博 士 学 位 論 文

新規ＥＰ３００／ＣＢＰアセチルトランスフェラーゼ阻害薬ＤＳ－９３００の創製研究

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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。

9-BBN	9-borabicyclo[3.3.1]nonane
Ac	acetyl
ADME	absorption, distribution, metabolism and excretion
APCI	atmospheric-pressure chemical ionization
Ar	aryl
AR	androgen receptor
Arg	arginine
Asp	aspartic acid
AUC	area under the curve
BA	bioavailability
β-CD	β-cyclodextrin
Bn	benzyl
Boc	*tert*-butoxycarbonyl
brs	broad singlet
BRD	bromodomain
Bu	butyl
CBP	CREB-binding protein
Cbz	benzyloxycarbonyl
CDI	carbonyldiimidazole
CH1/CH2/CH3	cysteine/histidine rich regions 1/2/3
CL	clearance
Cmax	maximum plasma concentration
CoA	coenzyme A
COMU	1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate
conc	concentration
CRD1	cyclin-dependent kinase inhibitor-reactive domain
CREB	cyclic adenosine monophosphate response element binding protein
CRPC	castration-resistant prostate cancer
CYP	cytochrome P450
d	doublet

DAST	*N*,*N*-diethylaminosulfur trifluoride
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DCM	dichloromethane
dd	double doublet
DIBALH	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMEDA	*N*,*N*'-dimethylethane-1,2-diamine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dppf	1,1'-bis(diphenylphosphino)ferrocene
E1A	adenovirus early region 1A
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immuno sorbent assay
EP300	E1A-associated protein p300
ESI	electrospray ionization
Et	ethyl
F	bioavailability
FDA	Food and Drug Administration
Fmoc	9-fluorenylmethyloxycarbonyl
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GCN5	general control non-depressible 5
GI_{50}	50% inhibitory concentration of cell growth
Gly	glycine
GnRH	gonadotropin releasing hormone
h	hour(s)
HAT	histone acetyltransferase
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HDAC	histone deacetylase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

His	histidine
HLM	human liver microsome
HRMS	high resolution mass spectrometry
HRP	horseradish peroxidase
HTS	high-throughput screening
IBiD	interferon binding domain
IC_{50}	50% inhibitory concentration
IgG	immunoglobulin G
Ile	isoleucine
IPA	isopropanol
iPr	isopropyl
IV	intravenous
KAT	lysine acetyltransferase
KIX	CREB-interacting kinase-inducible domain interacting
KLK3	kallikrein related peptidase 3
LC/MS	liquid chromatography/mass spectrometry
Leu	leucine
LHMDS	lithium bis(trimethylsilyl)amide
Log D	logarithm of octanol-water distribution coefficient
Lys	lysine
m	multiplet
m-CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MLM	mouse liver microsome
MOZ	monocytic leukemia zinc finger protein
mRNA	messenger ribonucleic acid
MS	metabolic stability
MYST	MOZ, YBF2/SAS3, SAS2, TIP60
n	normal
NMR	nuclear magnetic resonance
NRID	nuclear receptor interaction domain
NT	not tested
PAMPA	parallel artificial membrane permeability assay
PBS-T	phosphate buffered saline with Tween 20
PCAF	p300/CBP associated factor
PD	pharmacodynamics

PDB	protein data bank
PEG	polyethylene glycol
Phe	phenylalanine
Pin	pinacolato
PK	pharmacokinetics
PO	per os (oral administration)
Pro	proline
PSA	prostate-specific antigen
PTSA	*p*-toluenesulfonic acid
q	quantet
QD	quaque die (once a day)
qRT-PCR	quantitative real-time PCR
RPMI	Roswell Park Memorial Institute
rt	room temperature
s	singlet
SAR	structure-activity relationship
SBDD	structure-based drug design
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	standard error of the mean, 2-(trimethylsilyl)ethoxymethyl
Ser	serine
SOX2	SRY-box transcription factor 2
SPhos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
SRY	sex determining region Y
t, *ter*t-	tertiary
t1/2	elimination half-life
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TCEP	tris(2-carboxyethyl)phosphine
TEA	triethylamine
TFA	trifluoroacetic acid
TGI	tumor growth inhibition
THF	tetrahydrofuran
Thr	threonine
TIP60	tat interactive protein 60kDa
TLC	thin-layer chromatography

TMS	tetramethylsilane, trimethylsilyl
Tof MS	time of flight mass spectrometry
Tris	tris(hydroxymethyl)aminomethane
Troc	2,2,2-trichloroethoxycarbonyl
Trp	tryptophan
TSA	thermal shift assay
TTBP·HBF_4	tri-*tert*-butylphosphonium tetrafluoroborate
Tyr	tyrosine
UPLC	ultra-performance liquid chromatography
UV	ultraviolet
VS	virtual screening
XPhos	2-dicyclohexylphosphino-2’,4’,6’-triisopropylbiphenyl

序論

がんは 1981 年から我が国の死亡原因の第一位となり、2020 年には 37 万 8385 人、人口 10 万人当たりの死亡率は 306.6 であり、総死亡数の 27.6%を占めている[1]。治療法の進歩やがん予防、がん検診の普及等によってがん罹患者の 5 年生存率、及び死亡率は着実に改善している一方で、急速な高齢人口の増加に伴いがん罹患者数及びがん死亡者数は増加の一途を辿ることが予想される。そのような状況の中、依然として既存の治療法が著効を示さないがんが多く残されており、これらのアンメットメディカルニーズが高いがん種に対する新規薬剤の開発が求められている。

がんに対する薬物の開発においては、DNA 合成や細胞分裂を阻害する化学療法剤が長らく中心であったが、分子生物学の発展に伴い、がんの増殖に関与する特定のがん遺伝子やがん抑制遺伝子のジェネティックな異常を特異的に狙った分子標的薬の開発が進められている。2001 年にイマチニブが低分子の分子標的薬として最初に FDA 承認されて以降、100 剤近くの分子標的薬が承認されており、多くのがん種において治療選択肢を広げてきた[2,3]。しかし、いまだ有効な分子標的薬が存在しないがん種が存在するほか、一度奏功を示したとしても、薬剤耐性の発現により薬物治療抵抗性を獲得した悪性度の高い腫瘍へと進展する場合があり、新規メカニズムを有する薬剤の開発が強く望まれている。

近年、遺伝子変異や染色体異常などのジェネティックな異常だけでなく、DNA メチル化やヒストン修飾などのエピジェネティクス機構の異常が、がんの分子機構において重要な役割を果たすことが明らかとなっている[4,5]。エピジェネティクス機構の異常を標的とした治療薬としては、DNA メチル化酵素（DNA methyltransferases; DNMT）阻害薬やヒストン脱アセチル化酵素（histone deacetylases; HDAC）阻害薬等が FDA の承認を既に受けている (**Figure 1**)[6–11]。これらのエピジェネティクス治療薬はがんに関連する複数の遺伝子発現を制御することが可能で、これまでの分子標的薬とは異なり複数のシグナル経路制御によって抗腫瘍効果が発揮されることから、がん治療における新たな選択肢の一つとなっている。現時点では、がんのエピジェネティック異常に関与する多種多様な因子の中で臨床応用まで進められた標的分子は限られており、世界中で新たな標的分子の探索及び治療薬の開発が進められている。

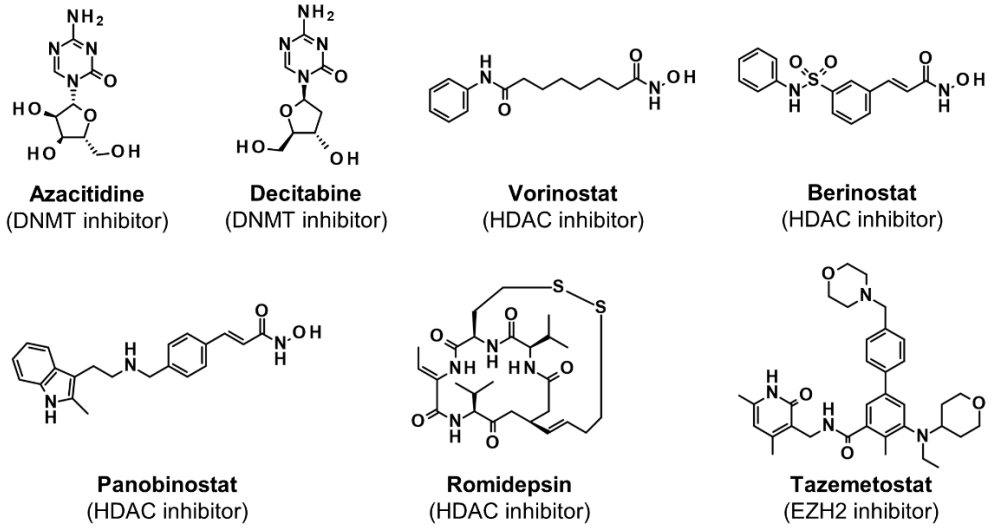

Figure 1. Chemical Structures of FDA approval drugs for epigenetic drugs.

EP300（E1A-associated protein p300）及び CBP（CREB-binding protein）は、ヒストンアセチルトランスフェラーゼ（Histone acetyltransferases; HAT）機能を有し、ヒストンをアセチル化することで遺伝子の発現を制御するエピジェネティック関連因子の一つである。EP300/CBP の過剰発現や変異は多くの腫瘍に見られ、がんの発生や進展に深く関与していることが報告されており、その阻害薬は新たながん治療の有望な選択肢の一つとなることが期待されている [12,13]。しかし、これまで承認された EP300/CBP 阻害薬はなく、新規薬剤の開発が強く望まれている。

著者は、インシリコスクリーニング及びハイスループットスクリーニング（High-throughput screening; HTS）による 2 つのアプローチから新規 EP300/CBP HAT 阻害薬の獲得を目指し、研究に着手した。第一章では、インシリコスクリーニングにより得られたヒット化合物からの誘導体展開による新規 4-ピリドン-3-カルボン酸誘導体の獲得について述べる。第二章では、HTS より得られたヒット化合物の誘導体展開、及び 1,4-オキサゼパン骨格のスキャフォールドホッピングによる *in vivo* 活性を有する DS17701585 の獲得について述べる。第三章では、EP300 HAT 阻害活性増強、及び薬物動態改善を指向した構造最適化研究による *in vivo* で強力な抗腫瘍効果を有する DS-9300 の獲得とその薬理評価について述べる。

以下、各章にて本研究内容を詳細に記述する。

本論

第一章　4-ピリドン-3-カルボン酸骨格を有する新規 EP300/CBP HAT 阻害薬の合成と構造活性相関

第一節　背景

ヒストンタンパク質は、核内で DNA と結合してクロマチン構造を形成し、ヒストン末端の化学修飾によるクロマチン構造の変化をトリガーとした遺伝子発現の制御に重要な役割を果たしている [14]。クロマチン構造の変化に大きく寄与する化学修飾としてはヒストンリジン残基のアセチル化が挙げられる。ヒストンのリジン残基はヒストンアセチルトランスフェラーゼ（histone acetyltransferases; HAT）を介してアセチル化され、正電荷が中和されることでヒストンと DNA との静電相互作用を弱める。これによってクロマチン構造が緩み、RNA ポリメラーゼ II や転写因子などの DNA 結合タンパク質と DNA が結合しやすくなることで遺伝子発現が活性化される [15]。一方で、ヒストン脱アセチル化酵素（histone deacetylase; HDAC）はリジン残基からアセチル基を除去する酵素であり、HAT と HDAC の二者によってヒストンリジン残基のアセチル化状態とエピジェネティックな遺伝子発現が制御されている（**Figure 1-1**）。

EP300 及び EP300 と構造的に高い相同性を示す CBP は、KAT3 ファミリーに属するヒストンアセチルトランスフェラーゼであり、細胞増殖や分化、恒常性維持などの様々な細胞プロセスに関与することが知られている [16,17]。EP300/CBP は主にヒストン H3K18 及び K27 をアセチル化するほか、アンドロゲン受容体や p53 などの非ヒストンタンパク質をアセチル化することで遺伝子の転写を調節している [18,19]。さらに EP300/CBP は転写コアクチベーターとしての機能を有し、数百のヒストン修飾タンパク質及び核内受容体と結合して多数のシグナル伝達経路の制御に関与している [20]。

