さいため、明確な SAR を確認できる。G9a/GLP の場合は、数 nM から数 μM までの活性の幅を評価できていた。また、ターゲットがわかっているため、そのターゲットとリガンドの共結晶構造が明らかであればそれを基に化合物デザインや活性結果の考察ができる（3-4 参照）。

次に、化合物の薬効評価の点について比較する。フェノタイプスクリーニングでは陽性対照が必須であるが、その陽性対照は一般的に標的疾患の治療薬ではなく最も感度のいい既知化合物が選抜されることが多く、化合物の薬効と臨床現場における薬効の相対的な比較が困難である。実際、本研究においても現在の SCD の標準治療薬であるヒドロキシ尿素では *in vitro* 試験において HbF 誘導が確認しづらく（1-1、3-6 参照）、デシタビンを陽性対照においていたため、どれくらいの薬効を示せば臨床現場においても十分なのかが不明瞭であった（デシタビンはあくまで臨床試験中の化合物である）。一方、ターゲットベースの場合では、ターゲット阻害活性を指標に SAR を取得し、最も有望な化合物に対して標準治療薬のヒドロキシ尿素と薬効比較ができるので薬効のポテンシャルがわかりやすい（3-7 参照）。

最後に、第 1 及び第 3 章で到達した化合物 **37**、**144** のヘモグロビン症治療薬候補化合物としてのメリット・デメリットについて比較議論する。化合物 **37** は、デシタビンより強い *in vivo* 薬効を示し、臨床でも治療効果を発揮できると期待できる。一方、標的が明らかとなっていないため、1-6 の研究を踏まえながら標的探索を実施する必要がある。化合物 **144** は、ヒドロキシ尿素と同等の *in vivo* 薬効であったので、臨床現場において標準治療薬を変革するためにはより活性の向上した化合物や薬物動態が改善した化合物を取得する必要がある。一方で、エピジェネティクスな作用を持つヘモグロビン症治療薬は未だ存在しないので、新規の作用機序として First-in-class の医薬品に繋がる可能性がある。

総論

本論文では、フェノタイプ及びターゲットベースドスクリーニングの2つのアプローチによって新規ヘモグロビン症治療薬の獲得を目指した。そして、フェノタイプスクリーニングによる研究では2-アザスピロ[3.3]ヘプタン誘導体**37**を取得し、ターゲットベースドスクリーニングによる研究ではG9a/GLP阻害剤DS79932728（**144**）を創出した。

第1章では、フェノタイプスクリーニングによって新規HbF誘導剤の取得を目指した。まず、ハイスループットのフェノタイプスクリーニングで約18,000の化合物を評価し、得られたヒット化合物から合成展開を実施することで*in vivo*試験においてHbF誘導活性を示す2-アザスピロ[3.3]ヘプタン誘導体**37**を取得した。また、化合物**37**の類縁体によるメカニズム解析により、既知のエピジェネティクスのモジュレーターに影響を与えることなく、Bcl11aやLRFなどのHbFの主たる転写調節因子をダウンレギュレートすることを明らかにした。

第2章では、Epizyme社から報告されたEPZ035544（**80**）を出発化合物として強力な新規G9a/GLP阻害剤の創製を目指し、EPZ035544の側鎖である3-（ピロリジン-1-イル）プロポキシ基をコンフォメーションがより固定化されるテトラヒドロアゼピン基に変換した化合物**89**を取得した。テトラヒドロアゼピン体**89**は、化合物**80**と比較してG9aに対して4倍、GLPに対して6倍活性が向上し、hERG阻害活性及びマウスにおけるPKプロファイルも改善した。
以上より、側鎖の設計、合成、活性評価、及び構造最適化により、化合物 **89** が同定され、化合物 **80** と比較して hERG 阻害活性が改善し、G9a/GLP 阻害活性が増加した。

第3章では、前章で取得した側鎖のテトラヒドロアゼピン基の汎用性を調べるために、この側鎖を既知のG9a/GLP阻害剤の種々中心コアに導入した。いずれもG9a/GLP阻害活性を示し、テトラヒドロアゼピン側鎖が様々な中心コアに適用可能で一般性のある置換基であることを明らかとした。また、インドール誘導体のSARを取得し、強いG9a/GLP阻害活性を示す化合物**144**（DS79932728）を得た。続いて、静脈切開されたカニクイザルモデルを用いて、*in vivo*にて化合物**144**のγ-グロビン産生の誘導を評価したところ、化合物**144**（15 mg/kg, 1日2回投与）は、ヒドロキシ尿素（35 mg/kg, 1日1回投与: 臨床的に使用される最高用量に相当）と同等の薬効を示した。また、*in vivo*試験を通して、骨髄抑制を含む安全性の問題は観察されなかった。
また、本研究を通してフェノタイプ及びターゲットベースドスクリーニングのメリット、デメリットについて考察した。

G9a/GLP と HbF 誘導との関連性は既に報告されているものの、G9a/GLP 阻害剤によって *in vivo* 試験において HbF 産生の誘導効果を示した例はこれが初めてであり、DS79932728（**144**）による HbF 誘導活性が見られたことは、今後の研究開発にとって重要な一歩である。本研究において得られた新たな知見が、今後の G9a/GLP 阻害剤の研究開発に繋がることを強く願っている。

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最後に、これまで長きに渡り筆者の研究生活を温かく支えて下さいました、家族、親族に心より感謝致します。

2021 年 9 月　片山勝史

実験項

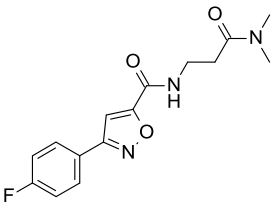
1. Chemistry

General Methods

Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a JEOL JNM-EX400 spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to the internal standard tetramethylsilane. Abbreviations of multiplicity are as follows: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, and br: broad. Data are presented as follows: chemical shift (multiplicity, integration, coupling constant). APCI/ESI mass spectra were recorded on Agilent Technologies Agilent 1100 or 1200 series LC/MS. HRMS was carried out by using an liquid chromatography-mass spectrometry (LC-MS) system composed of a Waters Xevo Quadrupole Time-of-Flight Mass Spectrometer and an Acquity UHPLC system. Elemental analyses were conducted by using Microcorder JM10 and Dionex ICS-1500 systems. Compound purity was confirmed to exceed 95% by the DAD signal area (%), which was calculated with an Agilent Infinity 1260 LC-MS system. The conditions used were: column, Develosil Combi-RP-5 2.0 mmID × 50 mmL; gradient elution, 0.1% HCO_2H-H_2O/0.1% HCO_2H-MeCN = 98/2 to 0/100 (v/v); flow rate, 1.2 mL/min; UV detection, 254 nm; column temperature, 40 °C; ionization, APCI/ESI. Purity ≥95% was determined by elemental analysis for each of the tested compounds. Chemical reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Fujifilm Wako Pure Chemical Industries, Ltd., Kanto Kagaku, or Nacalai Tesque and used without purification. Flash column chromatography was performed using Purif-Pack SI 30 μm and Purif-Pack NH 50 μm (Shoko Scientific), Chromatorex SI 30 μm and Chromatorex NH 60 μm (Fuji Silysia Chemical) or Merck silica gel 60 (230–400 mesh).

第一章

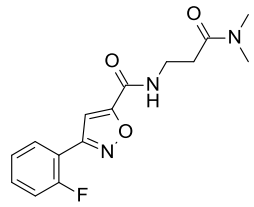
N-[3-(Dimethylamino)-3-oxo-propyl]-3-(4-fluorophenyl)isoxazole-5-carboxamide (10): General procedure A



A solution of 3-(4-fluorophenyl)isoxazole-5-carboxylic acid **43a** (300 mg, 1.4 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (250 mg, 2.2 mmol), and DIPEA (0.80 mL, 4.6 mmol) in DMF (5.0 mL) was treated with HBTU (660 mg, 1.7 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 0 : 100 to 10 : 90) to afford compound **10** (160 mg, 36%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.77 (2H, m), 7.60 (1H, br s), 7.20–7.13 (3H, m), 3.78 (2H, dd, J = 11.3, 6.3 Hz), 2.99 (3H, s), 2.98 (3H, s), 2.63 (2H, t, J = 5.5 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.1, 164.12, 164.05 (d, $^1J_{C,F}$ = 250 Hz), 162.2, 155.8, 128.9 (d, $^3J_{C,F}$ = 9.0 Hz), 124.4 (d, $^4J_{C,F}$ = 4.0 Hz), 116.2 (d, $^2J_{C,F}$ = 22 Hz), 104.7, 37.0, 35.3, 35.1, 32.8; ESIMS-LR *m/z* 306

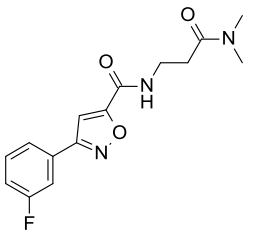
[(M+H)$^+$]; ESIMS-HR calcd for $C_{15}H_{17}FN_3O_3$ (M+H)$^+$ 306.1248, found 306.1253.

***N*-[3-(Dimethylamino)-3-oxo-propyl]-3-(2-fluorophenyl)isoxazole-5-carboxamide (11)**



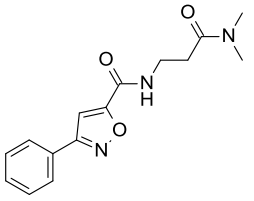
Following General procedure A using 3-(2-fluorophenyl)isoxazole-5-carboxylic acid **43b** (75 mg, 0.36 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (68 mg, 0.45 mmol), DIPEA (190 µL, 1.0 mmol), HBTU (180 mg, 0.47 mmol), and DMF (5.0 mL), compound **11** (59 mg, 53%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (1H, td, *J* = 7.5, 1.8 Hz), 7.68 (1H, br s), 7.48–7.42 (1H, m), 7.31–7.26 (1H, m), 7.24–7.18 (1H, m), 7.12 (1H, d, *J* = 3.8 Hz), 3.78 (2H, dd, *J* = 11.5, 6.3 Hz), 2.99 (3H, s), 2.98 (3H, s), 2.64 (2H, t, *J* = 5.6 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.1, 164.3, 163.0 (d, $^1J_{C,F}$ = 246 Hz), 162.2, 155.7, 130.8 (d, $^3J_{C,F}$ = 8.0 Hz), 122.7 (d, $^4J_{C,F}$ = 3.0 Hz), 117.5 (d, $^2J_{C,F}$ = 21 Hz), 113.9 (d, $^2J_{C,F}$ = 21 Hz), 104.8, 37.0, 35.4, 35.1, 32.8; ESIMS-LR *m/z* 306 [(M+H)$^+$]; ESIMS-HR calcd for $C_{15}H_{17}FN_3O_3$ (M+H)$^+$ 306.1248, found 306.1244.

***N*-[3-(Dimethylamino)-3-oxo-propyl]-3-(3-fluorophenyl)isoxazole-5-carboxamide (12)**



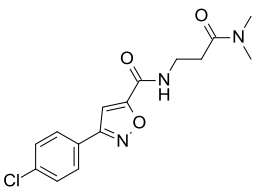
Following General procedure A using 3-(3-fluorophenyl)-1,2-oxazole-5-carboxylic acid **43c** (100 mg, 0.48 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (70 mg, 0.49 mmol), DIPEA (250 µL, 1.5 mmol), HBTU (220 mg, 0.58 mmol), and DMF (5.0 mL), compound **12** (42 mg, 29%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.66–7.52 (3H, m), 7.49–7.42 (1H, m), 7.20–7.14 (2H, m), 3.79 (2H, q, *J* = 5.7 Hz), 3.00 (3H, s), 2.99 (3H, s), 2.63 (2H, t, *J* = 5.7 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.1, 165.3, 159.4, 164.2 (d, $^1J_{C,F}$ = 254 Hz), 158.9, 132.1 (d, $^3J_{C,F}$ = 9.0 Hz), 127.6, 124.7 (d, $^4J_{C,F}$ = 4.0 Hz), 116.4 (d, $^2J_{C,F}$ = 21 Hz), 103.1 (d, $^3J_{C,F}$ = 10.0 Hz), 37.0, 35.3, 35.1, 32.9; ESIMS-LR *m/z* 306 [(M+H)$^+$]; ESIMS-HR calcd for $C_{15}H_{17}FN_3O_3$ (M+H)$^+$ 306.1248, found 306.1257.

***N*-[3-(Dimethylamino)-3-oxo-propyl]-3-phenyl-isoxazole-5-carboxamide (13)**



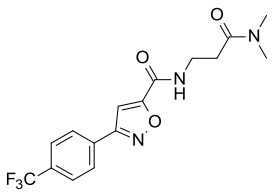
Following General procedure A using 3-phenyl-5-isoxazolecarboxylic acid **43d** (550 mg, 2.9 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (670 mg, 4.4 mmol), triethylamine (2.0 mL, 15 mmol), EDCI (840 mg, 4.4 mmol), and DMF (20 mL), compound **13** (270 mg, 32%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85–7.79 (2H, m), 7.58 (1H, br s), 7.50–7.45 (3H, m), 7.18 (1H, s), 3.78 (2H, dd, *J* = 11.3, 6.3 Hz), 2.99 (3H, s), 2.98 (3H, s), 2.63 (2H, t, *J* = 5.5 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.1, 164.0, 163.2, 130.5, 129.1, 128.1, 126.9, 104.9, 37.0, 35.3, 35.1, 32.8; ESIMS-LR *m/z* 288 [(M+H)$^+$]; ESIMS-HR calcd for $C_{15}H_{18}N_3O_3$ (M+H)$^+$ 288.1343, found 288.1352.

3-(4-Chlorophenyl)-*N*-[3-(dimethylamino)-3-oxo-propyl]isoxazole-5-carboxamide (14)



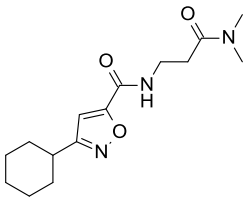
Following General procedure A using 3-(4-chlorophenyl)isoxazole-5-carboxylic acid **43e** (75 mg, 0.34 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (62 mg, 0.41 mmol), DIPEA (0.18 mL, 1.0 mmol), HBTU (170 mg, 0.45 mmol), and DMF (5.0 mL), compound **14** (48 mg, 45%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.72 (2H, dt, *J* = 9.0, 2.1 Hz), 7.68 (1H, br s), 7.46 (2H, dt, *J* = 9.0, 2.1 Hz), 6.92 (1H, s), 3.77 (2H, dd, *J* = 11.5, 6.3 Hz), 2.99 (3H, s), 2.97 (3H, s), 2.63 (2H, t, *J* = 5.6 Hz); ESIMS-LR *m/z* 323 [$(M+H)^+$]; ESIMS-HR calcd for $C_{15}H_{17}ClN_3O_3$ $(M+H)^+$ 322.0953, found 322.0950.

_N_-[3-(Dimethylamino)-3-oxo-propyl]-3-[4-(trifluoromethyl)phenyl]isoxazole-5-carboxamide (15)



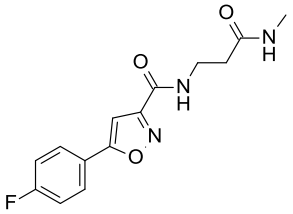
Following General procedure A using 3-(4-trifluorophenyl)isoxazole-5-carboxylic acid **43f** (150 mg, 0.58 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (80 mg, 0.69 mmol), DIPEA (0.30 mL, 1.8 mmol), HBTU (270 mg, 0.70 mmol), and DMF (5.0 mL), compound **15** (58 mg, 28%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (2H, d, *J* = 8.0 Hz), 7.74 (2H, d, *J* = 8.3 Hz), 7.64 (1H, br s), 7.21 (1H, s), 3.79 (2H, dd, *J* = 11.2, 6.1 Hz), 3.00 (3H, s), 2.99 (3H, s), 2.64 (2H, t, *J* = 5.5 Hz); ESIMS-LR *m/z* 356 [$(M+H)^+$]; ESIMS-HR calcd for $C_{16}H_{17}F_3N_3O_3$ $(M+H)^+$ 356.1217, found 356.1208.

3-Cyclohexyl-*N*-[3-(dimethylamino)-3-oxo-propyl]isoxazole-5-carboxamide (16)



Following General procedure A using 3-cyclohexylisoxazole-5-carboxylic acid **43g** (100 mg, 0.51 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (80 mg, 0.52 mmol), DIPEA (1.2 mL, 6.9 mmol), HBTU (1.2 g, 3.2 mmol), and DMF (5.0 mL), compound **16** (72 mg, 25%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.48 (1H, br s), 6.75 (1H, s), 3.76 (2H, dd, *J* = 11.3, 6.3 Hz), 2.99 (3H, s), 2.98 (3H, s), 2.84–2.75 (1H, m), 2.62 (2H, t, *J* = 5.5 Hz), 2.02–1.94 (2H, m), 1.88–1.80 (2H, m), 1.79–1.72 (1H, m), 1.51–1.38 (4H, m), 1.36–1.23 (1H, m); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.1, 169.2, 163.0, 156.2, 105.3, 37.0, 35.8, 35.3, 35.0, 32.8, 31.9, 25.8, 25.7; ESIMS-LR *m/z* 294 [$(M+H)^+$]; ESIMS-HR calcd for $C_{15}H_{24}N_3O_3$ $(M+H)^+$ 294.1812, found 294.1832.

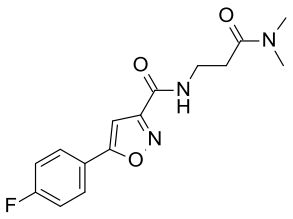
5-(4-Fluorophenyl)-*N*-[3-(methylamino)-3-oxo-propyl]isoxazole-3-carboxamide (35)



Following General procedure A using 3-cyclohexylisoxazole-5-carboxylic acid **43h** (70 mg, 0.51 mmol), 3-amino-*N*-methylpropanamide hydrochloride **45** (72 mg, 0.52 mmol), DIPEA (0.20 mL, 1.1 mmol), HBTU (170 mg, 0.44 mmol), and DMF (5.0 mL), compound **35** (45 mg, 61%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.83–7.77 (2H, m), 7.55 (1H, br s), 7.23–7.16 (2H, m), 6.90 (1H, s), 5.58 (1H, s), 3.78 (2H, q, *J* = 6.1 Hz), 2.85 (3H, d, *J* = 4.8 Hz), 2.54 (2H, t, *J* =

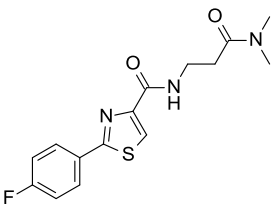
6.1 Hz); ESIMS-LR *m/z* 292 [(M+H)$^+$]; ESIMS-HR calcd for $C_{14}H_{15}FN_3O_3$ (M+H)$^+$ 292.1092, found 292.1103.

_N_-[3-(Dimethylamino)-3-oxo-propyl]-5-(4-fluorophenyl)isoxazole-3-carboxamide (17)



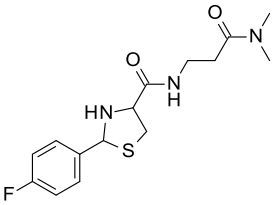
Following General procedure A using 3-cyclohexylisoxazole-5-carboxylic acid **43h** (100 mg, 0.48 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (80 mg, 0.52 mmol), DIPEA (0.25 mL, 1.5 mmol), HBTU (220 mg, 0.58 mmol), and DMF (5.0 mL), compound **17** (72 mg, 48%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.81–7.75 (2H, m), 7.68 (1H, br s), 7.21–7.14 (2H, m), 6.88 (1H, s), 3.77 (2H, q, *J* = 5.9 Hz), 2.99 (3H, s), 2.97 (2H, s), 2.63 (2H, t, *J* = 5.8 Hz); ESIMS-LR *m/z* 306 [(M+H)$^+$]; ESIMS-HR calcd for $C_{15}H_{17}FN_3O_3$ (M+H)$^+$ 306.1248, found 306.1244.

_N_-[3-(Dimethylamino)-3-oxo-propyl]-2-(4-fluorophenyl)thiazole-4-carboxamide (18)



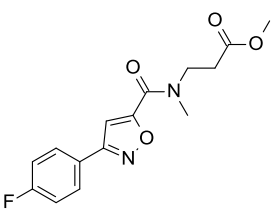
Following General procedure A using 2-(4-fluorophenyl)-1,3-thiazole-4-carboxylic acid **43i** (75 mg, 0.34 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (60 mg, 0.39 mmol), DIPEA (0.2 mL, 1.2 mmol), HBTU (160 mg, 0.42 mmol), and DMF (5.0 mL), compound **18** (65 mg, 60%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (1H, br s), 8.05 (1H, s), 8.00–7.94 (2H, m), 7.18–7.11 (2H, m), 3.79 (2H, dd, *J* = 11.7, 6.4 Hz), 2.99 (3H, s), 2.98 (3H, s), 2.66 (2H, t, *J* = 5.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.4, 166.9, 164.2 (d, $^1J_{C,F}$ = 250 Hz), 161.2, 150.9, 129.2 (d, $^4J_{C,F}$ = 3.0 Hz), 128.7 (d, $^3J_{C,F}$ = 7.0 Hz), 122.7, 116.2 (d, $^2J_{C,F}$ = 21 Hz), 37.0, 35.4, 35.1, 33.2; ESIMS-LR *m/z* 322 [(M+H)$^+$]; ESIMS-HR calcd for $C_{15}H_{17}FN_3O_2S$ (M+H)$^+$ 322.1020, found 322.1034.

_N_-[3-(Dimethylamino)-3-oxo-propyl]-2-(4-fluorophenyl)thiazolidine-4-carboxamide (19)



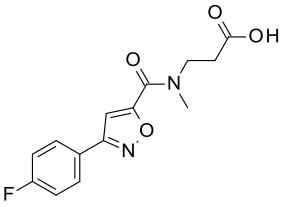
Following General procedure A using 2-(4-fluorophenyl)-1,3-thiazolidine-4-carboxylic acid **43j** (75 mg, 0.33 mmol), 3-amino-*N*,*N*-dimethylpropanamide hydrochloride **44** (65 mg, 0.43 mmol), DIPEA (0.2 mL, 1.2 mmol), HBTU (160 mg, 0.42 mmol), and DMF (5.0 mL), compound **19** (49 mg, 46%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz, 2 : 1 mixture of diastereomers, selected data for the major diastereomer): δ 7.91 (1H, br s), 7.53–7.48 (2H, m), 7.08–7.00 (2H, m), 5.37 (1H, d, *J* = 9.5 Hz), 4.32–4.24 (1H, m), 3.67–3.57 (3H, m), 3.39–3.32 (1H, m), 2.99 (3H, s), 2.95 (3H, s), 2.59–2.45 (2H, m); ESIMS-LR *m/z* 326 [(M+H)$^+$]; ESIMS-HR calcd for $C_{15}H_{21}FN_3O_2S$ (M+H)$^+$ 326.1333, found 326.1352.

Methyl 3-[[3-(4-fluorophenyl)isoxazole-5-carbonyl]-methyl-amino]propanoate (47)



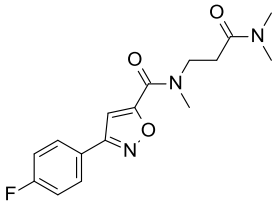
Following General procedure A using 3-(4-fluorophenyl)isoxazole-5-carboxylic acid **43a** (500 mg, 2.4 mmol), methyl 3-(methylamino)propanoate **46** (290 mg, 2.5 mmol), DIPEA (1.2 mL, 6.9 mmol), HBTU (1.2 g, 3.2 mmol), and DMF (5.0 mL), compound **47** (460 mg, 62%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz, 3 : 2 mixture of two rotamers at 25 °C) data for the major rotamer: δ 7.85–7.78 (2H, m), 7.20–7.14 (2H, m), 7.07 (1H, s), 3.83 (2H, t, *J* = 6.9 Hz), 3.72 (3H, s), 3.35 (3H, s), 2.73 (2H, t, *J* = 6.9 Hz); ESIMS-LR *m/z* 307 [(M+H)$^+$].

3-[[3-(4-Fluorophenyl)isoxazole-5-carbonyl]-methyl-amino]propanoic acid (48)



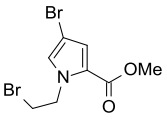
A solution of compound **47** (420 mg, 1.4 mmol) in THF (5.0 mL) and H_2O (5.0 mL) was treated with LiOH-H_2O (140 mg, 3.4 mmol) at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (50 mL) and 1 N HCl (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford compound **48** (210 mg, 52%) as a colorless solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.97–7.91 (2H, m), 7.29–7.21 (3H, m), 3.89–3.78 (2H, m), 3.30 (3H, s), 2.77–2.67 (2H, m); ESIMS-LR *m/z* 293 [(M+H)$^+$].

***N*-[3-(Dimethylamino)-3-oxo-propyl]-3-(4-fluorophenyl)-*N*-methyl-isoxazole-5-carboxamide (20)**



Following General procedure A using compound **48** (75 mg, 0.26 mmol), dimethylamine in THF (0.13 ml, 0.26 mmol), DIPEA (0.12 mL, 0.69 mmol), HBTU (120 mg, 0.32 mmol), and DMF (5.0 mL), compound **20** (42 mg, 51%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz, 3 : 2 mixture of two rotamers at 25 °C) data for the major rotamer: δ 7.84–7.78 (2H, m), 7.21–7.14 (2H, m), 7.05 (1H, s), 3.84 (2H, t, *J* = 7.0 Hz), 3.38 (3H, s), 3.05 (3H, s), 2.97 (3H, s), 2.75 (2H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz, a mixture of rotamers, selected data for the major rotamer): δ 170.6, 165.2, 164.1 (d, $^1J_{C,F}$ = 250 Hz), 161.4, 158.1, 128.9 (d, $^3J_{C,F}$ = 9.0 Hz), 124.3 (d, $^4J_{C,F}$ = 4.0 Hz), 116.3 (d, $^2J_{C,F}$ = 22 Hz), 106.3, 46.2, 37.3, 35.4, 34.6, 32.8; ESIMS-LR *m/z* 320 [(M+H)$^+$]; ESIMS-HR calcd for $C_{16}H_{19}FN_3O_3$ (M+H)$^+$ 320.1405, found 320.1406.

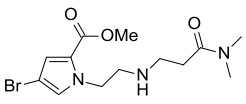
Methyl 4-bromo-1-(2-bromoethyl)pyrrole-2-carboxylate (51)



A solution of methyl 4-bromopyrrole-2-carboxylate **49** (1.2 g, 5.9 mmol) and NaH (430 mg, 60% dispersion in mineral oil, 11 mmol) in DMF (10 mL) was treated with 1,2-dibromoethane **50** (0.91 mL, 11 mmol) at room temperature, and the mixture was stirred at 80 °C for 1 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; from 0 : 100 to 30 : 70) to afford compound **51** (1.4 g, 77%) as a

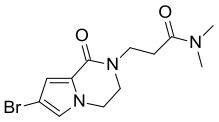
colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.96 (1H, d, *J* = 2.0 Hz), 6.91 (1H, d, *J* = 2.0 Hz), 4.63 (2H, t, *J* = 6.4 Hz), 3.82 (3H, s), 3.65 (2H, t, *J* = 6.4 Hz); ESIMS-LR *m/z* 310 [$(M+H)^+$].

Methyl 4-bromo-1-[2-[[3-(dimethylamino)-3-oxo-propyl]amino]ethyl]pyrrole-2-carboxylate (52)



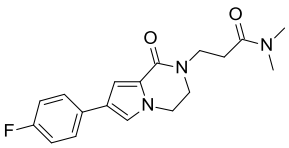
A solution of compound **51** (490 mg, 1.6 mmol) and K_2CO_3 (280 mg, 2.0 mmol) in DMF (3.0 mL) was treated with 3-amino-*N*,*N*-dimethylpropanamide (240 mg, 2.1 mmol) at room temperature, and the mixture was stirred at 80 °C for 2 h. The resulting mixture was partitioned between AcOEt (50 mL) and saturated aqueous $NaHCO_3$ (30 mL), and the organic phase was washed with H_2O (30 mL) and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; from 20 : 80 to 90 : 10) to afford compound **52** (67 mg, 12%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz):δ 6.93–6.89 (2H, m), 4.38 (2H, t, *J* = 6.3 Hz), 4.08–4.02 (1H, m), 3.91–3.85 (1H, m), 3.80 (3H, s), 2.99 (3H, s), 2.94 (3H, s), 2.87 (2H, t, *J* = 6.3 Hz), 2.47 (2H, t, *J* = 6.3 Hz); ESIMS-LR *m/z* 346, 348 [$(M+H)^+$].

3-(7-Bromo-1-oxo-3,4-dihydropyrrolo[1,2-a]pyrazin-2-yl)-*N*,*N*-dimethylpropanamide (53)



A solution of compound **52** (80 mg, 0.23 mmol) in DMSO (1.5 mL) was stirred at 150 °C for 4 h. The mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; from 40 : 60 to 100 : 0) to afford compound **53** (63 mg, 87%) as a colorless oil. ^{1}H NMR (DMSO-d_6, 400 MHz):δ 7.12 (1H, d, *J* = 1.8 Hz), 6.63 (1H, d, *J* = 1.8 Hz), 4.13–4.08 (2H, m), 3.72–3.67 (2H, m), 3.58 (2H, t, *J* = 7.3 Hz), 2.95 (3H, s), 2.81 (3H, s), 2.59 (2H, t, *J* = 7.3 Hz); ESIMS-LR *m/z* 314, 316 [$(M+H)^+$].

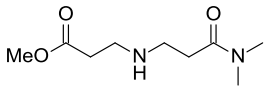
3-[7-(4-Fluorophenyl)-1-oxo-3,4-dihydropyrrolo[1,2-a]pyrazin-2-yl]-*N*,*N*-dimethylpropanamide (21)



A suspension of compound **53** (63 mg, 0.20 mmol), 4-fluorophenylboronic acid **54** (42 mg, 0.30 mmol), and K_2CO_3 (83 mg, 0.60 mmol) in dioxane (2.0 mL) and H_2O (1.0 mL) was treated with $Pd(PPh_3)_2Cl_2$ (14 mg, 0.020 mmol) at room temperature; the mixture was stirred at 120 °C for 1 h with μW irradiation and then cooled to room temperature. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH; from 40 : 60 to 100 : 0) to afford compound **21** (22 mg, 33%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45–7.41 (2H, m), 7.11 (1H, d, *J* = 1.9 Hz), 7.06–7.00 (2H, m), 6.93 (1H, d, *J* = 1.9 Hz), 4.13–4.08 (2H, m), 3.94–3.89 (2H, m), 3.79 (2H, t, *J* = 6.1 Hz), 3.01 (3H, s), 2.94 (3H, s), 2.76 (2H, t, *J* = 6.1 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.3, 161.5 (d, $^1J_{C,F}$ = 244 Hz), 159.7, 130.8 (d, $^4J_{C,F}$ = 3.0 Hz), 126.7

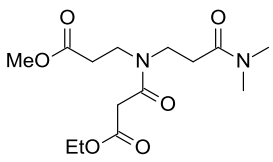
(d, $^3J_{C,F}$ = 8.0 Hz), 125.8, 125.3, 119.2, 115.6 (d, $^2J_{C,F}$ = 22 Hz), 110.1, 48.1, 44.2, 44.1, 37.3, 35.4, 32.5; ESIMS-LR *m/z* 330 [(M+H)$^+$]; ESIMS-HR calcd for $C_{18}H_{21}FN_3O_2$ (M+H)$^+$ 330.1612, found 330.1609.

Methyl 3-[[3-(dimethylamino)-3-oxo-propyl]amino]propanoate (57)



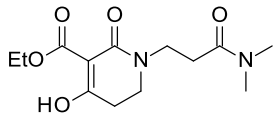
A suspension of methyl acrylate **55** (0.20 mL, 2.23 mmol) in MeOH (5.0 mL) was treated with compound **56** (31 mg, 2.7 mmol) at room temperature, and the mixture was stirred at 50 °C for 16 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; from 20 : 80 to 90 : 10) to afford compound **57** (22 mg, 33%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.68 (3H, s), 2.99 (3H, s), 2.94 (3H, s), 2.93–2.87 (4H, m), 2.54–2.48 (4H, m); ESIMS-LR *m/z* 203 [(M+H)$^+$].

Ethyl 3-[[3-(dimethylamino)-3-oxo-propyl]-(3-methoxy-3-oxo-propyl)amino]-3-oxo-propanoate (59)



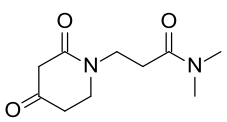
A solution of compound **57** (170 mg, 0.84 mmol) and DIPEA (0.17 mL, 1.0 mmol) in CH_2Cl_2 (10 mL) was treated with ethyl malonyl chloride **58** (0.13 mL, 1.0 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; from 30 : 70 to 90 : 10) to afford compound **59** (120 mg, 45%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.23–4.17 (2H, m), 3.71–3.65 (5H, m), 3.61 (2H, td, *J* = 7.0, 2.5 Hz), 3.55 (2H, d, *J* = 7.0 Hz), 3.01 (3H, d, *J* = 2.0 Hz), 2.94 (3H, d, *J* = 6.3 Hz), 2.69–2.59 (4H, m), 1.28 (3H, td, *J* = 7.2, 1.9 Hz); ESIMS-LR *m/z* 317 [(M+H)$^+$].

Ethyl 1-[3-(dimethylamino)-3-oxo-propyl]-4-hydroxy-6-oxo-2,3-dihydropyridine-5-carboxylate (60)



A solution of compound **59** (110 mg, 0.35 mmol) in EtOH (2.0 mL) was treated with NaH (17 mg, 60% dispersion in mineral oil, 0.43 mmol) at 0 °C, and the mixture was stirred at room temperature for 17 h. The resulting mixture was concentrated *in vacuo* to afford compound **60** (88 mg, 89%) as a colorless oil. This product was used in the next reaction without further purification; ESIMS-LR *m/z* 285 [(M+H)$^+$].

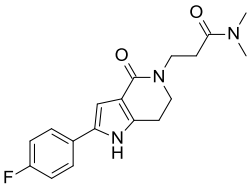
3-(2,4-Dioxo-1-piperidyl)-*N*,*N*-dimethylpropanamide (61)



A solution of compound **60** (88 mg, 0.31 mmol) in H_2O (1.0 mL) was treated with AcOH (0.5 mL) at room temperature, and the mixture was stirred at 90 °C for 3 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 1 : 99 to

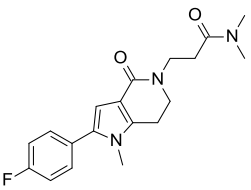
15 : 85) to afford compound **61** (66 mg, 100%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.81 (2H, t, *J* = 6.1 Hz), 3.75 (2H, t, *J* = 6.1 Hz), 3.31 (2H, s), 3.00 (3H, s), 2.94 (3H, s), 2.71 (2H, t, *J* = 6.0 Hz), 2.60 (2H, t, *J* = 6.0 Hz); ESIMS-LR *m/z* 213 [$(M+H)^+$].