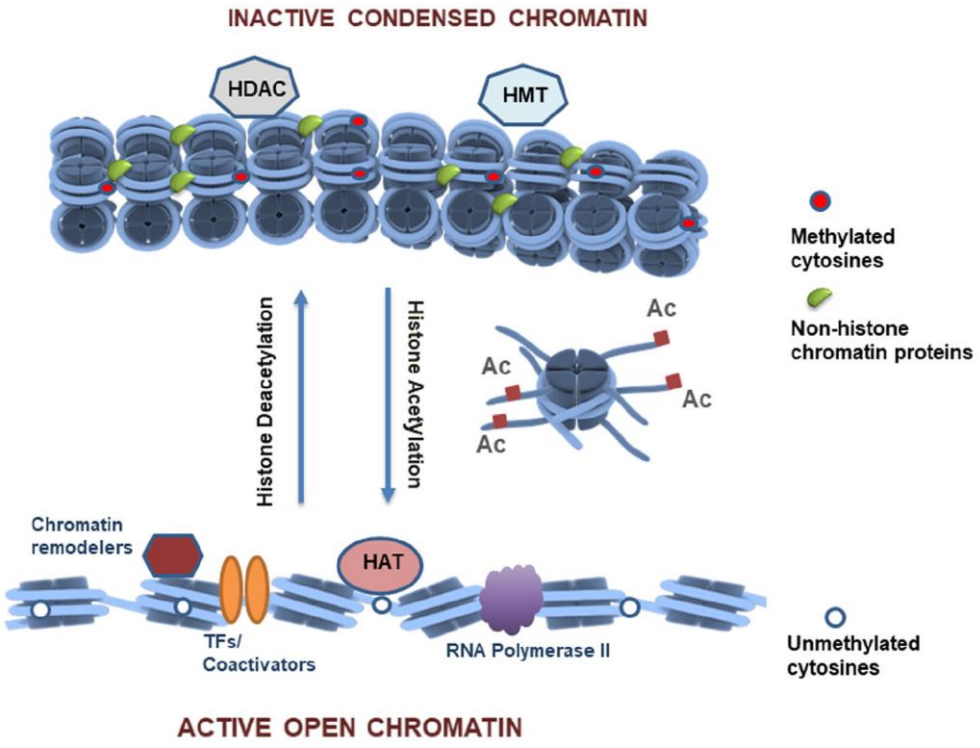

Figure 1-1. Regulation of chromatin structure and gene expression by HAT and HDAC.[21]

EP300/CBP の調節異常は、がんを含む様々な疾患に関連することが報告されている。前立腺がんにおいては、アンドロゲン受容体（androgen receptor; AR）シグナルが異常に活性化することで前立腺がんの増殖が促進されると考えられているが、前立腺がんで過剰発現している EP300/CBP はこの AR シグナルの活性化に関与していることが知られている。すなわち核内移行した AR と EP300/CBP が相互作用することで KLK3（PSA）などの AR シグナルによって制御されている遺伝子の発現を活性化し、これによって前立腺がん細胞の増殖が誘発される[22]。急性骨髄性白血病では、EP300/CBP は DNA の複製や修復、細胞周期進行に関わる遺伝子の転写を調節しており、急性骨髄性白血病の細胞増殖や不死化に深く関与している[23]。この他にも大腸がん[24]、肝細胞がん[25]、小細胞肺がん[26]の進行や予後不良との関連が報告されており、EP300/CBP が新たながん治療の標的となり得ると期待されている。

第二節　既知 EP300/CBP 阻害薬の総括

EP300/CBP の主要なタンパク質ドメインとしては、アセチルトランスフェラーゼ活性を持つ HAT ドメインの他に、核内受容体相互作用ドメイン（nuclear receptor interaction domain; NRID）、CREB-interacting kinase-inducible domain interacting（KIX）ドメイン、3 つのシステイン／ヒスチジンリッチドメイン（CH1/CH2/CH3）、細胞周期調節と転写抑制に関与するサイクリン依存性キナーゼ阻害反応ドメイン（cyclin-dependent kinase inhibitor-reactive domain; CRD1）、アセチル化リジンを認識するブロモドメイン（bromodomain; BRD）、インターフェロン結合ドメイン（interferon binding domain; IBiD）が挙げられる（**Figure 1-2**）[27]。

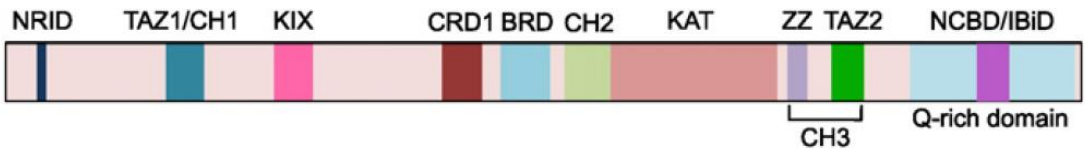

Figure 1-2. Domain structure of EP300/CBP.[27]

このうち、HAT ドメインとブロモドメインががん治療の有望な標的として認識されており、ブロモドメイン阻害薬では Inobrodib（CCS1477）[28]、FT-7051（構造非開示）[29] が血液がん、前立腺がんを対象として臨床試験が行われている。一方で、HAT ドメインを阻害する化合物は、Lys-CoA や、C646 などが知られているものの、臨床試験まで進んだ化合物は存在しない（**Figure 1-3**）[30]。この背景としては、これらの化合物は EP300/CBP HAT ドメインに対して強力な *in vitro* 阻害活性を有する一方で、選択性や細胞膜透過性に課題の残る化合物であるために *in vivo* において十分な抗腫瘍活性が発揮されない可能性が挙げられる。しかしごく最近になり、AbbVie 社、Constellation Pharmaceuticals 社からそれぞれ A-485、CPI-1612 という高活性な EP300/CBP HAT 阻害活性が報告された化合物が次々と見出されており、EP300/CBP HAT 阻害薬開発に対する関心が増々高まっている [31,32]。

そこで著者は、*in vivo* で顕著な薬効を示す EP300/CBP 阻害薬獲得を目的にイ

ンシリコスクリーニング及びハイスループットスクリーニングを実施することとし、まず初めに研究開始当時報告されていた唯一の共結晶構造である EP300 HAT ドメイン／Lys-CoA の構造情報を基にしたドッキングシミュレーションによる新規化合物探索を実施することとした[33]。

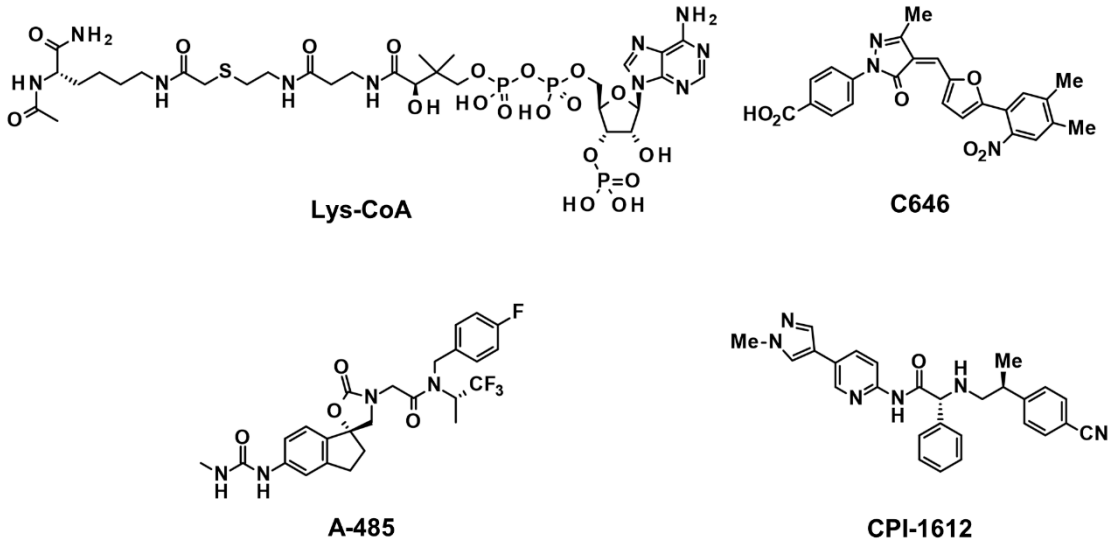

Figure 1-3. Structures of representative EP300/CBP HAT inhibitors.

第三節　バーチャルスクリーニングヒットの獲得

まず、公開されている EP300 HAT ドメインと Lys-CoA との共結晶構造[33]及び C646 のドッキングモデル[34]の解析から EP300 HAT 阻害に関与する結合様式の特定に取り組み、Figure 1-4 に示す①～③の 3 つのファーマコフォアを EP300 との結合に重要なファーアコフォアであるとの仮説を立てた。

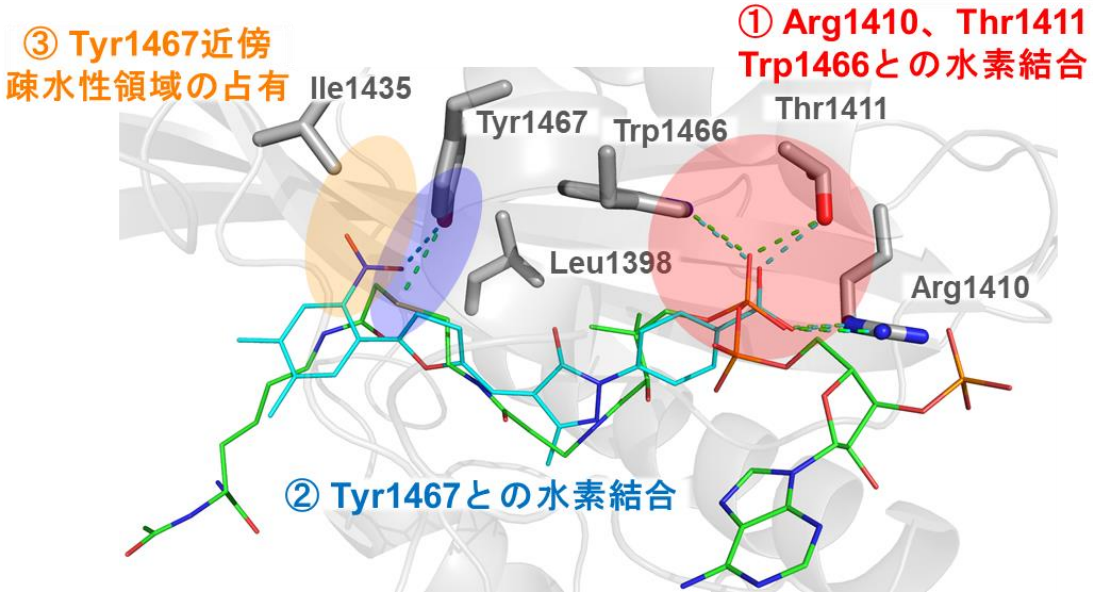

Figure 1-4. Pharmacophore mapping of C646 with EP300 HAT[34].

設定したファーマコフォアをもとに、市販の低分子バーチャルライブラリー400 万化合物をリガンドセットとした Glide によるバーチャルスクリーニングを実施し、新たな EP300 HAT 阻害剤探索を行った（**Figure 1-5**）。ドッキングの結果、2180 化合物が①と②のファーマコフォアに適合し、そのうち回転可能結合数が 5 以下の化合物として 699 化合物を絞り込んだ*[1]。さらに、Tyr1467 近傍の空間を占有している化合物を目視により選抜し、条件を満たす化合物として 20 化合物を選定した。

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*[1]
回転可能結合数が多いほど標的タンパク質と結合した際のエントロピー損失が大きくなるため、創薬探索研究を進める上では回転可能結合数を低減することが望まれる。今回は、現実的に目視での選抜が可能な化合物数まで絞り込むことを目的として、回転可能結合数のクライテリアを 5 以下と設定した。

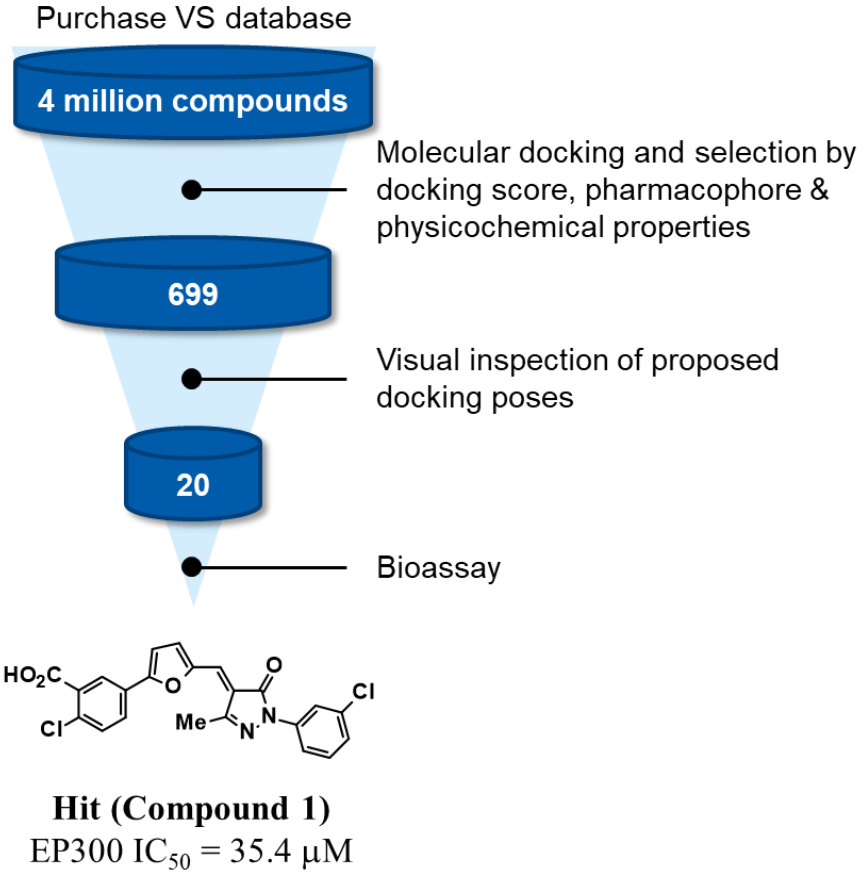


Figure 1-5. Virtual screening workflow of the discovery of the hit compound **1**.

次に、上記 20 化合物を購入し、EP300 HAT 阻害活性を AlphaLISA 法にて評価したところ、**Figure 1-5** に示す化合物 **1** のみが IC_{50} 値が算出できる程度の活性を示し、その値は 35.4 μM であった。この時、陽性対照化合物として設定した C646 は、50 μM で 29%の EP300 HAT 阻害活性を示すのみであった。

化合物 **1** と EP300 とのドッキングモデルを **Figure 1-6** に示す。化合物 **1** のカルボキシ基は Arg1410、Thr1411、及び Trp1466 の 3 つのアミノ酸残基と水素結合、静電相互作用を形成しており、結合能に重要であることが強く示唆された。さらにピラゾロン部位のカルボニル基は Tyr1467 との水素結合を形成しており、Tyr1467 近傍の空間はピラゾロン環、及び疎水性のクロロフェニル部位によって占められている。これらの結合様式は Lys-CoA の共結晶構造や C646 の推定ドッキングポーズと一致していた。

化合物 **1** は既知の EP300 HAT 阻害剤 C646 と構造的に類似度が高く、分子内に長い共役系を有する化合物であったが、毒性の原因構造となり得るニトロ基

を持たない点、C646 よりも強い EP300 HAT 阻害活性を有している点から有望なヒット化合物であると判断し、誘導体展開を実施することとした。

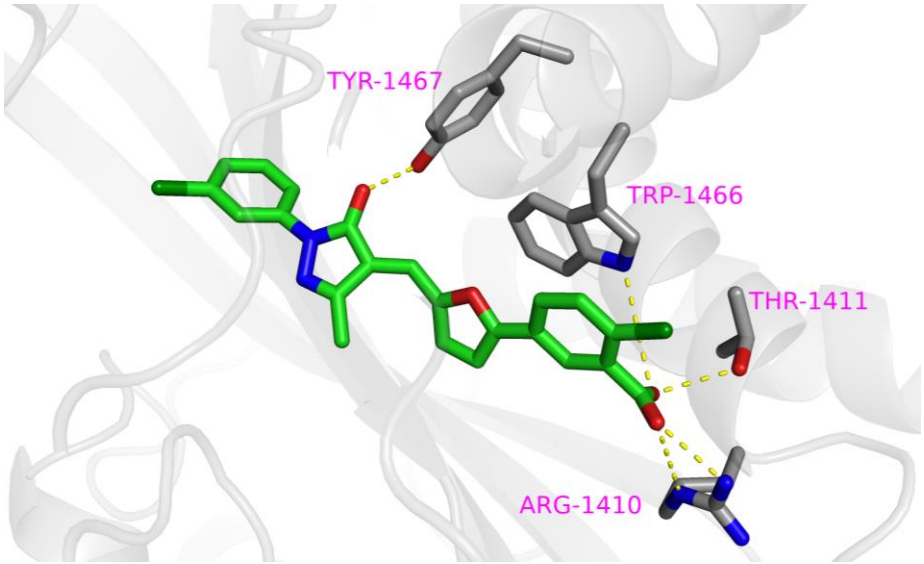
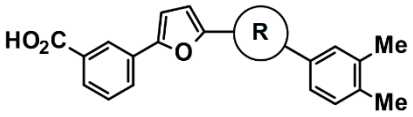


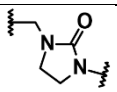
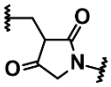
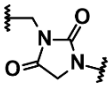
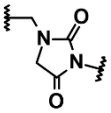
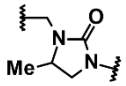
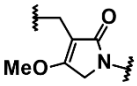
Figure 1-6. Proposed docking pose of compound **1** with EP300 HAT (PDB: 3BIY). Intermolecular H-bonds are shown by yellow lines. The figure was generated using PyMOL (http://www.pymol.org/).

第四節　ヒット化合物周辺の構造活性相関

ヒット化合物 **1** は、α,β-不飽和カルボニルを有する化合物であることから、C646 と同様にシステイン含有タンパク質との反応性が懸念されるほか、分子全体に共役系が広がる化合物である事から、高い蛍光性による蛍光ベースのアッセイ系への影響が想定される[35,36]。このことから、著者はまず共役系分断を目的としてピラゾロン骨格の種々5 員環複素環構造への変換を試みた（**Table 1-1**）。

Table 1-1. Structures and activity evaluation of the synthesized compounds **2a–f** for EP300 HAT inhibition.



Compound	R	IC_{50} (μM)
2a		>50 (47%)[a]
2b		>50 (47%)[a]
2c		>50 (27%)[a]
2d		>50 (34%)[a]
2e		37.3
2f		35.1
1		35.4
C646		>50 (29%)[a]

[a]% inhibition at 50 μM

イミダゾリジノン骨格（**2a**）、ピロリジンジオン骨格（**2b**）及びヒダントイン骨格（**2c**, **2d**）の導入により、50 μM での評価において C646 と同等以上の活性が認められた。さらに、バーチャルスクリーニングヒットと同様にメチル基やメトキシ基などの疎水性置換基を含む 5 員環を導入した化合物 **2e** や **2f** では、共役

系を分断した新規骨格でありながら、化合物 **1** と同等以上の EP300 HAT 阻害活性を示した。

5 員環部のスキャフォールドホッピングに続いて、更なる EP300 HAT 阻害活性の向上を志向して、末端芳香環及びフラン環の最適化を実施した（**Table 1-2**）。化合物 **1** のドッキングポーズを観察すると、クロロフェニル部位の先には Tyr1397 と Trp1436 によって形成された疎水性の間隙が存在することがわかる。そこで化合物 **2a** の 2-ジメチルフェニル基を 2-ナフチル基に変換した **2g** を合成すると、想定通り阻害活性が向上し、18.4 μM の IC_{50} 値を示した。さらに **2a** のフラン環をチオフェン環に変換した **2h** も阻害活性が 2.5 倍程度向上することが明らかとなった。そこで、これらの変換と **Table 1-1** で最も高活性を示した **2f** の骨格を組み合わせた **3a** では、**2f** から 12 倍以上活性が向上した（IC_{50} = 2.9 μM）。

以上のように、著者は最適な活性向上パーツの組み合わせにより一桁 μM の阻害活性を有する化合物を取得する事ができた。

次に、EP300 HAT 阻害に感受性のある 2 種の細胞株を用いて細胞増殖阻害活性を評価した [37]。化合物 **3a** の阻害活性を評価すると、LK2 細胞及び 2G2 細胞において有意な増殖阻害活性を確認することはできなかった。著者は **3a** が細胞内で阻害活性を示さなかった要因として安息香酸部位に起因する細胞膜透過性の不足にあると考え、カルボン酸部位の酸性度や脂溶性を変化させた化合物の合成を実施した（**3b**–**3f**）。安息香酸をピリジンカルボン酸とした **3b** は **3a** と同等の HAT 阻害活性（IC_{50} = 2.6 μM）を示したが、**3a** と同様に LK2 細胞及び 2G2 細胞の細胞増殖阻害活性を示さなかった。一方、ピリジンカルボン酸を *N*-メチル-4-ピリドン-3-カルボン酸構造とした **3c** においては、HAT 阻害活性は若干低下傾向であったが、2 種の細胞株に対して増殖阻害活性を示した（LK2: GI_{50} = 12.4 μM, 2G2: IC_{50} = 8.8 μM）。**3c** が細胞増殖抑制活性を示した理由としては、ピリドン骨格の酸素原子とカルボン酸のヒドロキシ基との分子内水素結合によりカルボン酸のプロトンがマスクされたことや、カルボン酸部位の酸性度が減少したことによって、細胞膜透過性が向上したことが考えられる。さらに、ピリドンの窒素原子上の置換基を種々変換したところ、脂肪族側鎖のエチル基（**3d**）やイソプロピル基（**3e**）はメチル基（**3c**）と同程度の HAT 阻害活性及び細胞増殖阻害活性であったが、ベンジル基を導入した **3f** は **3c** から 2 倍程度高い HAT 阻害活性を示し（IC_{50} = 4.9 μM）、それに対応して細胞増殖阻害活性も向上した（LK2: GI_{50}

= 2.7 μM, 2G2: IC_{50} = 2.4 μM）。以上の結果から、4-ピリドン-3-カルボン酸骨格は、安息香酸と同等の結合様式や活性を維持しつつ膜透過性を向上させることのできるバイオアイソスターとなり得ることが示された。

Table 1-2. Structures and inhibitory activity evaluation of the synthesized compounds **2g**–**2h** and **3a**–**3f** for EP300 HAT inhibition and cell growth inhibition.