3-[2-(4-Fluorophenyl)-4-oxo-6,7-dihydro-1H-pyrrolo[3,2-c]pyridin-5-yl]-*N*,*N*-dimethylpropanamide (22)



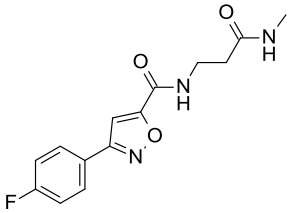
A solution of compound **61** (66 mg, 0.31 mmol) and 2-bromo-4′-fluoroacetophenone **62** (81 mg, 0.373 mmol) in EtOH (3.0 mL) was treated with ammonium acetate (120 mg, 1.6 mmol) at room temperature for 17 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; from 1 : 99 to 10 : 90) to afford compound **22** (17 mg, 17%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.87–7.81 (1H, m), 7.44–7.39 (2H, m), 7.09–7.03 (2H, m), 6.74 (1H, d, *J* = 2.8 Hz), 3.76 (4H, td, *J* = 6.8, 1.2 Hz), 3.03 (3H, s), 2.96–2.91 (5H, m), 2.72 (2H, t, *J* = 6.8 Hz); ESIMS-LR *m/z* 330 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{21}FN_3O_2$ $(M+H)^+$ 330.1612, found 330.1618.

3-[2-(4-Fluorophenyl)-1-methyl-4-oxo-6,7-dihydropyrrolo[3,2-c]pyridin-5-yl]-*N*,*N*-dimethylpropanamide (23)



A solution of compound **22** (6.8 mg, 0.021 mmol) and NaH (1.0 mg, 60% dispersion in mineral oil, 0.025 mmol) in DMF (1.5 mL) was treated with MeI (0.0013 mL, 0.021 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (50 mL) and saturated aqueous $NaHCO_3$ (30 mL), and the organic phase was washed with H_2O (30 mL) and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; from 50 : 50 to 100 : 0) to afford compound **23** (5.5 mg, 76%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35–7.30 (2H, m), 7.14–7.07 (2H, m), 6.54 (1H, s), 3.82–3.74 (4H, m), 3.47 (3H, s), 3.04 (3H, s), 2.94 (3H, s), 2.87 (2H, t, *J* = 6.8 Hz), 2.73 (2H, t, *J* = 6.8 Hz); ESIMS-LR *m/z* 344 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{21}FN_3O_2$ $(M+H)^+$ 344.1769, found 344.1775.

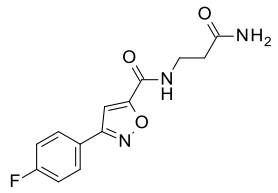
3-(4-Fluorophenyl)-*N*-[3-(methylamino)-3-oxo-propyl]isoxazole-5-carboxamide (24)



Following General procedure A using compound **43a** (7.0 g, 34 mmol), 3-amino-*N*-methylpropanamide hydrochloride **45** (5.0 g, 36 mmol), DIPEA (8.9 mL, 51 mmol), HBTU (1.5 g, 41 mmol), and DMF (50 mL), compound **24** (7.9 g, 80%) was obtained as a colorless solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.96–7.89 (2H, m), 7.35 (1H, s), 7.28–7.21 (2H, m), 3.64 (2H, t, *J* = 6.9 Hz), 2.73 (3H, s), 2.51 (2H, t, *J* = 6.9 Hz); ESIMS-LR *m/z* 292 [$(M+H)^+$]; ESIMS-HR calcd for $C_{14}H_{15}FN_3O_3$ $(M+H)^+$ 292.1092, found

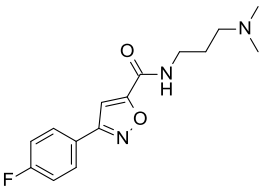
292.1099.

***N*-(3-Amino-3-oxo-propyl)-3-(4-fluorophenyl)isoxazole-5-carboxamide (25)**



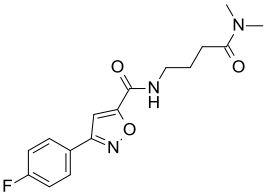
Following General procedure A using compound **43a** (250 mg, 1.2 mmol), β-alaninamide hydrochloride (100 mg, 1.1 mmol), DIPEA (0.62 mL, 3.7 mmol), HATU (600 mg, 1.6 mmol), and DMF (3.0 mL), compound **25** (93 mg, 28%) was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.99 (1H, t, J = 5.5 Hz), 8.02–7.95 (2H, m), 7.63 (1H, s), 7.43–7.34 (3H, m), 6.86 (1H, br s), 3.46 (2H, dd, J = 12.9, 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz); ESIMS-LR *m/z* 278 [(M+H)$^+$]; ESIMS-HR calcd for $C_{13}H_{13}FN_3O_3$ (M+H)$^+$ 278.0935, found 278.0944.

***N*-[3-(Dimethylamino)propyl]-3-(4-fluorophenyl)isoxazole-5-carboxamide (26)**



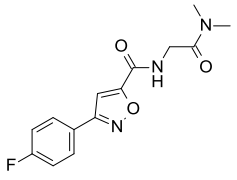
Following General procedure A using compound **43a** (120 mg, 0.58 mmol), *N*,*N*-dimethyl-1,3-propanediamine (0.080 mL, 0.63 mmol), DIPEA (0.30 mL, 1.8 mmol), HBTU (280 mg, 0.74 mmol), and DMF (5.0 mL), compound **26** (25 mg, 15%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.04 (1H, br s), 7.85–7.78 (2H, m), 7.20–7.13 (3H, m), 3.61–3.56 (2H, m), 2.51 (2H, t, J = 5.9 Hz), 2.32 (6H, s), 1.80–1.74 (2H, m); ESIMS-LR *m/z* 292 [(M+H)$^+$]. ESIMS-HR calcd for $C_{15}H_{19}FN_3O_2$ (M+H)$^+$ 292.1456, found 292.1455.

***N*-[4-(Dimethylamino)-4-oxo-butyl]-3-(4-fluorophenyl)isoxazole-5-carboxamide (27)**



Following General procedure A using compound **43a** (100 mg, 0.48 mmol), 4-amino-*N*,*N*-dimethylbutanamide (76 mg, 0.58 mmol), DIPEA (0.22 mL, 1.3 mmol), HBTU (220 mg, 0.58 mmol), and DMF (5.0 mL), compound **27** (46 mg, 30%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85–7.79 (2H, m), 7.68 (1H, br s), 7.21–7.13 (3H, m), 3.53 (2H, dd, J = 12.8, 5.8 Hz), 3.02 (3H, s), 2.98 (3H, s), 2.48 (2H, t, J = 6.5 Hz), 2.07–1.99 (2H, m); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 172.6, 164.2, 164.1 (d, $^1J_{C,F}$ = 250 Hz), 162.2, 156.1, 128.9 (d, $^3J_{C,F}$ = 9.0 Hz), 124.4 (d, $^4J_{C,F}$ = 3.0 Hz), 116.2 (d, $^2J_{C,F}$ = 22 Hz), 104.5, 39.9, 37.3, 35.7, 31.2, 23.7; ESIMS-LR *m/z* 320 [(M+H)$^+$]; ESIMS-HR calcd for $C_{16}H_{19}FN_3O_3$ (M+H)$^+$ 320.1405, found 320.1414.

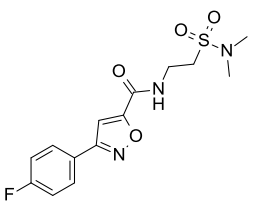
***N*-[2-(Dimethylamino)-2-oxo-ethyl]-3-(4-fluorophenyl)isoxazole-5-carboxamide (28)**



Following General procedure A using compound **43a** (100 mg, 0.48 mmol), 2-amino-*N*,*N*-dimethylacetamide (60 mg, 0.59 mmol), DIPEA (0.16 mL, 0.94 mmol), HATU (50 mg, 0.13 mmol), and DMF (1.5 mL), compound **28** (98 mg, 70%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.86–7.80 (2H, m), 7.75 (1H, br s), 7.21–7.15 (3H, m), 4.25 (2H, d, J = 4.0 Hz), 3.05 (6H, d, J = 1.0 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 166.8, 164.1 (d, $^1J_{C,F}$ = 250 Hz), 163.6, 162.3, 155.7, 128.9 (d, $^3J_{C,F}$ = 9.0 Hz), 124.3 (d, $^4J_{C,F}$ = 4.0 Hz), 116.2 (d, $^2J_{C,F}$ = 22 Hz),

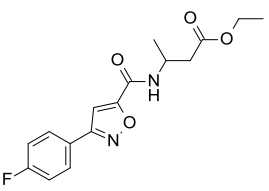
105.0, 41.3, 36.0, 35.7; ESIMS-LR *m/z* 292 [(M+H)$^+$]; ESIMS-HR calcd for $C_{14}H_{15}FN_3O_3$ (M+H)$^+$ 292.1092, found 292.1117.

_N_-[2-(Dimethylsulfamoyl)ethyl]-3-(4-fluorophenyl)isoxazole-5-carboxamide (33)



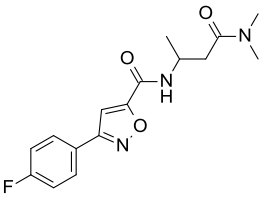
Following General procedure A using compound **43a** (75 mg, 0.36 mmol), 2-amino-*N*,*N*-dimethylethanesulfonamide (80 mg, 0.53 mmol), DIPEA (0.19 mL, 1.1 mmol), HBTU (180 mg, 0.47 mmol), and DMF (1.5 mL), compound **33** (38 mg, 31%) was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.19 (1H, br s), 8.04–7.97 (2H, m), 7.67 (1H, s), 7.43-7.36 (2H, m), 3.69–3.62 (2H, m), 3.35–3.30 (2H, m), 2.79 (6H, s); ESIMS-LR *m/z* 342 [(M+H)$^+$]; ESIMS-HR calcd for $C_{14}H_{17}FN_3O_4S$ (M+H)$^+$ 342.0918, found 342.0916.

Ethyl 3-[[3-(4-fluorophenyl)isoxazole-5-carbonyl]amino]butanoate (64a)



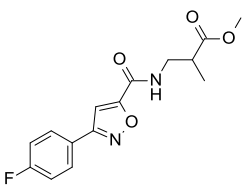
Following General procedure A using compound **43a** (200 mg, 0.97 mmol), ethyl 3-aminobutyrate **63a** (150 mg, 1.2 mmol), DIPEA (0.50 mL, 2.9 mmol), HATU (480 mg, 1.3 mmol), and DMF (5.0 mL), compound **64a** (70 mg, 23%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.79 (2H, m), 7.29–7.25 (1H, m), 7.20–7.14 (3H, m), 4.62–4.52 (1H, m), 4.20 (2H, q, *J* = 7.2 Hz), 2.65 (2H, dd, *J* = 5.4, 1.6 Hz), 1.38 (3H, d, *J* = 6.8 Hz), 1.29 (3H, t, *J* = 7.2 Hz); ESIMS-LR *m/z* 321 [(M+H)$^+$].

_N_-[3-(Dimethylamino)-1-methyl-3-oxo-propyl]-3-(4-fluorophenyl)isoxazole-5-carboxamide (29)



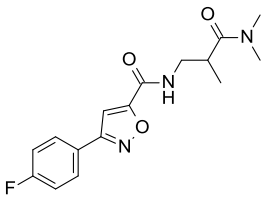
A solution of compound **64a** (70 mg, 0.22 mmol) in THF (1.5 mL) and H_2O (0.5 mL) was treated with LiOH-H_2O (20 mg, 0.48 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (50 mL) and 1 M aqueous HCl (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. A solution of the crude **65a**, DIPEA (0.11 mL, 0.65 mmol) and dimethylamine hydrochloride (22 mg, 0.27 mmol) in DMF (1.5 mL) was treated with HATU (110 mg, 0.29 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; from 40 : 60 to 100 : 0) to afford compound **29** (33 mg, 47% over 2 steps) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.04 (1H, d, *J* = 8.5 Hz), 7.84–7.78 (2H, m), 7.20–7.14 (2H, m), 7.13 (1H, s), 4.60–4.51 (1H, m), 3.04 (3H, s), 2.98 (3H, s), 2.73 (1H, dd, *J* = 16.1, 4.3 Hz), 2.60 (1H, dd, *J* = 16.1, 5.3 Hz), 1.41 (3H, d, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 170.9, 165.3, 164.3 (d, $^1J_{C,F}$ = 250 Hz), 162.2, 155.2, 128.9 (d, $^3J_{C,F}$ = 9.0 Hz), 124.5 (d, $^4J_{C,F}$ = 4.0 Hz), 116.2 (d, $^2J_{C,F}$ = 22 Hz), 104.6, 43.3, 37.4, 37.3, 35.3, 20.0; ESIMS-LR *m/z* 320 [(M+H)$^+$]; ESIMS-HR calcd for $C_{16}H_{19}FN_3O_3$ (M+H)$^+$ 320.1405, found 320.1411.

Methyl 3-[[3-(4-fluorophenyl)isoxazole-5-carbonyl]amino]-2-methyl-propanoate (64b)



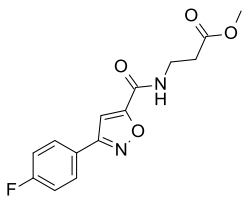
Following General procedure A using compound **43a** (150 mg, 0.73 mmol), methyl 3-amino-2-methylpropanoate hydrochloride **63b** (130 mg, 0.87 mmol), DIPEA (0.37 mL, 2.2 mmol), HATU (360 mg, 0.95 mmol), and DMF (3.0 mL), compound **64b** (96 mg, 44%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.79 (2H, m), 7.20–7.15 (3H, m), 7.11 (1H, br s), 3.77–3.70 (1H, m), 3.75 (3H, s), 3.59–3.51 (1H, m), 2.86–2.79 (1H, m), 1.27 (3H, d, J = 7.3 Hz); ESIMS-LR *m/z* 307 [$(M+H)^+$].

***N*-[3-(Dimethylamino)-2-methyl-3-oxo-propyl]-3-(4-fluorophenyl)isoxazole-5-carboxamide (30)**



A solution of compound **64b** (92 mg, 0.30 mmol) in THF (1.5 mL) and H_2O (0.5 mL) was treated with LiOH-H_2O (25 mg, 0.60 mmol) at room temperature for 15 h. The resulting mixture was partitioned between AcOEt (50 mL) and 1 M aqueous HCl (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. A solution of crude **65b**, DIPEA (0.15 mL, 0.90 mmol) and dimethylamine hydrochloride (30 mg, 0.37 mmol) in DMF (1.5 mL) was treated with HATU (150 mg, 0.40 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; from 20 : 80 to 80 : 20) to afford compound **30** (68 mg, 71% over 2 steps) as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.02 (1H, t, J = 5.6 Hz), 8.02–7.95 (2H, m), 7.64 (1H, s), 7.39 (2H, t, J = 8.9 Hz), 3.46–3.38 (1H, m), 3.28–3.20 (1H, m), 3.19–3.11 (1H, m), 3.04 (3H, s), 2.84 (3H, s), 1.03 (3H, d, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 174.6, 164.1 (d, $^1J_{C,F}$ = 250 Hz), 164.0, 162.3, 156.1, 128.9 (d, $^3J_{C,F}$ = 8.0 Hz), 124.3 (d, $^4J_{C,F}$ = 4.0 Hz), 116.2 (d, $^2J_{C,F}$ = 22 Hz), 104.8, 42.1, 37.1, 35.9, 35.6, 14.9; ESIMS-LR *m/z* 320 [$(M+H)^+$]; ESIMS-HR calcd for $C_{16}H_{19}FN_3O_3$ $(M+H)^+$ 320.1405, found 320.1399.

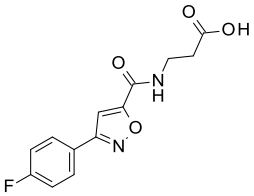
Methyl 3-[[3-(4-fluorophenyl) isoxazole-5-carbonyl]amino] propanoate (64c)



A solution of compound **43a** (10 g, 48 mmol) in CH_2Cl_2 (150 mL) and DMF (0.50 mL) was treated with $SOCl_2$ (5.7 mL, 78 mmol) at 0 °C and the mixture was stirred at 40 °C for 18 h. The solvent was then removed *in vacuo* to afford acid chloride as a yellow solid. The acid chloride was dissolved in CH_2Cl_2 (150 mL) and added dropwise to a mixture of compound **63c** (11 g, 79 mmol) and Et_3N (22 mL, 158 mmol) in CH_2Cl_2 (20 mL) at 0 °C, and the mixture was stirred at room temperature for 5 h. The solvent was then removed *in vacuo* and the residue was quenched with H_2O (10 mL). The resulting mixture was partitioned between AcOEt (200 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and

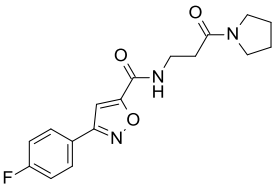
concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; 0 : 100 to 10 : 90) to afford compound **64c** (12 g, 85%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ: 7.84–7.79 (2H, m), 7.23–7.13 (4H, m), 3.79–3.72 (5H, m), 2.68 (2H, t, *J* = 6.0 Hz). ESIMS-LR *m/z* 293 [$(M+H)^+$].

3-[[3-(4-Fluorophenyl)isoxazole-5-carbonyl]amino] propanoic acid (65c)



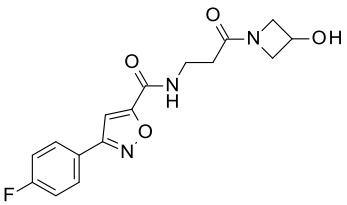
A solution of compound **64c** (12 g, 41 mmol) in THF (100 mL) and H_2O (30 mL) was treated with LiOH-H_2O (2.0 g, 84 mmol) at room temperature for 4 h. The resulting mixture was partitioned between AcOEt (200 mL) and 1 N HCl (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford compound **65c** (7.1 g, 62%) as a colorless solid. This material was used in the next reaction without further purification. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.94–7.87 (2H, m), 7.33 (1H, s), 7.27–7.19 (2H, m), 3.66 (2H, t, *J* = 6.9 Hz), 2.66 (2H, t, *J* = 6.9 Hz). ESIMS-LR *m/z* 279 [$(M+H)^+$].

3-(4-Fluorophenyl)-*N*-(3-oxo-3-pyrrolidin-1-yl-propyl)isoxazole-5-carboxamide (31)



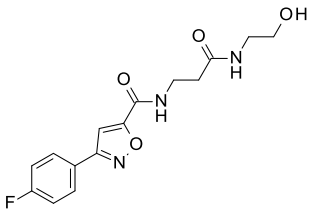
Following General procedure A using compound **65c** (150 mg, 0.80 mmol), pyrrolidine (0.20 mL, 2.4 mmol), DIPEA (0.28 mL, 1.6 mmol), HATU (330 mg, 0.88 mmol), and DMF (3.0 mL), compound **31** (200 mg, 76%) was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.94 (1H, t, *J* = 5.5 Hz), 8.02–7.96 (2H, m), 7.64 (1H, s), 7.42–7.34 (2H, m), 3.49 (2H, dd, *J* = 12.8, 7.3 Hz), 3.39 (2H, t, *J* = 6.8 Hz), 3.30–3.27 (2H, m), 2.55 (2H, t, *J* = 7.2 Hz), 1.90–1.83 (2H, m), 1.80–1.73 (2H, m); ESIMS-LR *m/z* 332 [$(M+H)^+$]; ESIMS-HR calcd for $C_{17}H_{19}FN_3O_3$ $(M+H)^+$ 332.1405, found 320.1422.

3-(4-Fluorophenyl)-*N*-[3-(3-hydroxyazetidin-1-yl)-3-oxo-propyl]isoxazole-5-carboxamide (32)



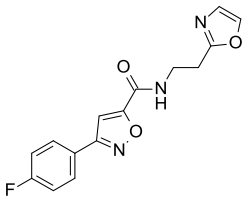
Following General procedure A using compound **65c** (100 mg, 0.36 mmol), 3-hydroxyazetidine hydrochloride (48 mg, 0.44 mmol), DIPEA (0.20 mL, 1.1 mmol), HBTU (170 mg, 0.44 mmol), and DMF (5.0 mL), compound **32** (45 mg, 38%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): 7.86–7.80 (2H, m), 7.54 (1H, br s), 7.22–7.15 (3H, m), 4.29–4.17 (3H, m), 4.00 (1H, dd, *J* = 8.8, 2.0 Hz), 3.93 (1H, t, *J* = 7.5 Hz), 3.77 (2H, ddd, *J* = 11.8, 6.0, 2.3 Hz), 2.44 (2H, t, *J* = 6.0 Hz); ESIMS-LR *m/z* 334 [$(M+H)^+$]; ESIMS-HR calcd for $C_{16}H_{17}FN_3O_4$ $(M+H)^+$ 334.1198, found 334.1190.

3-(4-Fluorophenyl)-*N*-[3-(2-hydroxyethylamino)-3-oxo-propyl]isoxazole-5-carboxamide (66)



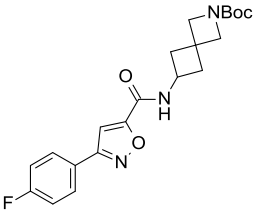
Following General procedure A using compound **65c** (40 mg, 0.14 mmol), 2-aminoethanol (0.010 mL, 0.17 mmol), DIPEA (0.073 mL, 0.43 mmol), HATU (71 mg, 0.19 mmol), and DMF (3.0 mL), compound **66** (43 mg, 93%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.79 (2H, m), 7.20–7.14 (3H, m), 3.81–3.72 (4H, m), 3.47 (2H, dd, *J* = 10.3, 5.5 Hz), 2.57 (2H, t, *J* = 5.9 Hz), 2.14 (1H, t, *J* = 5.1 Hz); ESIMS-LR *m/z* 322 [$(M+H)^+$].

3-(4-Fluorophenyl)-*N*-(2-oxazol-2-ylethyl)isoxazole-5-carboxamide (34)



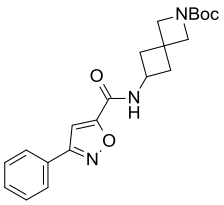
A solution of compound **66** (40 mg, 0.13 mmol), hexachloroethane (80 mg, 0.34 mmol) and Et_3N (100 μL, 0.72 mmol) in CH_2Cl_2 (1.0 mL) was treated with triphenylphosphine (100 mg, 0.38 mmol) at room temperature for 16 h. The resulting mixture was partitioned between AcOEt (50 mL) and saturated aqueous $NaHCO_3$ (30 mL), and the organic phase was washed with H_2O (30 mL), and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; from 50 : 50 to 100 : 0) to afford compound **34** (12 mg, 32%) as a colorless solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.91–7.84 (2H, m), 7.76 (1H, d, *J* = 1.0 Hz), 7.64 (1H, s), 7.25–7.17 (2H, m), 7.07 (1H, d, *J* = 1.0 Hz), 3.82 (2H, t, *J* = 6.9 Hz), 3.15 (2H, t, *J* = 6.9 Hz); ESIMS-LR *m/z* 302 [$(M+H)^+$]; ESIMS-HR calcd for $C_{15}H_{13}FN_3O_3$ $(M+H)^+$ 302.0935, found 302.0927.

tert-Butyl 6-[[3-(4-fluorophenyl)isoxazole-5-carbonyl]amino]-2-azaspiro[3.3]heptane-2-carboxylate (68)



A solution of compound **43a** (2.0 g, 9.7 mmol), compound **67** (2.3 g, 11 mmol), and Et_3N (5.4 mL, 39 mmol) in DMF (50 mL) was treated with COMU (6.2 g, 15 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The resulting mixture was partitioned between AcOEt (50 mL) and saturated aqueous $NaHCO_3$ (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford compound **68** (2.2 g, 56%) as a colorless solid. This product was used in the next reaction without further purification; ESIMS-LR *m/z* 346 [$(M-^tBu+H)^+$].

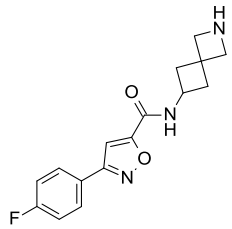
tert-Butyl 6-[(3-phenylisoxazole-5-carbonyl)amino]-2-azaspiro[3.3]heptane-2-carboxylate (69)



A solution of compound **43d** (5.5 g, 29 mmol), DMF (0.1 mL) in CH_2Cl_2 (50 mL) was treated with $SOCl_2$ (2.6 mL, 37.8 mmol) at room temperature, and the mixture was stirred at 50 °C for 4 h. The resulting mixture was concentrated *in vacuo*. A solution of the acid chloride and triethylamine (16 mL, 120 mmol) in CH_2Cl_2 (50 mL) was treated with compound **67** (7.4 g, 35 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The resulting mixture was

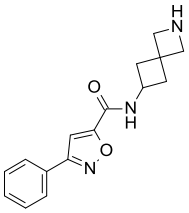
partitioned between AcOEt (100 mL) and saturated aqueous $NaHCO_3$ (50 mL), and the organic phase was washed with H_2O (50 mL) and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; from 3 : 7 to 8 : 2) to afford compound **69** (10 g, 91%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.78 (2H, m), 7.51–7.46 (3H, m), 7.21 (1H, s), 6.77 (1H, d, *J* = 7.5 Hz), 4.45 (1H, q, *J* = 8.3 Hz), 4.01 (2H, s), 3.90 (2H, s), 2.75–2.66 (2H, m), 2.28–2.20 (2H, m), 1.44 (9H, s); ESIMS-LR *m/z* 328 [(M-^{t}Bu+H)$^+$].

_N_-(2-Azaspiro[3.3]heptan-6-yl)-3-(4-fluorophenyl)isoxazole-5-carboxamide (70)



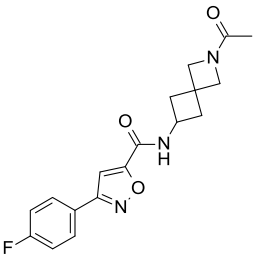
A solution of compound **68** (2.2 g, 5.4 mmol) in CH_2Cl_2 (50 mL) was treated with TFA (10 mL) at 0 °C for 3 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; from 1 : 99 to 13 : 87) to afford compound **70** (880 mg, 54%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.17 (1H, d, *J* = 7.3 Hz), 8.01–7.95 (2H, m), 7.61 (1H, s), 7.42–7.35 (2H, m), 4.22 (1H, dd, *J* = 15.9, 7.9 Hz), 3.51 (2H, s), 3.39 (2H, s), 2.48–2.41 (2H, m), 2.18 (2H, td, *J* = 9.0, 2.8 Hz); ESIMS-LR *m/z* 302 [(M+H)$^+$].

_N_-(2-Azaspiro[3.3]heptan-6-yl)-3-phenyl-isoxazole-5-carboxamide (71)



A solution of compound **69** (11 g, 29 mmol) in CH_2Cl_2 (20 mL) was treated with TFA (11 mL, 140 mmol) at 0 °C for 3 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2; MeOH/$CHCl_3$; from 1 : 99 to 15 : 85) to afford compound **71** (6.9 g, 85%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.79 (2H, m), 7.51–7.46 (3H, m), 7.20 (1H, s), 6.67 (1H, br s), 4.43 (1H, dd, *J* = 16.4, 8.7 Hz), 3.72 (2H, s), 3.61 (2H, s), 2.76–2.71 (2H, m), 2.20–2.11 (2H, m); ESIMS-LR *m/z* 284 [(M+H)$^+$].

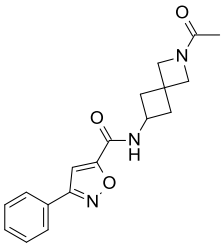
_N_-(2-Acetyl-2-azaspiro[3.3]heptan-6-yl)-3-(4-fluorophenyl)isoxazole-5-carboxamide (36)



A solution of the compound **70** (310 mg, 1.0 mmol) and pyridine (420 µL, 5.1 mmol) in CH_2Cl_2 (10 mL) was treated with acetyl chloride (110 µL, 1.5 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 1 : 99 to 13 : 87) to afford compound **36** (310 mg, 88%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (1H, dd, *J* = 7.3, 2.5 Hz), 8.02–7.95 (2H, m), 7.63 (1H, d, *J* = 2.3 Hz), 7.42–7.34 (2H, m), 4.29 (1H, dd, *J* = 16.1, 8.0 Hz), 4.17 (1H, s), 4.06 (1H, s), 3.89 (1H, s), 3.78 (1H, s), 2.57–2.52 (2H, m), 2.35–2.28 (2H, m), 1.72 (3H, d, *J* = 4.3 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz, a mixture of rotamers, selected data for the major rotamer): δ 170.4, 164.2 (d, $^1J_{C,F}$ = 250 Hz), 163.5, 162.5, 155.1, 128.9

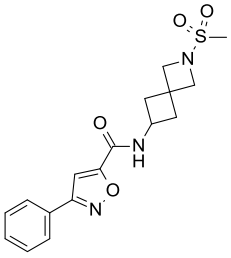
(d, $^3J_{C,F}$ = 8.0 Hz), 124.1 (d, $^4J_{C,F}$ = 3.0 Hz), 116.3 (d, $^2J_{C,F}$ = 22 Hz), 105.2, 62.7, 61.1, 60.0, 58.3, 41.2, 39.9, 18.8; ESIMS-LR *m/z* 344 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{19}FN_3O_3$ $(M+H)^+$ 344.1405, found 344.1414.

_N_-(2-Acetyl-2-azaspiro[3.3]heptan-6-yl)-3-phenyl-isoxazole-5-carboxamide (37)



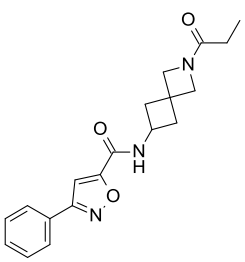
A suspension of compound **71** (6.4 g, 23 mmol) and pyridine (9.1 mL, 110 mmol) in CH_2Cl_2 (100 mL) was treated with acetyl chloride (2.4 mL, 34 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (300 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 1 : 99 to 15 : 85) to afford compound **37** (5.9 g, 80%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.79 (2H, m), 7.51–7.47 (3H, m), 7.22 (1H, d, *J* = 2.5 Hz), 6.69 (1H, br s), 4.49 (1H, dd, *J* = 16.6, 7.8 Hz), 4.21 (1H, s), 4.11 (1H, s), 4.08 (1H, s), 3.98 (1H, s), 2.77–2.71 (2H, m), 2.30 (2H, td, *J* = 9.4, 3.3 Hz), 1.87 (3H, d, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz, a mixture of rotamers, selected data for the major rotamer): δ 170.5, 163.41, 163.36, 155.2, 130.7, 129.2, 127.8, 126.9, 105.3, 60.6, 41.2, 39.9, 31.5, 18.8; ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{20}N_3O_3$ $(M+H)^+$ 326.1499, found 326.1516.

_N_-(2-Methylsulfonyl-2-azaspiro[3.3]heptan-6-yl)-3-phenyl-isoxazole-5-carboxamide (38)



A solution of compound **71** (50 mg, 0.18 mmol) and pyridine (71 μL, 0.88 mmol) in CH_2Cl_2 (1.0 mL) was treated with methanesulfonyl chloride (20 μL, 0.26 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; from 30 : 70 to 100 : 0) to afford compound **38** (45 mg, 71%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85–7.78 (2H, m), 7.51–7.46 (3H, m), 7.22 (1H, s), 6.71 (1H, d, *J* = 7.3 Hz), 4.53–4.42 (1H, m), 4.01 (2H, s), 3.92 (2H, s), 2.86 (3H, s), 2.79–2.72 (2H, m), 2.35–2.26 (2H, m); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 163.5, 163.3, 155.1, 130.7, 129.1, 127.8, 126.9, 105.4, 61.6, 60.3, 40.9, 39.9, 36.7, 31.4; ESIMS-LR *m/z* 362 [$(M+H)^+$]; ESIMS-HR calcd for $C_{17}H_{20}N_3O_4S$ $(M+H)^+$ 362.1169, found 362.1174.

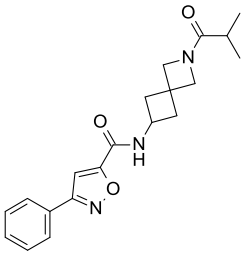
3-Phenyl-_N_-(2-propanoyl-2-azaspiro[3.3]heptan-6-yl)isoxazole-5-carboxamide (39)



A solution of compound **71** (80 mg, 0.28 mmol), pyridine (110 μL, 1.4 mmol) in CH_2Cl_2 (2.0 mL), and DMF (1.0 mL) was treated with propionyl chloride (37 μL, 0.42 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The resulting mixture was partitioned between AcOEt (50 mL) and saturated aqueous $NaHCO_3$ (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*.

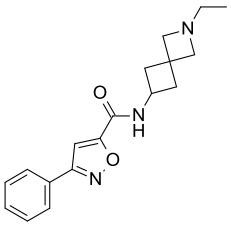
The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 1 : 99 to 13 : 87) to afford compound **39** (86 mg, 90%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.79 (2H, m), 7.51–7.47 (3H, m), 7.22 (1H, d, *J* = 2.3 Hz), 6.71 (1H, t, *J* = 8.2 Hz), 4.53–4.43 (1H, m), 4.19 (1H, s), 4.09 (2H, d, *J* = 2.3 Hz), 3.98 (1H, s), 2.77–2.71 (2H, m), 2.29 (2H, dt, *J* = 13.8, 4.4 Hz), 2.14–2.05 (2H, m), 1.12 (3H, td, *J* = 7.6, 2.4 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz, a mixture of rotamers, selected data for the major rotamer): δ 173.9, 163.40, 163.37, 155.2, 130.7, 129.1, 127.8, 126.9, 105.4, 62.3, 60.7, 60.0, 58.3, 41.3, 40.0, 31.9, 24.7; ESIMS-LR *m/z* 340 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{22}N_3O_3$ $(M+H)^+$ 340.1656, found 340.1659.

***N*-[2-(2-Methylpropanoyl)-2-azaspiro[3.3]heptan-6-yl]-3-phenyl-isoxazole-5-carboxamide (40)**



A solution of compound **71** (70 mg, 0.25 mmol), pyridine (100 μL, 1.2 mmol) in CH_2Cl_2 (2.0 mL), and DMF (1.0 mL) was treated with isobutyryl chloride (39 μL, 0.37 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The resulting mixture was partitioned between AcOEt (50 mL) and saturated aqueous $NaHCO_3$ (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 1 : 99 to 13 : 87) to afford compound **40** (84 mg, 96%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.80 (2H, m), 7.51–7.47 (3H, m), 7.22 (1H, d, *J* = 2.3 Hz), 6.71 (1H, t, *J* = 8.0 Hz), 4.53–4.44 (1H, m), 4.23 (1H, s), 4.13 (1H, s), 4.08 (1H, s), 3.97 (1H, s), 2.78–2.71 (2H, m), 2.47–2.38 (1H, m), 2.33–2.26 (2H, m), 1.10 (3H, d, *J* = 2.5 Hz), 1.09 (3H, d, *J* = 2.5 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz, a mixture of rotamers, selected data for the major rotamer): δ 176.9, 163.41, 163.37, 155.2, 130.7, 129.1, 127.8, 126.9, 105.4, 62.4, 60.8, 60.0, 58.3, 41.3, 40.0, 31.9, 29.8, 18.8; ESIMS-LR *m/z* 354 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{24}N_3O_3$ $(M+H)^+$ 354.1812, found 354.1832.