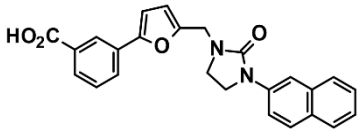
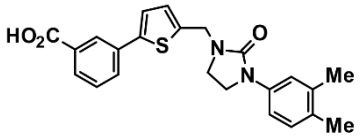
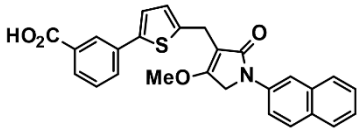
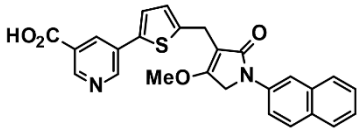
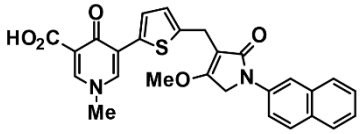
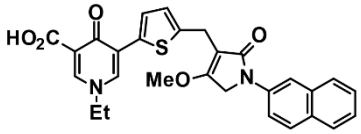
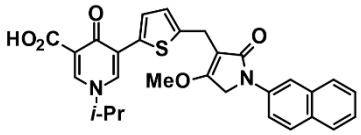
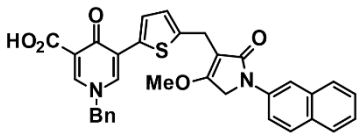
Compound	Structure	EP300 HAT IC_{50} (μM)	Cell growth inhibition	
			LK2 GI_{50} (μM)	2G2 GI_{50} (μM)
2g		18.4	-	-
2h		18.9	-	-
3a		2.9	>25	>25
3b		2.6	>25	>25
3c		10.6	12.4	8.8
3d		9.3	9.1	12.2
3e		11.8	11.8	9.3
3f		4.9	2.7	2.4

Figure 1-7 に、化合物 **3c** と EP300 HAT ドメイン（PDB: 3BIY）とのドッキングスタディの結果を示す。化合物 **3c** はバーチャルスクリーニングヒット **1** と同様の結合様式をとっており、カルボキシ基は Arg1410、Thr1411 及び Trp1466 と相互作用を形成し、ジヒドロピロロン環のカルボニル基は Tyr1467 と水素結合を形成することが示唆された。さらに、**3c** のナフチル基は Tyr1397 と Trp1436 に囲まれた疎水性間隙に沿って位置する結果が得られた。EP300 HAT 阻害評価では、この間隙の方向に疎水性のナフチル基を伸長することで阻害活性の向上を実現していることから、**3c** のドッキングポーズは妥当であることが示唆された。

次に、得られた 4-ピリドン-3-カルボン酸骨格を有する代表化合物 **3c**、**3f** 及び陽性対照化合物 **C646** の HAT ファミリーに対する選択性を評価した（**Table 1-3**）。**C646** は EP300 及び CBP よりも他の HAT ファミリーに対して強い阻害活性を有することが明らかとなった。化合物 **3c** 及び **3f** は、EP300/CBP ファミリーに対して阻害活性を有する一方、MYST2、MYST4、PCAF、GCN5、TIP60 に対しては阻害活性を示さず、EP300/CBP 選択的な阻害剤であることが明らかとなった。

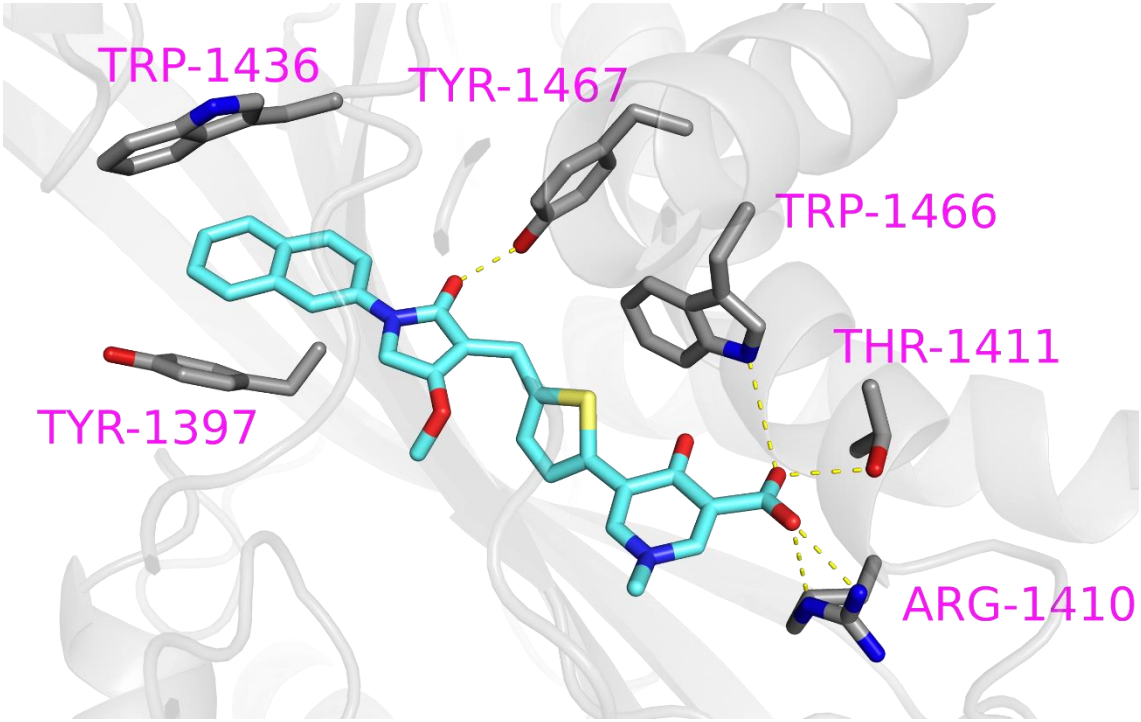


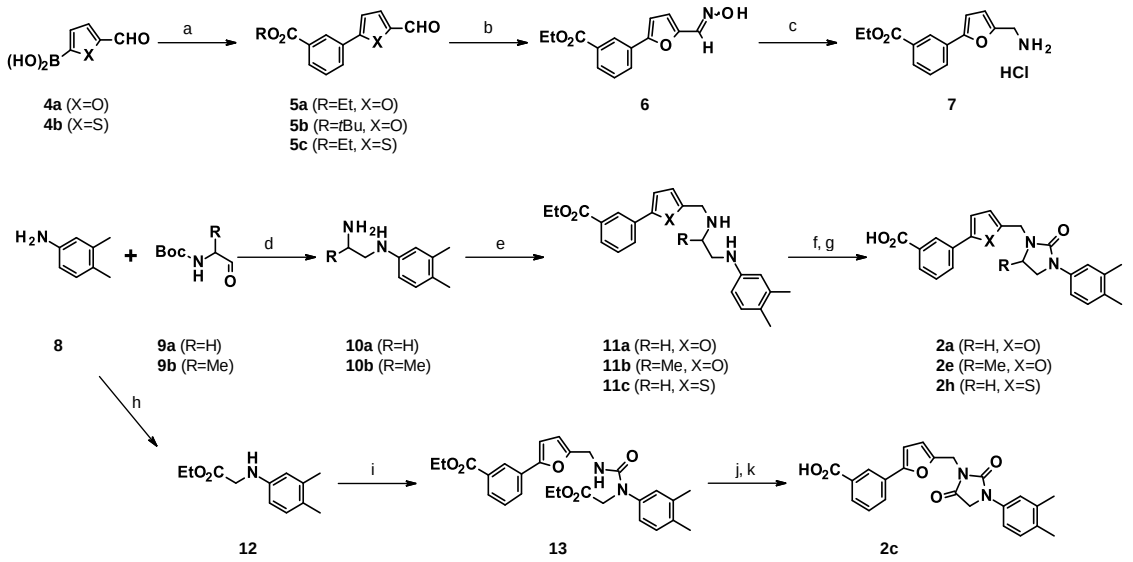
Figure 1-7. Proposed docking pose of compound **3c** with EP300 HAT (PDB: 3BIY). Intermolecular H-bonds are shown by yellow lines. The figure was generated using PyMOL (http://www.pymol.org/).

Table 1-3. *In Vitro* selectivity profiling of compounds **3c**, **3f** against other histone acetyltransferases.

Compound	IC_{50} (μM)						
	EP300	CBP	MYST2	MYST4	PCAF	GCN5	TIP60
3c	10.6	3.4	>50	>50	>50	>50	>50
3f	4.9	1.3	>50	>50	>50	>50	>50
C646	>50	>50	37.2	7.81	21.0	38.9	47.4

第五節　誘導体の合成法

ヒット化合物 **1** のピラゾロン骨格をイミダゾリジノン骨格に変換した化合物の合成経路を **Scheme 1-1** に示した。

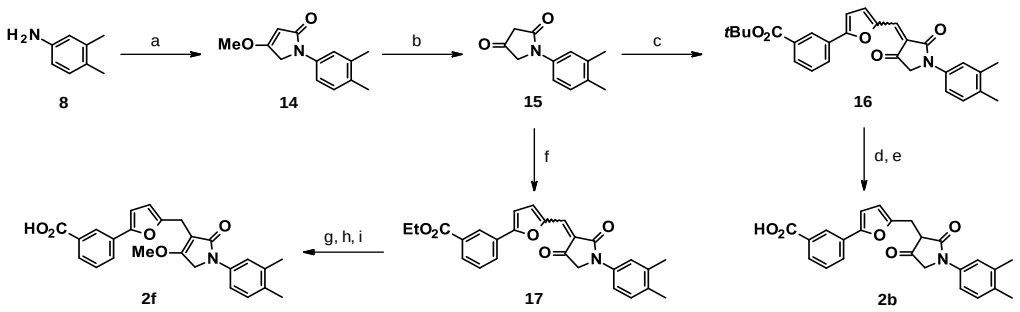


Scheme 1-1. Preparation of compounds **2a**, **2e**, **2h**. Reagents and conditions: (a) Ethyl 3-bromobenzoate or *tert*-butyl 3-bromobenzoate, $PdCl_2(PPh_3)_2$, $NaHCO_3$, 1,4-dioxane, EtOH, H_2O, 70 °C, 3.5 h, **5a** (92%) or **5b** (91%) or **5c** (71%); (b) NaOAc, hydroxylammonium chloride, EtOH, 70 °C, 1 h, 97%; (c) 1 mol/L HCl/H_2O, 10% Pd/C, H_2, rt, 1.5 h, 72%; (d) (i) $NaBH(OAc)_3$, DCM, rt, 6–19 h, (ii) TFA, DCM, 0 °C to rt, 1–2 h, **10a** (78%) or **10b** (71%); (e) **5a** or **5c**, $NaBH(OAc)_3$, DCM, rt, 3.5–64 h, **11a** (36%) or **11b** (40%) or **11c** (37%); (f) triphosgene, TEA, DCE, rt to 70 °C, 3.5 h; (g) 5 mol/L NaOH/H_2O, EtOH, 70 °C, 3 h, **2a** (33%) or **2e** (48%), **2h** (49%) for 2 steps; (h) Ethyl glyoxylate, $NaBH(OAc)_3$, DCM, rt, 18 h, 71%; (i) **7**, triphosgene, TEA, DCE, 0 °C to 70 °C, 3 h, 70%; (j) DIPEA, DMSO, 100 °C, 20 h; (k) 5 mol/L NaOH/H_2O, EtOH, 70 °C, 0.5 h, 12% (two steps).

まず、出発原料のボロン酸 **4a**、**4b** を対応するブロモ安息香酸エステルと鈴木-宮浦カップリングを行うことで化合物 **5a**–**5c** を得た。続いて化合物 **5a** をヒドロキシルアミン存在下にて加熱することでオキシム **6** を得た後、酸性条件下にて水素添加反応を行うことでアミン **7** を得た。化合物 **2a**、**2e**、**2h** の合成は、市販のアニリン **8** とアルデヒド **9a**、**9b** との還元的アミノ化と続く酸性条件下での Boc 基の脱保護によって **10a**、**10b** を得た後、対応するアルデヒドとの還元的アミノ化、さらにトリエチルアミン存在下トリホスゲンで処理することでイミダ

ゾロン環を構築した後、エステルの加水分解によって目的とする **2a**、**2e**、**2h** を得た。化合物 **2c** は、化合物 **8** とグリオキシル酸エチルとの還元的アミノ化と、続いてトリホスゲンとアミン **7** を作用させることでウレア **13** を得た。得られた **13** を塩基性条件下加熱することで環化した後、エステルの加水分解によって目的の化合物 **2c** へと誘導した。

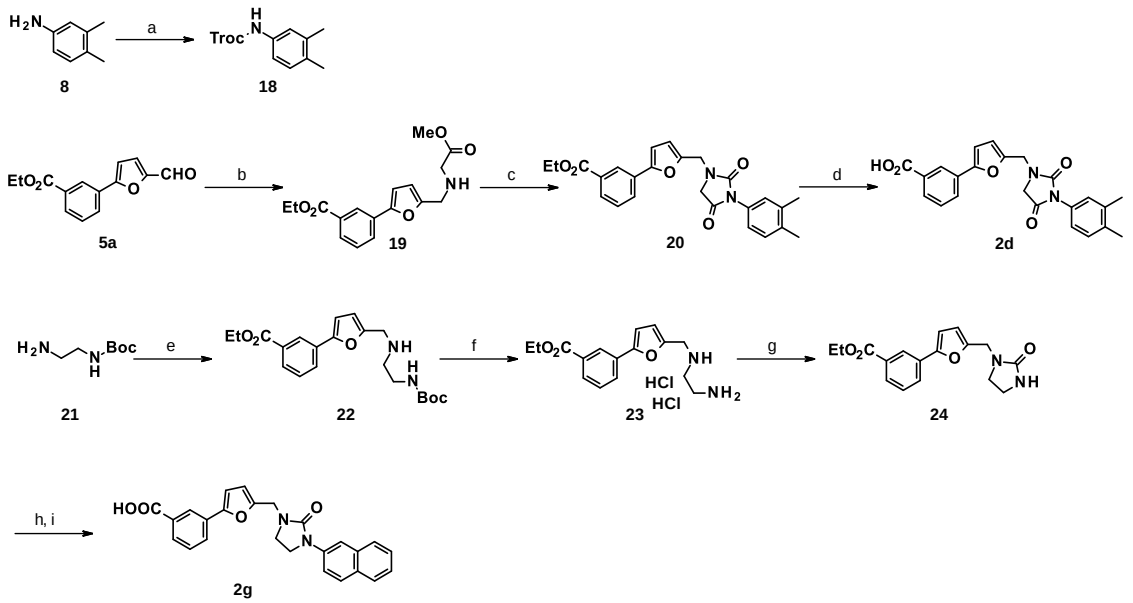
化合物 **2b** 及び **2f** は、**Scheme 1-2** に示す通り合成した。アニリン **8** に塩基性条件下にて(*E*)-4-クロロ-3-メトキシ-2-ブテン酸メチルを作用させることでピロロン環を構築した後、濃塩酸で処理することでピロリジンジオン **15** へと導いた。続いて **15** とアルデヒド **5b** を Knoevenagel 縮合で **16** としたのち、Hantzsch エステルによるオレフィンの還元と、続く酸性条件下での *t*Bu エステルの脱保護により目的物 **2b** を得た。化合物 **2f** は、**15** とアルデヒド **5a** をジエチルアミン存在下加熱することで **17** としたのち、Hantzsch エステルによるオレフィンの還元と、次いで TMS ジアゾメタンと反応させることでメチルエノールエーテルとし、さらにエステルを加水分解することで合成した。