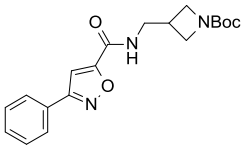
***N*-(2-Ethyl-2-azaspiro[3.3]heptan-6-yl)-3-phenyl-isoxazole-5-carboxamide (41)**



A solution of compound **71** (50 mg, 0.18 mmol), acetaldehyde (5 M in THF solution, 190 μL, 0.95 mmol) in THF (10 mL) was treated with sodium triacetoxyborohydride (140 mg, 0.64 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 1 : 99 to 13 : 87) to afford compound **41** (60 mg, 61%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.79 (2H, m), 7.50–7.46 (3H, m), 7.20 (1H, s), 6.66 (1H, d, *J* = 7.5 Hz), 4.46 (1H, q, *J* = 8.3 Hz), 3.25 (2H, s), 3.15 (2H, s), 2.71–2.64 (2H, m), 2.42 (2H, q, *J* = 7.2 Hz), 2.19–2.12 (2H, m), 0.95 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 163.6, 163.4, 155.0, 130.6, 129.1, 127.9, 126.9, 105.2, 66.0, 64.9, 53.6, 41.2, 40.6, 33.0, 12.4; ESIMS-LR *m/z* 312 [$(M+H)^+$]; ESIMS-HR

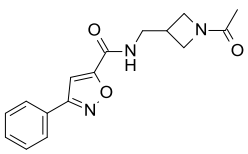
calcd for $C_{18}H_{22}N_3O_2$ $(M+H)^+$ 312.1707, found 312.1703.

***tert*-Butyl 3-[[(3-phenylisoxazole-5-carbonyl)amino]methyl]azetidine-1-carboxylate (73)**



Following General procedure A using compound **43d** (40 mg, 0.14 mmol), *tert*-butyl 3-(aminomethyl)azetidine-1-carboxylate **72** (500 mg, 2.7 mmol), DIPEA (1.2 mL, 7.1 mmol), HATU (1.1 g, 2.9 mmol), and DMF (30 mL), compound **73** (370 mg, 41%) was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84–7.78 (2H, m), 7.21–7.15 (3H, m), 6.72 (1H, br s), 4.06 (2H, t, *J* = 8.5 Hz), 3.70 (4H, dd, *J* = 8.9, 5.1 Hz), 2.90–2.79 (1H, m), 1.44 (9H, s); ESIMS-LR *m/z* 320 [(M-^{t}Bu+H)$^+$].

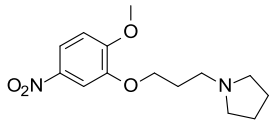
***N*-[(1-Acetylazetidin-3-yl)methyl]-3-phenyl-isoxazole-5-carboxamide (42)**



A solution of compound **73** (3.1 g, 8.7 mmol) in CH_2Cl_2 (10 mL) was treated with TFA (3.3 mL, 43 mmol) at 0 °C for 3 h. The resulting mixture was partitioned between AcOEt (50 mL) and saturated aqueous $NaHCO_3$ (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford the free amine (1.4 g, 61%) as a colorless solid. A solution of the free amine (280 mg, 1.1 mmol) and pyridine (440 μL, 5.4 mmol) in CH_2Cl_2 (1.0 mL) was treated with acetyl chloride (120 μL, 1.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$; from 1 : 99 to 13 : 87) to afford compound **42** (280 mg, 86%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85–7.79 (2H, m), 7.52–7.47 (3H, m), 7.24 (1H, s), 6.94 (1H, br s), 4.25 (1H, t, *J* = 8.4 Hz), 4.14 (1H, t, *J* = 9.2 Hz), 3.94 (1H, dd, *J* = 8.7, 5.1 Hz), 3.83–3.75 (2H, m), 3.70–3.62 (1H, m), 2.99–2.89 (1H, m), 1.87 (3H, s); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 170.9, 163.5, 163.3, 156.4, 130.7, 129.2, 127.8, 126.9, 105.5, 53.7 50.8, 42.5, 28.2, 18.7; ESIMS-LR *m/z* 300 [$(M+H)^+$]; ESIMS-HR calcd for $C_{16}H_{18}N_3O_3$ $(M+H)^+$ 300.1343, found 300.1338.

第二章

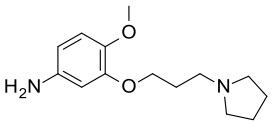
1-[3-(2-Methoxy-5-nitro-phenoxy)propyl]pyrrolidine (101)



A solution of compound **100** (3.0 g, 18 mmol) and K_2CO_3 (3.2 g, 23 mmol) in MeCN (30 mL) was treated with 1-chloro-3-iodopropane (2.2 mL, 21 mmol) at room temperature, and the mixture was stirred at 80 ºC for 2 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford crude **101'** (4.3 g, 18 mmol) as a brown solid. A suspension of crude **101'** (4.3 g, 18 mmol), NaI (5.3 g, 35 mmol), TBAI (260 mg, 0.70 mmol), and K_2CO_3 (7.3 g, 53 mmol) in MeCN (30 mL) was treated with pyrrolidine (2.9 mL, 35 mmol) at room temperature, and the mixture was stirred at 80 ºC for 3 h. The resulting mixture was partitioned between AcOEt

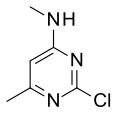
(100 mL) and saturated aqueous $NaHCO_3$ (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; 0:100 to 40:60) to afford compound **101** (3.3 g, 68% over 2 steps) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.90 (1H, dd, *J* = 8.7, 2.6 Hz), 7.80 (1H, d, *J* = 2.6 Hz), 6.90 (1H, d, *J* = 8.7 Hz), 4.18 (2H, t, *J* = 6.7 Hz), 3.96 (3H, s), 2.64 (2H, t, *J* = 7.0 Hz), 2.55–2.46 (4H, m), 2.14–2.05 (2H, m), 1.82–1.75 (4H, m). ESIMS-LR *m/z* 281 [$(M+H)^+$].

4-Methoxy-3-(3-pyrrolidin-1-ylpropoxy)aniline (102)



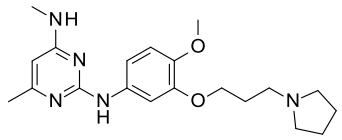
A solution of compound **101** (2.0 g, 7.1 mmol) in EtOH (30 mL) was treated with 10% Pd/C (760 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 3 h. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **102** (1.3 g, 74%) as a pale brown oil. This material was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.71 (1H, d, *J* = 8.3 Hz), 6.35 (1H, d, *J* = 2.4 Hz), 6.23 (1H, dd, *J* = 8.3, 2.4 Hz), 4.04 (2H, t, *J* = 7.0 Hz), 3.79 (3H, s), 3.42 (2H, br s), 2.62 (2H, t, *J* = 7.0 Hz), 2.55–2.48 (4H, m), 2.09–2.02 (2H, m), 1.82–1.75 (4H, m). ESIMS-LR *m/z* 251 [$(M+H)^+$].

2-Chloro-*N*,6-dimethyl-pyrimidin-4-amine (104)



A suspension of 2,4-dichloro-6-methyl-pyrimidine **103** (5.2 g, 32 mmol) and K_2CO_3 (13 g, 92 mmol) in DMF (50 mL) was treated with methylamine hydrochloride (2.1 g, 32 mmol) at room temperature for 5 h. The resulting mixture was partitioned between AcOEt (300 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 20:80 to 80:20) to afford compound **104** (2.8 g, 56%) as a colorless solid. ^{1}H NMR (DMSO–d_6, 400 MHz) δ: 7.69 (1H, s), 6.27 (1H, s), 2.75 (3H, s), 2.15 (3H, s). ESIMS-LR *m/z* 158 [$(M+H)^+$].

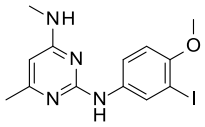
***N*2-[4-Methoxy-3-(3-pyrrolidin-1-ylpropoxy)phenyl]-*N*4,6-dimethyl-pyrimidine-2,4-diamine (80): General procedure B**



A solution of compound **104** (189 mg, 1.2 mmol) and compound **102** (300 mg, 1.2 mmol) in 2-propanol (5.0 mL) was treated with TFA (0.37 mL, 4.8 mmol) at room temperature, and the mixture was stirred at 140 °C for 1 h with μW irradiation; then, the mixture was cooled to room temperature. The precipitates were collected by filtration and washed twice with AcOEt (10 mL) to afford compound **80** (420 mg, 95%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.52 (1H, br s), 6.92 (1H, dd, *J* = 8.5, 2.4 Hz), 6.80 (1H, d, *J* = 8.5 Hz), 6.70 (1H, s), 5.71 (1H, s), 4.78 (1H, br s), 4.11 (2H, t, *J* = 7.0 Hz), 3.84 (3H, s), 2.93 (3H, d, *J* = 5.5 Hz), 2.62 (2H, t, *J* = 7.6 Hz), 2.54–2.49 (4H, m), 2.25 (3H, s), 2.13–2.04 (2H, m), 1.80–1.75 (4H, m). ESIMS-LR

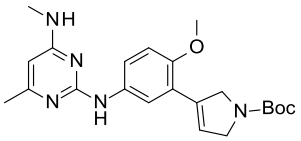
m/z 372 [(M+H)$^+$]; ESIMS-HR calcd for $C_{20}H_{30}N_5O_2$ (M+H)$^+$ 372.2394, found 372.2392.

_N_2-(3-Iodo-4-methoxy-phenyl)-_N_4,6-dimethyl-pyrimidine-2,4-diamine (106)



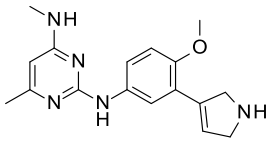
Following general procedure B using compound **104** (470 mg, 3.0 mmol), compound **105** (740 mg, 3.0 mmol), TFA (0.91 mL, 12 mmol), and 2-propanol (4.0 mL), the title compound, **106** (1.1 g, quant.), was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.27 (1H, s), 9.01 (1H, s), 8.15 (1H, s), 7.51 (1H, dd, *J* = 9.1, 2.1 Hz), 7.04 (1H, d, *J* = 9.1 Hz), 6.04 (1H, s), 3.82 (3H, s), 2.91 (3H, d, *J* = 4.3 Hz), 2.25 (3H, s). ESIMS-LR *m/z* 371 [(M+H)$^+$].

***tert*-Butyl 3-[2-methoxy-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,5-dihydropyrrole-1-carboxylate (108): General procedure C**



A solution of compound **106** (100 mg, 0.27 mmol), compound **107** (96 mg, 0.32 mmol), and K_2CO_3 (75 mg, 0.54 mmol) in 1,4-dioxane (3.0 mL) and H_2O (0.4 mL) was treated with Pd(dppf)Cl_2 (40 mg, 0.054 mmol) at room temperature. The mixture was stirred at 120 °C for 1 h with μW irradiation, followed by cooling to room temperature. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; 30:70 to 90:10) to afford compound **108** (110 mg, 98%) as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.62–7.53 (1H, m), 7.39 (1H, ddd, *J* = 16.3, 8.8, 2.7 Hz), 6.86 (1H, dd, *J* = 8.8, 2.7 Hz), 6.70 (1H, br s), 6.51–6.35 (1H, m), 5.71 (1H, d, *J* = 6.1 Hz), 4.76–4.61 (1H, m), 4.53 (2H, d, *J* = 22.6 Hz), 4.31 (2H, d, *J* = 27.5 Hz), 3.85 (3H, d, *J* = 6.1 Hz), 2.94 (3H, d, *J* = 4.9 Hz), 2.25 (3H, d, *J* = 3.7 Hz), 1.51 (9H, d, *J* = 5.5 Hz). ESIMS-LR *m/z* 412 [(M+H)$^+$].

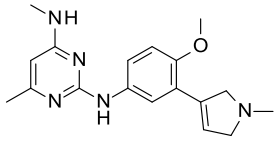
_N_2-[3-(2,5-Dihydro-1_H_-pyrrol-3-yl)-4-methoxy-phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (109): General procedure D



A solution of compound **108** (99 mg, 0.24 mmol) in CH_2Cl_2 (6.0 mL) was treated with TFA (2.0 mL, 26 mmol) at room temperature for 0.5 h. The reaction was quenched by Et_3N (1.8 mL, 13 mmol) at room temperature. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 0:100 to 10:90) to afford compound **109** (67 mg, 89%) as a pale brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.48 (1H, d, *J* = 2.5 Hz), 7.39 (1H, dd, *J* = 8.9, 2.5 Hz), 7.04 (1H, br s), 6.83 (1H, d, *J* = 9.2 Hz), 6.51 (1H, t, *J* = 1.8 Hz), 5.69 (1H, s), 4.96 (1H, br s), 4.14–4.09 (2H, m), 3.96–3.91 (2H, m), 3.83 (3H, s), 2.90 (3H, d, *J* = 4.9 Hz), 2.23 (3H, s). ESIMS-LR *m/z* 312 [(M+H)$^+$].

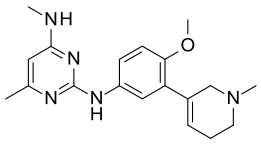
_N_2-[4-Methoxy-3-(1-methyl-2,5-dihydropyrrol-3-yl)phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (82): General procedure E

A solution of compound **109** (34 mg, 0.11 mmol), formaldehyde solution (37%) (13 mg, 0.16 mmol),



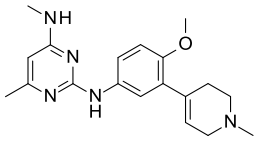
and AcOH (0.013 mL, 0.21 mmol) in CH_2Cl_2 (1.0 mL) was treated with $NaBH(OAc)_3$ (93 mg, 0.44 mmol) at room temperature for 1 h. The reaction was quenched by saturated aqueous $NaHCO_3$ (2.0 mL) at room temperature. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 1:99 to 10:90) to afford compound **82** (29 mg, 82%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.44 (1H, s), 7.41 (1H, dd, *J* = 8.5, 2.4 Hz), 6.88 (1H, s), 6.83 (1H, d, *J* = 8.5 Hz), 6.36 (1H, t, *J* = 1.8 Hz), 5.69 (1H, s), 4.82 (1H, br s), 3.88–3.84 (2H, m), 3.83 (3H, s), 3.67–3.62 (2H, m), 2.91 (3H, d, J = 4.9 Hz), 2.54 (3H, s), 2.23 (3H, s). ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{24}N_5O$ $(M+H)^+$ 326.1975, found 326.1974.

_N_2-[4-Methoxy-3-(1-methyl-3,6-dihydro-2_H_-pyridin-5-yl)phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (83)



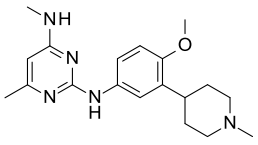
Following general procedure C using compound **106** (100 mg, 0.27 mmol), compound **110** (72 mg, 0.32 mmol), K_2CO_3 (75 mg, 0.54 mmol), Pd(dppf)Cl_2 (40 mg, 0.054 mmol), 1,4-dioxane (1.5 mL), and H_2O (0.2 mL), the title compound, **83** (82 mg, 89%), was obtained as a brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51 (1H, dd, *J* = 9.0, 2.6 Hz), 7.34 (1H, d, *J* = 2.6 Hz), 6.80 (1H, d, *J* = 9.0 Hz), 6.69 (1H, br s), 5.84–5.80 (1H, m), 5.70 (1H, s), 4.70 (1H, br s), 3.78 (3H, s), 3.23 (2H, dd, *J* = 4.5, 2.1 Hz), 2.92 (3H, d, *J* = 4.9 Hz), 2.59 (2H, t, *J* = 5.8 Hz), 2.40 (3H, s), 2.39–2.33 (2H, m), 2.24 (3H, s). ESIMS-LR *m/z* 340 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_5O$ $(M+H)^+$ 340.2132, found 340.2138.

_N_2-[4-Methoxy-3-(1-methyl-3,6-dihydro-2_H_-pyridin-4-yl)phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (84)



Following general procedure C using compound **106** (100 mg, 0.27 mmol), compound **111** (72 mg, 0.32 mmol), K_2CO_3 (75 mg, 0.54 mmol), Pd(dppf)Cl_2 (40 mg, 0.054 mmol), 1,4-dioxane (1.5 mL), and H_2O (0.2 mL), the title compound, **84** (88 mg, 96%), was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.49 (1H, dd, *J* = 8.9, 2.5 Hz), 7.33 (1H, d, *J* = 2.5 Hz), 6.82–6.76 (2H, m), 5.80–5.76 (1H, m), 5.68 (1H, s), 4.82 (1H, br s), 3.77 (3H, s), 3.11–3.07 (2H, m), 2.90 (3H, d, *J* = 4.9 Hz), 2.66–2.61 (2H, m), 2.60–2.55 (2H, m), 2.40 (3H, s), 2.23 (3H, s). ESIMS-LR *m/z* 340 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_5O$ $(M+H)^+$ 340.2132, found 340.2145.

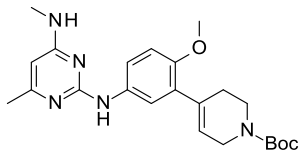
_N_2-[4-Methoxy-3-(1-methyl-4-piperidyl)phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (86)



A solution of compound **84** (45 mg, 0.13 mmol) in MeOH (1.3 mL) was treated with 20% $Pd(OH)_2$/C (19 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 12 h. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2,

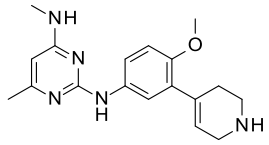
AcOEt/hexane; 30:70 to 70:30) to afford compound **86** (20 mg, 44%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.44 (1H, d, *J* = 3.1 Hz), 7.38 (1H, dd, *J* = 8.9, 2.1 Hz), 6.82–6.73 (2H, m), 5.68 (1H, s), 4.79 (1H, br s), 3.80 (3H, s), 3.00–2.86 (6H, m), 2.32 (3H, s), 2.24 (3H, s), 2.09 (2H, td, *J* = 11.3, 3.5 Hz), 1.84–1.74 (4H, m). ESIMS-LR *m/z* 342 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{28}N_5O$ $(M+H)^+$ 342.2288, found 342.2290.

tert-Butyl 4-[2-methoxy-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-3,6-dihydro-2*H*-pyridine-1-carboxylate (113)



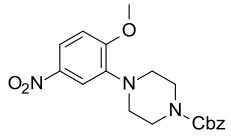
Following general procedure C using compound **106** (200 mg, 0.54 mmol), compound **112** (200 mg, 0.65 mmol), K_2CO_3 (150 mg, 1.1 mmol), $Pd(dppf)Cl_2$ (80 mg, 0.11 mmol), 1,4-dioxane (3.0 mL), and H_2O (0.4 mL), the title compound, **113** (220 mg, 94%), was obtained as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.48 (1H, d, *J* = 8.8 Hz), 7.37 (1H, s), 6.82 (1H, d, *J* = 8.8 Hz), 6.67 (1H, s), 5.80 (1H, s), 5.71 (1H, s), 4.65 (1H, br s), 4.07–4.01 (2H, m), 3.79 (3H, s), 3.63–3.55 (2H, m), 2.93 (3H, d, *J* = 4.9 Hz), 2.55–2.46 (2H, m), 2.25 (3H, s), 1.50 (9H, s). ESIMS-LR *m/z* 426 [$(M+H)^+$].

***N*2-[4-Methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)phenyl]-*N*4,6-dimethyl-pyrimidine-2,4-diamine (85)**



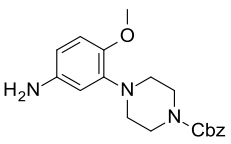
Following general procedure D using compound **113** (210 mg, 0.49 mmol), TFA (1.0 mL, 13 mmol), and CH_2Cl_2 (3.0 mL), the title compound, **85** (230 mg, 87%), was obtained as a pale brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.50 (1H, dd, *J* = 9.0, 2.5 Hz), 7.33 (1H, d, *J* = 2.5 Hz), 6.81 (1H, d, *J* = 9.0 Hz), 6.67 (1H, s), 5.87–5.82 (1H, m), 5.70 (1H, s), 4.66 (1H, br s), 3.79 (3H, s), 3.52–3.48 (2H, m), 3.06 (2H, t, *J* = 5.8 Hz), 2.93 (3H, d, *J* = 5.5 Hz), 2.47–2.41 (2H, m), 2.25 (3H, s). ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{24}N_5O$ $(M+H)^+$ 326.1975, found 326.1974.

Benzyl 4-(2-methoxy-5-nitro-phenyl)piperazine-1-carboxylate (115)



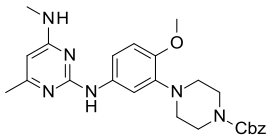
A suspension of 2-bromo-1-methoxy-4-nitro-benzene **114** (800 mg, 3.4 mmol), Xantphos (240 mg, 0.41 mmol), 1-Z-piperazine (840 mg, 3.8 mmol), and ^{t}BuONa (500 mg, 5.2 mmol) in toluene (17 mL) was treated with $Pd_2(dba)_3$ (320 mg, 0.34 mmol) at room temperature, and the mixture was stirred at 100 ºC for 2 h. The resulting mixture was filtered through a pad of Celite and washed with chloroform. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 20:80 to 80:20) to afford compound **115** (850 mg, 66%) as a brown amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.97 (1H, dd, *J* = 9.0, 2.9 Hz), 7.77 (1H, d, *J* = 2.9 Hz), 7.43–7.32 (5H, m), 6.92 (1H, d, *J* = 9.0 Hz), 5.18 (2H, s), 3.98 (3H, s), 3.75–3.68 (4H, m), 3.14–3.02 (4H, m). ESIMS-LR *m/z* 372 [$(M+H)^+$].

Benzyl 4-(5-amino-2-methoxy-phenyl)piperazine-1-carboxylate (116)



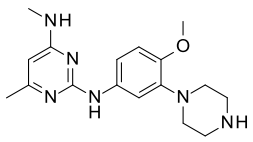
A solution of compound **115** (830 mg, 2.2 mmol) and NH_4Cl (600 mg, 11 mmol) in MeOH (30 mL) and H_2O (10 mL) was treated with iron powder (440 mg, 7.8 mmol) at room temperature, and the mixture was stirred at 80 ºC for 3 h. The resulting mixture was filtered through a pad of Celite and washed with MeOH. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 10:90 to 50:50) to afford compound **116** (560 mg, 73%) as a brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.40–7.30 (5H, m), 6.69 (1H, d, *J* = 8.5 Hz), 6.35 (1H, dd, *J* = 8.5, 2.4 Hz), 6.30 (1H, d, *J* = 2.4 Hz), 5.17 (2H, s), 3.80 (3H, s), 3.68 (4H, t, *J* = 5.2 Hz), 3.41 (2H, br s), 3.05–2.94 (4H, m). ESIMS-LR *m/z* 342 [$(M+H)^+$].

Benzyl 4-[2-methoxy-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]piperazine-1-carboxylate (117)



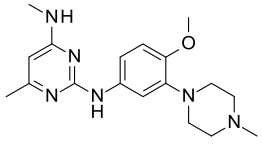
Following general procedure B using compound **116** (140 mg, 0.90 mmol), compound **104** (280 mg, 0.82 mmol), TFA (0.25 mL, 3.3 mmol), and 2-propanol (4.0 mL), the title compound, **117** (480 mg, quant.), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.40–7.29 (5H, m), 7.10 (1H, dd, *J* = 8.5, 2.4 Hz), 6.79 (1H, d, *J* = 8.5 Hz), 6.72 (1H, br s), 5.71 (1H, s), 5.17 (2H, s), 4.64 (1H, br s), 3.84 (3H, s), 3.70 (4H, t, *J* = 4.9 Hz), 3.10–2.98 (4H, m), 2.93 (3H, d, *J* = 4.9 Hz), 2.25 (3H, s). ESIMS-LR *m/z* 463 [$(M+H)^+$].

***N*2-(4-Methoxy-3-piperazin-1-yl-phenyl)-*N*4,6-dimethyl-pyrimidine-2,4-diamine (118)**



A solution of compound **117** (380 mg, 0.82 mmol) and acetic acid (0.10 mL, 1.6 mmol) in MeOH (4.1 mL) was treated with 20% $Pd(OH)_2/C$ (115 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 3 h. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 0:99 to 10:90) to afford compound **118** (250 mg, 93%) as a colorless amorphous. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.60 (1H, s), 7.49 (1H, s), 7.26 (1H, d, *J* = 8.8 Hz), 6.84 (1H, br s), 6.74 (1H, d, *J* = 8.8 Hz), 5.71 (1H, s), 3.70 (3H, s), 2.90–2.77 (11H, m), 2.08 (3H, s). ESIMS-LR *m/z* 329 [$(M+H)^+$].

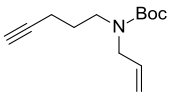
***N*2-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl]-*N*4,6-dimethyl-pyrimidine-2,4-diamine (87)**



Following general procedure E using compound **118** (48 mg, 0.15 mmol), formaldehyde solution (37%) (18 mg, 0.22 mmol), AcOH (0.017 mL, 0.29 mmol), $NaBH(OAc)_3$ (120 mg, 0.58 mmol), and CH_2Cl_2 (2.0 mL), the title compound, **87** (43 mg, 86%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.40 (1H, d, *J* = 2.4 Hz), 7.09 (1H, dd, *J* = 8.5, 2.4 Hz), 6.81–6.72 (2H, m), 5.69 (1H, s), 4.67 (1H, br s), 3.84 (3H, s), 3.12 (4H, br s), 2.93 (3H, d, *J* = 4.9 Hz), 2.63 (4H, br s), 2.36 (3H, s), 2.24 (3H, s). ESIMS-LR *m/z* 343 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{27}N_6O$

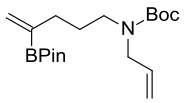
$(M+H)^+$ 343.2241, found 343.2246.

***tert*-Butyl *N*-allyl-*N*-pent-4-ynyl-carbamate (126)**



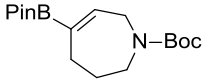
A suspension of compound **125** (1.6 g, 8.7 mmol) and allyl iodide (0.87 mL, 9.6 mmol) in DMF (20 mL) was treated with NaH (460 mg, 55% dispersion of mineral oil, 10 mmol) at room temperature for 3 days. The resulting mixture was partitioned between AcOEt (200 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 40:60) to afford compound **126** (1.5 g, 77%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.84–5.72 (1H, m), 5.12 (2H, d, *J* = 11.6 Hz), 3.82 (2H, d, *J* = 15.9 Hz), 3.33–3.22 (2H, m), 2.19 (2H, td, *J* = 7.0, 2.4 Hz), 1.96 (1H, t, *J* = 2.7 Hz), 1.75 (2H, t, *J* = 6.1 Hz), 1.46 (9H, s). ESIMS-LR *m/z* 168 [(M-^{t}Bu+H)$^+$].

***tert*-Butyl *N*-allyl-*N*-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pent-4-enyl]carbamate (127)**



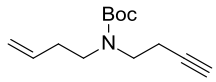
A suspension of LiCl (340 mg, 8.1 mmol) in DMF (30 mL) was treated with copper (I) chloride (800 mg, 8.1 mmol) at room temperature for 3 h. AcOK (790 mg, 8.1 mmol), bis(pinacolato)diboron (2.0 g, 8.1 mmol), and compound **126** (1.5 g, 6.7 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 2 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (200 mL) and NH_4Cl (100 mL), and the organic phase was washed with H_2O (100 mL), saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 15:85) to afford compound **127** (1.5 g, 63%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.83–5.72 (2H, m), 5.61 (1H, s), 5.16–5.05 (2H, m), 3.88–3.73 (2H, m), 3.24–3.10 (2H, m), 2.15–2.06 (2H, m), 1.69–1.57 (2H, m), 1.45 (9H, s), 1.26 (12H, s). ESIMS-LR *m/z* 252 [(M-^{t}BuCO$_2$+H)$^+$].

***tert*-Butyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (128)**



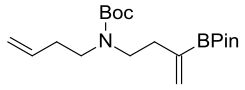
A solution of compound **127** (1.5 g, 4.2 mmol) in CH_2Cl_2 (210 mL) was treated with Grubbs catalyst, 2nd generation (180 mg, 0.21 mmol), at room temperature for 15 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **128** (1.2 g, 84%) as a pale brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.52 (1H, t, *J* = 4.3 Hz), 4.09–4.04 (1H, m), 4.00–3.94 (1H, m), 3.53 (1H, t, *J* = 6.1 Hz), 3.46 (1H, t, *J* = 6.1 Hz), 2.36–2.28 (2H, m), 1.82–1.74 (2H, m), 1.45 (9H, d, *J* = 4.9 Hz), 1.25 (12H, s). ESIMS-LR *m/z* 268 [(M-^{t}Bu+H)$^+$].

***tert*-Butyl *N*-allyl-*N*-but-3-ynyl-carbamate (131)**



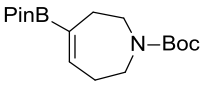
A suspension of but-3-en-1-amine hydrochloride **129** (3.0 g, 28 mmol) and K_2CO_3 (17 g, 120 mmol) in MeCN (200 mL) was treated with 3-butynyl p-toluenesulfonate **130** (4.2 mL, 24 mmol) at room temperature; the mixture was stirred at 85 °C for 1 day, after which it was cooled to room temperature. Boc_2O (6.4 g, 29 mmol) was added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 1 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 5:95) to afford compound **131** (4.2 g, 68%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.84–5.70 (1H, m), 5.10–5.00 (2H, m), 3.42–3.26 (4H, m), 2.47–2.38 (2H, m), 2.34–2.24 (2H, m), 2.00–1.95 (1H, m), 1.46 (9H, s).

tert-Butyl _N_-but-3-enyl-_N_-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-enyl]carbamate (132)



A suspension of LiCl (970 mg, 23 mmol) in DMF (190 mL) was treated with copper (I) chloride (2.3 g, 23 mmol) at room temperature for 1 h. AcOK (2.2 g, 23 mmol), bis(pinacolato)diboron (5.8 g, 23 mmol), and compound **131** (4.2 g, 19 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 2 days. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (200 mL) and NH_4Cl (100 mL), and the organic phase was washed with H_2O (100 mL), saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 5:95) to afford compound **132** (3.9 g, 58%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.87–5.71 (2H, m), 5.65 (1H, d, J = 3.1 Hz), 5.06 (1H, d, J = 17.1 Hz), 5.00 (1H, d, J = 8.5 Hz), 3.38–3.14 (4H, m), 2.36–2.27 (4H, m), 1.46 (9H, s), 1.27 (12H, s).

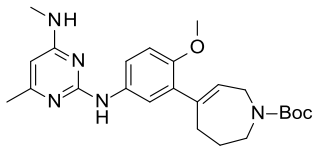
tert-Butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,6,7-tetrahydroazepine-1-carboxylate (133)



A solution of compound **132** (3.3 g, 9.4 mmol) in CH_2Cl_2 (950 mL) was treated with Grubbs catalyst, 2nd generation (400 mg, 0.47 mmol), at room temperature for 3 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 10:90) to afford compound **133** (3.9 g, 92%) as a pale brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.70–6.66 (1H, br s), 3.49–3.38 (4H, m), 2.48–2.33 (4H, m), 1.45 (9H, s), 1.26 (12H, s). ESIMS-LR *m/z* 268 [(M-^{t}Bu+H)$^+$].

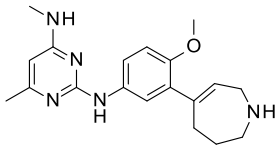
tert-Butyl 5-[2-methoxy-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (134)

Following general procedure C using compound **106** (300 mg, 0.81 mmol), compound **128** (320 mg, 0.97 mmol), K_2CO_3 (220 mg, 1.6 mmol), Pd(dppf)Cl_2 (120 mg, 0.16 mmol), 1,4-dioxane (4.0 mL),



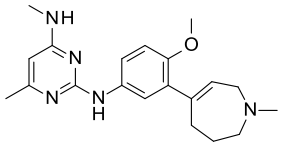
and H_2O (1.0 mL), the title compound, **134** (350 mg, 98%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.54–7.38 (2H, m), 6.83–6.67 (2H, m), 5.83 (1H, d, *J* = 5.5 Hz), 5.70 (1H, s), 4.85–4.63 (1H, m), 4.05 (1H, d, *J* = 4.3 Hz), 3.96 (1H, d, *J* = 4.9 Hz), 3.77 (3H, s), 3.67–3.61 (1H, m), 3.61–3.55 (1H, m), 2.92 (3H, d, *J* = 4.3 Hz), 2.58–2.53 (2H, m), 2.24 (3H, s), 1.90–1.83 (2H, m), 1.47 (9H, d, *J* = 4.9 Hz). ESIMS-LR *m/z* 440 [$(M+H)^+$].

_N_2-[4-Methoxy-3-(2,3,4,7-tetrahydro-1_H_-azepin-5-yl)phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (89)



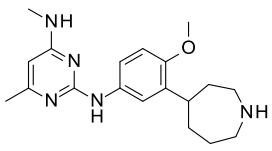
Following general procedure D using compound **134** (340 mg, 0.77 mmol), TFA (2.0 mL, 26 mmol), and CH_2Cl_2 (6.0 mL), the title compound, **89** (230 mg, 87%), was obtained as a colorless amorphous. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.69 (1H, s), 7.63–7.46 (2H, m), 6.95–6.83 (1H, m), 6.81 (1H, d, *J* = 9.2 Hz), 5.76–5.70 (2H, m), 3.70 (3H, s), 3.28 (2H, d, *J* = 5.5 Hz), 2.95 (2H, t, *J* = 5.5 Hz), 2.80 (3H, d, *J* = 3.7 Hz), 2.47–2.42 (2H, m), 2.07 (3H, s), 1.68–1.60 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 164.3, 159.9, 151.8, 145.9, 134.9, 133.2, 131.1, 121.1, 119.1, 110.0, 77.2, 55.8, 52.7, 47.7, 33.3, 29.4, 28.2, 24.0. ESIMS-LR *m/z* 340 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_5O$ $(M+H)^+$ 340.2132, found 340.2149.

_N_2-[4-Methoxy-3-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (88)



Following general procedure E using compound **89** (60 mg, 0.18 mmol), formaldehyde solution (37%) (22 mg, 0.27 mmol), AcOH (0.021 mL, 0.35 mmol), $NaBH(OAc)_3$ (150 mg, 0.71 mmol), and CH_2Cl_2 (2.0 mL), the title compound, **88** (46 mg, 74%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51 (1H, dd, *J* = 8.5, 2.4 Hz), 7.30 (1H, s), 6.79 (1H, d, *J* = 8.5 Hz), 6.65 (1H, br s), 5.80 (1H, t, *J* = 6.1 Hz), 5.69 (1H, s), 4.70 (1H, br s), 3.79 (3H, s), 3.22 (2H, d, *J* = 6.1 Hz), 2.93 (3H, d, *J* = 4.9 Hz), 2.85 (2H, t, *J* = 5.5 Hz), 2.56 (2H, t, *J* = 5.5 Hz), 2.39 (3H, s), 2.24 (3H, s), 1.84–1.74 (2H, m). ESIMS-LR *m/z* 354 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_5O$ $(M+H)^+$ 354.2288, found 354.2316.