Scheme 1-2. Preparation of compounds **2b**, **2f**. (a) Methyl 4-chloro-3-methoxy-2(*E*)-butenoate, KOAc, MeCN, rt to reflux, 12 h, 54%; (b) c.HCl, 1,4-dioxane, rt, 1 h, 90%; (c) **5b**, diethylamine, toluene, 100 °C, 2 h, 65%; (d) Diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, EtOH, toluene, rt, 4 h; (e) TFA, DCM, rt, overnight, 38% (two steps); (f) **5a**, diethylamine, toluene, 100 °C, 2 h, 73%; (g) Diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, EtOH, toluene, rt, 24 h; (h) $TMSCHN_2$, toluene, MeOH, rt, 3 h; (i) 1 mol/L $NaOH/H_2O$, THF, MeOH, rt, overnight, 77% (three steps).

化合物 **2d** 及び **2g** は、**Scheme 1-3** に示す通り合成した。アニリン **8** を 2,2,2-トリクロロエチルカルボニル （Troc）基により保護し、化合物 **18** を得た。化合物 **5a** とグリシンメチルエステルとの還元的アミノ化反応を行うことで化合物 **19** と

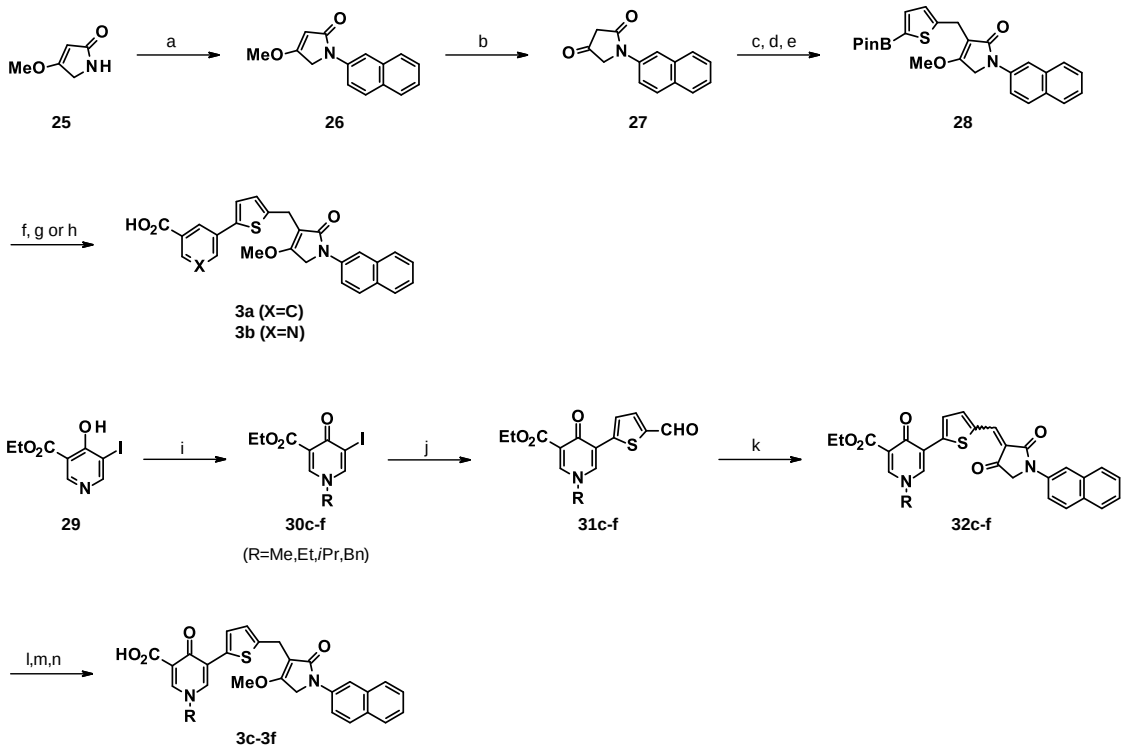
したのち、化合物 **18** と塩基性条件下で加熱した後、さらに DMSO 中で加熱することでヒダントイン環を構築し、さらにエステルを加水分解することで目的の化合物 **2d** を得た。化合物 **2g** は、化合物 **5a** と化合物 **21** との還元的アミノ化でアミン **22** としたのち、Boc 基の脱保護、カルボニルジイミダゾール（CDI）を用いた分子内環化反応によりイミダゾリジノン環を構築し、化合物 **24** を得た。次いで、銅触媒を用いた C-N カップリング反応によりナフタレン環を導入した後、エステルを加水分解することで目的物 **2g** を得た。



Scheme 1-3. Preparation of compounds **2d**, **2g**. (a) 2,2,2-Trichloroethyl chloroformate, TEA, DCM, 0 °C to rt, 2 h, 51%; (b) Glycine methyl ester hydrochloride, $NaBH(OAc)_3$, DCM, rt, 2.5 h, 44%; (c) **18**, DIPEA, toluene, reflux, 4 h, then DMSO, 120 °C, 10.5 h, 74%; (d) 5 mol/L $NaOH/H_2O$, EtOH, 70 °C, 0.5 h, 38%; (e) **5a**, $NaBH(OAc)_3$, DCM, rt, 18 h, 62%; (f) 4 mol/L HCl/1,4-dioxane, rt, 0.5 h, 91%; (g) CDI, TEA, THF, 70 °C, 1 h, 90%; (h) 2-Bromonaphthalene, CuI, *trans*-1,2-cyclohexanediamine, K_2CO_3, 1,4-dioxane, 110 °C, 4.5 h, 71%; (i) 5 mol/L $NaOH/H_2O$, EtOH, 70 °C, 0.5 h, 78%.

化合物 **3a–3f** は、**Scheme 1-4** に示す通り合成した。市販の化合物 **25** に対して、銅触媒を用いた C-N クロスカップリング反応によってナフチル基を導入した後に濃塩酸で処理することでピロリジンジオン **27** へと導いた。続いて **27** とアルデヒド **4c** を Knoevenagel 反応によって縮合したのちに、Hantzsch エステルによるエキソメチレンの還元と次いで TMS ジアゾメタンと反応させることでメチル

エノールエーテル **28** を得た。さらに、対応するブロモ安息香酸エステルと鈴木-宮浦カップリングと続くエステルの加水分解によって化合物 **3a** を得た。化合物 **3b** は、化合物 **28** と 5-ブロモピリジン-3-カルボン酸との鈴木-宮浦カップリングにより得た。



Scheme 1-4. Preparation of **3a**–**f**. (a) 2-Bromonaphthalene, CuI, DMEDA, K_2CO_3, toluene, 100 °C, 8 h, 66%; (b) c.HCl, 1,4-dioxane, rt, 17 h, 96%; (c) **4c**, diethylamine, EtOH, 90 °C, 2 h; (d) Diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, EtOH, 90 °C, 4 h, 64% (two steps); (e) $TMSCHN_2$, toluene, MeOH, AcOH, rt, 16 h, 59%; (f) Ethyl 3-bromobenzoate, $Pd(OAc)_2$, SPhos, K_3PO_4, 1,4-dioxane, H_2O, 60 °C, 1 h; (g) 1 mol/L NaOH/H_2O, THF, MeOH, rt, 18 h, **3a** (29%, two steps); (h) 5-Bromopyridine-3-carboxylic acid, $Pd(OAc)_2$, SPhos, K_3PO_4, 1,4-dioxane, H_2O, 80 °C, 4 h, **3b** (92%); (i) R-X (X=Br,I), Cs_2CO_3, DMF, rt to 50 °C, 2–8 h, **30c** (90%) or **30d** (89%) or **30e** (50%) or **30f** (81%); (j) 5-(6-Methyl-4,8-dioxo-1,3,6,2-dioxazaborocan-2-yl)thiophene-2-carbaldehyde, $Pd(OAc)_2$, SPhos, K_3PO_4, 1,4-dioxane, H_2O, 60 °C, 3.5–24 h, **31c** (60%) or **31d** (28%) or **31e** (46%) or **31f** (85%); (k) **27**, diethylamine, EtOH, reflux, 3–4 h; (l) Diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, EtOH, chloroform, reflux, 1.5–24 h; (m) $TMSCHN_2$, toluene, MeOH, rt, 12–15 h; (n) 1 mol/L NaOH/H_2O, THF, MeOH, rt, 2–17 h, **3c** (39%) or **3d** (40%) or **3e** (43%) or **3f** (10%) (four steps).

第六節　小括

本章では、EP300 HAT ドメインを選択的に阻害する新規骨格の獲得を目指して、バーチャルスクリーニングを実施した。獲得したバーチャルスクリーニングヒット化合物 **1** は分子全体に伸長する共役系とα,β-不飽和カルボニルを含有する化合物であり、システイン含有タンパク質との反応性の懸念や評価系への干渉が想定される化合物であることから、まずは共役系を分断する誘導体展開を実施した。その結果、ピラゾロン骨格からイミダゾロン骨格及びジヒドロピロロン骨格に変換した化合物において化合物 **1** と同等以上の EP300 HAT 阻害活性を示すことを見出し、共役系を分断する骨格を複数獲得した。さらに、細胞増殖阻害活性の向上を目的に、カルボン酸部位の酸性度や脂溶性を調節する誘導体展開を実施し、安息香酸に代えて 4-ピリドン-3-カルボン酸骨格を有する化合物 **3c**–**3f** を獲得した。化合物 **3f** は安息香酸部位を有する化合物 **3a** と比較して、EP300 HAT 阻害活性は同程度であるにもかかわらず、細胞増殖阻害活性が 9 倍以上向上しており、4-ピリドン-3-カルボン酸骨格が、安息香酸のバイオアイソスターとなり得ることが示された。

また、化合物 **3c** 及び **3f** の各種 HAT ファミリーに対する選択性を評価したところ、**C646** と比較して高い EP300/CBP ファミリーを有することが明らかとなった。これらのことから、4-ピリドン-3-カルボン酸骨格を有する新規 EP300/CBP HAT 阻害剤 **3c** 及び **3f** は **C646** よりも優れた EP300/CBP HAT 阻害研究のツール化合物となり得ることが示された。

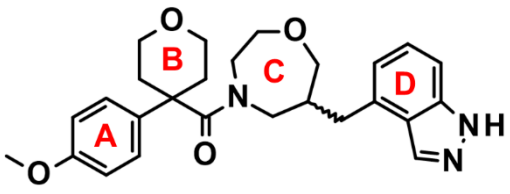
第二章　HTS ヒットからの *in vivo* ツール化合物の獲得

第一節　HTS ヒットの獲得

前章において、バーチャルスクリーニングヒットから 4-ピリジン-3-カルボン酸誘導体へと合成展開することで、EP300 HAT 阻害活性及び細胞増殖抑制活性を向上した化合物を獲得することができた。さらに 4-ピリジン-3-カルボン酸誘導体は *in vitro* の各種 HAT ファミリーに対する選択性評価において EP300/CBP ファミリー選択的な阻害活性を示し、獲得した化合物が EP300/CBP HAT 阻害研究における有用な *in vitro* ツールとなることを示した。

続いて著者は、*in vivo* 試験、更には臨床において経口投与可能な化合物獲得を目指すにあたり、自社の化合物ライブラリーを活用したハイスループットスクリーニング（HTS）により、前章で得られた化合物とは異なる新規骨格を有する阻害剤を探索することとした。

まず、EP300 HAT 阻害活性を指標として、社内化合物ライブラリー約 46 万化合物について HTS を実施したところ、阻害活性を示す約 3000 個の化合物を見出した。さらに、これらの化合物について EP300 HAT 阻害活性の濃度依存性試験、及び thermal shift assay（TSA）試験を実施した結果、化合物 **33** に代表される 1,4-オキサゼパン誘導体がスクリーニングヒットとして得られた。化合物 **33** は EP300 HAT に対して nM オーダーの阻害活性（IC_{50} = 0.61 μM）を示し、さらに LK2 細胞を用いた細胞内での H3K27 に対するアセチル化阻害活性を評価したところ、IC_{50} 値は 2.24 μM であった（**Figure 2-1**）。以上の結果から、化合物 **33** は細胞内においても EP300 HAT 阻害作用を示す有望なヒット化合物であると考えられたため、**Figure 2-1** に示すように、化合物をメトキシフェニル部位（A 環）、テトラヒドロピラン部位（B 環）、オキサゼパン部位（C 環）、インダゾール部位（D 環）の 4 つの部分構造に分割して、それぞれ構造活性相関情報を取得することとした。



33 (HTS hit)

EP300 HAT inhibition IC_{50} 0.61 μM
H3K27ac inhibition (LK2 cells) IC_{50} 2.24 μM

Figure 2-1. Structure and properties of HTS hit **33**.