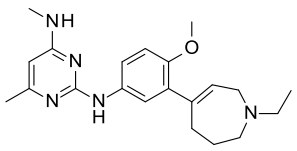
_N_2-[3-(Azepan-4-yl)-4-methoxy-phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (91)



A solution of compound **89** (20 mg, 0.059 mmol) in MeOH (1.0 mL) was treated with 20% $Pd(OH)_2$/C (8.2 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 12 h. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 1:99 to 12:88) to afford compound **91** (16 mg, 80%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.48 (1H, d, *J* = 2.4 Hz), 7.34 (1H, dd, *J* = 8.5, 2.4 Hz), 6.98 (1H, br s), 6.78 (1H, d, *J* = 8.5 Hz), 5.68 (1H, s), 4.87 (1H, br s), 3.79 (3H, s), 3.25–3.05 (3H, m), 3.00–2.86 (5H,

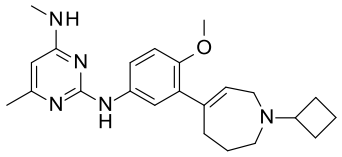
m), 2.24 (3H, s), 2.04–1.88 (3H, m), 1.86–1.66 (3H, m). ESIMS-LR *m/z* 342 [(M+H)$^+$]; ESIMS-HR calcd for $C_{19}H_{28}N_5O$ (M+H)$^+$ 342.2288, found 342.2284.

_N_2-[3-(1-Ethyl-2,3,4,7-tetrahydroazepin-5-yl)-4-methoxy-phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (92)



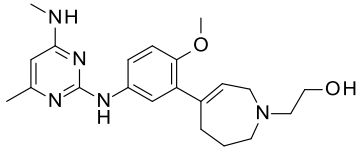
Following general procedure E using compound **89** (30 mg, 0.088 mmol), acetaldehyde (7.0 μL, 0.14 mmol), AcOH (0.011 mL, 0.18 mmol), $NaBH(OAc)_3$ (75 mg, 0.35 mmol), and CH_2Cl_2 (2.0 mL), the title compound, **92** (27 mg, 83%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.47 (1H, dd, *J* = 8.9, 2.7 Hz), 7.33 (1H, d, *J* = 3.1 Hz), 6.83–6.75 (2H, m), 5.79 (1H, t, *J* = 6.1 Hz), 5.68 (1H, s), 4.86 (1H, br s), 3.78 (3H, s), 3.32 (2H, d, *J* = 6.1 Hz), 2.98 (2H, t, *J* = 5.2 Hz), 2.90 (3H, d, *J* = 4.3 Hz), 2.65–2.55 (4H, m), 2.24 (3H, s), 1.79–1.71 (2H, m), 1.10 (3H, t, *J* = 7.3 Hz). ESIMS-LR *m/z* 368 [(M+H)$^+$]; ESIMS-HR calcd for $C_{21}H_{30}N_5O$ (M+H)$^+$ 368.2445, found 368.2462.

_N_2-[3-(1-Cyclobutyl-2,3,4,7-tetrahydroazepin-5-yl)-4-methoxy-phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (93)



Following general procedure E using compound **89** (36 mg, 0.11 mmol), cyclobutanone (0.013 mL, 0.16 mmol), AcOH (0.013 mL, 0.21 mmol), $NaBH(OAc)_3$ (89 mg, 0.42 mmol), and CH_2Cl_2 (2.0 mL), the title compound, **93** (32 mg, 77%), was obtained as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.49 (1H, dd, *J* = 8.5, 2.4 Hz), 7.31 (1H, d, *J* = 3.1 Hz), 6.80 (1H, br s), 6.77 (1H, d, *J* = 9.2 Hz), 5.78 (1H, t, *J* = 6.4 Hz), 5.68 (1H, s), 4.88 (1H, br s), 3.77 (3H, s), 3.12 (2H, d, *J* = 6.1 Hz), 3.09–3.00 (1H, m), 2.90 (3H, d, *J* = 4.9 Hz), 2.77 (2H, t, *J* = 5.5 Hz), 2.56 (2H, t, *J* = 5.5 Hz), 2.24 (3H, s), 2.09–2.01 (2H, m), 1.93–1.82 (2H, m), 1.77–1.57 (4H, m). ESIMS-LR *m/z* 394 [(M+H)$^+$]; ESIMS-HR calcd for $C_{23}H_{32}N_5O$ (M+H)$^+$ 394.2601, found 394.2619.

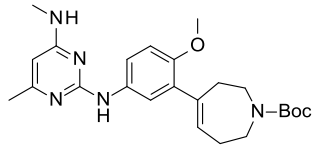
2-[5-[2-Methoxy-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,3,4,7-tetrahydroazepin-1-yl]ethanol (94)



A solution of compound **89** (36 mg, 0.11 mmol), Cs_2CO_3 (69 mg, 0.21 mmol), and NaI (32 mg, 0.21 mmol) in MeCN (1.0 mL) was treated with 2-bromoethanol (0.020 mL, 0.16 mmol) at room temperature, and the mixture was stirred at 80 °C for 2 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 1:99 to 10:90) to afford compound **94** (28 mg, 69%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.43 (1H, dd, *J* = 8.9, 3.1 Hz), 7.37 (1H, d, *J* = 2.5 Hz), 6.91 (1H, br s), 6.77 (1H, d, *J* = 9.2 Hz), 5.76 (1H, t, *J* = 6.1 Hz), 5.68 (1H, s), 4.95 (1H, br s), 3.78 (3H, s), 3.60 (2H, t, *J* = 5.5 Hz), 3.35 (2H, d, *J* = 6.1 Hz), 3.01 (2H, t, *J* = 5.8 Hz), 2.90 (3H, d, *J* = 4.9 Hz), 2.73 (2H, t, *J* = 5.2 Hz), 2.58 (2H, t, *J* = 5.2 Hz), 2.23 (3H, s), 1.78–1.71

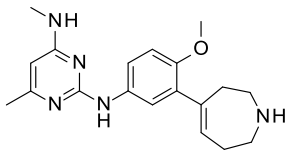
(2H, m). ESIMS-LR *m/z* 384 [(M+H)$^+$]; ESIMS-HR calcd for $C_{21}H_{30}N_5O_2$ (M+H)$^+$ 384.2394, found 384.2400.

tert-Butyl 4-[2-methoxy-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,3,6,7-tetrahydroazepine-1-carboxylate (135)



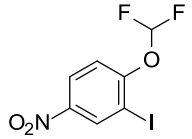
Following general procedure C using compound **106** (300 mg, 0.81 mmol), compound **133** (310 mg, 0.97 mmol), K_2CO_3 (220 mg, 1.6 mmol), Pd(dppf)Cl_2 (120 mg, 0.16 mmol), 1,4-dioxane (4.0 mL), and H_2O (1.0 mL), the title compound, **135** (330 mg, 93%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46 (1H, dd, *J* = 8.9, 2.7 Hz), 7.36–7.30 (1H, m), 6.78 (1H, d, *J* = 9.2 Hz), 6.74–6.68 (1H, m), 5.83 (1H, br s), 5.70 (1H, s), 4.70 (1H, br s), 3.79 (3H, s), 3.62–3.49 (4H, m), 2.92 (3H, d, *J* = 4.9 Hz), 2.65–2.54 (2H, m), 2.45–2.34 (2H, m), 2.24 (3H, s), 1.48 (9H, s). ESIMS-LR *m/z* 440 [(M+H)$^+$].

***N*2-[4-Methoxy-3-(2,3,6,7-tetrahydro-1*H*-azepin-4-yl)phenyl]-*N*4,6-dimethyl-pyrimidine-2,4-diamine (90)**



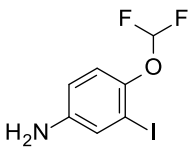
Following general procedure D using compound **135** (330 mg, 0.76 mmol), TFA (2.0 mL, 26 mmol), and CH_2Cl_2 (6.0 mL), the title compound, **90** (210 mg, 82%), was obtained as a colorless amorphous. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.44 (2H, dd, *J* = 9.0, 2.1 Hz), 6.83 (1H, d, *J* = 9.0 Hz), 5.92 (1H, t, *J* = 6.4 Hz), 5.75 (1H, s), 3.78 (3H, s), 2.96–2.91 (2H, m), 2.90 (3H, s), 2.87–2.82 (2H, m), 2.60 (2H, t, *J* = 4.9 Hz), 2.39 (2H, dd, *J* = 10.4, 6.1 Hz), 2.16 (3H, s). ESIMS-LR *m/z* 340 [(M+H)$^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_5O$ (M+H)$^+$ 340.2132, found 340.2130.

1-(Difluoromethoxy)-2-iodo-4-nitro-benzene (137)



A solution of 2-iodo-4-nitrophenol **136** (920 mg, 3.5 mmol) and KOH (2.3 g, 42 mmol) in MeCN (30 mL) and H_2O (30 mL) was treated with diethyl (bromodifluoromethyl)phosphonate (1.9 mL, 10 mmol) at 0 ºC for 3 h. The resulting mixture was partitioned between AcOEt (200 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **137** (860 mg, 79%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.73 (1H, d, *J* = 3.1 Hz), 8.26 (1H, dd, *J* = 9.2, 2.4 Hz), 7.28 (1H, d, *J* = 9.2 Hz), 6.67 (1H, t, *J* = 71.7 Hz).

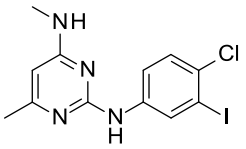
4-(Difluoromethoxy)-3-iodo-aniline (138d)



A solution of compound **137** (850 mg, 2.7 mmol) and NH_4Cl (720 mg, 13 mmol) in MeOH (10 mL) and H_2O (5.0 mL) was treated with iron powder (530 mg, 9.4 mmol) at room temperature, and the mixture was stirred at 80 ºC for 3 h. The resulting mixture was filtered through a pad of Celite and washed with MeOH.

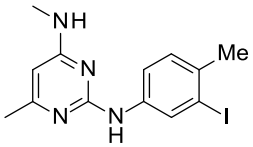
The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 10:90 to 50:50) to afford compound **138d** (710 mg, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.14 (1H, d, *J* = 3.1 Hz), 6.96 (1H, d, *J* = 8.5 Hz), 6.62 (1H, dd, *J* = 8.5, 2.4 Hz), 6.38 (1H, t, *J* = 74.2 Hz), 3.66 (2H, br s). ESIMS-LR *m/z* 286 [(M+H)$^+$].

***N*2-(4-Chloro-3-iodo-phenyl)-*N*4,6-dimethyl-pyrimidine-2,4-diamine (139a)**



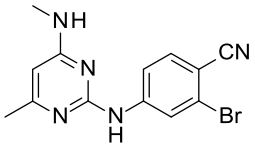
Following general procedure D using compound **104** (300 mg, 1.9 mmol), compound **138a** (480 mg, 1.9 mmol), TFA (0.91 mL, 7.6 mmol), and 2-propanol (10 mL), the title compound, **139a** (690 mg, 96%), was obtained as a colorless amorphous. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.22 (1H, br s), 8.98 (1H, br s), 8.41 (1H, s), 7.60–7.52 (2H, m), 6.08 (1H, s), 2.96–2.90 (3H, m), 2.27 (3H, s). ESIMS-LR *m/z* 375 [(M+H)$^+$].

***N*2-(3-Iodo-4-methyl-phenyl)-*N*4,6-dimethyl-pyrimidine-2,4-diamine (139b)**



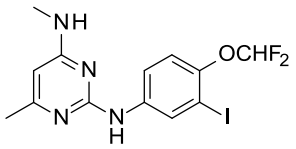
Following general procedure D using compound **104** (270 mg, 1.7 mmol), compound **138b** (480 mg, 2.1 mmol), TFA (0.91 mL, 6.9 mmol), and 2-propanol (10 mL), the title compound, **139b** (570 mg, 94%), was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.16 (1H, br s), 9.00 (1H, br s), 8.29 (1H, s), 7.43 (1H, dd, *J* = 7.9, 1.8 Hz), 7.34 (1H, d, *J* = 7.9 Hz), 6.05 (1H, s), 2.93 (3H, d, *J* = 4.3 Hz), 2.35 (3H, s), 2.26 (3H, s). ESIMS-LR *m/z* 355 [(M+H)$^+$].

2-Bromo-4-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]benzonitrile (139c)



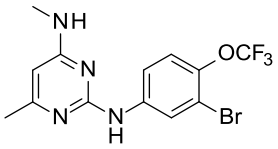
Following general procedure D using compound **104** (270 mg, 1.7 mmol), compound **138c** (480 mg, 2.1 mmol), TFA (0.91 mL, 6.8 mmol), and 2-propanol (10 mL), the title compound, **139c** (570 mg, 94%), was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.82 (1H, s), 8.60 (1H, br s), 7.83 (1H, s), 7.73 (1H, d, *J* = 8.5 Hz), 7.28 (1H, br s), 5.93 (1H, s), 2.85 (3H, s), 2.17 (3H, s). ESIMS-LR *m/z* 318, 320 [(M+H)$^+$].

***N*2-[4-(Difluoromethoxy)-3-iodo-phenyl]-*N*4,6-dimethyl-pyrimidine-2,4-diamine (139d)**



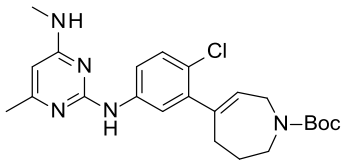
Following general procedure D using compound **104** (340 mg, 2.2 mmol), compound **138d** (680 mg, 2.4 mmol), TFA (0.66 mL, 8.6 mmol), and 2-propanol (10 mL), the title compound, **139d** (610 mg, 70%), was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.29–10.11 (1H, m), 8.98 (1H, br s), 8.32 (1H, s), 7.59 (1H, d, *J* = 8.5 Hz), 7.26 (1H, d, *J* = 9.2 Hz), 7.23 (1H, t, *J* = 73.5 Hz), 6.07 (1H, s), 2.92 (3H, s), 2.27 (3H, s). ESIMS-LR *m/z* 407 [(M+H)$^+$].

***N*2-[3-Bromo-4-(trifluoromethoxy)phenyl]-*N*4,6-dimethyl-pyrimidine-2,4-diamine (139e)**



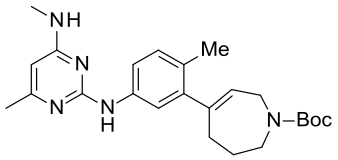
Following general procedure D using compound **104** (250 mg, 1.6 mmol), compound **138e** (0.26 mL, 1.7 mmol), TFA (0.49 mL, 6.3 mmol), and 2-propanol (10 mL), the title compound, **139e** (480 mg, 80%), was obtained as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.61 (1H, br s), 9.09 (1H, br s), 8.25 (1H, d, *J* = 1.8 Hz), 7.65 (1H, dd, *J* = 9.2, 2.4 Hz), 7.58 (1H, d, *J* = 9.2 Hz), 6.11 (1H, s), 2.94 (3H, d, *J* = 4.9 Hz), 2.28 (3H, s). ESIMS-LR *m/z* 377, 379 [(M+H)$^+$].

tert-Butyl 5-[2-chloro-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (140a)



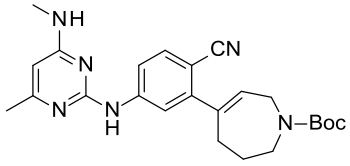
Following general procedure C using compound **139a** (300 mg, 0.80 mmol), compound **128** (310 mg, 0.96 mmol), K_2CO_3 (220 mg, 1.6 mmol), Pd(dppf)Cl_2 (120 mg, 0.16 mmol), 1,4-dioxane (3.0 mL), and H_2O (0.4 mL), the title compound, **140a** (350 mg, 98%), was obtained as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.60–7.38 (2H, m), 7.24–7.18 (1H, m), 6.82 (1H, br s), 5.85–5.76 (1H, m), 5.74 (1H, s), 4.95–4.68 (1H, m), 4.05 (1H, d, *J* = 4.9 Hz), 3.97 (1H, d, *J* = 4.9 Hz), 3.65 (1H, t, *J* = 6.1 Hz), 3.60 (1H, d, *J* = 6.1 Hz), 2.94 (3H, d, *J* = 4.9 Hz), 2.60–2.53 (2H, m), 2.25 (3H, s), 2.00–1.89 (2H, m), 1.48 (9H, d, *J* = 3.1 Hz). ESIMS-LR *m/z* 444 [(M+H)$^+$].

tert-Butyl 5-[2-methyl-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (140b)



Following general procedure C using compound **139b** (300 mg, 0.85 mmol), compound **128** (330 mg, 1.0 mmol), K_2CO_3 (230 mg, 1.7 mmol), Pd(dppf)Cl_2 (120 mg, 0.17 mmol), 1,4-dioxane (3.0 mL), and H_2O (0.4 mL), the title compound, **140b** (310 mg, 86%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46–7.33 (2H, m), 7.05 (1H, t, *J* = 7.0 Hz), 6.74 (1H, br s), 5.73–5.66 (2H, m), 4.69 (1H, br s), 4.04 (1H, d, *J* = 4.3 Hz), 3.95 (1H, d, *J* = 5.5 Hz), 3.66 (1H, t, *J* = 5.8 Hz), 3.60 (1H, d, *J* = 5.8 Hz), 2.94 (3H, d, *J* = 4.9 Hz), 2.52–2.45 (2H, m), 2.24 (3H, s), 2.20 (3H, s), 1.95–1.84 (2H, m), 1.48 (9H, d, *J* = 6.1 Hz). ESIMS-LR *m/z* 424 [(M+H)$^+$].

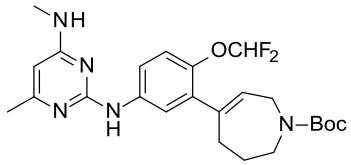
tert-Butyl 5-[2-cyano-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (140c)



Following general procedure C using compound **139c** (200 mg, 0.63 mmol), compound **128** (240 mg, 0.75 mmol), K_2CO_3 (170 mg, 1.3 mmol), Pd(dppf)Cl_2 (92 mg, 0.13 mmol), 1,4-dioxane (3.0 mL), and H_2O (0.4 mL), the title compound, **140c** (270 mg, 98%), was obtained as a yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68–7.47 (3H, m), 7.10 (1H, s), 5.98 (1H, t, *J* = 4.9 Hz), 5.82 (1H, s), 4.80 (1H, s), 4.10 (1H, d, *J* = 4.3 Hz), 4.05 (1H, d, *J* = 4.3 Hz), 3.67 (1H, t, *J* = 6.1 Hz), 3.62 (1H, d, *J* = 6.1 Hz), 2.96 (3H, d, *J* = 4.9 Hz), 2.70–2.62 (2H, m),

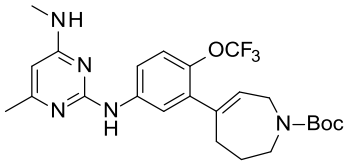
2.27 (3H, s), 2.03–1.94 (2H, m), 1.48 (9H, d, *J* = 2.4 Hz). ESIMS-LR *m/z* 435 [(M+H)$^+$].

tert-Butyl 5-[2-(difluoromethoxy)-5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]phenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (140d)



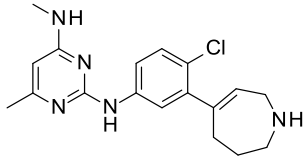
Following general procedure B using compound **139d** (220 mg, 0.54 mmol), compound **128** (210 mg, 0.65 mmol), K_2CO_3 (150 mg, 1.1 mmol), $Pd(dppf)Cl_2$ (80 mg, 0.11 mmol), 1,4-dioxane (3.0 mL), and H_2O (0.4 mL), the title compound, **140d** (260 mg, 99%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.53 (1H, dt, *J* = 30.9, 12.2 Hz), 7.34–7.22 (1H, m), 7.01 (1H, t, *J* = 10.4 Hz), 6.77 (1H, br s), 6.34 (1H, t, *J* = 75.4 Hz), 5.87 (1H, d, *J* = 18.3 Hz), 5.75 (1H, s), 4.68 (1H, br s), 4.04 (1H, d, *J* = 4.3 Hz), 3.97 (1H, d, *J* = 5.5 Hz), 3.64 (1H, t, *J* = 5.5 Hz), 3.59 (1H, d, *J* = 5.8 Hz), 2.94 (3H, d, *J* = 4.9 Hz), 2.56 (2H, t, *J* = 5.5 Hz), 2.25 (3H, s), 1.93–1.86 (2H, m), 1.47 (9H, d, *J* = 3.7 Hz). ESIMS-LR *m/z* 476 [(M+H)$^+$].

tert-Butyl 5-[5-[[4-methyl-6-(methylamino)pyrimidin-2-yl]amino]-2-(trifluoromethoxy)phenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (140e)



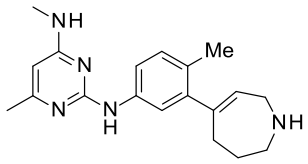
Following general procedure C using compound **139e** (260 mg, 0.69 mmol), compound **128** (270 mg, 0.83 mmol), K_2CO_3 (190 mg, 1.4 mmol), $Pd(dppf)Cl_2$ (100 mg, 0.14 mmol), 1,4-dioxane (3.0 mL), and H_2O (0.4 mL), the title compound, **140e** (330 mg, 97%), was obtained as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.62–7.37 (2H, m), 7.11 (1H, t, *J* = 8.5 Hz), 6.87 (1H, br s), 5.87 (1H, dt, *J* = 17.4, 4.7 Hz), 5.76 (1H, s), 4.79 (1H, d, *J* = 43.7 Hz), 4.04 (1H, d, *J* = 4.9 Hz), 3.97 (1H, d, *J* = 4.9 Hz), 3.64 (1H, t, *J* = 6.1 Hz), 3.59 (1H, d, *J* = 5.8 Hz), 2.95 (3H, d, *J* = 4.9 Hz), 2.55 (2H, t, *J* = 5.5 Hz), 2.26 (3H, s), 1.94–1.85 (2H, m), 1.47 (9H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 494 [(M+H)$^+$].

_N_2-[4-Chloro-3-(2,3,4,7-tetrahydro-1_H_-azepin-5-yl)phenyl]-_N_4,6-dimethyl-pyrimidine-2,4-diamine (95)



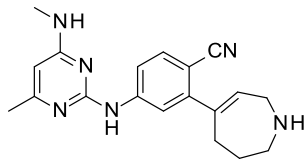
Following general procedure D using compound **140a** (180 mg, 0.41 mmol), TFA (2.0 mL, 26 mmol), and CH_2Cl_2 (6.0 mL), the title compound, **95** (90 mg, 65%), was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.53 (1H, dd, *J* = 8.5, 2.4 Hz), 7.40 (1H, br s), 7.22 (1H, d, *J* = 8.5 Hz), 6.79 (1H, br s), 5.85 (1H, t, *J* = 5.5 Hz), 5.75 (1H, s), 4.73 (1H, br s), 3.48 (2H, d, *J* = 5.5 Hz), 3.13 (2H, t, *J* = 5.5 Hz), 2.95 (3H, d, *J* = 4.9 Hz), 2.60 (2H, t, *J* = 5.2 Hz), 2.25 (3H, s), 1.88–1.82 (2H, m). ESIMS-LR *m/z* 344 [(M+H)$^+$]; ESIMS-HR calcd for $C_{18}H_{23}ClN_5$ (M+H)$^+$ 344.1636, found 344.1643.

_N_4,6-Dimethyl-_N_2-[4-methyl-3-(2,3,4,7-tetrahydro-1_H_-azepin-5-yl)phenyl]pyrimidine-2,4-diamine (96)



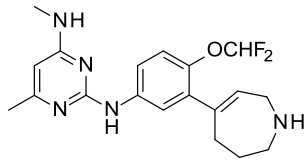
Following general procedure D using compound **140b** (310 mg, 0.72 mmol), TFA (2.0 mL, 26 mmol), and CH_2Cl_2 (6.0 mL), the title compound, **96** (180 mg, 76%), was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.44 (1H, dd, *J* = 8.5, 2.4 Hz), 7.30 (1H, br s), 7.05 (1H, d, *J* = 8.5 Hz), 6.74 (1H, br s), 5.75 (1H, t, *J* = 5.5 Hz), 5.71 (1H, s), 4.70 (1H, br s), 3.47 (2H, d, *J* = 5.5 Hz), 3.13 (2H, t, *J* = 5.8 Hz), 2.94 (3H, d, *J* = 4.9 Hz), 2.54 (2H, t, *J* = 5.2 Hz), 2.24 (3H, s), 2.23 (3H, s), 1.85–1.78 (2H, m). ESIMS-LR *m/z* 324 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_5$ $(M+H)^+$ 324.2182, found 324.2189.

4-[[4-Methyl-6-(methylamino)pyrimidin-2-yl]amino]-2-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)benzonitrile (97)



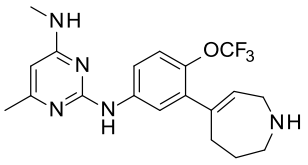
Following general procedure D using compound **140c** (260 mg, 0.60 mmol), TFA (2.0 mL, 26 mmol), and CH_2Cl_2 (6.0 mL), the title compound, **97** (170 mg, 85%), was obtained as a colorless solid. ^{1}H NMR ((DMSO-d_6, 400 MHz) δ: 9.54 (1H, s), 8.10 (1H, br s), 7.72 (1H, br s), 7.56 (1H, d, *J* = 8.5 Hz), 7.18 (1H, br s), 5.93 (1H, t, *J* = 5.5 Hz), 5.88 (1H, s), 3.38–3.31 (2H, m), 2.96 (2H, t, *J* = 5.8 Hz), 2.83 (3H, s), 2.61 (2H, t, *J* = 5.2 Hz), 2.15 (3H, s), 1.80–1.73 (2H, m). ESIMS-LR *m/z* 335 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{23}N_6$ $(M+H)^+$ 335.1979, found 335.1982.

***N*2-[4-(Difluoromethoxy)-3-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)phenyl]-*N*4,6-dimethylpyrimidine-2,4-diamine (98)**



Following general procedure D using compound **140d** (260 mg, 0.54 mmol), TFA (1.0 mL, 13 mmol), and CH_2Cl_2 (3.0 mL), the title compound, **98** (170 mg, 84%), was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.57 (1H, dd, *J* = 8.5, 2.4 Hz), 7.39 (1H, s), 7.01 (1H, d, *J* = 8.5 Hz), 6.82 (1H, br s), 6.36 (1H, t, *J* = 75.1 Hz), 5.91 (1H, t, *J* = 5.5 Hz), 5.75 (1H, s), 4.75 (1H, s), 3.47 (2H, d, *J* = 5.5 Hz), 3.13 (2H, t, *J* = 5.8 Hz), 2.95 (3H, d, *J* = 4.9 Hz), 2.60 (2H, t, *J* = 5.2 Hz), 2.26 (3H, s), 1.84–1.76 (2H, m). ESIMS-LR *m/z* 376 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{24}F_2N_5O$ $(M+H)^+$ 376.1943, found 394.1926.

***N*4,6-Dimethyl-*N*2-[3-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)-4(trifluoromethoxy)phenyl]pyrimidine-2,4-diamine (99)**

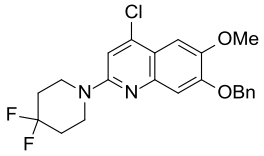


Following general procedure C using compound **140e** (310 mg, 0.63 mmol), TFA (1.0 mL, 13 mmol), and CH_2Cl_2 (3.0 mL), the title compound, **99** (220 mg, 89%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, dd, *J* = 9.0, 2.7 Hz), 7.43 (1H, s), 7.12 (1H, dd, *J* = 9.0, 1.2 Hz), 6.80 (1H, br s), 5.92 (1H, t, *J* = 5.5 Hz), 5.76 (1H, s), 4.71 (1H, br s), 3.47 (2H, d, *J* = 5.5 Hz), 3.13 (2H, t, *J* = 5.8 Hz), 2.95 (3H, d, *J* = 4.9 Hz), 2.60 (2H, t, *J* = 5.2 Hz), 2.26 (3H, s), 1.84–1.77 (2H, m). ESIMS-LR *m/z* 394 [$(M+H)^+$];

ESIMS-HR calcd for $C_{19}H_{23}F_3N_5O$ $(M+H)^+$ 394.1849, found 394.1864.

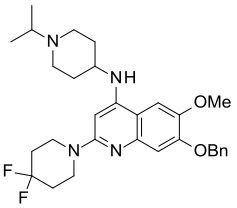
第三章

7-Benzyloxy-4-chloro-2-(4,4-difluoro-1-piperidyl)-6-methoxy-quinoline (159)



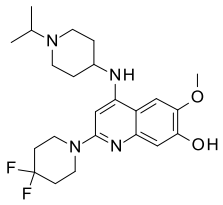
A suspension of compound **158** (400 mg, 1.2 mmol), 4,4-difluoropiperidine (159 mg, 1.3 mmol), Xantphos (69 mg, 0.12 mmol) and NaO^{t}Bu (130 mg, 1.3 mmol) in toluene (12 mL) was treated with $Pd_2(dba)_3$ (55 mg, 0.060 mmol) at room temperature, and the mixture was stirred at 80 °C for 1 h, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, AcOEt/hexane; 0:100 to 20:80) to afford compound **159** (340 mg, 68%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.49 (2H, t, *J* = 7.6 Hz), 7.43–7.36 (2H, m), 7.36–7.30 (1H, m), 7.15 (1H, s), 6.97 (1H, s), 5.27 (2H, s), 4.00 (3H, s), 3.81 (4H, t, *J* = 5.5 Hz), 2.12–1.99 (4H, m). ESIMS-LR *m/z* 419 $[(M+H)^+]$.

7-Benzyloxy-2-(4,4-difluoro-1-piperidyl)-*N*-(1-isopropyl-4-piperidyl)-6-methoxy-quinolin-4-amine (160)



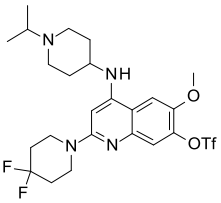
A suspension of compound **159** (340 mg, 0.82 mmol), 4-amino-1-isopropylpiperidine (0.52 mL, 3.3 mmol), Xantphos (47 mg, 0.082 mmol) and NaO^{t}Bu (160 mg, 1.6 mmol) in 1,4-dioxane (10 mL) was treated with $Pd_2(dba)_3$ (37 mg, 0.041 mmol) at room temperature, and the mixture was stirred at 120 °C for 1.5 h with μW irradiation, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; 0:100 to 50:50) to afford compound **160** (190 mg, 44%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.49 (2H, d, *J* = 7.3 Hz), 7.38 (2H, t, *J* = 7.3 Hz), 7.32 (1H, t, *J* = 7.0 Hz), 7.13 (1H, s), 6.77 (1H, s), 5.94 (1H, s), 5.24 (2H, s), 4.36 (1H, d, *J* = 7.3 Hz), 3.96 (3H, s), 3.77 (4H, t, *J* = 5.8 Hz), 3.48 (2H, q, *J* = 6.9 Hz), 2.92 (2H, d, *J* = 12.2 Hz), 2.84–2.75 (1H, m), 2.38 (2H, t, *J* = 11.6 Hz), 2.18 (2H, d, *J* = 11.6 Hz), 2.12–2.00 (4H, m), 1.08 (6H, d, *J* = 6.1 Hz). ESIMS-LR *m/z* 525 $[(M+H)^+]$.

2-(4,4-Difluoro-1-piperidyl)-4-[(1-isopropyl-4-piperidyl)amino]-6-methoxy-quinolin-7-ol (161)



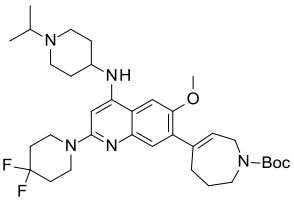
A solution of compound **160** (190 mg, 0.36 mmol) in MeOH (10 mL) was treated with 10% Pd/C (50 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 1 h. The catalyst was filtered through a pad of Celite and the filtrate was concentrated *in vacuo* to afford compound **161** (160 mg, quant.) as a colorless oil. This material was used in the next reaction without further purification. ESIMS-LR *m/z* 435 $[(M+H)^+]$.

[2-(4,4-Difluoro-1-piperidyl)-4-[(1-isopropyl-4-piperidyl)amino]-6-methoxy-7-quinolyl] trifluoromethanesulfonate (162)



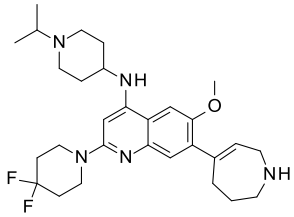
A solution of compound **161** (160 mg, 0.36 mmol) and K_2CO_3 (100 mg, 0.72 mmol) in DMF (5.0 mL) was treated with $PhNTf_2$ (150 mg, 0.43 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (300 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 20:80 to 50:50) to afford compound **162** (130 mg, 64%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51 (1H, s), 6.89 (1H, s), 6.03 (1H, s), 4.41 (1H, d, *J* = 7.3 Hz), 4.00 (3H, s), 3.81 (4H, t, *J* = 5.8 Hz), 3.53–3.44 (1H, m), 2.97–2.88 (2H, m), 2.84–2.76 (1H, m), 2.38 (2H, t, *J* = 11.3 Hz), 2.18 (2H, d, *J* = 11.6 Hz), 2.13–1.99 (5H, m), 1.09 (6H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 567 [$(M+H)^+$].

***tert*-Butyl 5-[2-(4,4-difluoro-1-piperidyl)-4-[(1-isopropyl-4-piperidyl)amino]-6-methoxy-7-quinolyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (163)**



A solution of compound **162** (130 mg, 0.23 mmol), compound **128** (110 mg, 0.34 mmol) and K_3PO_4 (92 mg, 0.46 mmol) in 1,4-dioxane (10 mL), H_2O (1.0 mL) was treated with XPhos Pd G2 (18 mg, 0.023 mmol) at room temperature, and the mixture was stirred at 90 °C for 1 h, and then the mixture was cooled to room temperature. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; 0:100 to 50:50) to afford compound **163** (130 mg, 92%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41 (1H, d, *J* = 11.6 Hz), 6.69 (1H, s), 6.01 (1H, s), 5.91 (1H, t, *J* = 4.9 Hz), 4.43–4.37 (1H, m), 4.11–4.07 (1H, m), 3.99 (1H, d, *J* = 4.9 Hz), 3.87 (3H, s), 3.78 (4H, t, *J* = 5.5 Hz), 3.68–3.63 (1H, m), 3.62–3.56 (1H, m), 3.54–3.44 (1H, m), 2.92 (2H, d, *J* = 12.2 Hz), 2.83–2.75 (1H, m), 2.58–2.52 (2H, m), 2.38 (2H, t, *J* = 10.7 Hz), 2.19 (2H, d, *J* = 9.8 Hz), 2.12–2.01 (5H, m), 1.94–1.85 (2H, m), 1.49 (9H, d, *J* = 4.3 Hz), 1.08 (6H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 558 [$(M-tBu+H)^+$].

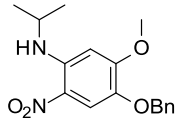
2-(4,4-Difluoro-1-piperidyl)-*N*-(1-isopropyl-4-piperidyl)-6-methoxy-7-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)quinolin-4-amine (141)



Following general procedure D using compound **163** (130 mg, 0.21 mmol), TFA (3.0 mL, 39 mmol), and CH_2Cl_2 (3.0 mL), the title compound, **141** (110 mg, 99%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.43 (1H, s), 6.69 (1H, s), 6.03–5.98 (2H, m), 4.40 (1H, d, *J* = 7.3 Hz), 3.90 (3H, s), 3.78 (4H, t, *J* = 5.5 Hz), 3.52–3.45 (4H, m), 3.15 (2H, t, *J* = 5.5 Hz), 2.92 (2H, d, *J* = 12.2 Hz), 2.84–2.75 (1H, m), 2.60 (2H, t, *J* = 5.2 Hz), 2.38 (2H, t, *J* = 10.4 Hz), 2.19 (2H, d, *J* = 11.0 Hz), 2.12–

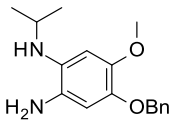
2.01 (4H, m), 1.83–1.75 (2H, m), 1.09 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ: 157.2, 152.6, 148.7, 145.6, 145.5, 143.5, 127.9, 122.6 (t, $J_{C,F}$ = 241 Hz), 97.9, 95.8, 93.0, 86.5, 55.8, 54.6, 52.5, 49.8, 47.6, 47.5, 43.1 (t, $J_{C,F}$ = 5.0 Hz), 33.7 (t, $J_{C,F}$ = 22 Hz), 33.1, 32.3, 18.3. ESIMS-LR *m/z* 514 [(M-tBu+H)$^+$]. ESIMS-HR calcd for $C_{29}H_{42}F_2N_5O$ (M+H)$^+$ 514.3352, found 514.3361.

4-Benzyloxy-*N*-isopropyl-5-methoxy-2-nitroaniline (165)



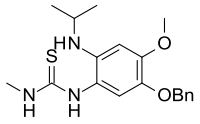
A suspension of 1-benzyloxy-4-fluoro-2-methoxy-5-nitro-benzene **164** (1.8 g, 6.4 mmol) and Cs_2CO_3 (4.4 g, 13 mmol) in MeCN (15 mL) was treated with isopropylamine (1.7 mL, 19 mmol) at room temperature, and the mixture was stirred at 50 °C for 1 h with μW irradiation, and then the mixture was cooled to room temperature. The resulting mixture was partitioned between AcOEt (200 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, CH_2Cl_2/hexane; 0:100 to 10:20) to afford compound **165** (0.32 g, 16%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.48–8.41 (1H, br s), 7.69 (1H, s), 7.46–7.29 (5H, m), 6.18 (1H, s), 5.07 (2H, s), 3.94 (3H, s), 3.76–3.74 (1H, m), 1.34 (6H, d, J = 6.8 Hz). ESIMS-LR *m/z* 317 [(M+H)$^+$].

4-Benzyloxy-*N*1-isopropyl-5-methoxybenzene-1, 2-diamine (166*)*



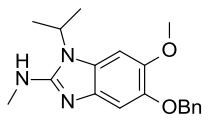
A solution of compound **165** (1.3 g, 4.2 mmol) and NH_4Cl (1.1 g, 21 mmol) in MeOH (32 mL) and H_2O (8.0 mL) was treated with iron powder (1.2 g, 21 mmol) at room temperature, and the mixture was stirred at 80 ºC for 1.5 h. The resulting mixture was filtered through a pad of Celite and washed with CH_2Cl_2. The filtrate was concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford compound **166** (1.2 g, quant.) as a dark reddish brown oil. This material was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46–7.41 (2H, m), 7.39–7.33 (2H, m), 7.32–7.27 (1H, m), 6.41 (1H, s), 6.40 (1H, s), 5.04 (2H, s), 3.83 (3H, s), 3.55–3.44 (1H, m), 1.21 (6H, d, J = 6.4 Hz).

1-[5-Benzyloxy-2-(isopropylamino)-4-methoxyphenyl]-3-methylthiourea (167) *The position at which thiourea is introduced is estimated.



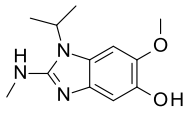
A solution of compound **166** (1.2 g, 4.3 mmol) in THF (50 mL) was treated with methyl isothiocyanate (450 mg, 6.1 mmol) at room temperature for 18 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, CH_2Cl_2/AcOEt; 0:100 to 90:10) to afford compound **167** (1.03 g, 67%) as a brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41–7.27 (5H, m), 6.91–6.86 (1H, br s), 6.59 (1H, s), 6.30 (1H, s), 5.70–5.61 (1H, br s), 5.02 (2H, s), 3.90 (3H, s), 3.63–3.53 (m, 2H), 3.04 (3H, d, J = 4.8 Hz), 1.18 (6H, d, J = 6.4 Hz). ESIMS-LR *m/z* 360 [(M+H)$^+$].

5-Benzyloxy-1-isopropyl-6-methoxy-*N*-methyl-benzimidazol-2-amine (168)



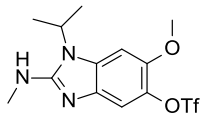
A solution of compound **167** (0.61 g, 1.7 mmol) in MeCN (30 mL) and CH_2Cl_2 (10 mL) was treated with iodomethane (0.14 mL, 2.2 mmol) at room temperature for 21 h. The resulting mixture was concentrated *in vacuo* to afford crude compound (0.74 g). A solution of this crude compound (0.74 g) in MeOH (75 mL) was stirred at 55 ºC for 5 h. The resulting mixture was partitioned between CH_2Cl_2 (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/CH_2Cl_2; 0:100 to 1:99) to afford compound **168** (0.33 g, 60%) as a brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.49–7.43 (2H, m), 7.40–7.23 (3H, m), 7.11 (1H, s), 6.84 (1H, s), 5.16 (2H, s), 4.70 (1H, br), 4.36–4.24 (1H, m), 3.92 (3H, s), 3.09 (3H, d, *J* = 4.8 Hz), 1.56 (6H, d, *J* = 6.8 Hz). ESIMS-LR *m/z* 326 [$(M+H)^+$].

1-Isopropyl-6-methoxy-2-(methylamino)benzimidazol-5-ol (169)



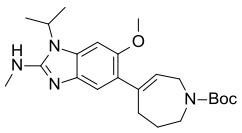
A solution of compound **168** (0.16 g, 0.49 mmol) in EtOH (6.0 mL) and EtOAc (2.0 mL) was treated with 10% Pd/C (0.25 g), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 2 h. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **169** (96 mg, 83%) as a brown solid. This material was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.15 (1H, br), 6.87 (1H, s), 6.67 (1H, s), 6.20 (1H, br), 4.52–4.40 (1H, m), 3.76 (3H, s), 2.82 (3H, d, *J* = 4.8 Hz), 1.43 (6H, d, *J* = 7.2 Hz). ESIMS-LR *m/z* 236 [$(M+H)^+$].

[1-Isopropyl-6-methoxy-2-(methylamino)benzimidazol-5-yl] trifluoromethanesulfonate (170)



A suspension of compound **169** (95 mg, 0.40 mmol) and Cs_2CO_3 (0.11 g, 0.83 mmol) in DMF (3.0 mL) was treated with *N*-phenylbis(trifluoromethanesulfonimide) (0.17 g, 0.48 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, MeOH/CH_2Cl_2; 0:100 to 3:97) to afford compound **170** (0.15 g, quant.) as a brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.34 (1H, s), 6.85 (1H, s), 4.94 (3H, s), 4.42–4.28 (1H, m), 4.06 (1H, br), 3.13 (3H, d, *J* = 5.2 Hz), 1.59 (6H, d, *J* = 6.8 Hz). ESIMS-LR *m/z* 368 [$(M+H)^+$].

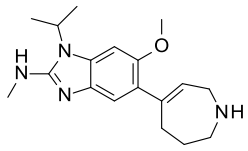
***tert*-Butyl 5-[1-isopropyl-6-methoxy-2-(methylamino)benzimidazol-5-yl]-2, 3, 4, 7-tetrahydro-azepine-1-carboxylate (171)**



A solution of compound **170** (300 mg, 0.81 mmol), compound **128** (270 mg., 0.85 mmol) and K_3PO_4 (530 mg, 2.5 mmol) in 1,4-dioxane (15 mL) and H_2O (1.5 mL) was treated with XPhos Pd G2 (63 mg, 0.080 mmol) at room temperature, and the mixture was stirred at 80 °C for 0.5 h, and then the mixture was cooled to room temperature. The resulting mixture was partitioned between AcOEt

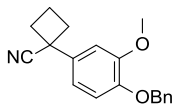
(100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/CH_2Cl_2; 0:100 to 2:98) to afford compound **171** (250 mg, 74%) as a brown amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.23 (1H, br), 6.73 (1H, s), 5.81 (1H, dd, *J* = 5.6 Hz, *J* = 4.8 Hz), 4.40–4.27 (1H, m), 4.05 (1H, br), 3.99–3.89 (2H, m), 3.81 (3H, s), 3.69–3.54 (2H, m), 3.12 (3H, d, *J* = 5.2 Hz), 2.61–2.53 (2H, m), 1.90–1.80 (2H, m), 1.57 (6H, d, *J* = 7.2 Hz), 1.47 (9H, s). ESIMS-LR *m/z* 415 [$(M+H)^+$].

1-Isopropyl-6-methoxy-*N*-methyl-5-(2, 3, 4, 7-tetrahydro-1*H*-azepin-5-yl)benzimidazol-2-amine (143)



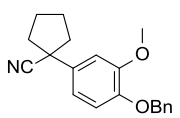
Following general procedure D using compound **171** (250 mg, 0.61 mmol), TFA (2.5 mL, 33 mmol), and CH_2Cl_2 (7.6 mL), the title compound, **143** (99 mg, 51%), was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.25 (1H, s), 6.73 (1H, s), 5.88 (1H, t, *J* = 6.0 Hz), 4.39–4.28 (1H, m), 3.96 (1H, br), 3.84 (1H, s), 3.54–3.47 (2H, m), 3.20–3.14 (2H, m), 3.12 (3H, d, *J* = 4.8 Hz), 2.67–2.58 (2H, m), 1.85–1.71 (2H, m), 1.57 (6H, d, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ: 176.6, 152.5, 146.9, 146.1, 139.8, 134.5, 131.0, 116.6, 105.2, 56.4, 53.0, 52.7, 47.7, 33.6, 31.4, 29.4, 16.9. ESIMS-LR *m/z* 315 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{27}N_4O$ $(M+H)^+$ 315.2179, found 315.2190.

1-(4-Benzyloxy-3-methoxy-phenyl)cyclobutanecarbonitrile (173a): General Procedure F



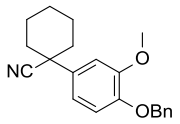
A suspension of compound **172** (10 g, 39 mmol), 1,3-dibromopropane (4.8 mL, 47 mmol) in DMF (200 mL) was treated with NaH (4.3 g, 55% dispersion of mineral oil, 99 mmol) at 0 ºC, and the mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (300 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **173a** (7.8 g, 67%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46–7.41 (2H, m), 7.40–7.34 (2H, m), 7.34–7.28 (1H, m), 6.92–6.85 (3H, m), 5.16 (2H, s), 3.92 (3H, s), 2.83–2.75 (2H, m), 2.64–2.53 (2H, m), 2.46–2.35 (1H, m), 2.10–1.99 (1H, m).

1-(4-Benzyloxy-3-methoxy-phenyl)cyclopentanecarbonitrile (173b)



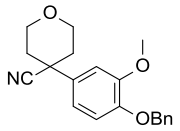
Following general procedure F using compound **172** (600 mg, 2.4 mmol), 1,4-dibromobutane (0.34 mL, 2.8 mmol), NaH (260 mg, 55% dispersion of mineral oil, 5.9 mmol), and DMF (25 mL), the title compound, **173b** (550 mg, 76%), was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.43 (2H, d, *J* = 7.3 Hz), 7.37 (2H, t, *J* = 7.3 Hz), 7.34–7.28 (1H, m), 6.98 (1H, d, *J* = 2.4 Hz), 6.90 (1H, dd, *J* = 8.5, 1.8 Hz), 6.85 (1H, d, *J* = 8.5 Hz), 5.16 (2H, s), 3.92 (3H, s), 2.50–2.39 (2H, m), 2.09–1.98 (4H, m), 1.97–1.87 (2H, m).

1-(4-Benzyloxy-3-methoxy-phenyl)cyclohexanecarbonitrile (173c)



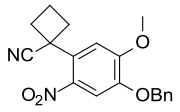
Following general procedure A using compound **172** (600 mg, 2.4 mmol), 1,5-dibromopentane (0.39 mL, 2.8 mmol), NaH (260 mg, 55% dispersion of mineral oil, 5.9 mmol), and DMF (25 mL), the title compound, **173c** (570 mg, 75%), was obtained as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.43 (2H, d, *J* = 7.3 Hz), 7.37 (2H, t, *J* = 7.6 Hz), 7.33–7.28 (1H, m), 7.02 (1H, d, *J* = 2.4 Hz), 6.93 (1H, dd, *J* = 8.5, 2.4 Hz), 6.86 (1H, d, *J* = 8.5 Hz), 5.16 (2H, s), 3.92 (3H, s), 2.14 (2H, d, *J* = 12.8 Hz), 1.92–1.79 (5H, m), 1.78–1.66 (2H, m), 1.33–1.19 (1H, m).

4-(4-Benzyloxy-3-methoxy-phenyl)tetrahydropyran-4-carbonitrile (173d)



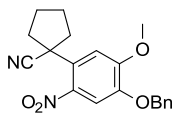
Following general procedure A using compound **172** (600 mg, 2.4 mmol), 2-bromoethyl ether (0.36 mL, 2.8 mmol), NaH (260 mg, 55% dispersion of mineral oil, 5.9 mmol), and DMF (25 mL), the title compound, **173d** (540 mg, 70%), was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.44 (2H, d, *J* = 7.3 Hz), 7.38 (2H, t, *J* = 7.3 Hz), 7.35–7.29 (1H, m), 7.01 (1H, d, *J* = 2.4 Hz), 6.94 (1H, dd, *J* = 8.5, 2.4 Hz), 6.89 (1H, d, *J* = 8.5 Hz), 5.17 (2H, s), 4.08 (2H, dd, *J* = 11.9, 2.7 Hz), 3.93 (3H, s), 3.92–3.85 (2H, m), 2.16–1.98 (4H, m).

1-(4-Benzyloxy-5-methoxy-2-nitrophenyl)cyclobutanecarbonitrile (174a): General Procedure G



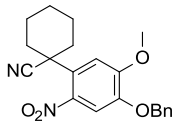
A solution of compound **173a** (4.2 g, 14 mmol) in AcOH (16 mL) and Ac_2O (16 mL) was treated with 69% nitric acid (1.2 mL, 29 mmol) at 0 ºC for 30 min. The resulting mixture was partitioned between AcOEt (300 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 20:80 to 60:40) to afford compound **174a** (2.9 g, 59%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.64 (1H, s), 7.47–7.35 (5H, m), 6.75 (1H, s), 5.20 (2H, s), 3.98 (3H, s), 2.99–2.93 (2H, m), 2.52–2.47 (3H, m), 1.97–1.91 (1H, m).

1-(4-Benzyloxy-5-methoxy-2-nitrophenyl)cyclopentanecarbonitrile (174b)



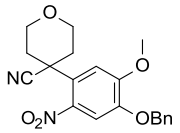
Following general procedure G using compound **173b** (530 mg, 1.7 mmol), AcOH (5.0 mL), Ac_2O (5.0 mL), and 69% nitric acid (0.20 mL, 5.2 mmol), the title compound, **174b** (440 mg, 72%), was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51 (1H, s), 7.46–7.33 (5H, m), 6.98 (1H, s), 5.18 (2H, s), 3.97 (3H, s), 2.76–2.64 (2H, m), 2.10–1.99 (4H, m), 1.95–1.83 (2H, m).

1-(4-Benzyloxy-5-methoxy-2-nitrophenyl)cyclohexanecarbonitrile (174c)



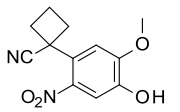
Following general procedure G using compound **173c** (530 mg, 1.6 mmol), AcOH (5.0 mL), Ac_2O (5.0 mL), and 69% nitric acid (0.20 mL, 4.9 mmol), the title compound, **174c** (500 mg, 83%), was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46–7.33 (6H, m), 7.07 (1H, s), 5.17 (2H, s), 3.98 (3H, s), 2.39 (2H, d, *J* = 7.9 Hz), 1.95–1.82 (7H, m), 1.35–1.24 (1H, m).

4-(4-Benzyloxy-5-methoxy-2-nitrophenyl)tetrahydropyran-4-carbonitrile (174d)



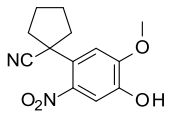
Following general procedure G using compound **173d** (420 mg, 1.6 mmol), AcOH (5.0 mL), Ac_2O (5.0 mL), and 69% nitric acid (0.20 mL, 4.9 mmol), the title compound, **174d** (420 mg, 88%), was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.50 (1H, s), 7.46–7.33 (5H, m), 6.98 (1H, s), 5.19 (2H, s), 4.09 (2H, dd, *J* = 11.6, 3.7 Hz), 4.03–3.94 (2H, m), 3.98 (3H, s), 2.37 (2H, d, *J* = 11.6 Hz), 2.18 (2H, td, *J* = 12.5, 4.1 Hz).

1-(4-Hydroxy-5-methoxy-2-nitrophenyl)cyclobutanecarbonitrile (175a): General Procedure H



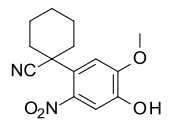
A solution of compound **174a** (8.0 g, 24 mmol), 1,4-cyclohexadiene (11 mL, 120 mmol) in EtOH (60 mL) and AcOEt (40 mL) was treated with 10% Pd/C (4.0 g) at room temperature, and the mixture was stirred at 80 °C for 0.5 h, and then the mixture was cooled to room temperature. The catalyst was filtered through a pad of Celite and the filtrate was concentrated *in vacuo* to afford compound **175a** (5.9 g, quant.) as a brown solid. This material was used in the next reaction without further purification.

1-(4-Hydroxy-5-methoxy-2-nitrophenyl)cyclopentanecarbonitrile (175b)



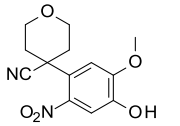
Following general procedure H using compound **174b** (430 mg, 1.2 mmol), 1,4-cyclohexadiene (0.57 mL. 6.1 mmol), EtOH (5.0 mL), AcOEt (5.0 mL), and 10% Pd/C (200 mg), the title compound, **175b** (260 mg, 81%), was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.48 (1H, s), 6.97 (1H, s), 5.81 (1H, br s), 4.00 (3H, s), 2.75–2.64 (2H, m), 2.10–1.98 (4H, m), 1.95-1.84 (2H, m).

1-(4-Hydroxy-5-methoxy-2-nitrophenyl)cyclohexanecarbonitrile (175c)



Following general procedure H using compound **174c** (490 mg, 1.3 mmol), 1,4-cyclohexadiene (0.63 mL. 6.7 mmol), EtOH (5.0 mL), AcOEt (5.0 mL), and 10% Pd/C (240 mg), the title compound, **175c** (320 mg, 87%), was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.35 (1H, s), 7.07 (1H, s), 5.80 (1H, br s), 4.01 (3H, s), 2.41–2.31 (2H, m), 1.95–1.80 (7H, m), 1.35-1.23 (1H, m).

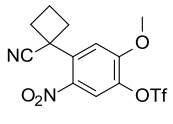
4-(4-Hydroxy-5-methoxy-2-nitrophenyl)tetrahydropyran-4-carbonitrile (175d)



Following general procedure H using compound **174d** (460 mg, 1.2 mmol), 1,4-cyclohexadiene (0.58 mL. 6.2 mmol), EtOH (5.0 mL), AcOEt (5.0 mL), and 10% Pd/C (230 mg), the title compound, **175d** (250 mg, 72%), was obtained as a yellow

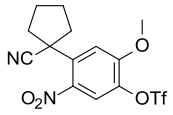
solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.47 (1H, s), 6.97 (1H, s), 5.86 (1H, br s), 4.09 (2H, dd, *J* = 12.3, 4.3 Hz), 4.02 (3H, s), 4.02–3.93 (2H, m), 2.36 (2H, d, *J* = 13.4 Hz), 2.19 (2H, td, *J* = 12.3, 4.3 Hz).

[4-(1-Cyanocyclobutyl)-2-methoxy-5-nitrophenyl] trifluoromethanesulfonate (176a): General Procedure I



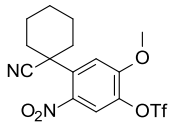
A solution of compound **175a** (5.9 g, 24 mmol), Et_3N (5.0 mL, 36 mmol) in CH_2Cl_2 (120 mL) was treated with trifluoromethanesulfonic anhydride (4.8 mL, 29 mmol) at 0 ºC for 30 min. The resulting mixture was partitioned between AcOEt (300 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 20:80 to 50:50) to afford compound **176a** (6.5 g, 72%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.00 (1H, s), 6.94 (1H, s), 4.06 (3H, s), 3.02-2.96 (2H, m), 2.60–2.49 (3H, m), 2.03–1.95 (1H, m).

[4-(1-Cyanocyclopentyl)-2-methoxy-5-nitrophenyl] trifluoromethanesulfonate (176b)



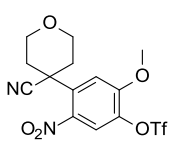
Following general procedure I using compound **175b** (260 mg, 0.99 mmol), Et_3N (0.21 mL, 1.5 mmol), trifluoromethanesulfonic anhydride (0.20 mL, 1.2 mmol), and CH_2Cl_2 (5.0 mL), the title compound, **176b** (340 mg, 87%), was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.85 (1H, s), 7.20 (1H, s), 4.05 (3H, s), 2.79–2.67 (2H, m), 2.16–2.04 (4H, m), 2.00–1.89 (2H, m).

[4-(1-Cyanocyclohexyl)-2-methoxy-5-nitrophenyl] trifluoromethanesulfonate (176c)



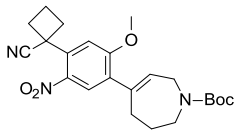
Following general procedure I using compound **175c** (310 mg, 1.1 mmol) and Et_3N (0.23 mL, 1.7 mmol), trifluoromethanesulfonic anhydride (0.22 mL, 1.3 mmol), and CH_2Cl_2 (5.0 mL), the title compound, **176c** (420 mg, 92%), was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.73 (1H, s), 7.28 (1H, s), 4.05 (3H, s), 2.41 (2H, d, *J* = 6.1 Hz), 1.98–1.84 (7H, m), 1.38–1.24 (1H, m).

[4-(4-Cyanotetrahydropyran-4-yl)-2-methoxy-5-nitrophenyl] trifluoromethanesulfonate (176d)



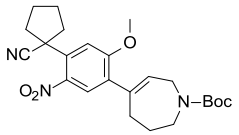
Following general procedure I using compound **175d** (210 mg, 0.75 mmol), Et_3N (0.16 mL, 1.1 mmol), trifluoromethanesulfonic anhydride (0.15 mL, 0.91 mmol), and CH_2Cl_2 (5.0 mL), the title compound, **176d** (300 mg, 96%), was obtained as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (1H, s), 7.19 (1H, s), 4.13 (2H, dd, *J* = 12.5, 4.0 Hz), 4.07 (3H, s), 4.00 (2H, t, *J* = 12.2 Hz), 2.40 (2H, d, *J* = 12.2 Hz), 2.23 (2H, td, *J* = 12.5, 4.3 Hz).

***tert*-Butyl 5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (177a)**



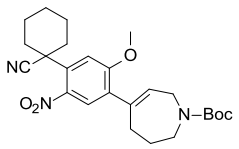
Following general procedure C using compound **176a** (500 mg, 1.3 mmol), compound **128** (510 mg, 1.7 mmol), K_2CO_3 (360 mg, 2.6 mmol), Pd(dppf)Cl_2 (190 mg, 0.26 mmol), 1,4-dioxane (5.0 mL) and H_2O (1.0 mL), the title compound, **177a** (520 mg, 93%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.81 (1H, s), 6.71 (1H, s), 5.87 (1H, t, *J* = 4.9 Hz), 4.08 (1H, d, *J* = 4.3 Hz), 3.98 (1H, d, *J* = 4.9 Hz), 3.91 (3H, s), 3.68–3.63 (1H, m), 3.62–3.57 (1H, m), 3.02–2.93 (2H, m), 2.56–2.45 (5H, m), 1.97–1.85 (3H, m), 1.48 (9H, d, *J* = 5.5 Hz).

tert-Butyl 5-[4-(1-cyanocyclopentyl)-2-methoxy-5-nitrophenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (177b)



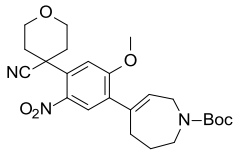
Following general procedure C using compound **176b** (100 mg, 0.25 mmol), compound **128** (98 mg, 0.36 mmol), K_2CO_3 (70 mg, 0.51 mmol), Pd(dppf)Cl_2 (37 mg, 0.051 mmol), 1,4-dioxane (3.0 mL) and H_2O (1.0 mL), the title compound, **177b** (88 mg, 79%), was obtained as a brown amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.67 (1H, s), 6.95 (1H, s), 5.86 (1H, t, *J* = 4.6 Hz), 4.08 (1H, d, *J* = 4.3 Hz), 3.97 (1H, d, *J* = 4.3 Hz), 3.91 (3H, s), 3.68–3.62 (1H, m), 3.61–3.55 (1H, m), 2.76–2.64 (2H, m), 2.52–2.44 (2H, m), 2.13–2.00 (4H, m), 1.98–1.85 (4H, m), 1.50–1.43 (9H, m).

tert-Butyl 5-[4-(1-cyanocyclohexyl)-2-methoxy-5-nitrophenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (177c)



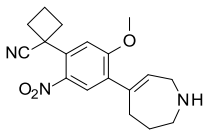
Following general procedure C using compound **176c** (100 mg, 0.25 mmol), compound **128** (95 mg, 0.29 mmol), K_2CO_3 (68 mg, 0.49 mmol), Pd(dppf)Cl_2 (36 mg, 0.049 mmol), 1,4-dioxane (3.0 mL) and H_2O (1.0 mL), the title compound, **177c** (92 mg, 82%), was obtained as a brown amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.54 (1H, s), 7.04 (1H, s), 5.86 (1H, t, *J* = 4.9 Hz), 4.07 (1H, d, *J* = 4.9 Hz), 3.97 (1H, d, *J* = 4.9 Hz), 3.91 (3H, s), 3.66–3.62 (1H, m), 3.61–3.55 (1H, m), 2.52–2.45 (2H, m), 2.41–2.35 (2H, m), 1.96–1.82 (9H, m), 1.49–1.44 (9H, m), 1.35–1.24 (1H, m).

tert-Butyl 5-[4-(4-cyanotetrahydropyran-4-yl)-2-methoxy-5-nitrophenyl]-2,3,4,7-tetrahydroazepine-1-carboxylate (177d)



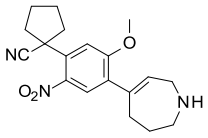
Following general procedure C using compound **176d** (100 mg, 0.24 mmol), compound **128** (94 mg, 0.29 mmol), K_2CO_3 (67 mg, 0.49 mmol), Pd(dppf)Cl_2 (36 mg, 0.049 mmol), 1,4-dioxane (3.0 mL) and H_2O (1.0 mL), the title compound, **177d** (106 mg, 95%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.66 (1H, s), 6.94 (1H, s), 5.87 (1H, t, *J* = 4.9 Hz), 4.15–4.06 (3H, m), 4.04–3.95 (3H, m), 3.92 (3H, s), 3.67–3.62 (1H, m), 3.61–3.56 (1H, m), 2.53–2.46 (2H, m), 2.38 (2H, d, *J* = 13.4 Hz), 2.22 (2H, td, *J* = 12.4, 4.3 Hz), 1.93–1.85 (2H, m), 1.48 (9H, d, *J* = 6.1 Hz).

1-[5-Methoxy-2-nitro-4-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)phenyl]cyclobutanecarbonitrile (178a)



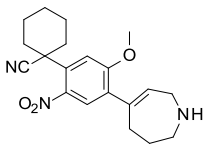
Following general procedure D using compound **177a** (520 mg, 1.2 mmol), TFA (3.0 mL, 39 mmol), and CH_2Cl_2 (6.0 mL), the title compound, **178a** (380 mg, 95%), was obtained as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (1H, s), 6.71 (1H, s), 5.93 (1H, t, *J* = 5.8 Hz), 3.93 (3H, s), 3.54 (2H, d, *J* = 5.5 Hz), 3.20 (2H, t, *J* = 5.8 Hz), 3.03–2.92 (2H, m), 2.59–2.54 (3H, m), 2.53–2.49 (2H, m), 2.00–1.92 (1H, m), 1.88–1.81 (2H, m).

1-[5-Methoxy-2-nitro-4-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)phenyl]cyclopentanecarbonitrile (178b)



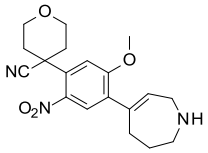
Following general procedure D using compound **177b** (30 mg, 0.068 mmol), CH_2Cl_2 (3.0 mL), and TFA (1.0 mL, 13 mmol), the title compound, **178b** (21 mg, 91%), was obtained as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.71 (1H, s), 6.95 (1H, s), 5.94 (1H, t, *J* = 5.8 Hz), 3.93 (3H, s), 3.49 (2H, d, *J* = 5.5 Hz), 3.15 (2H, t, *J* = 5.8 Hz), 2.76–2.66 (2H, m), 2.58–2.50 (2H, m), 2.14–2.02 (4H, m), 1.96–1.86 (2H, m), 1.84–1.76 (2H, m).

1-[5-Methoxy-2-nitro-4-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)phenyl]cyclohexanecarbonitrile (178c)



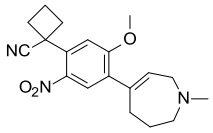
Following general procedure D using compound **177c** (40 mg, 0.087 mmol), CH_2Cl_2 (3.0 mL) and TFA (1.0 mL, 13 mmol), the title compound, **178c** (24 mg, 77%), was obtained as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.59 (1H, s), 7.04 (1H, s), 5.94 (1H, t, *J* = 5.8 Hz), 3.93 (3H, s), 3.48 (2H, d, *J* = 5.5 Hz), 3.14 (2H, t, *J* = 5.8 Hz), 2.54 (2H, t, *J* = 5.2 Hz), 2.39 (2H, d, *J* = 7.9 Hz), 1.98–1.85 (7H, m), 1.82–1.74 (2H, m), 1.37–1.24 (1H, m).

4-[5-Methoxy-2-nitro-4-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)phenyl]tetrahydropyran-4-carbonitrile (178d)



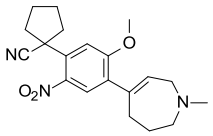
Following general procedure D using compound **177d** (50 mg, 0.11 mmol), CH_2Cl_2 (3.0 mL) and TFA (1.0 mL, 13 mmol), the title compound, **178d** (38 mg, 97%), was obtained as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.71 (1H, s), 6.94 (1H, s), 5.95 (1H, t, *J* = 5.5 Hz), 4.10 (2H, dd, *J* = 12.5, 3.4 Hz), 4.00 (2H, t, *J* = 11.6 Hz), 3.94 (3H, s), 3.49 (2H, d, *J* = 6.1 Hz), 3.14 (2H, t, *J* = 5.8 Hz), 2.55 (2H, t, *J* = 5.5 Hz), 2.38 (2H, d, *J* = 12.2 Hz), 2.22 (2H, td, *J* = 12.5, 4.1 Hz), 1.83–1.75 (2H, m).

1-[5-Methoxy-4-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)-2-nitrophenyl]cyclobutanecarbonitrile (179a)



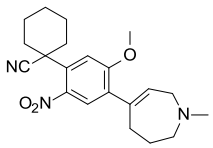
Following general procedure E using compound **178a** (5.2 g, 16 mmol), formaldehyde (1.8 mL, 37% solution, 24 mmol), AcOH (5.0 mL, 79 mmol), $NaBH(OAc)_3$ (5.4 g, 26 mmol) and CH_2Cl_2 (80 mL), the title compound, **179a** (5.1 g, 94%), was obtained as a pale yellow solid. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.88 (1H, s), 6.70 (1H, s), 5.86 (1H, t, *J* = 6.1 Hz), 3.92 (3H, s), 3.22 (2H, d, *J* = 6.1 Hz), 3.01–2.93 (2H, m), 2.84 (2H, t, *J* = 5.5 Hz), 2.57–2.47 (5H, m), 2.40 (3H, s), 1.99–1.90 (1H, m), 1.86–1.78 (2H, m).

1-[5-Methoxy-4-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)-2-nitrophenyl]cyclopentanecarbonitrile (179b)



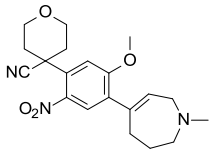
Following general procedure E using compound **178b** (21 mg, 0.062 mmol), formaldehyde (7.5 µL, 37% solution, 0.092 mmol), AcOH (7.4 µL, 0.12 mmol), $NaBH(OAc)_3$ (52 mg, 0.25 mmol) and CH_2Cl_2 (1.0 mL), the title compound, **S-179b** (19 mg, 87%), was obtained as a pale brown oil. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.73 (1H, s), 6.94 (1H, s), 5.85 (1H, t, *J* = 5.8 Hz), 3.91 (3H, s), 3.21 (2H, d, *J* = 6.1 Hz), 2.84 (2H, t, *J* = 5.8 Hz), 2.75–2.65 (2H, m), 2.53–2.48 (2H, m), 2.40 (3H, s), 2.13–2.00 (4H, m), 1.96–1.86 (2H, m), 1.85–1.77 (2H, m).

1-[5-Methoxy-4-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)-2-nitrophenyl]cyclohexanecarbonitrile (179c)



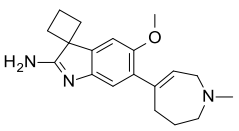
Following general procedure E using compound **178c** (24 mg, 0.067 mmol), formaldehyde (8.2 µL, 37% solution, 0.10 mmol), AcOH (8.1 µL, 0.14 mmol), $NaBH(OAc)_3$ (57 mg, 0.27 mmol) and CH_2Cl_2 (1.0 mL), the title compound, **179c** (21 mg, 84%), was obtained as a pale brown oil. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.61 (1H, s), 7.03 (1H, s), 5.85 (1H, t, *J* = 6.1 Hz), 3.92 (3H, s), 3.21 (2H, d, *J* = 6.1 Hz), 2.84 (2H, t, *J* = 5.5 Hz), 2.51 (2H, t, *J* = 5.5 Hz), 2.43–2.36 (5H, m), 1.98–1.85 (7H, m), 1.84–1.77 (2H, m), 1.37–1.23 (1H, m).