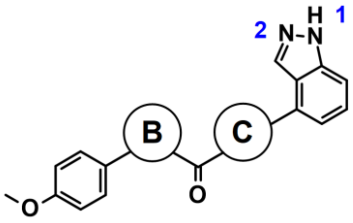
第二節　HTS ヒット化合物周辺の構造活性相関

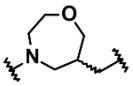
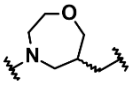
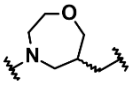
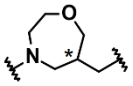
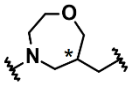
まず、テトラヒドロピラン環（B 環）部位の変換を実施した（**Table 2-1**）。テトラヒドロピラン環をシクロアルキル環に変換すると（**34**, **35**）、EP300 HAT 活性に対する阻害活性は 4 倍向上した。特に、シクロペンチル体 **35** は、LK2 細胞における H3K27 アセチル化阻害評価においても良好な阻害活性を示した（IC_{50} = 0.38 μM）。続いて、オキサゼパン環（C 環）部位の立体化学の阻害活性に対する影響を評価するために化合物 **35** の光学分割を実施したところ、(+)体がユートマーであり、HAT 阻害活性は 0.050 μM の IC_{50} 値を示し、細胞内の H3K27 アセチル化阻害活性評価においても 0.13 μM の IC_{50} 値と強力な阻害活性を示した。一方で、(−)体は H3K27 アセチル化阻害評価でほとんど阻害活性を示さず、HAT ドメインが C 環部の立体を厳密に認識していることが明らかとなった。

次に、見出された強力な EP300 HAT 阻害活性を有する新規オキサゼパン誘導体の結合様式を解析するために、化合物 **(+)-35** と EP300 HAT の X 線共結晶構造を決定した（**Figure 2-2**）。化合物 **(+)-35** のインダゾール環の N_1H 基及び 2 位の窒素原子は、それぞれ Asp1444 の主鎖カルボニル基及び Tyr1446 の主鎖 NH 基と水素結合を形成している。また、インダゾール環と His1451 の間には π-π 相互作用の形成が見られ、カルボニル基は Ser1400 の側鎖 OH 基と水素結合を形成していることが示唆された。

この共結晶構造に基づいて **Table 2-1** の構造活性相関を考察した。化合物 **33** のテトラヒドロピラン環の酸素原子が位置している部位は、Phe1374、Leu1398、Tyr1414、Leu1418 によって構成されている疎水性のポケットである。そのため、テトラヒドロピラン環をシクロアルキル環に変換することで疎水性相互作用が増加し、それに伴って HAT 阻害活性が向上したと考えられる。

Table 2-1. SAR exploration from HTS hit **1**.[a]



Compound	B ring	C ring	EP300 HAT IC_{50} (μM)	LK2 H3K27ac IC_{50} (μM)
33			0.61	2.24
34			0.14	0.61
35			0.13	0.38
(−)-35			NT	>25
(+)-35			0.050	0.13

[a] NT: Not Tested.

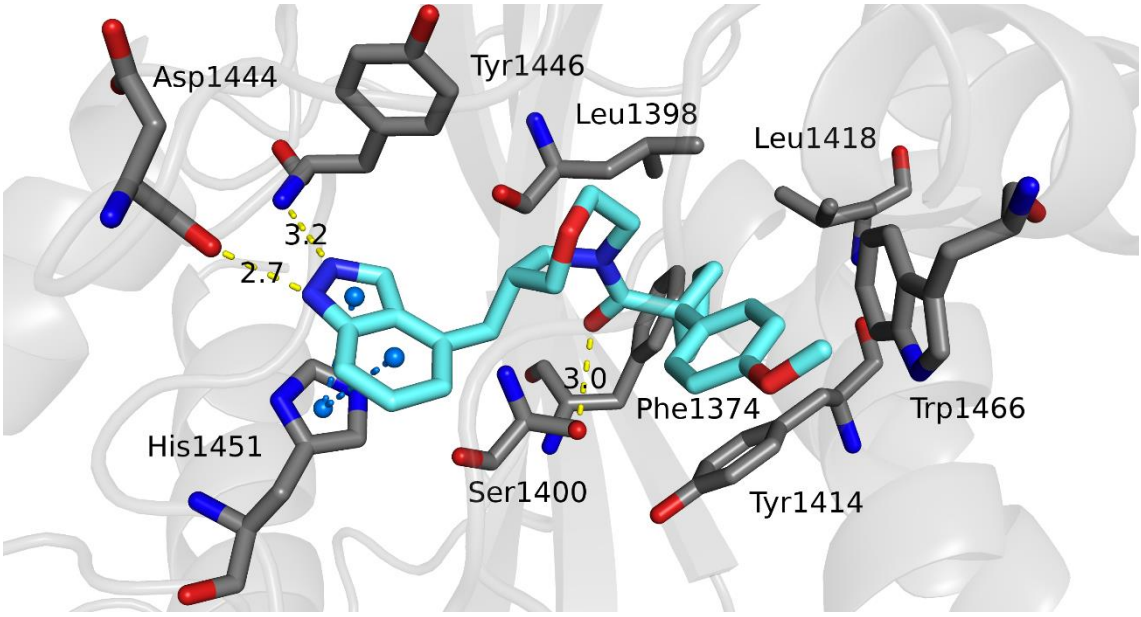


Figure 2-2. Crystal structure of compound **(+)-35** bound to EP300 (PDB ID: 7VHY). H-bonding interactions (yellow) and π-π stacking interactions (blue) are shown as dashed lines.

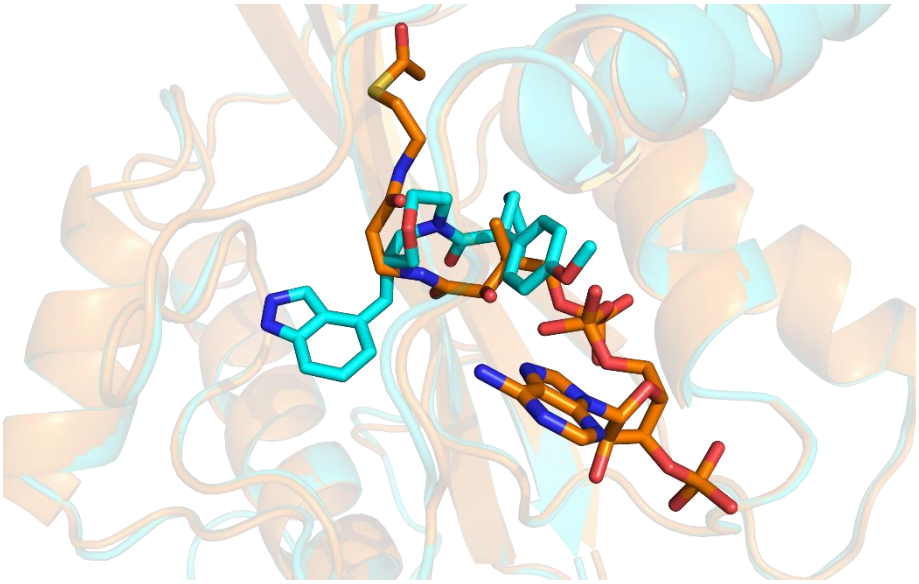


Figure 2-3. Superposition of **(+)-35** and acetyl-CoA (PDB ID: 4PZS, orange) bound to EP300.

また、この共結晶構造と、公開されている Ac-CoA の共結晶構造を重ね合わせると、EP300 HAT ドメインに結合した**(+)-35** は Ac-CoA のリン酸部分からアミド部分に重なり、**(+)-35** のオキサゼパン環の酸素原子は、Ac-CoA のアミドの酸素原子に重なることが確認された（**Figure 2-3**）。

化合物 **(+)-35** の更なる高次評価として、LK2 細胞を Balb/c ヌードマウスに移植した xenograft モデルにおいて、EP300 HAT 阻害の薬力学的バイオマーカーとして考えられる転写因子 SRY-box transcription factor 2（SOX2）の mRNA 発現抑制活性を評価した[38–40]。しかし、経口投与後に**(+)-35** は血中暴露を示さず、SOX2 mRNA の明確な発現抑制は認められなかった（data not shown）。

(+)-35 が血中暴露を示さなかった要因として、オキサゼパン環の酸化代謝が想定されたため、続いてオキサゼパン環上の酸素原子の炭素原子への置換や窒素原子への置換、環サイズの調節を実施し、阻害活性を保持しつつ、経口薬を目指すうえで指標の一つとなる代謝安定性を改善する化合物の獲得を目指した。しかし、いずれの化合物も EP300 HAT 阻害活性が消失する結果となり（data not shown）、オキサゼパン環の酸素原子が EP300 HAT 阻害活性に重要で、オキサゼパン環の最適化によって阻害活性を維持する化合物を得るのは非常に困難であることが示唆された。

第三節　スキャフォールドホッピングと SBDD アプローチによるツール化合物の獲得

前節では HTS ヒットから B 環の変換によって、EP300 HAT 阻害活性が二桁 nM の IC_{50} 値を示す化合物 **(+)-35** を獲得した。一方で、**(+)-35** は経口投与によって血中暴露と *in vivo* 薬効を示さず、続くオキサゼパン環上の置換基変換では阻害活性、及び代謝安定性を向上させた化合物の取得には至らなかった。そこで著者は、*in vivo* 試験で薬効を発現する化合物を取得するためには、オキサゼパン骨格からの脱却が必要であると考え、C 環部のスキャフォールドホッピングを実施した（**Table 2-2**）。

堅牢な合成法で、構造的に多様な置換基導入が可能なアミノ酸をベースとしたスキャフォールドホッピングによって簡便かつ迅速な構造活性相関情報の取得を図ることとし、*N*-メチルグリシン（**36**）、*N*-メチル-D-アラニン（**37**）、D-プロリン誘導体（**38**、**39**）を合成したところ、これらの化合物は HTS ヒット周辺化合物と同程度の EP300 HAT 阻害活性を示すことが確認された。特に、D-プロリン誘導体は細胞内での H3K27 アセチル化阻害活性についても良好な値を示し、アミノ酸ベースのスキャフォールドホッピングによってオキサゼパン骨格からの変換が可能であることを示した。

続いて、プロリン誘導体からの活性向上の可能性を探索するために、化合物 **39** と EP300 との X 線共結晶構造を取得した。化合物 **(+)-35** と **39** の共結晶構造を比較すると、ほとんど同様の結合様式を維持しており、特に**(+)-35** のオキサゼパン環と化合物 **39** のプロリン環が同じ位置にあることが明らかとなった（**Figure 2-4**）。前章に記載したように、オキサゼパン環の酸素原子は EP300 HAT 阻害活性に重要な役割を果たすことから、さらなる阻害活性の向上を目的として、**39** のプロリン環上へのヘテロ原子の導入を検討することとした（**Figure 2-5**）。