4-[5-Methoxy-4-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)-2-nitrophenyl]tetrahydropyran-4-carbonitrile (179d)



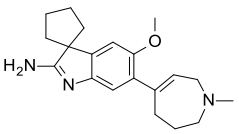
Following general procedure E using compound **178d** (38 mg, 0.11 mmol), formaldehyde (13 µL, 37% solution, 0.16 mmol), AcOH (13 µL, 0.21 mmol), $NaBH(OAc)_3$ (90 mg, 0.43 mmol) and CH_2Cl_2 (1.0 mL), the title compound, **179d** (35 mg, 89%), was obtained as a pale brown solid. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.73 (1H, s), 6.93 (1H, s), 5.86 (1H, t, *J* = 6.1 Hz), 4.10 (2H, dd, *J* = 12.5, 3.4 Hz), 3.99 (2H, t, *J* = 11.6 Hz), 3.92 (3H, s), 3.22 (2H, d, *J* = 6.1 Hz), 2.84 (2H, t, *J* = 5.8 Hz), 2.51 (2H, t, *J* = 5.2 Hz), 2.42–2.35 (5H, m), 2.22 (2H, td, *J* = 12.2, 3.9 Hz), 1.85–1.78 (2H, m).

5'-Methoxy-6'-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)spiro[cyclobutane-1,3'-indole]-2'-amine (144)



A solution of compound **179a** (5.1 g, 15 mmol) in THF (100 mL) and AcOH (17 mL) was treated with Zn (4.9 g, 75 mmol) at room temperature, and the mixture was stirred at 40 °C for 5 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **144'** (4.3 g, 87%) as a colorless solid. This material was used in the next reaction without further purification. A solution of crude **144'** (1.0 g, 3.1 mmol) and Ac_2O (0.29 mL, 3.1 mmol) in THF (110 mL), was treated with 10% Pd/C (500 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, MeOH/$CHCl_3$; 0:100 to 10:90) to afford compound **144** (670 mg, 71%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.02 (1H, s), 6.94 (1H, s), 5.78 (1H, t, *J* = 6.4 Hz), 3.85 (3H, s), 3.21 (2H, d, *J* = 6.7 Hz), 2.85 (2H, t, *J* = 5.8 Hz), 2.56 (2H, t, *J* = 5.2 Hz), 2.51 (4H, t, *J* = 7.6 Hz), 2.43–2.32 (4H, m), 2.26–2.13 (1H, m), 1.83–1.74 (2H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ: 176.6, 152.5, 147.2, 147.0, 139.9, 134.3, 127.1, 116.7, 105.2, 62.0, 56.4, 55.9, 53.0, 46.0, 33.5, 31.4, 26.0, 16.9. ESIMS-LR *m/z* 312 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_3O$ $(M+H)^+$ 312.2070, found 312.2074.

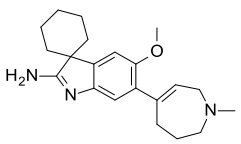
5'-Methoxy-6'-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)spiro[cyclopentane-1,3'-indole]-2'-amine (147)



A solution of compound **179b** (19 mg, 0.053 mmol) in THF (1.0 mL) and AcOH (0.064 mL) was treated with Zn (17 mg, 0.27 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **147'** (16 mg, 88%) as a colorless solid. This material was used in the next reaction without further purification. A solution of crude **147'** (16 mg, 0.047 mmol) and Ac_2O (0.0044 mL, 0.047 mmol) in THF (1.0 mL), was treated with 10% Pd/C (8.0 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 10 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 0:100 to 15:85] to afford compound **147** (8.9 mg, 58%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.97 (1H, s), 6.71 (1H, s), 5.79 (1H, t, *J* = 6.4 Hz), 3.79 (3H, s), 3.22 (2H, d, *J* = 6.1 Hz), 2.85 (2H, t, *J* = 5.5 Hz), 2.57 (2H, t, *J* = 5.2 Hz), 2.39 (3H, s), 2.13–2.04 (2H, m), 2.03–1.93 (6H, m), 1.83–1.74 (2H, m). ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_3O$ $(M+H)^+$ 326.2226, found 326.2231.

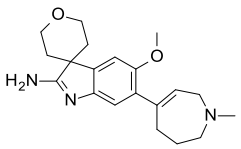
5'-Methoxy-6'-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)spiro[cyclohexane-1,3'-indole]-2'-amine (148)

A solution of compound **179c** (21 mg, 0.057 mmol) in THF (2.0 mL) and AcOH (0.068 mL) was treated with Zn (19 mg, 0.28 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered



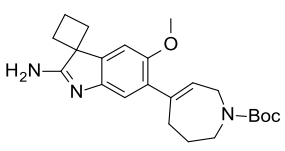
through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **148'** (17 mg, 84%) as a colorless solid. This material was used in the next reaction without further purification. A solution of crude **148'** (17 mg, 0.048 mmol) and Ac_2O (0.0045 mL, 0.048 mmol) in THF (1.0 mL), was treated with 10% Pd/C (19 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 10 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 0:100 to 15:85] to afford compound **148** (7.8 mg, 48%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.07 (1H, s), 7.01 (1H, s), 5.80 (1H, t, *J* = 6.1 Hz), 3.82 (3H, s), 3.23 (2H, d, *J* = 6.7 Hz), 2.86 (2H, t, *J* = 5.8 Hz), 2.58 (2H, t, *J* = 5.5 Hz), 2.39 (3H, s), 1.98–1.86 (3H, m), 1.84–1.74 (4H, m), 1.68–1.57 (4H, m), 1.56–1.44 (1H, m). ESIMS-LR *m/z* 340 [$(M+H)^+$]; ESIMS-HR calcd for $C_{21}H_{30}N_3O$ $(M+H)^+$ 340.2383, found 340.2392.

5-Methoxy-6-(1-methyl-2,3,4,7-tetrahydroazepin-5-yl)spiro[indole-3,4'-tetrahydropyran]-2-amine (149)



A solution of compound **179d** (44 mg, 0.12 mmol) in THF (2.0 mL) and AcOH (0.14 mL) was treated with Zn (39 mg, 0.59 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **149'** (26 mg, 61%) as a colorless solid. This material was used in the next reaction without further purification. A solution of crude **149'** (20 mg, 0.056 mmol) and Ac_2O (0.0052 mL, 0.056 mmol) in THF (2.0 mL), was treated with 10% Pd/C (10 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 10 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 0:100 to 15:85) to afford compound **149** (11 mg, 58%) as a colorless amorphous. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.26 (1H, s), 6.89 (1H, s), 5.77 (1H, t, *J* = 6.4 Hz), 4.17–4.01 (4H, m), 3.85 (3H, s), 3.27 (2H, d, *J* = 6.1 Hz), 2.91 (2H, t, *J* = 5.5 Hz), 2.58 (2H, t, *J* = 5.2 Hz), 2.38 (3H, s), 2.15 (2H, td, *J* = 12.8, 5.2 Hz), 1.89–1.79 (2H, m), 1.52 (2H, d, *J* = 13.4 Hz). ESIMS-LR *m/z* 342 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_3O_2$ $(M+H)^+$ 342.2176, found 342.2184.

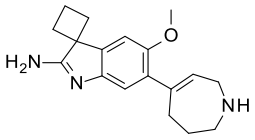
***tert*-Butyl 5-(2'-amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (181)**



A solution of compound **177a** (110 mg, 0.12 mmol) in THF (5.0 mL) and AcOH (0.30 mL) was treated with Zn (80 mg, 1.2 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **181'**

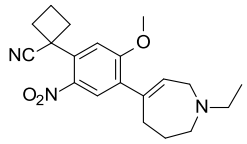
(85 mg, 69%) as a colorless amorphous. This material was used in the next reaction without further purification. A solution of crude **181'** (80 mg, 0.19 mmol) and Ac_2O (0.018 mL, 0.19 mmol) in THF (5.0 mL), was treated with 10% Pd/C (40 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 10 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 0:100 to 12:88] to afford compound **181** (53 mg, 69%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.03 (1H, s), 6.86 (1H, s), 5.81 (1H, s), 4.08–4.02 (1H, m), 3.95 (1H, d, J = 4.9 Hz), 3.84 (3H, s), 3.68–3.55 (2H, m), 2.59–2.47 (6H, m), 2.44–2.32 (1H, m), 2.24–2.12 (1H, m), 1.92–1.81 (2H, m), 1.50–1.45 (9H, m); ESIMS-LR *m/z* 398 [$(M+H)^+$].

5'-Methoxy-6'-(2,3,4,7-tetrahydro-1*H*-azepin-5-yl)spiro[cyclobutane-1,3'-indole]-2'-amine (142)



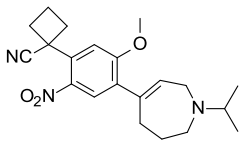
A solution of compound **181** (74 mg, 1.2 mmol) in CH_2Cl_2 (3.0 mL) was treated with TFA (0.5 mL, 7.0 mmol) at room temperature for 12 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 1:99 to 12:88) to afford compound **142** (41 mg, 74%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.03 (1H, s), 6.90 (1H, s), 5.88 (1H, t, *J* = 5.8 Hz), 3.87 (3H, s), 3.46 (2H, d, *J* = 5.5 Hz), 3.13 (2H, t, *J* = 5.5 Hz), 2.60 (2H, t, *J* = 5.2 Hz), 2.55–2.48 (4H, m), 2.45–2.34 (1H, m), 2.25–2.15 (1H, m), 1.80–1.74 (2H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ: 176.6, 152.5, 146.9, 146.1, 139.8, 134.5, 131.0, 116.6, 105.2, 56.4, 53.0, 52.7, 47.7, 33.6, 31.4, 29.4, 16.9. ESIMS-LR *m/z* 298 [$(M+H)^+$]. ESIMS-HR calcd for $C_{18}H_{24}N_3O$ $(M+H)^+$ 298.1914, found 298.1922.

1-[4-(1-Ethyl-2,3,4,7-tetrahydroazepin-5-yl)-5-methoxy-2-nitrophenyl]cyclobutanecarbonitrile (180a)



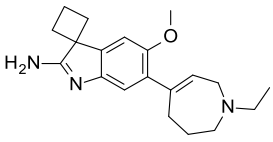
A suspension of compound **178a** (100 mg, 0.31 mmol) and K_2CO_3 (93 mg, 0.67 mmol) in MeCN (2.0 mL) was treated with EtI (0.032 mL, 0.40 mmol) at 0 ºC, and the mixture was stirred at room temperature for 2 h. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; 20:80 to 80:20) to afford compound **180a** (81 mg, 75%) as a pale brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.87 (1H, s), 6.69 (1H, s), 5.87 (1H, t, *J* = 6.1 Hz), 3.92 (3H, s), 3.31 (2H, d, *J* = 6.1 Hz), 3.00–2.91 (4H, m), 2.61 (2H, q, *J* = 7.3 Hz), 2.55–2.48 (4H, m), 1.98–1.91 (1H, m), 1.82–1.75 (1H, m), 1.11 (3H, t, *J* = 7.0 Hz).

1-[4-(1-Isopropyl-2,3,4,7-tetrahydroazepin-5-yl)-5-methoxy-2-nitrophenyl]cyclobutanecarbonitrile (180b)



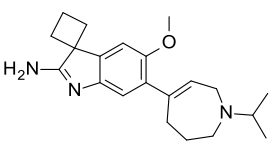
A solution of compound **178a** (80 mg, 0.24 mmol), acetone (30 mg, 0.49 mmol) and AcOH (0.029 mL, 0.49 mmol) in CH_2Cl_2 (1.0 mL) was treated with $NaBH(OAc)_3$ (210 mg, 0.98 mmol) at room temperature for 0.5 h. The reaction was quenched by H_2O (2.0 mL) at room temperature, and the whole mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, AcOEt/hexane; 10:90 to 70:30) to afford compound **180b** (48 mg, 53%) as a pale brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.87 (1H, s), 6.69 (1H, s), 5.89 (1H, t, *J* = 6.1 Hz), 3.92 (3H, s), 3.31 (2H, d, *J* = 6.1 Hz), 3.01–2.92 (5H, m), 2.55–2.46 (5H, m), 1.98–1.91 (1H, m), 1.82–1.73 (2H, m), 1.07 (6H, d, *J* = 6.1 Hz).

6'-(1-Ethyl-2,3,4,7-tetrahydroazepin-5-yl)-5'-methoxy-spiro[cyclobutane-1,3'-indole]-2'-amine (145)



A solution of compound **180a** (74 mg, 0.21 mmol) in THF (2.0 mL) and AcOH (0.25 mL) was treated with Zn (68 mg, 1.0 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **145'** (61 mg, 86%) as a colorless solid. This material was used in the next reaction without further purification. A solution of crude **145'** (60 mg, 0.18 mmol) and Ac_2O (0.017 mL, 0.18 mmol) in THF (2.0 mL), was treated with 10% Pd/C (30 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 10 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 0:100 to 12:88] to afford compound **145** (27 mg, 47%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.02 (1H, s), 6.94 (1H, s), 5.78 (1H, t, *J* = 6.4 Hz), 3.86 (3H, s), 3.33 (2H, d, *J* = 6.1 Hz), 2.99 (2H, t, *J* = 5.5 Hz), 2.66–2.57 (4H, m), 2.54–2.47 (4H, m), 2.44–2.32 (1H, m), 2.25–2.13 (1H, m), 1.79–1.71 (2H, m), 1.09 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ: 176.5, 152.6, 147.23, 147.20, 140.0, 134.2, 127.3, 116.8, 105.2, 59.1, 56.4, 53.0, 52.4, 49.2, 33.9, 31.4, 24.6, 16.9, 12.5. ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_3O$ $(M+H)^+$ 326.2226, found 326.2236.

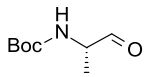
6'-(1-Isopropyl-2,3,4,7-tetrahydroazepin-5-yl)-5'-methoxy-spiro[cyclobutane-1,3'-indole]-2'-amine (146)



A solution of compound **180b** (44 mg, 0.12 mmol) in THF (2.0 mL) and AcOH (0.14 mL) was treated with Zn (39 mg, 0.60 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **146'** (38 mg, 90%) as a colorless solid. This material was used in the next reaction without further purification. A solution of crude **146'** (38 mg, 0.11 mmol) and Ac_2O (0.010 mL, 0.11 mmol) in THF (2.0 mL), was treated with 10% Pd/C (19 mg), and the mixture was vigorously stirred under H_2

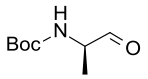
atmosphere (balloon pressure) at room temperature for 10 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 0:100 to 12:88) to afford compound **146** (18 mg, 50%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.02 (1H, s), 6.92 (1H, s), 5.81 (1H, t, *J* = 6.1 Hz), 3.85 (3H, s), 3.35 (2H, d, *J* = 6.1 Hz), 3.06–2.97 (3H, m), 2.59 (2H, t, *J* = 5.5 Hz), 2.51 (4H, t, *J* = 7.9 Hz), 2.44–2.32 (1H, m), 2.24–2.13 (1H, m), 1.78–1.70 (2H, m), 1.07 (6H, d, *J* = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ: 176.5, 152.6, 147.0, 146.6, 139.8, 134.3, 127.9, 116.7, 105.2, 56.4, 55.2, 53.0, 52.0, 50.3, 33.9, 31.4, 25.2, 19.3, 16.9. ESIMS-LR *m/z* 340 [$(M+H)^+$]; ESIMS-HR calcd for $C_{21}H_{30}N_3O$ $(M+H)^+$ 340.2383, found 340.2388.

tert-Butyl _N_-[(1_S_)-1-methyl-2-oxo-ethyl]carbamate (183a)



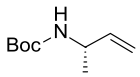
A solution of compound **182a** (800 mg, 4.6 mmol) in CH_2Cl_2 (22 mL) was treated with Dess-Martin periodinane (2.3 g, 5.5 mmol) at room temperature for 0.5 h. The resulting mixture was partitioned between $CHCl_3$ (200 mL) and saturated aqueous $NaHCO_3$ (100 mL), and the organic phase was washed with H_2O (30 mL), and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 40:60) to afford compound **183a** (650 mg, 82%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.57 (1H, s), 5.17–5.01 (1H, m), 4.24 (1H, t, *J* = 6.4 Hz), 1.46 (9H, s), 1.34 (3H, d, *J* = 7.3 Hz).

tert-Butyl _N_-[(1_R_)-1-methyl-2-oxo-ethyl]carbamate (183b)



A solution of compound **182b** (1.2 g, 6.8 mmol) in CH_2Cl_2 (34 mL) was treated with Dess-Martin periodinane (3.5 g, 8.2 mmol) at room temperature for 0.5 h. The resulting mixture was partitioned between $CHCl_3$ (200 mL) and saturated aqueous $NaHCO_3$ (100 mL), and the organic phase was washed with H_2O (30 mL), and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 40:60) to afford compound **183b** (1.0 g, 84%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.57 (1H, s), 5.17–5.01 (1H, m), 4.30–4.20 (1H, m), 1.46 (9H, s), 1.34 (3H, d, *J* = 6.7 Hz).

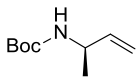
tert-Butyl _N_-[(1_S_)-1-methylallyl]carbamate (184a)



A suspension of potassium *tert*-butoxide (260 mg, 2.3 mmol) in THF (20 mL) was treated with methyltriphenylphosphonium bromide (820 mg, 2.3 mmol) at 0 °C for 0.5 h. Compound **183a** (200 mg, 1.2 mmol) was added to the mixture at 0 °C, and the whole mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, AcOEt/hexane; 0:100 to 20:80) to afford compound **184a** (160 mg, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.89–5.76 (1H, m), 5.14 (1H, d, *J* = 17.6 Hz), 5.06 (1H, d, *J* = 10.3 Hz), 4.45 (1H, br s), 4.29–4.16 (1H, m), 1.45 (9H, s), 1.21 (3H,

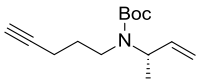
d, *J* = 6.7 Hz).

tert-Butyl _N_-[(1_R_)-1-methylallyl]carbamate (184b)



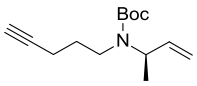
A suspension of potassium *tert*-butoxide (260 mg, 2.3 mmol) in THF (20 mL) was treated with methyltriphenylphosphonium bromide (820 mg, 2.3 mmol) at 0 °C for 0.5 h. Compound **183b** (200 mg, 1.2 mmol) was added to the mixture at 0 °C, and the whole mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, AcOEt/hexane; 0:100 to 20:80) to afford compound **184b** (150 mg, 77%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.89–5.75 (1H, m), 5.14 (1H, d, *J* = 17.1 Hz), 5.06 (1H, d, *J* = 10.3 Hz), 4.45 (1H, br s), 4.27–4.14 (1H, m), 1.45 (9H, s), 1.21 (3H, d, *J* = 7.3 Hz).

tert-Butyl _N_-[(1_S_)-1-methylallyl]-_N_-pent-4-ynyl-carbamate (185a)



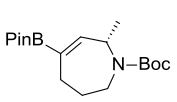
A suspension of compound **184a** (140 mg, 0.82 mmol) and potassium *tert*-butoxide (100 mg, 0.90 mmol) in DMF (1.5 mL) was treated with 5-chloro-1-pentyne (0.094 mL, 0.90 mmol) at room temperature, and the mixture was stirred at 70 ºC for 5 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 10:90) to afford compound **185a** (76 mg, 39%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.92–5.80 (1H, m), 5.13 (1H, d, *J* = 4.3 Hz), 5.09 (1H, d, *J* = 12.1 Hz), 4.83–4.48 (1H, m), 3.22–3.02 (2H, m), 2.21–2.13 (2H, m), 1.95 (1H, t, *J* = 2.4 Hz), 1.85–1.66 (2H, m), 1.47 (9H, s), 1.25 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 182 [(M-^{t}Bu+H)$^+$].

tert-Butyl _N_-[(1_R_)-1-methylallyl]-_N_-pent-4-ynyl-carbamate (185b)



A suspension of compound **184b** (140 mg, 0.82 mmol) and potassium *tert*-butoxide (100 mg, 0.90 mmol) in DMF (1.5 mL) was treated with 5-chloro-1-pentyne (0.12 mL, 1.1 mmol) at room temperature, and the mixture was stirred at 70 ºC for 5 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 10:90) to afford compound **185b** (33 mg, 17%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.92–5.80 (1H, m), 5.12 (1H, d, *J* = 4.3 Hz), 5.09 (1H, d, *J* = 11.6 Hz), 4.82–4.48 (1H, m), 3.22–3.01 (2H, m), 2.21–2.13 (2H, m), 1.95 (1H, t, *J* = 2.7 Hz), 1.84–1.66 (2H, m), 1.47 (9H, s), 1.25 (3H, d, *J* = 7.3 Hz). ESIMS-LR *m/z* 182 [(M-^{t}Bu+H)$^+$].

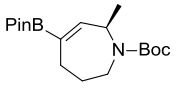
tert-Butyl (7_S_)-7-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (186a)



Step 1. A suspension of LiCl (6.9 mg, 0.16 mmol) in DMF (1.0 mL) was treated with copper (I) chloride (16 mg, 0.16 mmol) at room temperature for 1 h. AcOK (16 mg, 0.16 mmol), bis(pinacolato)diboron (41 mg, 0.16 mmol), and compound **185a** (32 mg, 0.13 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 5 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (50 mL) and saturated aqueous NH_4Cl (30 mL), and the organic phase was washed with H_2O (30 mL), saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 20:80) to afford compound **186a'** (14 mg, 28%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.92–5.80 (1H, m), 5.77 (1H, d, *J* = 3.1 Hz), 5.61 (1H, s), 5.12–5.02 (2H, m), 4.81–4.62 (1H, m), 3.12–2.88 (2H, m), 2.09 (2H, t, *J* = 6.7 Hz), 1.73–1.56 (2H, m), 1.46 (9H, s), 1.32–1.20 (15H, m). ESIMS-LR *m/z* 266 [(M-^{t}BuCO$_2$+H)$^+$].

Step 2. A solution of compound **186a'** (12 mg, 0.032 mmol) in CH_2Cl_2 (1.7 mL) was treated with Grubbs Catalyst, 2nd Generation (1.4 mg, 0.0016 mmol) at room temperature for 16 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **186a** (9.7 mg, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.22–6.13 (1H, m), 5.12–4.82 (1H, m), 3.77–3.53 (1H, m), 3.09–2.94 (1H, m), 2.29–2.18 (2H, m), 2.12–1.90 (2H, m), 1.62–1.54 (1H, m), 1.46 (9H, s), 1.28–1.18 (15H, m). ESIMS-LR *m/z* 282 [(M-^{t}Bu+H)$^+$].

tert-Butyl (7*R*)-7-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (186b)

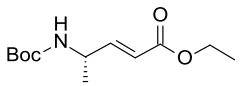


Step 1. A suspension of LiCl (7.0 mg, 0.17 mmol) in DMF (1.5 mL) was treated with copper (I) chloride (17 mg, 0.17 mmol) at room temperature for 1 h. AcOK (16 mg, 0.16 mmol), bis(pinacolato)diboron (42 mg, 0.17 mmol), and compound **185b** (33 mg, 0.14 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 5 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (50 mL) and saturated aqueous NH_4Cl (30 mL), and the organic phase was washed with H_2O (30 mL), saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 20:80) to afford compound **186b'** (45 mg, 89%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.92–5.80 (1H, m), 5.77 (1H, d, *J* = 3.1 Hz), 5.61 (1H, s), 5.12–5.03 (2H, m), 4.81–4.62 (1H, m), 3.12–2.88 (2H, m), 2.09 (2H, t, *J* = 6.7 Hz), 1.72–1.56 (2H, m), 1.46 (9H, s), 1.32–1.20 (15H, m). ESIMS-LR *m/z* 266 [(M-^{t}BuCO$_2$+H)$^+$].

Step 2. A solution of compound **186b'** (45 mg, 0.032 mmol) in CH_2Cl_2 (6.1 mL) was treated with Grubbs Catalyst, 2nd Generation (5.2 mg, 0.050 mmol) at room temperature for 16 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **186b** (40 mg, 96%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.22–6.13 (1H, m), 5.12–4.82 (1H, m), 3.79–3.51 (1H, m), 3.08–2.92 (1H, m), 2.28–2.18 (2H, m), 2.12–1.89 (1H, m), 1.66–1.55 (1H, m), 1.46 (9H, s), 1.29–1.18 (15H, m).

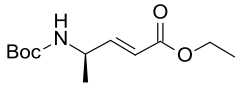
ESIMS-LR *m/z* 282 [(M-^{t}Bu+H)$^+$].

Ethyl (*E*,4*S*)-4-(*tert*-butoxycarbonylamino)pent-2-enoate (187a)



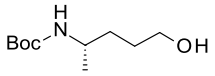
A solution of compound **183a** (460 mg, 2.7 mmol) in THF (20 mL) was treated with [(ethoxycarbonyl)methylene]triphenylphosphorane (1.1 g, 3.2 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **187a** (550 mg, 85%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.90–6.84 (1H, m), 5.93–5.89 (1H, m), 4.51–4.38 (1H, m), 4.20 (2H, q, *J* = 7.1 Hz), 1.45 (9H, s), 1.31–1.26 (6H, m).

Ethyl (*E*,4*R*)-4-(tert-butoxycarbonylamino)pent-2-enoate (187b)



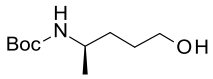
A solution of compound **183b** (760 mg, 4.4 mmol) in THF (20 mL) was treated with [(ethoxycarbonyl)methylene]triphenylphosphorane (1.8 g, 5.3 mmol) at room temperature for 1 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **187b** (820 mg, 77%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.90–6.84 (1H, m), 5.93–5.89 (1H, m), 4.51–4.38 (1H, m), 4.20 (2H, q, *J* = 7.1 Hz), 1.45 (9H, s), 1.32–1.24 (6H, m).

***tert*-Butyl *N*-[(1*S*)-4-hydroxy-1-methyl-butyl]carbamate (188a)**



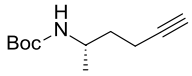
A solution of compound **187a** (520 mg, 2.1 mmol) in MeOH (20 mL) was treated with 10 % Pd/C (110 mg) at room temperature, and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at a room temperature for 1 h. The catalyst was filtered off through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **188a'** (520 mg, 99%) as a colorless oil. This material was used in the next reaction without further purification. A solution of compound **188a'** (520 mg, 2.1 mmol) in THF (20 mL) was treated with lithium borohydride (140 mg, 6.4 mmol) at 0 ºC, and the mixture was stirred at 50 ºC for 3 h. The reaction was quenched by saturated aqueous NH_4Cl (30 mL) at 0 ºC. The resulting mixture was partitioned between AcOEt (50 mL × 3) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 40:60 to 100:0) to afford compound **188a** (420 mg, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.37 (1H, br s), 3.74–3.62 (3H, m), 1.65–1.56 (2H, m), 1.55–1.46 (2H, m), 1.44 (9H, s), 1.13 (3H, d, *J* = 6.7 Hz).

***tert*-Butyl *N*-[(1*R*)-4-hydroxy-1-methyl-butyl]carbamate (188b)**



A solution of compound **187b** (760 mg, 3.1 mmol) in MeOH (20 mL) was treated with 10 % Pd/C (110 mg) at room temperature, and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at a room temperature for 1 h. The catalyst was filtered off through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **188b'** (670 mg, 87%) as a colorless oil. This material was used in the next reaction without further purification. A solution of **188b'** (670 mg, 2.7 mmol) in THF (20 mL) was treated with lithium borohydride (180 mg, 8.2 mmol) at 0 ºC, and the mixture was stirred at 50 ºC for 3 h. The reaction was quenched by saturated aqueous NH_4Cl (30 mL) at 0 ºC. The resulting mixture was partitioned between AcOEt (50 mL × 3) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 40:60 to 100:0) to afford compound **188b** (420 mg, 76%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.37 (1H, br s), 3.75–3.61 (3H, m), 1.65–1.57 (2H, m), 1.53–1.47 (2H, m), 1.44 (9H, s), 1.13 (3H, d, *J* = 6.7 Hz).

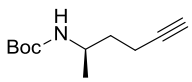
tert-Butyl _N_-[(1_S_)-1-methylpent-4-ynyl]carbamate (189a)



Step 1. A solution of compound **188a** (420 mg, 2.1 mmol) in CH_2Cl_2 (10 mL) was treated with Dess-Martin periodinane (1.0 g, 1.4 mmol) at room temperature for 0.5 h. The resulting mixture was partitioned between $CHCl_3$ (200 mL) and saturated aqueous $NaHCO_3$ (100 mL), and the organic phase was washed with H_2O (30 mL), and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 40:60) to afford the aldehyde (280 mg, 67%) as a colorless oil.

Step 2. A suspension of the aldehyde (240 mg, 1.2 mmol) and K_2CO_3 (0.33 g, 2.4 mmol) in MeOH (10 mL) was treated with dimethyl (1-diazo-2-oxopropyl)phosphonate (0.20 mL, 1.3 mmol) at room temperature for 15 h. The resulting mixture was partitioned between AcOEt (100 mL) and saturated aqueous $NaHCO_3$ (50 mL), and the organic phase was washed with H_2O (50 mL), and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **189a** (120 mg, 51%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.36 (1H, br s), 3.81–3.68 (1H, m), 2.24 (2H, td, *J* = 7.6, 2.4 Hz), 1.96 (1H, t, *J* = 2.4 Hz), 1.70–1.62 (2H, m), 1.44 (9H, s), 1.15 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 142 [(M-^{t}Bu+H)$^+$].

tert-Butyl _N_-[(1_R_)-1-methylpent-4-ynyl]carbamate (189b)

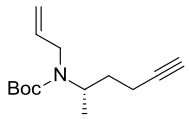


Step 1. A solution of compound **188b** (420 mg, 2.1 mmol) in CH_2Cl_2 (10 mL) was treated with Dess-Martin periodinane (1.0 g, 1.4 mmol) at room temperature for 0.5 h. The resulting mixture was partitioned between $CHCl_3$ (200 mL) and saturated aqueous $NaHCO_3$ (100 mL), and the organic phase was washed with H_2O (30 mL), and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 40:60) to afford the

aldehyde (330 mg, 79%) as a colorless oil.

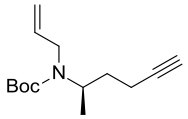
Steps 2. A suspension of the aldehyde (300 mg, 1.5 mmol) and K_2CO_3 (0.42 g, 3.0 mmol) in MeOH (10 mL) was treated with dimethyl (1-diazo-2-oxopropyl)phosphonate (0.25 mL, 1.6 mmol) at room temperature for 15 h. The resulting mixture was partitioned between AcOEt (100 mL) and saturated aqueous $NaHCO_3$ (50 mL), and the organic phase was washed with H_2O (50 mL), and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **189b** (74 mg, 25%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.36 (1H, br s), 3.82–3.68 (1H, m), 2.24 (2H, td, *J* = 7.3, 2.4 Hz), 1.96 (1H, t, *J* = 2.4 Hz), 1.72–1.61 (2H, m), 1.44 (9H, s), 1.15 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 142 [(M-^{t}Bu+H)$^+$].

tert-Butyl _N_-allyl-_N_-[(1_S_)-1-methylpent-4-ynyl]carbamate (190a)



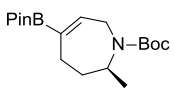
A suspension of compound **189a** (120 mg, 0.61 mmol) and allyl iodide (0.061 mL, 0.67 mmol) in DMF (4.0 mL) was treated with NaH (33 mg, 55% dispersion of mineral oil, 0.72 mmol) at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 15:85) to afford compound **190a** (120 mg, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.92–5.75 (1H, m), 5.20–5.02 (2H, m), 4.31–4.13 (1H, m), 3.83–3.59 (2H, m), 2.16 (2H, t, *J* = 7.0 Hz), 1.95 (1H, t, *J* = 2.7 Hz), 1.87–1.76 (1H, m), 1.67–1.57 (1H, m), 1.46 (9H, s), 1.15 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 182 [(M-^{t}Bu+H)$^+$].

tert-Butyl _N_-allyl-_N_-[(1_R_)-1-methylpent-4-ynyl]carbamate (190b)



A suspension of compound **189b** (74 mg, 0.38 mmol) and allyl iodide (0.037 mL, 0.41 mmol) in DMF (2.0 mL) was treated with NaH (20 mg, 55% dispersion of mineral oil, 0.45 mmol) at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 15:85) to afford compound **190b** (64 mg, 72%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.91–5.76 (1H, m), 5.19–5.04 (2H, m), 4.30–4.13 (1H, m), 3.83–3.59 (2H, m), 2.21–2.11 (2H, m), 1.99–1.91 (1H, m), 1.88–1.76 (1H, m), 1.67–1.57 (1H, m), 1.46 (9H, s), 1.15 (3H, d, *J* = 6.1 Hz). ESIMS-LR *m/z* 182 [(M-^{t}Bu+H)$^+$].

tert-Butyl (2_S_)-2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (191a)

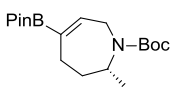


Step 1. A suspension of LiCl (26 mg, 0.61 mmol) in DMF (5.0 mL) was treated with copper (I) chloride (61 mg, 0.61 mmol) at room temperature for 1 h. AcOK (60 mg, 0.61 mmol), bis(pinacolato)diboron (150 mg, 0.61 mmol), and compound

190a (120 mg, 0.51 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 15 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (50 mL) and saturated aqueous NH_4Cl (30 mL), and the organic phase was washed with H_2O (30 mL), saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 20:80) to afford compound **191a'** (110 mg, 60%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.91–5.80 (1H, m), 5.76 (1H, s), 5.60 (1H, s), 5.17–5.00 (2H, m), 4.26–4.13 (1H, m), 3.87–3.60 (2H, m), 2.15–2.04 (2H, m), 1.45 (9H, s), 1.26 (12H, s), 1.15–1.07 (3H, m). ESIMS-LR *m/z* 266 [(M-^{t}BuCO$_2$+H)$^+$].

Step 2. A solution of compound **191a'** (90 mg, 0.25 mmol) in CH_2Cl_2 (12 mL) was treated with Grubbs Catalyst, 2nd Generation (10 mg, 0.012 mmol) at room temperature for 2 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **191a** (42 mg, 50%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.47 (1H, d, *J* = 9.8 Hz), 4.36 (1H, d, *J* = 11.3 Hz), 4.13 (1H, d, *J* = 11.3 Hz), 3.60–3.56 (1H, m), 2.48–2.33 (1H, m), 2.21–2.06 (1H, m), 1.93–1.77 (1H, m), 1.64–1.57 (1H, m), 1.44 (9H, d, *J* = 12.8 Hz), 1.25 (12H, s), 1.09 (3H, d, *J* = 6.1 Hz). ESIMS-LR *m/z* 282 [(M-^{t}Bu+H)$^+$].

tert-Butyl (2*R*)-2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (191b)

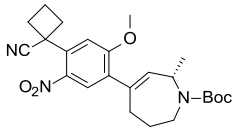


Step 1. A suspension of LiCl (13 mg, 0.30 mmol) in DMF (2.0 mL) was treated with copper (I) chloride (30 mg, 0.30 mmol) at room temperature for 1 h. AcOK (30 mg, 0.30 mmol), bis(pinacolato)diboron (77 mg, 0.30 mmol), and compound **190b** (60 mg, 0.25 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 5 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (50 mL) and saturated aqueous NH_4Cl (30 mL), and the organic phase was washed with H_2O (30 mL), saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 20:80) to afford compound **191b'** (58 mg, 63%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.92–5.80 (1H, m), 5.76 (1H, s), 5.60 (1H, s), 5.18–5.00 (2H, m), 4.28–4.12 (1H, m), 3.85–3.61 (2H, m), 2.15–2.05 (2H, m), 1.45 (9H, s), 1.26 (12H, s), 1.16–1.07 (3H, m). ESIMS-LR *m/z* 266 [(M-^{t}BuCO$_2$+H)$^+$].

Step 2. A solution of compound **191b'** (50 mg, 0.14 mmol) in CH_2Cl_2 (6.8 mL) was treated with Grubbs Catalyst, 2nd Generation (5.8 mg, 0.050 mmol) at room temperature for 2 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 30:70) to afford compound **191b** (29 mg, 63%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.47 (1H, d, *J* = 9.2 Hz), 4.42–4.31 (1H, m), 4.18–4.07 (1H, m), 3.60–3.56 (1H, m), 2.46–2.33 (1H, m), 2.21-2.06 (1H, m), 1.92–1.78 (1H, m), 1.65–1.57 (1H, m), 1.44 (9H, d, *J* = 12.8 Hz), 1.25 (12H, s), 1.09 (3H, d, *J* = 6.1 Hz). ESIMS-LR *m/z* 282 [(M-^{t}Bu+H)$^+$].

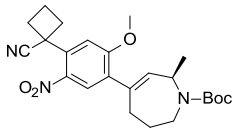
tert-Butyl (7*S*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-7-methyl-2,3,4,7-

tetrahydroazepine-1-carboxylate (192a)



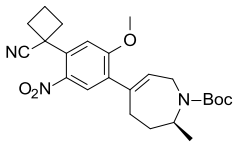
Following general procedure C using compound **176a** (12 mg, 0.032 mmol), compound **186a** (9.1 mg, 0.027 mmol), K_2CO_3 (7.5 mg, 0.054 mmol), $Pd(dppf)Cl_2$ (3.9 mg, 0.0054 mmol), 1,4-dioxane (3.0 mL) and H_2O (1.0 mL), the title compound, **192a** (7.7 mg, 65%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.82 (1H, s), 6.71 (1H, s), 5.43 (1H, s), 5.18–4.89 (1H, m), 3.92 (3H, s), 3.86–3.63 (1H, m), 3.23–3.12 (1H, m), 3.02–2.89 (2H, m), 2.70–2.59 (1H, m), 2.55–2.44 (3H, m), 2.16–2.01 (2H, m), 1.97–1.89 (1H, m), 1.80–1.69 (1H, m), 1.50 (9H, s), 1.31–1.22 (3H, m). ESIMS-LR *m/z* 342 [(M-^{t}BuCO$_2$+H)$^+$].

***tert*-Butyl (7*R*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-7-methyl-2,3,4,7-tetrahydroazepine-1-carboxylate (192b)**



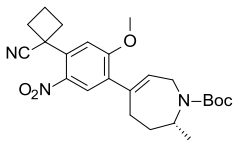
Following general procedure C using compound **176a** (54 mg, 0.14 mmol), compound **186b** (40 mg, 0.12 mmol), K_2CO_3 (33 mg, 0.24 mmol), $Pd(dppf)Cl_2$ (17 mg, 0.024 mmol), 1,4-dioxane (3.0 mL) and H_2O (1.0 mL), the title compound, **192b** (52 mg, 99%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.82 (1H, s), 6.71 (1H, s), 5.43 (1H, s), 5.18–4.89 (1H, m), 3.92 (3H, s), 3.88–3.63 (1H, m), 3.24–3.12 (1H, m), 3.01–2.89 (2H, m), 2.70–2.58 (1H, m), 2.55–2.44 (3H, m), 2.16–2.00 (2H, m), 2.00–1.90 (1H, m), 1.81–1.69 (1H, m), 1.50 (9H, s), 1.30–1.23 (3H, m). ESIMS-LR *m/z* 342 [(M-^{t}BuCO$_2$+H)$^+$].

***tert*-Butyl (2*S*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-2-methyl-2,3,4,7-tetrahydroazepine-1-carboxylate (192c)**



Following general procedure C using compound **176a** (46 mg, 0.12 mmol), compound **191a** (34 mg, 0.10 mmol), K_2CO_3 (28 mg, 0.20 mmol), $Pd(dppf)Cl_2$ (15 mg, 0.020 mmol), 1,4-dioxane (3.0 mL) and H_2O (1.0 mL), the title compound, **192c** (34 mg, 76%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.81 (1H, d, *J* = 11.0 Hz), 6.69 (1H, s), 5.74 (1H, br s), 4.53–4.31 (1H, m), 4.29–4.05 (1H, m), 3.89 (3H, s), 3.68–3.64 (1H, m), 3.00-2.91 (2H, m), 2.58–2.44 (4H, m), 2.35–2.21 (1H, m), 2.01–1.89 (2H, m), 1.85–1.76 (1H, m), 1.51–1.43 (9H, m), 1.16 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 342 [(M-^{t}BuCO$_2$+H)$^+$].

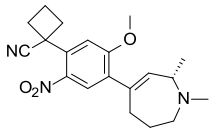
***tert*-Butyl (2*R*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-2-methyl-2,3,4,7-tetrahydroazepine-1-carboxylate (192d)**



Following general procedure C using compound **176a** (42 mg, 0.11 mmol), compound **191b** (31 mg, 0.092 mmol), K_2CO_3 (25 mg, 0.18 mmol), $Pd(dppf)Cl_2$ (14 mg, 0.018 mmol), 1,4-dioxane (3.0 mL) and H_2O (1.0 mL), the title compound, **192d** (38 mg, 93%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.81 (1H, d, *J* = 11.0 Hz), 6.70 (1H, s), 5.81–5.70 (1H, m), 4.52–4.33 (1H, m), 4.29–4.06 (1H, m), 3.89 (3H, s), 3.68–3.64 (1H, m), 3.01–2.91 (2H, m),

2.59–2.44 (4H, m), 2.36–2.21 (1H, m), 2.01–1.90 (2H, m), 1.85–1.76 (1H, m), 1.51–1.43 (9H, m), 1.16 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 342 [(M-^{t}BuCO$_2$+H)$^+$].

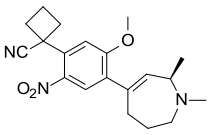
1-[4-[(7*S*)-1,7-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5-methoxy-2-nitrophenyl]cyclobutanecarbonitrile (193a)



Step 1. Following general procedure D using compound **192a** (7.0 mg, 0.016 mmol), CH_2Cl_2 (2.0 mL) and TFA (1.0 mL, 13 mmol), the title compound, **S-193a'** (5.6 mg, quant.), was obtained as a colorless oil.

Step 2. Following general procedure E using compound **193a'** (5.6 mg, 0.016 mmol), formaldehyde (2.0 μL, 37% solution, 0.025 mmol), AcOH (2.0 μL, 0.033 mmol), $NaBH(OAc)_3$ (14 mg, 0.066 mmol) and CH_2Cl_2 (1.0 mL), the title compound, **193a** (3.8 mg, 65%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.85 (1H, s), 6.71 (1H, s), 5.54 (1H, d, *J* = 4.3 Hz), 3.94 (3H, s), 3.81–3.72 (1H, m), 3.25–3.10 (2H, m), 3.03–2.91 (2H, m), 2.68 (1H, t, *J* = 13.1 Hz), 2.58–2.47 (3H, m), 2.45–2.35 (1H, m), 2.37 (3H, s), 2.03–1.90 (2H, m), 1.47–1.36 (1H, m), 1.28 (3H, d, *J* = 7.3 Hz). ESIMS-LR *m/z* 356 [(M+H)$^+$].

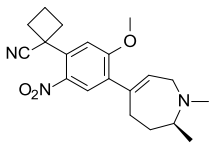
1-[4-[(7*R*)-1,7-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5-methoxy-2-nitrophenyl]cyclobutanecarbonitrile (193b)



Step 1. Following general procedure D using compound **192b** (26 mg, 0.059 mmol), CH_2Cl_2 (2.0 mL) and TFA (1.0 mL, 13 mmol), the title compound, **S-193b'** (19 mg, 95%), was obtained as a colorless oil.

Step 2. Following general procedure E using compound **193b'** (19 mg, 0.056 mmol), formaldehyde (6.8 μL, 37% solution, 0.083 mmol), AcOH (6.7 μL, 0.11 mmol), $NaBH(OAc)_3$ (47 mg, 0.22 mmol) and CH_2Cl_2 (1.0 mL), the title compound, **193b** (18 mg, 91%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.85 (1H, s), 6.71 (1H, s), 5.54 (1H, d, *J* = 5.5 Hz), 3.94 (3H, s), 3.81–3.73 (1H, m), 3.24–3.10 (2H, m), 3.01–2.91 (2H, m), 2.68 (1H, t, *J* = 13.1 Hz), 2.57–2.47 (3H, m), 2.45–2.36 (1H, m), 2.37 (3H, s), 2.03–1.90 (2H, m), 1.45–1.36 (1H, m), 1.28 (3H, d, *J* = 7.3 Hz). ESIMS-LR *m/z* 356 [(M+H)$^+$].

1-[4-[(2*S*)-1,2-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5-methoxy-2-nitrophenyl]cyclobutanecarbonitrile (193c)

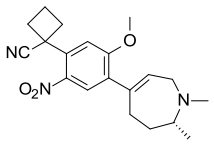


Step 1. Following general procedure D using compound **192c** (16 mg, 0.036 mmol), CH_2Cl_2 (2.0 mL) and TFA (1.0 mL, 13 mmol), the title compound, **193c'** (11 mg, 89%), was obtained as a colorless oil.

Step 2. Following general procedure E using compound **193c'** (11 mg, 0.032 mmol), formaldehyde (3.9 μL, 37% solution, 0.048 mmol), AcOH (3.9 μL, 0.064 mmol), $NaBH(OAc)_3$ (27 mg, 0.13 mmol) and CH_2Cl_2 (1.0 mL), the title compound, **193c** (9.8 mg, 86%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.87 (1H, s), 6.71 (1H, s), 5.77 (1H, t, *J* = 5.2 Hz), 3.93 (3H, s), 3.51–3.33 (1H, m), 3.01–2.88 (3H, m), 2.58–2.44 (5H, m), 2.38 (3H, s), 1.99–1.89 (1H, m), 1.82–1.73 (1H, m), 1.71–1.61 (1H, m), 1.15 (3H, d, *J* = 6.7 Hz).

ESIMS-LR *m/z* 356 [(M+H)$^+$].

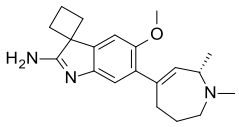
1-[4-[(2*R*)-1,2-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5-methoxy-2-nitrophenyl]cyclobutanecarbonitrile (193d)



Step 1. Following general procedure D using compound **192d** (15 mg, 0.036 mmol), CH_2Cl_2 (2.0 mL) and TFA (1.0 mL, 13 mmol), the title compound, **193d'** (10 mg, 86%), was obtained as a colorless oil.

Step 2. Following general procedure E using compound **193d'** (11 mg, 0.032 mmol), formaldehyde (3.9 μL, 37% solution, 0.048 mmol), AcOH (3.9 μL, 0.064 mmol), $NaBH(OAc)_3$ (27 mg, 0.13 mmol) and CH_2Cl_2 (1.0 mL), the title compound, **193d** (9.6 mg, 84%), was obtained as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.87 (1H, s), 6.71 (1H, s), 5.77 (1H, t, *J* = 5.5 Hz), 3.93 (3H, s), 3.51–3.33 (1H, m), 3.01–2.89 (3H, m), 2.57–2.45 (5H, m), 2.38 (3H, s), 2.00–1.89 (1H, m), 1.83–1.73 (1H, m), 1.71–1.62 (1H, m), 1.15 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 356 [(M+H)$^+$].

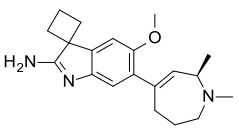
6'-[(7*S*)-1,7-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5'-methoxy-spiro[cyclobutane-1,3'-indole]-2'-amine (150)



A solution of compound **193a** (3.8 mg, 0.011 mmol) in THF (1.0 mL) and AcOH (0.013 mL) was treated with Zn (3.5 mg, 0.053 mmol) at room temperature, and the mixture was stirred at 50 °C for 20 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford the crude (3.3 mg) as a colorless solid. This material was used in the next reaction without further purification. A solution of the crude (3.3 mg, 0.0097 mmol) and Ac_2O (0.9 μL, 0.0097 mmol) in THF (1.0 mL), was treated with 10% Pd/C (2 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt= 1:1); 0:100 to 20:80] to afford compound **150** (1.9 mg, 54% over 2 steps) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, s), 6.93 (1H, s), 5.45 (1H, d, *J* = 4.9 Hz), 3.87 (3H, s), 3.85–3.77 (1H, m), 3.27–3.12 (2H, m), 2.73 (1H, t, *J* = 13.1 Hz), 2.56–2.45 (1H, m), 2.44–2.36 (1H, m), 2.37 (3H, s), 2.26–2.14 (1H, m), 2.08–1.95 (1H, m), 1.39–1.28 (1H, m), 1.26 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 326 [(M+H)$^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_3O$ (M+H)$^+$ 326.2232, found 326.2218.

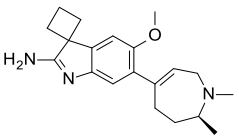
6'-[(7*R*)-1,7-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5'-methoxy-spiro[cyclobutane-1,3'-indole]-2'-amine (151)

A solution of compound **193b** (18 mg, 0.051 mmol) in THF (1.0 mL) and AcOH (0.060 mL) was treated with Zn (17 mg, 0.25 mmol) at room temperature, and the mixture was stirred at 50 °C for 20 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford the crude (16 mg) as a



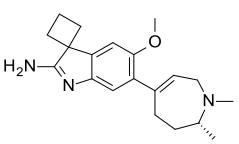
colorless solid. This material was used in the next reaction without further purification. A solution of the crude (16 mg, 0.050 mmol) and Ac_2O (4.7 μL, 0.050 mmol) in THF (1.0 mL), was treated with 10% Pd/C (4 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt= 1:1); 0:100 to 20:80] to afford compound **151** (13 mg, 74% over 2 steps) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, s), 6.93 (1H, s), 5.45 (1H, d, *J* = 5.5 Hz), 3.87 (3H, s), 3.85–3.77 (1H, m), 3.26–3.12 (2H, m), 2.73 (1H, t, *J* = 13.4 Hz), 2.56–2.45 (1H, m), 2.44–2.34 (1H, m), 2.36 (3H, s), 2.26–2.14 (1H, m), 2.06–1.93 (1H, m), 1.37–1.28 (1H, m), 1.25 (3H, d, *J* = 7.3 Hz). ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_3O$ $(M+H)^+$ 326.2232, found 326.2236.

6'-[(2S)-1,2-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5'-methoxy-spiro[cyclobutane-1,3'-indole]-2'-amine (152)



A solution of compound **193c** (9.1 mg, 0.026 mmol) in THF (1.0 mL) and AcOH (0.031 mL) was treated with Zn (8.4 mg, 0.13 mmol) at room temperature, and the mixture was stirred at 50 °C for 20 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford the crude (7.7 mg) as a colorless solid. This material was used in the next reaction without further purification. A solution of the crude (7.7 mg, 0.023 mmol) and Ac_2O (2.8 μL, 0.023 mmol) in THF (1.0 mL), was treated with 10% Pd/C (4 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt= 1:1); 0:100 to 20:80] to afford compound **152** (5.7 mg, 69% over 2 steps) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, s), 6.93 (1H, s), 5.69 (1H, t, *J* = 5.8 Hz), 3.86 (3H, s), 3.49 (1H, dd, *J* = 15.3, 6.1 Hz), 3.39 (1H, dd, *J* = 15.6, 5.2 Hz), 2.99–2.92 (1H, m), 2.59–2.48 (6H, m), 2.45–2.38 (1H, m), 2.36 (3H, s), 2.25–2.13 (1H, m), 1.72–1.64 (2H, m), 1.15 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_3O$ $(M+H)^+$ 326.2232, found 326.2232.

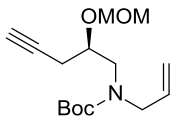
6'-[(2*R*)-1,2-Dimethyl-2,3,4,7-tetrahydroazepin-5-yl]-5'-methoxy-spiro[cyclobutane-1,3'-indole]-2'-amine (153)



A solution of compound **193d** (8.8 mg, 0.025 mmol) in THF (1.0 mL), AcOH (0.030 mL) was treated with Zn (8.1 mg, 0.12 mmol) at room temperature, and the mixture was stirred at 50 °C for 20 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford the crude (8.2 mg) as a colorless solid. This material was used in the next reaction without further purification. A solution of the crude (8.2

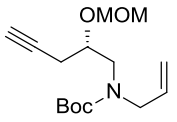
mg, 0.024 mmol) and Ac_2O (2.3 μL, 0.024 mmol) in THF (1.0 mL), was treated with 10% Pd/C (4 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/(hexane:AcOEt= 1:1); 0:100 to 20:80) to afford compound **153** (6.6 mg, 81% over 2 steps) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.03 (1H, s), 6.93 (1H, s), 5.69 (1H, t, *J* = 5.8 Hz), 3.86 (3H, s), 3.49 (1H, dd, *J* = 15.7, 6.1 Hz), 3.40 (1H, dd, *J* = 15.7, 5.2 Hz), 3.00–2.92 (1H, m), 2.59-2.48 (6H, m), 2.45–2.38 (1H, m), 2.36 (3H, s), 2.25-2.14 (1H, m), 1.72-1.64 (2H, m), 1.15 (3H, d, *J* = 6.7 Hz). ESIMS-LR *m/z* 326 [$(M+H)^+$]; ESIMS-HR calcd for $C_{20}H_{28}N_3O$ $(M+H)^+$ 326.2232, found 326.2230.

***tert*-Butyl *N*-allyl-*N*-[(2*R*)-2-(methoxymethoxy)pent-4-ynyl]carbamate (195a)**



A solution of compound **194a** (1.9 g, 13 mmol), allylamine hydrochloride (5.0 g, 53 mmol) and AcOH (1.6 mL, 27 mmol) in CH_2Cl_2 (100 mL) was treated with $NaBH(OAc)_3$ (5.7 g, 27 mmol) at room temperature for 0.5 h. The reaction was quenched by $NaHCO_3$ (10 mL) at room temperature, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO2, AcOEt/hexane; 10:90 to 70:30) to afford free amine (1.2 g, 49%) as a colorless oil. A solution of the free amine (1.5 g, 8.2 mmol) and Et_3N (1.4 mL, 9.8 mmol) in THF (40 mL) was treated with Boc_2O (2.0 g, 9.0 mmol) at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (200 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, AcOEt/hexane; 0:100 to 35:65) to afford compound **195a** (2.0 g, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.85–5.70 (1H, m), 5.18–5.05 (2H, m), 4.77–4.60 (2H, m), 4.10–3.91 (2H, m), 3.84 (1H, d, *J* = 14.6 Hz), 3.53–3.44 (1H, m), 3.38 (3H, s), 3.33–3.15 (1H, m), 2.55–2.38 (2H, m), 2.02 (1H, t, *J* = 2.7 Hz), 1.46 (9H, d, *J* = 9.8 Hz). ESIMS-LR *m/z* 184 [$(M-tBuCO_2+H)^+$].

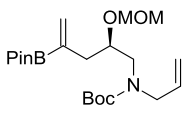
***tert*-Butyl *N*-allyl-*N*-[(2*S*)-2-(methoxymethoxy)pent-4-ynyl]carbamate (195b)**



A solution of compound **194b** (550 mg, 3.9 mmol), allylamine hydrochloride (1.4 g, 15 mmol) and AcOH (0.46 mL, 7.7 mmol) in CH_2Cl_2 (30 mL) was treated with $NaBH(OAc)_3$ (1.6 g, 7.7 mmol) at room temperature for 0.5 h. The reaction was quenched by $NaHCO_3$ (10 mL) at room temperature, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO2, AcOEt/hexane; 10:90 to 70:30) to afford free amine (510 mg, 72%) as a colorless oil. A solution of the free amine (510 mg, 3.3 mmol) and Et_3N (0.46 mL, 3.3 mmol) in THF (10 mL) was treated with Boc_2O (670 mg, 3.1 mmol) at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (200 mL) and H_2O (100 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, AcOEt/hexane; 0:100 to 35:65) to afford compound **195b** (520 mg, 65%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.86–5.70 (1H, m), 5.20–5.05 (2H, m), 4.73 (1H, d, *J* = 6.1 Hz),

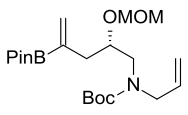
4.70–4.62 (1H, m), 4.12-3.90 (2H, m), 3.89–3.78 (1H, m), 3.53–3.44 (1H, m), 3.38 (3H, s), 3.34–3.16 (1H, m), 2.55–2.38 (2H, m), 2.02 (1H, t, *J* = 2.4 Hz), 1.46 (9H, d, *J* = 9.8 Hz). ESIMS-LR *m/z* 184 [(M-tBuCO$_2$+H)$^+$].

tert-Butyl *N*-allyl-*N*-[(2*R*)-2-(methoxymethoxy)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pent-4-enyl]carbamate (196a)



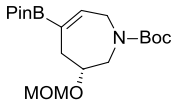
A suspension of LiCl (170 mg, 4.0 mmol) in DMF (30 mL) was treated with copper (I) chloride (400 mg, 4.0 mmol) at room temperature for 1 h. AcOK (390 mg, 4.0 mmol), bis(pinacolato)diboron (1.0 g, 4.0 mmol), and compound **195a** (940 mg, 3.3 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 5 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (200 mL) and saturated aqueous NH_4Cl (100 mL), and the organic phase was washed with H_2O (100 mL) and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 25:75) to afford compound **196a** (1.3 g, 95%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.88–5.65 (2H, m), 5.19–5.03 (2H, m), 4.76–4.54 (2H, m), 4.11–3.78 (2H, m), 3.51–3.16 (5H, m), 2.55-2.23 (2H, m), 1.46 (9H, d, *J* = 11.0 Hz), 1.27 (12H, s). ESIMS-LR *m/z* 312 [(M-^{t}BuCO$_2$+H)$^+$].

tert-Butyl *N*-allyl-*N*-[(2*S*)-2-(methoxymethoxy)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pent-4-enyl]carbamate (196b)



A suspension of LiCl (84 mg, 2.0 mmol) in DMF (10 mL) was treated with copper (I) chloride (200 mg, 2.0 mmol) at room temperature for 1 h. AcOK (200 mg, 2.0 mmol), bis(pinacolato)diboron (500 mg, 2.0 mmol), and compound **195b** (470 mg, 1.7 mmol) were added to the mixture at room temperature, and the whole mixture was stirred at room temperature for 5 h. The resulting mixture was filtered through a pad of Celite and washed with AcOEt. The filtrate was partitioned between AcOEt (200 mL) and saturated aqueous NH_4Cl (100 mL), and the organic phase was washed with H_2O (100 mL) and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 25:75) to afford compound **196b** (530 mg, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.88–5.67 (3H, m), 5.18–5.04 (2H, m), 4.75–4.54 (2H, m), 4.04–3.78 (2H, m), 3.49–3.10 (5H, m), 2.46–2.20 (2H, m), 1.46 (9H, d, *J* = 13.4 Hz), 1.26 (12H, s). ESIMS-LR *m/z* 312 [(M-^{t}BuCO$_2$+H)$^+$].

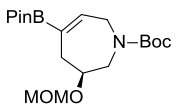
tert-Butyl (3*R*)-3-(methoxymethoxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (197a)



A solution of compound **196a** (1.3 g, 3.2 mmol) in CH_2Cl_2 (160 mL) was treated with Grubbs Catalyst, 2nd Generation (130 mg, 0.16 mmol) at room temperature for 2 days. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 25:75) to afford compound

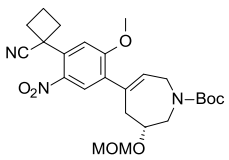
197a (370 mg, 31%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.59–6.49 (1H, m), 4.71 (1H, dd, *J* = 6.7, 3.1 Hz), 4.61 (1H, t, *J* = 6.7 Hz), 4.37–4.18 (1H, m), 4.02–3.78 (3H, m), 3.38 (3H, s), 3.25–3.17 (1H, m), 2.58–2.49 (2H, m), 1.45 (9H, d, *J* = 7.3 Hz), 1.25 (12H, s). ESIMS-LR *m/z* 284 [(M-^{t}BuCO$_2$+H)$^+$].

tert-Butyl (3*S*)-3-(methoxymethoxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3,4,7-tetrahydroazepine-1-carboxylate (197b)



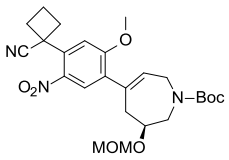
A solution of compound **196b** (530 mg, 1.3 mmol) in CH_2Cl_2 (60 mL) was treated with Grubbs Catalyst, 2nd Generation (55 mg, 0.064 mmol) at room temperature for 2 days. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 0:100 to 25:75) to afford compound **197b** (290 mg, 59%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.54 (1H, d, *J* = 11.6 Hz), 4.71 (1H, dd, *J* = 6.7, 2.4 Hz), 4.61 (1H, t, *J* = 6.4 Hz), 4.37–4.18 (1H, m), 4.02–3.78 (3H, m), 3.38 (3H, s), 3.26–3.15 (1H, m), 2.60–2.49 (2H, m), 1.45 (9H, d, *J* = 7.3 Hz), 1.25 (12H, s). ESIMS-LR *m/z* 284 [(M-^{t}BuCO$_2$+H)$^+$].

tert-Butyl (3*R*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-3-(methoxymethoxy)-2,3,4,7-tetrahydroazepine-1-carboxylate (198a)



A solution of compound **197a** (130 mg, 0.34 mmol), compound **176a** (160 mg., 0.41 mmol) and K_2CO_3 (94 mg, 0.68 mmol) in 1,4-dioxane (4.0 mL) and H_2O (1.0 mL) was treated with Pd(dppf)Cl_2 (50 mg, 0.068 mmol) at room temperature, and the mixture was stirred at 120 °C for 1 h with μW irradiation, and then the mixture was cooled to room temperature. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, AcOEt/hexane; 20:80 to 80:20) to afford compound **198a** (160 mg, 97%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.87 (1H, d, *J* = 5.5 Hz), 6.72 (1H, d, *J* = 4.3 Hz), 5.98 (1H, t, *J* = 4.9 Hz), 4.70–4.62 (2H, m), 4.19–4.09 (1H, m), 4.04–3.97 (1H, m), 3.93 (3H, s), 3.87–3.76 (1H, m), 3.38–3.26 (1H, m), 3.36 (3H, s), 3.01–2.92 (2H, m), 2.73-2.65 (2H, m), 2.56–2.47 (3H, m), 1.98–1.91 (2H, m), 1.48 (9H, d, *J* = 9.2 Hz). ESIMS-LR *m/z* 388 [(M-^{t}BuCO$_2$+H)$^+$].

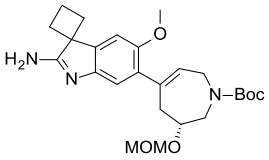
tert-Butyl (3*S*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-3-(methoxymethoxy)-2,3,4,7-tetrahydroazepine-1-carboxylate (198b)



A solution of compound **197b** (280 mg, 0.73 mmol), compound **176a** (330 mg., 0.88 mmol) and K_2CO_3 (210 mg, 1.5 mmol) in 1,4-dioxane (5.0 mL) and H_2O (1.0 mL) was treated with Pd(dppf)Cl_2 (110 mg, 0.15 mmol) at room temperature, and the mixture was stirred at 120 °C for 1 h with MW irradiation, and then the mixture was cooled to room temperature. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, AcOEt/hexane; 20:80 to 80:20) to afford compound **198b** (330 mg, 93%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.87 (1H, d, *J* = 5.5 Hz), 6.72 (1H, d, *J* = 4.3 Hz), 5.98 (1H, t, *J* = 5.2 Hz), 4.70–4.62 (2H,

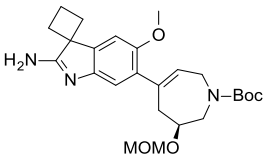
m), 4.18–4.09 (1H, m), 4.03–3.97 (1H, m), 3.93 (3H, s), 3.86–3.75 (1H, m), 3.37–3.26 (1H, m), 3.36 (3H, s), 3.02–2.93 (2H, m), 2.73–2.66 (2H, m), 2.57–2.46 (3H, m), 1.96–1.91 (2H, m), 1.48 (9H, d, *J* = 9.2 Hz). ESIMS-LR *m/z* 388 [(M-^{t}BuCO$_2$+H)$^+$].

***tert*-Butyl (3*R*)-5-(2'-amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-3-(methoxymethoxy)-2,3,4,7-tetrahydroazepine-1-carboxylate (199a)**



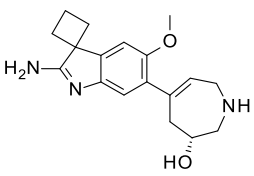
A solution of compound **198a** (30 mg, 0.061 mmol) in THF (2.0 mL) and AcOH (0.074 mL) was treated with Zn (20 mg, 0.31 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **199a'** (23 mg, 79%) as a colorless amorphous. This material was used in the next reaction without further purification. A solution of crude **199a'** (21 mg, 0.044 mmol) and Ac$_2$O (0.0041 mL, 0.044 mmol) in THF (2.0 mL), was treated with 10% Pd/C (5.0 mg), and the mixture was vigorously stirred under H$_2$ atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO$_2$, MeOH/(hexane:AcOEt = 1:1); 0:100 to 15:85] to afford compound **199a** (11 mg, 54%) as a colorless amorphous. ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 7.03 (1H, d, *J* = 4.9 Hz), 6.89 (1H, d, *J* = 4.3 Hz), 5.92 (1H, t, *J* = 5.5 Hz), 4.73–4.60 (2H, m), 4.22–3.93 (3H, m), 3.86 (3H, s), 3.36 (3H, s), 3.34–3.23 (1H, m), 2.79–2.71 (2H, m), 2.55–2.48 (4H, m), 2.43–2.33 (2H, m), 2.28–2.15 (1H, m), 1.47 (9H, d, *J* = 9.8 Hz). ESIMS-LR *m/z* 458 [(M+H)$^+$].

***tert*-Butyl (3*S*)-5-(2'-amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-3-(methoxymethoxy)-2,3,4,7-tetrahydroazepine-1-carboxylate (199b)**



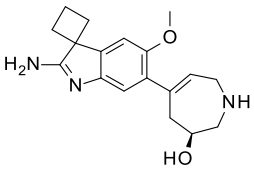
A solution of compound **198b** (200 mg, 0.41 mmol) in THF (2.0 mL) and AcOH (0.50 mL) was treated with Zn (140 mg, 2.1 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford compound **199b'** (120 mg, 62%) as a colorless solid. This material was used in the next reaction without further purification. A solution of crude **199b'** (120 mg, 0.25 mmol) and Ac$_2$O (0.024 mL, 0.25 mmol) in THF (5.0 mL), was treated with 10% Pd/C (20 mg), and the mixture was vigorously stirred under H$_2$ atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO$_2$, MeOH/CHCl$_3$; 0:100 to 12:88) to afford compound **199b** (98 mg, 85%) as a colorless amorphous. ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 7.03 (1H, d, *J* = 4.9 Hz), 6.89 (1H, d, *J* = 4.3 Hz), 5.92 (1H, t, *J* = 5.8 Hz), 4.73–4.61 (2H, m), 4.19–3.95 (3H, m), 3.86 (3H, s), 3.36 (3H, s), 3.34–3.23 (1H, m), 2.80–2.71 (2H, m), 2.55–2.49 (4H, m), 2.46–2.32 (2H, m), 2.27–2.15 (1H, m), 1.47 (9H, d, *J* = 9.8 Hz). ESIMS-LR *m/z* 458 [(M+H)$^+$].

(3*R*)-5-(2'-Amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-2,3,4,7-tetrahydro-1*H*-azepin-3-ol (154)



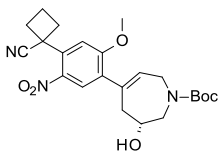
A solution of compound **199a** (4.4 mg, 0.0096 mmol) in MeOH (0.5 mL) was treated with 4N HCl/dioxane (1.0 mL) at room temperature for 2 h and the resulting mixture was concentrated *in vacuo*. The whole mixture was quenched by saturated aqueous $NaHCO_3$ (3.0 mL), and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 0:100 to 18:82) to afford compound **154** (2.2 mg, 73%) as a colorless solid. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.21 (1H, s), 6.73 (1H, s), 5.90 (1H, t, *J* = 5.5 Hz), 3.91–3.83 (4H, m), 3.44–3.38 (1H, m), 3.34 (1H, d, *J* = 5.5 Hz), 2.87–2.77 (2H, m), 2.73–2.59 (3H, m), 2.52–2.43 (1H, m), 2.41–2.33 (2H, m), 2.31–2.22 (1H, m). ESIMS-LR *m/z* 314 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{24}N_3O_2$ $(M+H)^+$ 314.1863, found 314.1869.

(3*S*)-5-(2'-Amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-2,3,4,7-tetrahydro-1*H*-azepin-3-ol (155)



A solution of compound **199b** (92 mg, 0.20 mmol) in MeOH (0.5 mL) was treated with 4N HCl/dioxane (2.0 mL) at room temperature for 2 h and the resulting mixture was concentrated *in vacuo*. The whole mixture was quenched by saturated aqueous $NaHCO_3$ (3.0 mL), and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 0:100 to 18:82) to afford compound **155** (55 mg, 87%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, s), 6.89 (1H, s), 6.00 (1H, t, *J* = 5.8 Hz), 3.98–3.92 (1H, m), 3.91 (3H, s), 3.56–3.51 (1H, m), 3.41–3.36 (1H, m), 3.30–3.26 (1H, m), 3.08–3.04 (1H, m), 2.86 (2H, d, *J* = 5.5 Hz), 2.56–2.49 (4H, m), 2.45–2.34 (1H, m), 2.27–2.15 (1H, m). ESIMS-LR *m/z* 314 [$(M+H)^+$]; ESIMS-HR calcd for $C_{18}H_{24}N_3O_2$ $(M+H)^+$ 314.1863, found 314.1865.