共結晶構造の解析から、オキサゼパン環の酸素原子は化合物 **39** のプロリン環 4 位付近に位置することがわかる。そこで、プロリン環 4 位にヘテロ原子としてフッ素原子、ヒドロキシ基、スルホニル基を導入することにした（**Table 2-3**）。

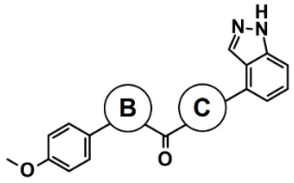
フッ素原子を導入した 4*R*-フルオロ-D-プロリン **40** は、無置換プロリン **39** よりも強力な HAT 阻害活性を示した（EP300 HAT IC_{50} = 0.056 μM）。オキサゼパン環の酸素原子側にヒドロキシ基を導入した 4*R*-ヒドロキシ-D-プロリン **42** は、も

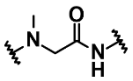
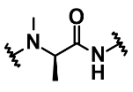
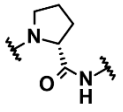
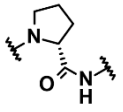
う一方のジアステレオマーである 4*S*-ヒドロキシ-D-プロリン **41** よりも高活性を示したが、**39** と同等の EP300 HAT 阻害活性であった。プロリン環にスルホニル基を置換した **43** は無置換プロリン **39** と比較して、HAT 阻害活性及び H3K27 アセチル化阻害活性がともに活性向上傾向であった。

続いて生体内での化合物のクリアランスを推定するために、マウス肝ミクロソームを用いた *in vitro* 代謝安定性試験を実施した。フルオロプロリン誘導体 **40** は一連の化合物で最も強力な HAT 阻害活性を示したが、無置換プロリン **39** と同様に代謝安定性は低値を示した。一方で、ヒドロキシ基及びスルホニル基を置換した化合物（**40**–**43**）は、マウス肝ミクロソーム中での代謝安定性が向上した。

以上の結果から、プロリン誘導体にフッ素原子を導入することで HAT 阻害活性が数倍向上することがわかり、さらにスルホニル基を導入した化合物 **43**（**DS17701585**）が HAT 阻害活性、細胞内での H3K27 アセチル化阻害活性及び代謝安定性のバランスが優れていることが明らかとなった。そこでまず、プロリン環へのフッ素原子導入によって HAT 阻害活性が向上した要因を考察することとし、続いて化合物 **43**（**DS17701585**）の更なる高次評価を実施することとした。

Table 2-2. SAR of scaffold hopping approach.



Compound	B ring	C ring	EP300 HAT IC_{50} (μM)	LK2 H3K27ac IC_{50} (μM)
36			0.19	1.01
37			0.22	5.02
38			0.17	0.42
39			0.18	0.69

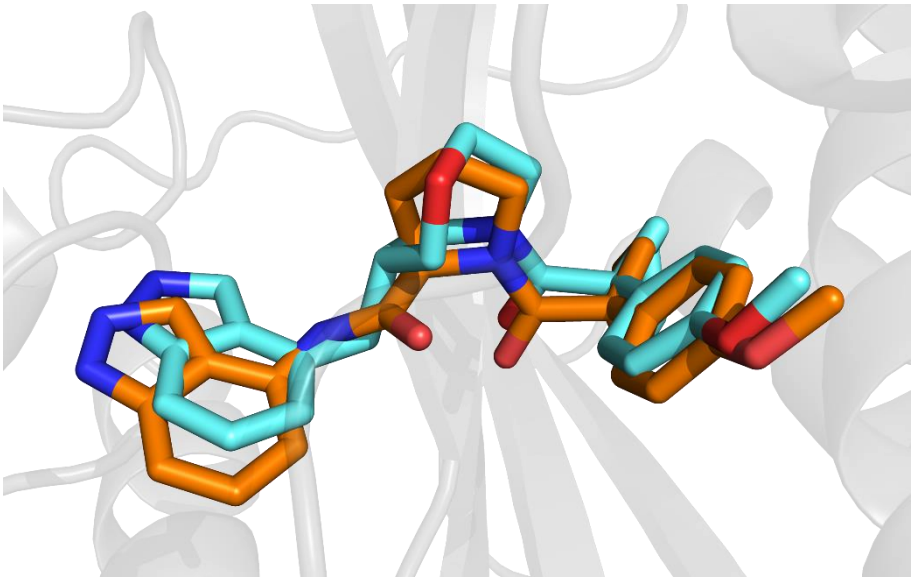


Figure 2-4. Superposition of compound **(+)-35** (PDB ID: 7VHY, cyan) and compound **39** (PDB ID: 7VHZ, orange) bound to EP300.

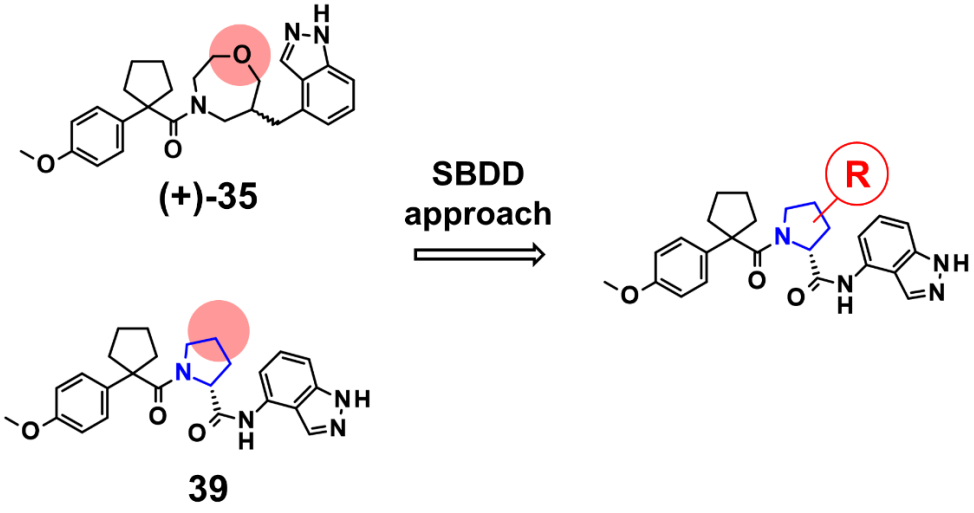
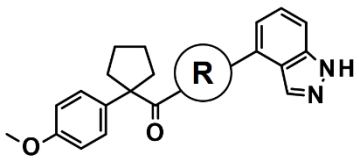
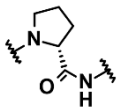
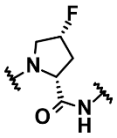
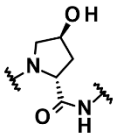
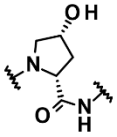
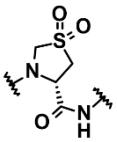


Figure 2-5. Structures of compound **(+)-35** and **39** highlighting the strategy of additional substitutions on the proline ring of **39**.

Table 2-3. SAR of the proline derivatives.



Compound	R	EP300 HAT IC_{50} (μM)	LK2 H3K27ac IC_{50} (μM)	Metabolic stability (MS, %)[a]
39		0.18	0.69	10
40		0.056	0.46	5
41		0.91	1.45	57
42		0.26	1.03	47
43 (DS17701585)		0.15	0.45	37

[a]The percentage (%) of the tested compound remaining after 0.5 h of incubation with mouse liver microsomes (0.1 mg/mL).

第四節　化合物 40 の *in vitro* 活性向上要因についての考察

前節のオキサゼパン環の酸素原子を模倣した置換基導入 (化合物 **41**–**43**)では明確な HAT 阻害活性の向上は認められなかったが、フッ素原子を導入した化合物 **40** では 3 倍の阻害活性向上が認められた。このことから、フッ素原子導入による *in vitro* 活性向上の要因として、立体電子効果による配座制御が想定された。

プロリン環 4 位にフッ素原子を導入したフルオロプロリンは、$\sigma_{C\text{-}H} \rightarrow \sigma^*_{C\text{-}F}$ や $\sigma_{C\text{-}H} \rightarrow \sigma^*_{C\text{-}N}$ の超共役効果によって gauche 配座が安定化される（**Figure 2-5**）[41]。また、化合物 **39** と EP300 との X 線共結晶構造中（**Figure 2-4**）のプロリン環の活性配座に着目すると、無置換プロリン誘導体 **39** においてはプロリン環のγ炭素が *endo* 配座を取っており、この配座が活性配座である事が示唆される（**Figure 2-6**）。

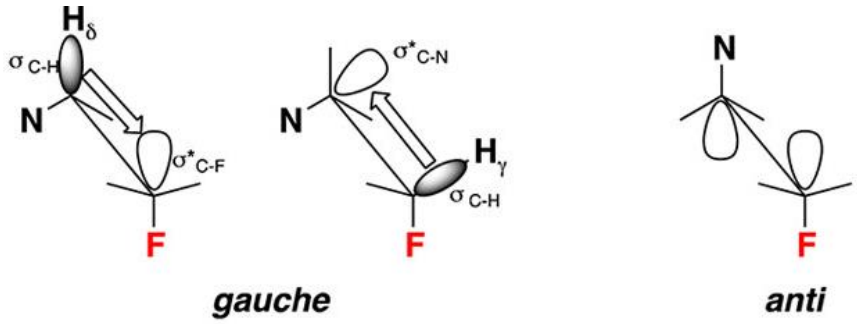


Figure 2-5. Stereoelectronic effects in 4-fluoroproline.[41]

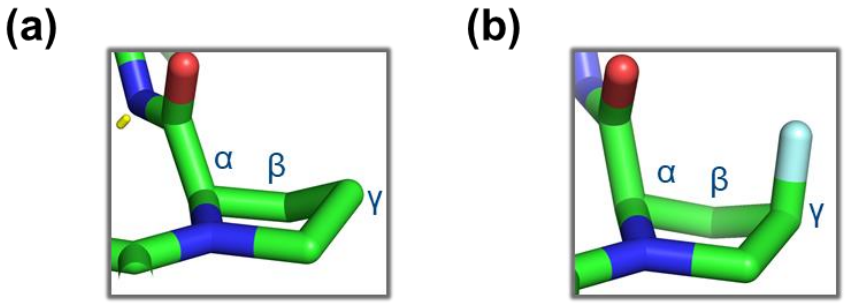


Figure 2-6. (a) Active conformation of unsubstituted-D-proline. (b) Active conformation of 4*R*-fluoro-D-proline.

4*R*-フルオロ-D-プロリンにおける *exo* 体と *endo* 体の立体配座を Newman 投影式で示すと、*exo* 体は窒素原子とフッ素原子が *anti* の関係となる一方、*endo* 体は gauche 配座となり、$\sigma_{C\text{-}H} \rightarrow \sigma^*_{C\text{-}F}$ 及び $\sigma_{C\text{-}H} \rightarrow \sigma^*_{C\text{-}N}$ の超共役効果によって安定化される（**Figure 2-7**）。以上のことから、化合物 **39** のプロリン環へフッ素原子を導入した化合物 **40** はプロリン環が立体電子効果によって活性配座である *endo* 配座に制御され、それによって EP300 HAT 阻害活性が向上したものと考察した。

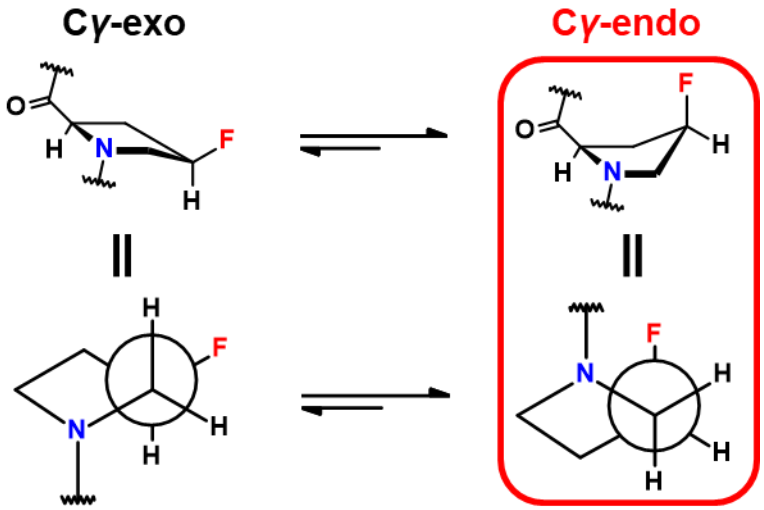


Figure 2-7. Ring conformations of 4*R*-fluoro-D-proline.