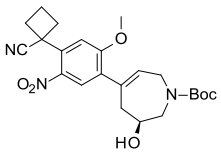
***tert*-Butyl (3*R*)-5-[4-(1-Cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-3-hydroxy-2,3,4,7-tetrahydroazepine-1-carboxylate (200a)**



A solution of compound **198a** (110 mg, 0.23 mmol) in MeOH (0.5 mL) was treated with 4N HCl/dioxane (1.0 mL) at room temperature for 2 h and the resulting mixture was concentrated *in vacuo* to afford free amine (77 mg) as a colorless solid. This material was used in the next reaction without further purification. A solution of free amine (77 mg, 0.22 mmol) and Et_3N (0.037 mL, 0.27 mmol) in THF (5.0 mL) was treated with Boc_2O (54 mg, 0.25 mmol) at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 30:70 to 70:30) to afford compound **200a** (79 mg, 79%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.85 (1H, s), 6.73 (1H, s), 5.69 (1H, s), 4.46–4.40 (1H, m), 4.28–4.17 (1H, m), 3.94 (3H, s), 3.91–3.74 (2H, m), 3.65–3.58 (1H, m), 3.01–2.94 (2H, m), 2.71–2.47 (5H, m), 1.98–1.91 (1H, m), 1.51 (9H, s). ESIMS-LR

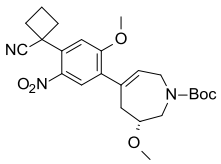
m/z 344 [(M-^{t}BuCO$_2$+H)$^+$].

***tert*-Butyl (3*S*)-5-[4-(1-Cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-3-hydroxy-2,3,4,7-tetrahydroazepine-1-carboxylate (200b)**



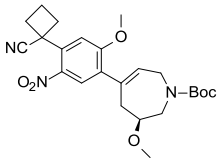
A solution of compound **198b** (30 mg, 0.23 mmol) in MeOH (0.5 mL) was treated with 4N HCl/dioxane (1.0 mL) at room temperature for 2 h and the resulting mixture was concentrated *in vacuo* to afford free amine (21 mg) as a colorless solid. This material was used in the next reaction without further purification. A solution of free amine (21 mg, 0.061 mmol) and Et_3N (0.010 mL, 0.073 mmol) in THF (3.0 mL) was treated with Boc_2O (15 mg, 0.067 mmol) at room temperature for 2 h. The resulting mixture was partitioned between AcOEt (100 mL) and H_2O (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 30:70 to 70:30) to afford compound **200b** (22 mg, 81%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.85 (1H, s), 6.73 (1H, s), 5.69 (1H, s), 4.46–4.40 (1H, m),, 4.31–4.15 (1H, m), 3.94 (3H, s), 3.92–3.73 (2H, m), 3.65–3.56 (1H, m), 3.01–2.93 (2H, m), 2.73–2.46 (5H, m), 2.01–1.89 (1H, m), 1.51 (9H, s). ESIMS-LR *m/z* 344 [(M-^{t}BuCO$_2$+H)$^+$].

***tert*-Butyl (3*R*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-3-methoxy-2,3,4,7-tetrahydroazepine-1-carboxylate (201a)**



A suspension of compound **200a** (20 mg, 0.045 mmol) and NaH (3.0 mg, 55% dispersion of mineral oil, 0.067 mmol) in DMF (1.0 mL) was treated with MeI (0.0042 mL, 0.068 mmol) at room temperature for 15 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 10:90 to 70:30) to afford compound **201a** (19 mg, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (1H, d, *J* = 4.3 Hz), 6.72 (1H, d, *J* = 5.5 Hz), 5.98–5.90 (1H, m), 4.32–4.15 (2H, m), 4.00–3.85 (4H, m), 3.61–3.51 (1H, m), 3.40–3.16 (4H, m), 3.02–2.92 (2H, m), 2.75–2.62 (2H, m), 2.59–2.46 (3H, m), 2.01–1.89 (1H, m), 1.49 (9H, d, *J* = 9.8 Hz). ESIMS-LR *m/z* 458 [(M+H)$^+$].

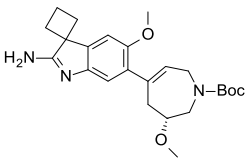
***tert*-Butyl (3*S*)-5-[4-(1-cyanocyclobutyl)-2-methoxy-5-nitrophenyl]-3-methoxy-2,3,4,7-tetrahydroazepine-1-carboxylate (201b)**



A suspension of compound **200b** (20 mg, 0.045 mmol) and NaH (3.0 mg, 55% dispersion of mineral oil, 0.067 mmol) in DMF (1.0 mL) was treated with MeI (0.0042 mL, 0.068 mmol) at room temperature for 15 h. The resulting mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane; 10:90 to 70:30) to afford compound **201b** (16 mg, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (1H, d,

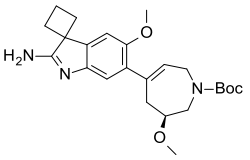
J = 4.3 Hz), 6.73 (1H, d, *J* = 5.5 Hz), 5.98–5.90 (1H, m), 4.33–4.14 (2H, m), 4.00–3.71 (4H, m), 3.61–3.51 (1H, m), 3.41–3.15 (4H, m), 3.03–2.91 (2H, m), 2.75–2.62 (2H, m), 2.59–2.44 (3H, m), 1.99–1.88 (1H, m), 1.49 (9H, d, *J* = 9.8 Hz). ESIMS-LR *m/z* 458 [$(M+H)^+$].

***tert*-Butyl (3*R*)-5-(2'-amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-3-methoxy-2,3,4,7-tetrahydroazepine-1-carboxylate (202a)**



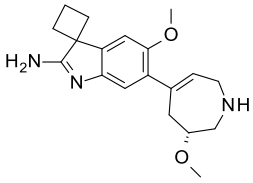
A solution of compound **201a** (18 mg, 0.039 mmol) in THF (2.0 mL) and AcOH (0.047 mL) was treated with Zn (13 mg, 0.20 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford the crude (12 mg) as a colorless solid. This material was used in the next reaction without further purification. A solution of the crude (12 mg, 0.027 mmol) and Ac_2O (0.0028 mL, 0.027 mmol) in THF (2.0 mL), was treated with 10% Pd/C (4.0 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 0:100 to 12:88) to afford compound **202a** (6.3 mg, 54%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, d, J = 4.3 Hz), 6.88 (1H, d, J = 5.5 Hz), 5.96–5.87 (1H, m), 4.29–3.95 (2H, m), 3.88–3.64 (4H, m), 3.55–3.48 (1H, m), 3.40–3.35 (3H, m), 3.34–3.09 (1H, m), 2.83–2.66 (2H, m), 2.56–2.48 (4H, m), 2.44–2.32 (1H, m), 2.26–2.14 (1H, m), 1.50–1.44 (9H, m). ESIMS-LR *m/z* 428 [$(M+H)^+$].

***tert*-Butyl (3*S*)-5-(2'-amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-3-methoxy-2,3,4,7-tetrahydroazepine-1-carboxylate (202b)**



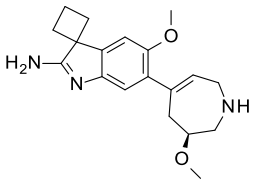
A solution of compound **201b** (16 mg, 0.035 mmol) in THF (2.0 mL) and AcOH (0.047 mL) was treated with Zn (13 mg, 0.20 mmol) at room temperature, and the mixture was stirred at 50 °C for 10 min, and then the mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford the crude (11 mg) as a colorless solid. This material was used in the next reaction without further purification. A solution of the crude (11 mg, 0.027 mmol) and Ac_2O (0.0023 mL, 0.025 mmol) in THF (2.0 mL), was treated with 10% Pd/C (4.0 mg), and the mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 20 min. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (NH-SiO_2, MeOH/$CHCl_3$; 0:100 to 12:88) to afford compound **202b** (4.5 mg, 30% over 2 steps) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, d, *J* = 3.7 Hz), 6.88 (1H, d, *J* = 6.1 Hz), 5.96–5.87 (1H, m), 4.28–3.96 (2H, m), 3.88–3.65 (4H, m), 3.55–3.47 (1H, m), 3.39–3.09 (4H, m), 2.82–2.65 (2H, m), 2.57–2.48 (4H, m), 2.45–2.32 (1H, m), 2.27–2.12 (1H, m), 1.50–1.42 (9H, m). ESIMS-LR *m/z* 428 [$(M+H)^+$].

(3*R*)-5-(2'-Amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-2,3,4,7-tetrahydro-1*H*-azepin-3-ol (156)



A solution of compound **202a** (5.1 mg, 0.012 mmol) in CH_2Cl_2 (2.0 mL) was treated with TFA (1.0 mL, 13 mmol) at room temperature for 1 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 1:99 to 20:80] to afford compound **156** (3.9 mg, quant.) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, s), 6.92 (1H, s), 5.92 (1H, t, *J* = 5.5 Hz), 3.88 (3H, s), 3.46–3.36 (4H, m), 3.35 (3H, s), 2.96 (1H, dd, *J* = 14.6, 9.2 Hz), 2.79 (2H, d, *J* = 4.9 Hz), 2.58–2.50 (4H, m), 2.46–2.35 (1H, m), 2.27–2.17 (1H, m). ESIMS-LR *m/z* 328 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_3O_2$ $(M+H)^+$ 328.2020, found 328.2014.

(3*S*)-5-(2'-Amino-5'-methoxy-spiro[cyclobutane-1,3'-indole]-6'-yl)-2,3,4,7-tetrahydro-1*H*-azepin-3-ol (157)



A solution of compound **202b** (3.5 mg, 0.0082 mmol) in CH_2Cl_2 (2.0 mL) was treated with TFA (1.0 mL, 13 mmol) at room temperature for 1 h. The resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography [NH-SiO_2, MeOH/(hexane:AcOEt = 1:1); 1:99 to 20:80] to afford compound **157** (1.4 mg, 52%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.04 (1H, s), 6.92 (1H, s), 5.93 (1H, t, *J* = 5.5 Hz), 3.88 (3H, s), 3.47–3.36 (5H, m), 3.35 (3H, s), 2.96 (1H, dd, *J* = 13.7, 8.2 Hz), 2.80 (2H, d, *J* = 4.9 Hz), 2.58–2.48 (4H, m), 2.46–2.33 (1H, m), 2.28–2.15 (1H, m). ESIMS-LR *m/z* 328 [$(M+H)^+$]; ESIMS-HR calcd for $C_{19}H_{26}N_3O_2$ $(M+H)^+$ 328.2020, found 328.2020.

2. Biological evaluation

第一章

2-1 HbF induction assay using human $CD34^+$ cells

The fetal hemoglobin (HbF) induction assay using human $CD34^+$ cells (Lonza USA) was performed for the study. Briefly, 1×10^4 cells/mL were cultured in Stemline II Hematopoietic Stem Cell Expansion Medium (S0192; Sigma-Aldrich Japan) supplemented with stem cell factor, FLT-3 ligand (126-FL-010; R&D Systems USA), and interleukin (IL)-3 (203-IL; R&D Systems USA), at a final concentration of 50 ng/mL each, at 37 °C and 5% CO_2 for 7 days to induce differentiation into proerythroid cells. The cells were then resuspended in Stemline II medium with various concentrations of test compounds or decitabine as a positive control and seeded at a density of 1×10^4 cells/100 μL in 96-well plates. After 5 days incubation, cells were harvested and fixed with 0.05% glutaraldehyde, followed by treatment with 0.1% Triton X-100 for 5 min. The treated cells were then stained with fluorescein isothiocyanate (FITC)-conjugated anti-fetal hemoglobin (HBF-1) monoclonal antibody (MHFH01; ThermoFisher Scientific Japan) or FITC-conjugated mouse IgG1 κ isotype control antibody (400108; BioLegend USA). Mean fluorescence was measured at 488 nm and the percentage of γ-globin-positive cells was determined using flow cytometry (MACSQuant; Miltenyi Biotec Japan). The E_{max} (i.e. the drug concentration at which the maximum effect is observed) of each compound was calculated as a percentage of the maximum effect of decitabine. The EC_{50} was defined as the concentration of a compound that resulted in a half-maximal HbF induction response relative to the effect of decitabine, which was set at 100%.

2-2 Cell viability measured using propidium iodide viability assay

BM $CD34^+$ cells isolated from human bone marrow (Lonza) were seeded at a density of 2000 cells per well in differentiation medium containing 50 ng/ml stem cell factor (SCF), 50 ng/ml Flt3 ligand (FL), and 50 ng/ml thrombopoietin (TPO). After 3 days of incubation at 37 °C in a 5% CO_2 humidified atmosphere, test compounds were added to the culture medium. After further incubation for 4 days, cell viability was measured by propidium iodide staining. Cell viability of 100% was calculated from cells without test compounds.

2-3 Pharmacokinetic profile of chemicals

In order to measure drug clearance and the ability of drug exposure to predict pharmacokinetic (PK) profiles, blood samples were collected from two cynomolgus monkeys at 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after oral administration of the lead compounds **10**, **24**, **32**, **25**, **36** and **37** (at concentrations of 1 or 10 mg/kg). The blood samples were then centrifuged, and the resulting plasma fractions were analyzed using liquid chromatography/mass spectrometry (Acquity Ultra performance LC, Acquity TQ Detector, and Acquity UPLC BEH C18 column 1.7 μm, 2.1 × 50 mM Waters Corporation) to measure the amount of test compound in the blood.

2-4 *In vivo* studies in non-human primates

An *in vivo* study was performed by monitoring HbF population changes in bone marrow erythroid cells of cynomolgus monkeys before and after the administration of test compounds. All animal experiments were approved by the Ethics Committee for Animal Experiments of Asubio Pharma Co. Ltd and were performed in the Shinyaku Research Center (Approval no. #171205B). All cynomolgus monkeys were maintained in a stress-free environment and only monkeys that had adapted well to the research environment were used in the experiments. The experiments were designed based on the PK profiles and performed as follows. In the first experiment, 5 and 15 mg/kg lead compound **24** was administered orally once a day (qd) to three monkeys each for 5 d. Three monkeys administered with PBS, served as vehicle controls. The results observed were compared with those after orally administering the positive control compound decitabine (15 mg/ kg qd [n = 3]). In the second experiment, the lead compound **24** was administered orally (7.5 mg/kg twice a day (bid) [n = 3], 15 mg/kg bid [n = 3]). Three monkeys administered with PBS, served as vehicle controls. Detailed information regarding the cynomolgus monkeys used in these 2 experiments is as follows, 1st experiment: age, 3–9 years old; sex, 6 males and 6 females; body weight, 2.49–5.64 kg; 2nd experiment: age, 3–9 year old; sex, 5 males and 4 females; body weight, 2.61–6.04 kg. In the third experiment, four monkeys each were administered 15 or 30 mg/kg, p.o., Compound **37** twice daily; another four monkeys were administered an equal volume of PBS and served as the vehicle controls. The cynomolgus monkeys (5 males, 7 females) used in the experiments were 3–9 years old and weighed 2.6–6.01 kg. On the day before the first administration of test compound (Day 0) and after the last administration (Day 6), each animal was anaesthetized using a mixture of ketamine hydrochloride (5 mg/kg; 9001961; Cayman Chemical USA) and xylazine hydrochloride (1 mg/kg; X-1251; Sigma-Aldrich Japan), and a 2-mL sample of bone marrow cells was extracted from the pelvis. These samples were then subjected to real-time polymerase chain reaction (PCR) to determine γ-globin (*HBG*) and β-globin (*HBB*) mRNA expression. *HBG* and *HBB* expression before administration was quantified as "pre" values, whereas expression after the 6 successive days of oral administration was quantified as "post" values. The ratio of post/pre expression was calculated for *HBG* and *HBB* separately to investigate changes in the expression of each gene individually. In addition, we determined the ratio of post/pre *HBG* expression to post/pre *HBB* expression as an indicator of HbF induction.

第二章

2-5 Enzymatic assay procedure

IC_{50} values of compounds inhibiting G9a were determined by Amplified Luminescence Proximity Homogeneous Assay technology (AlphaLISA; PerkinElmer) by detecting H3K9 dimethylation. Test compounds were incubated with 5 pM recombinant human G9a (amino acid Nos. 913–1193 tagged with His Tev, Lot # T1452) produced by Daiichi Sankyo RD Novare, 15 nM biotinylated H3K9me1 peptide (EpiCypher, # 12-0010), and 45 μM SAM (Sigma, # A7007) in 50 mM Tris-HCl buffer (pH 9.0) containing 10 mM NaCl, 0.01% Tween 20, 1 mM dithiothreitol, and 0.01% bovine serum albumin (BSA), with a total volume of 5 μL in each well of a 384-well plate (PerkinElmer, # 6008359) for 40 min at room temperature (RT). The enzymatic reaction was

stopped by the addition of 0.5 μM A-366, 0.05% Anti-Dimethyl-Histone H3K9 rabbit monoclonal antibody (CST, # 4658S), 5 μg/mL Protein A AlphaLISA Acceptor Beads (PerkinElmer, # AL101M), and 5 μg/mL AlphaScreen Streptavidin-coated Donor Beads (PerkinElmer, # 6760002), in AlphaLISA Epigenetics Buffer (PerkinElmer, # AL008F) with a total volume of 7.5 μL. After incubation for 120 min at RT, fluorescence (Ex 680 nm, Em 615 nm) of each well in the microplate was measured with an EnVision Multilabel Plate Reader (PerkinElmer). For the determination of IC_{50} values for GLP (recombinant human GLP, amino acid Nos. 982–1266 tagged with His Tev, Lot # T1700, produced by Daiichi Sankyo RD Novare), reactions were conducted under the same conditions as in the G9a assay, except for the concentrations of SAM and BSA (2.5 μM SAMWithout BSA) in the reaction buffer and the concentration of A-366 (3 μM) in the stopping buffer. Data (n = 4) were analyzed by the program Graphpad Prism 6 (Graphpad Software Inc.). IC_{50} values of the test compounds on G9a and GLP were calculated by regression analysis using the four-parameter logistic equation of Prism 6 sigmoidal dose-response (variable slope). More precisely, the following equation was used for the calculation of IC_{50} values.

Y=Bottom + (Top−Bottom)/(1+10^[(LogIC_{50}−X)*HillSlope)]

Top: Constant equal to 100

Bottom: Constant equal to 0

The values of inter-assay variance in the G9a assay and GLP assay evaluated at A-366 are 23.3% and 18.0%, respectively.

2-6 Globin switching activity in hematopoietic progenitor cells

The activity of the compounds was tested by evaluating switching of the globin mRNA expression from adult-type β-globin to fetal-type γ-globin using human bone marrow-derived hematopoietic progenitor CD34+ cells. Human bone marrow-derived CD34+ cells (Lonza Japan, Tokyo, Japan) were cultured with a hematopoietic progenitor cell expansion DXF medium (PromoCell, Heidelberg, Germany) supplemented with Cytokine Mix E containing thrombopoietin, stem cell factor, flt-3 ligand, and interleukin-3 (PromoCell) in a CO_2 incubator for 9 days. The cells were further cultured with Stemline II Hematopoietic Stem Cell Expansion Medium (Sigma-Aldrich, MO, USA) supplemented with Cytokine Mix E for 8 days. The cultured cells were suspended in DXF medium supplemented with 4 U/mL human erythropoietin (R&D Systems, MN, USA), 0.8 mg/mL human holo-transferrin (Fujifilm Wako Pure Chemicals, Osaka, Japan), and 0.1, 0.3, and 1 μM test compounds at a cell density of 2×10^6/mL and inoculated into each well of 24-well plates (0.25 mL/well). The cells were cultured in a CO_2 incubator for 14 days by exchanging the old medium with fresh medium containing test compound three times a week. After cultivation, the cells were harvested for the extraction of mRNA and subjected to the evaluation of globin mRNA expression by quantitative RT-PCR. The amounts of α-, β-, and γ-globin and β-actin mRNA were measured and the level of each globin's mRNA expression was expressed as the ratio of globin mRNA to β-actin mRNA. Data were analyzed from two biological replicates in each experimental group.

2-7 Monkey F-cell flow cytometric analysis

All experimental procedures for animals were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

Mononuclear cells from cynomolgus monkey bone marrow were prepared with Lymphoprep (Abbott Diagnostic Technologies AS, Oslo, Norway), in accordance with the manufacturer's instructions. The cells were suspended in Stemline II Hematopoietic Stem Cell Expansion Medium (Sigma-Aldrich, MO) supplemented with 5 U/mL human erythropoietin (R&D Systems, MN), 0.8 mg/mL human holo-transferrin (Fujifilm Wako Pure Chemicals, Osaka, Japan), and serially diluted test compounds (decitabine, hydroxyurea, or compound **144**) at a cell density of 4×10^5/mL and inoculated into each well of 96-well microplates (0.1 mL/well). The cells were cultured in a CO_2 incubator for 2 days, supplemented with 0.1 mL of fresh medium containing test compound, and additionally cultured for 3 days.

After cultivation, γ-globin and β-globin expression in the cells was evaluated by flow cytometric analysis. Briefly, the cells in 96-well microplates were fixed with 4% paraformaldehyde (Fujifilm Wako Pure Chemicals) at room temperature for 30 min. The fixed cells were treated with 0.05% Triton X-100 solution in Dulbecco's phosphate-buffered saline (D-PBS) containing 0.5% bovine serum albumin (BSA, Fraction V pH 7.0; Fujifilm Wako Pure Chemicals) and 2 mM EDTA at room temperature for 30 min. The cells were washed with D-PBS, 0.5% BSA, and 2 mM EDTA (BSA/EDTA/D-PBS) and re-suspended in eBioscience Flow Cytometry Staining Buffer (Thermo Fisher Scientific, MS). The cells were labeled with 1/10-diluted Brilliant Violet 421 Goat Anti-mouse IgG (minimal x-reactivity) Antibody (Biolegend, CA) and 1/10-diluted Fetal Hemoglobin Mouse Anti-Human Antibody, Unlabeled, Clone:2D12 (Becton Dickinson NJ), at room temperature for 20 min under shaded conditions. The cells in the negative wells were stained with 1/10-diluted Brilliant Violet 421 Mouse IgG1, k Isotype Ctrl Antibody (Biolegend) under the same conditions. The cells were washed with BSA/EDTA/D-PBS, resuspended in Flow Cytometry Staining Buffer, and labeled with 1/10 Hemoglobin β Antibody (37-8) PE (Santa Cruz Biotechnology, TX) at room temperature for 20 min under shaded conditions. The cells in the negative wells were stained with 1/10-diluted PE Mouse IgG1, κ Isotype Ctrl Antibody (Biolegend) under the same conditions. The labeled cells were washed with BSA/EDTA/D-PBS and subjected to flow cytometric analysis with a MACS Quant flow cytometer (Miltenyi Biotech, Bergisch Gladbach, Germany), in accordance with the manufacturer's instructions. Data were analyzed from three biological replicates in each experimental group.

2-8 *In vitro* and *in vivo* DMPK and toxicity assay.

Log D, solubility, microsomal stability, MBI and hERG inhibition assay were performed as previously described.[89–91]

2-9 Pharmacokinetic evaluation in mice

The test compounds were administered intravenously (IV) or orally (PO) to 7-week-old male

C57BL/6J mice at doses of 1 mg/kg (IV) or 10 mg/kg (PO). The IV solution was prepared with dimethyl sulfoxide (Wako Pure Chemical Industries)/Polysorbate 80 (HX2) (NOF CORPORATION)/saline (Otsuka Pharmaceutical Co., Ltd.) in the proportion of 1/1/8, and the PO solution was prepared with 0.5% (w/v) methyl cellulose 400 solution (Wako Pure Chemical Industries). Blood samples were collected from the peripheral vein at several time points after dosing and centrifuged at 14,000 rpm for 3 min at 4 °C to obtain plasma. Plasma samples were stored at −20 °C until analysis. The plasma concentration was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an API 4000 QTRAP system (Applied Biosystems/MDS SCIEX). PK parameters were calculated via non-compartmental analysis by using the BioBook function of the E-WorkBook Suite (IDBusiness Solutions Ltd.).

2-10 Pharmacokinetic evaluation in monkeys

The test compounds were administered to 3- to 5-year-old male cynomolgus monkeys at doses of 0.5 mg/kg (IV) or 5 mg/kg (PO). The IV solution was prepared with *N*,*N*-dimethylacetamide (Nacalai Tesque, Inc.)/Polysorbate 80 (Nacalai Tesque, Inc.)/saline (Otsuka Pharmaceutical Co., Ltd.) in the proportion of 1/1/8, and the PO solution was prepared with 0.5% (w/v) methyl cellulose 400 solution (Wako Pure Chemical Industries). Blood samples were collected from the peripheral vein at several time points after dosing and centrifuged at 14,000 rpm for 3 min at 4 °C to obtain plasma. Plasma samples were stored at −20 °C until analysis. The plasma concentration was determined by LC-MS/MS using an API 4000 QTRAP system (Applied Biosystems/MDS SCIEX). PK parameters were calculated via non-compartmental analysis by using the BioBook function of the E-WorkBook Suite (IDBusiness Solutions Ltd.).

2-11 *In vivo* fetal hemoglobin inducing study

2-11-1 Protocol for building *in vivo* phlebotomized cynomolgus monkey model

Six Cynomolgus macaques (age 3-4 years old, male) were phlebotomized from femoral venous 3 times a week (Monday, Wednesday, and Friday) under conscious condition. Phlebotomy volumes were 10 or 13 mL/kg to keep the Hb level between 8 to 11 g/dL throughout the experiment. CBC analysis was performed using phlebotomized blood samples three times a week. Plasma iron level was monitored weekly, and iron was supplemented weekly as described elsewhere.[4]

2-11-2 Administration method

DS79932728 and hydroxyurea (TCI) were suspended or dissolved in 0.5w/v% Methyl Cellulose 400 (Wako). 35 mg/kg of hydroxyurea once daily (N=3), or 15 mg/kg of DS79932728 twice daily (N=3) were orally administered for 5 consecutive days.

2-11-3 F-reticulocytes (fetal hemoglobin-expressing reticulocytes) in flow cytometric analysis

Whole blood cells collected from cynomolgus monkeys were suspended in 4% paraformaldehyde solution in PBS and incubated for 30 min at room temperature. Cells were centrifuged at 800 g for 3 min. After removing the supernatant, cells were suspended in 0.05%

glutaraldehyde solution in PBS and immediately centrifuged at 800 g for 3 min at room temperature. Cells were then washed with PBS containing 0.5% BSA and 2 mM EDTA before permeabilization with 0.05% Triton X-100 in PBS for 3–5 min at room temperature. Cells were washed twice with PBS containing 0.5% BSA and 2 mM EDTA and re-suspended in 200 µL of PBS containing 0.5% BSA and 2 mM EDTA. The cell suspension was added to eBioscience Flow Cytometry Staining Buffer (Thermo Fisher Scientific, Waltham, MA, USA). F-reticulocytes among the cells were analyzed by flow cytometry using the MACS Quant Analyzer (Miltenyi Biotec, Bergisch Gladbach, Germany) after staining with thiazole orange using BD Retic-Count and BV421-conjugated anti-HbF antibody, clone 2D12 (BD Bioscience, San Jose, CA, USA). Data analysis was performed using FlowJo software (Becton Dickinson, Franklin Lakes, NJ, USA). After gating the red blood cell population, thiazole orange and HbF dual-positive F-reticulocytes were evaluated. Data were analyzed from three biological replicates in each experimental group.

3. X-ray crystallography

3-1 Protein production and purification

A DNA fragment encoding human G9a (residues 913–1193) was amplified by PCR and inserted into the pET15b vector (Novagen) to produce an *N*-terminal 6×His-tagged G9a. The expression was performed in *E. coli* strain BL21 (DE3). After sonication and centrifugation of the cells, the supernatant was applied to a HisTrap FF crude column (GE Healthcare), and the protein was eluted with a gradient from 20 to 500 mM imidazole. Purified protein was subjected to TEV protease reaction and the released 6×His-tag was removed by second Ni-affinity chromatography. Untagged G9a was further purified by gel filtration chromatography using a HiLoad 26/600 Superdex 200 pg column (GE Healthcare) with buffer consisting of 20 mM Tris-HCl pH 8.0, 150 mM NaCl, and 0.2 mM TCEP. Fractions containing hG9a were collected, concentrated to 21 mg/ml, and stored at −80 °C.

3-2. Co-crystallization

Buffer of purified 6×His-tagged G9a (residues 913–1193) was exchanged to 20 mM Tris-HCl (pH 8) / 150 mM NaCl / 0.2 mM TCEP (pH 6.8) by repeated cycles of concentration and dilution using a Viva-spin ultra-filtration unit (Sartorius, Germany). At the final cycle, an excess amount of SAM and the test compound were added. The mixed sample was concentrated to 18–20 mg/ml, aliquoted, and stored at −80 °C. After the addition of 0.02 U/μL Turbo-TEV (Accelagen, CA) for in situ tag removal, thawed sample was subjected to the sitting drop vapor diffusion method at 20 °C.

Co-crystals of compound **80**, SAM, and G9a were grown under conditions of 0.2 M ammonium acetate / 20% polyethyleneglycol #3350. Obtained crystals were cryo-protected with 0.167 M ammonium acetate / 16.7% polyethyleneglycol #3350 / 16.7% glycerol and vitrified in liquid nitrogen.

Co-crystals of compound **89**, SAM, and G9a were grown under conditions of 0.2 M magnesium acetate / 20% polyethyleneglycol #3350. Obtained crystals were cryo-protected with 0.167 M magnesium acetate / 16.7% polyethyleneglycol #3350 / 16.7% glycerol and vitrified in liquid nitrogen.

Co-crystals of compound **144**, SAM, and G9a were grown under conditions of 0.2 M sodium acetate, pH 7, and 20% polyethylene glycol # 3350. Obtained crystals were cryo-protected with 0.2 M sodium acetate, pH 7, and 35% PEG3350, and vitrified in a cold nitrogen flow.

3-3 Data collection

Diffraction data of compound **80** / SAM / G9a co-crystal were collected on the XtalLab SynergyCustom system (Rigaku, Tokyo, Japan) and processed with *CrysAlis*^{pro} (Rigaku Oxford Diffraction, 2016) and *aimless*.[92] Diffraction data of compound **89** / SAM / G9a co-crystal were collected at the SWISS LIGHT SOURCE (SLS) on beamline X10SA and processed with *mosflm*[93] and *aimless*.[92] Diffraction data of compound **144** / SAM / G9a co-crystal were collected on the XtalLab SynergyCustom system (Rigaku, Tokyo, Japan) and processed with *CrysAlis*^{pro} (Rigaku

Oxford Diffraction, 2016) and *aimless.*[92]

3-4. Structure determination

The initial phase was obtained by the molecular replacement method with *Phaser*[92] using the in-house G9a crystal structure as a starting model. Phase refinement and model building were carried out in accordance with the standard protocol using *Refmac5*[94] and *coot*[95]. The statistics for data processing and phase refinement are summarized in **Table E1**.

Table E1. Statistics for data collection and phase refinement.

	Compound **80**	Compound **89**	Compound **144**
PDB code	7BTV	7BUC	7DCF
Data Collection			
X-ray source	MicroMax 007	SLS X10SA	MicroMax 007
Detector	HyPix 6000HE	EIGER X16M	HyPix 6000HE
Wavelength (Å)	1.542	1.000	1.542
Temperature (K)	100	100	100
Space group	$P2_1$	$P2_1$	$P2_1$
Unit cell dimensions	a = 56.4 Å, b = 79.1 Å, c = 72.5 Å, α = 90°, β = 91.8°, γ = 90°	a = 56.5 Å, b = 78.4 Å, c = 73.0 Å, α = 90°, β = 91.6°, γ = 90°	a = 55.9 Å, b = 72.7 Å, c = 63.6 Å, α = 90°, β = 92.7°, γ = 90°
Resolution (Å)	26.6–2.0 (2.05–2.0)	78.4–2.6 (2.72–2.6)	20.6–1.8 (1.86–1.8)
Total No. of observations	134485 (7956)	60135 (7484)	168791 (6074)
Unique reflections	42628 (2865)	19580 (2367)	47106 (2706)
Multiplicity	3.2 (2.8)	3.1 (3.2)	3.6 (2.2)
Completeness (%)	98.8 (91.4)	99.3 (99.5)	99.8 (98.1)
I/σ(I)	20.6 (2.9)	6.8 (1.7)	10.2 (1.0)
R_{merge}[a]	0.068 (0.845)	0.116 (0.678)	0.086 (0.891)
Refinement			
Resolution (Å)	25–2.0	25–2.6	20.6–1.8
No. of reflections	40516	18627	44794
RMS bonds (Å)	0.007	0.002	0.007
RMS angles (°)	1.538	0.796	1.567
No. of atoms			
protein	4347	4212	4126
water and Zn ion	323	32	306
ligand (including SAM)	108	104	100

Average B value (Å^2)	36.1	49.0	24.3
protein	36.6	50.7	24.3
water and solvent	38.1	49.3	26.8
ligand (include SAM)	32.5	37.5	17.7
R-value[b]	0.194	0.212	0.200
R_{free}[b]	0.244	0.260	0.246

[a] R_{merge} = ΣhΣj |<I(h)> − I(h)j | / ΣhΣj <I(h)>, where <I(h)> is the mean intensity of symmetry-related reflections.

[b] *R*-value = Σ | |Fobs| − |Fcalc| | / Σ |Fobs|. R_{free} for 5% of reflections excluded from refinement. Values in parentheses are for the highest-resolution shell.

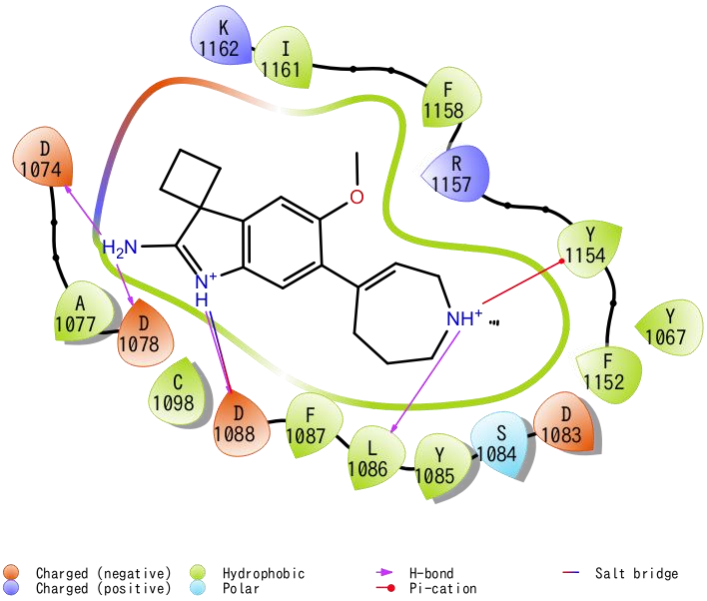


Figure E1. Ligand interaction diagram of compound **144** with G9a.

Ligand interaction diagram of compound **144** with G9a was generated by using Maestro (Schrödinger Release 2019-4: Maestro, Schrödinger, LLC, New York, NY, 2019). Amino acid residues are represented as colored teardrop shapes, labeled with 1-letter code and residue number, and colored according to their properties; negatively charged (red), positively charged (blue), hydrophobic (green), and cyan (polar). The point on the teardrop shape is oriented in the direction of the side chain of amino acid residues. The chain is represented as a black curve connecting residues. Black dots on this curve represented the residues are not within 4.0 Å of compound **144**. Interactions between the residues and compound **144** are drawn as lines, colored by interaction type; hydrogen bond (magenta), π-cation interaction (red), and salt-bridge (blue-red gradient). Residues without these lines are involved in nonspecific hydrophobic interactions with compound **144**. The binding pocket is indicated by a thicker curve drawn around compound **144**, colored by the color of the nearest residue.

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主論文目録

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