第五節　DS17701585 の高次評価

DS17701585 と EP300 複合体の X 線結晶構造を **Figure 2-8** に示す。**DS17701585** は、化合物 **39** に見られた EP300 との結合様式を維持しているのに加えて、スルホニル基の酸素原子が Tyr1446 の側鎖 OH 基と水分子を介した水素結合を形成していることが確認された。

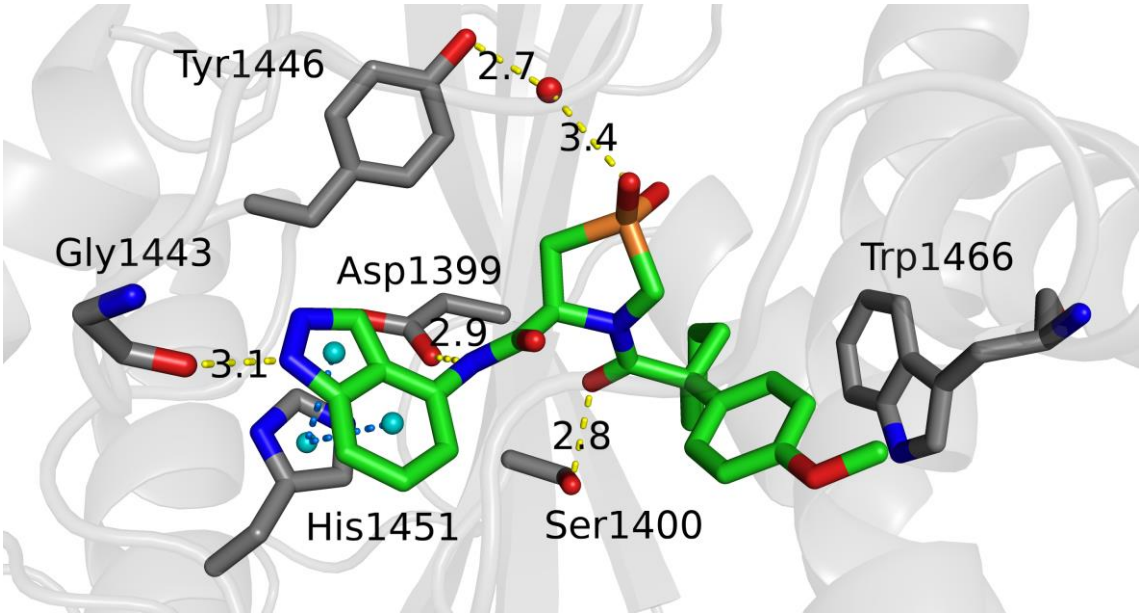


Figure 2-8. Crystal structure of compound **43** (**DS15501585**) bound to EP300. H-bonding interactions (yellow) and π-π stacking interactions (blue) are shown as dashed lines.

次に、**DS17701585** の HAT ファミリーに対する選択性を評価した（**Table 2-4**）。**DS17701585** は、EP300/CBP ファミリーを構成する EP300 及び CBP に対しては強力な阻害活性を示す一方、他の HAT ファミリーに対しては阻害活性を示さず、前章で示した化合物と同様に高い EP300/CBP 選択性を有する化合物であることが明らかとなった。

Table 2-4. *In Vitro* selectivity profiling of **DS17701585** against other histone acetyltransferases.

IC_{50} (μM)						
EP300	CBP	MYST2	MYST4	PCAF	GCN5	TIP60
0.15	0.040	>50	>50	>50	>50	>50

続いて、**DS17701585** の *in vitro* 薬理活性及び ADME プロファイルを **Table 2-5** に示す。**DS17701585** は、HAT 阻害活性、H3K27 アセチル化阻害活性に加えて、LK2 細胞を用いた SOX2 mRNA 発現抑制評価においても良好な阻害活性を示した。また、*in vitro* ADME 評価においても適度な溶解性、脂溶性、膜透過性及び血漿中タンパク結合率を示すことがわかった。以上より、**DS17701585** は *in vivo* 試験に資する化合物であると判断し、更なる高次評価を実施することとした。

Table 2-5. Summary of *in vitro* potency and ADME properties of **DS17701585**.

Compound	**43 (DS17701585)**
EP300 HAT IC_{50} (μM)	0.15
H3K27ac (LK2 cells) IC_{50} (μM)	0.45
SOX2 mRNA expression (LK2 cells) IC_{50} (μM)	0.70
Solubility (mg/mL) [pH 5.0/ pH 7.4]	63/50
Log D	3.2
PAMPA (10^{-6} cm/s) [pH 5.0/ pH 7.4]	>50/>50
Mouse MS (% remaining)	37
Plasma protein binding in mouse (%)	95.2

第六節　DS17701585 の *in vivo* 評価

LK2 細胞株を Balb/c ヌードマウスの皮下に移植した xenograft モデルを用いて、**DS17701585** の *in vivo* での SOX2 mRNA 発現抑制活性を経口投与にて評価した（**Figure 2-9**）。50 mg/kg 及び 200 mg/kg の単回投与 6 時間後における SOX2 mRNA 発現レベルを対照群と比較した。その結果、**DS17701585** は血漿中及び腫瘍中薬物濃度の増加と相関して用量依存的な SOX2 mRNA 発現抑制活性を示すことが明らかとなった。そして、**DS17701585** は EP300/CBP ファミリー選択的な HAT 阻害活性を有していることから、EP300/CBP HAT 阻害活性に基づく *in vivo* 薬効であることも示唆された。

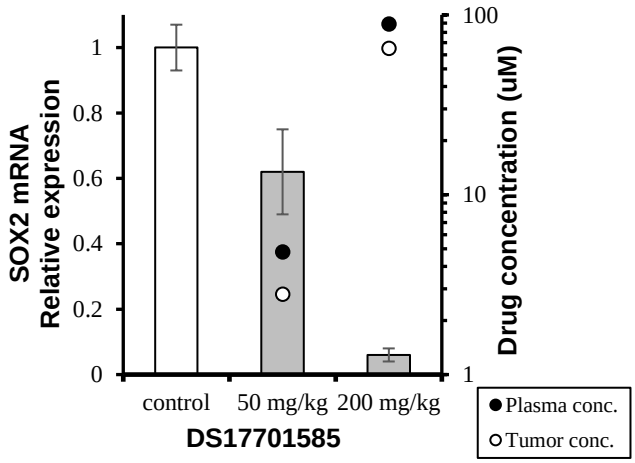
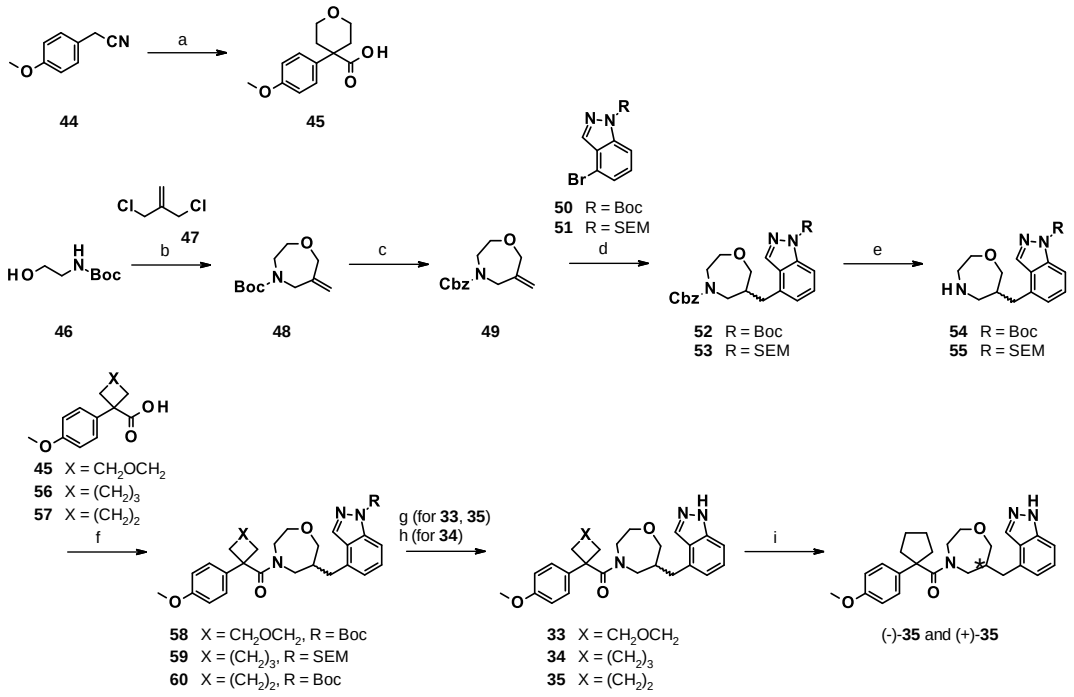


Figure 2-9. Dose-dependent *in vivo* inhibition of SOX2 mRNA expression by **DS17701585** (*N* = 3).

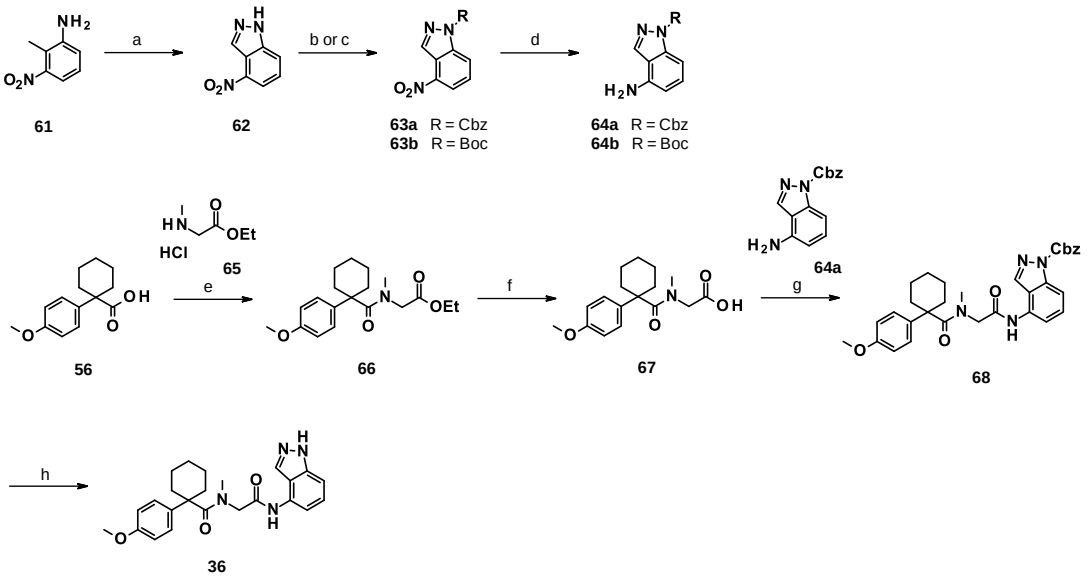
第七節　評価化合物の合成

HTS ヒット化合物 **33** 及び B 環部を変換した化合物の合成法を **Scheme 2-1** に示す。市販のニトリル **44** を出発原料とし、ベンジル位をジアルキル化することで A 環、B 環を連結した中間体 **45** を得た。次に、市販の *N*-Boc エタノールアミン **46** を **47** でジアルキル化することでオキサゼパン環を構築し、保護基の変換によって化合物 **49** を得た。続いて、9-BBN を用いた位置選択的なヒドロホウ素化とそれに続くブロモインダゾール **50** 及び **51** との鈴木カップリングにより、化合物 **52**、**53** を得た。水素雰囲気下で Cbz 基を除去し、対応するカルボン酸と縮合した後、インダゾール上の保護基を酸性条件下で除去することで目的の化合物 **33**–**35** を得た。**35** はキラルカラムを用いた光学分割を行い、化合物**(–)-35** と **(+)-35** をそれぞれ単離した。



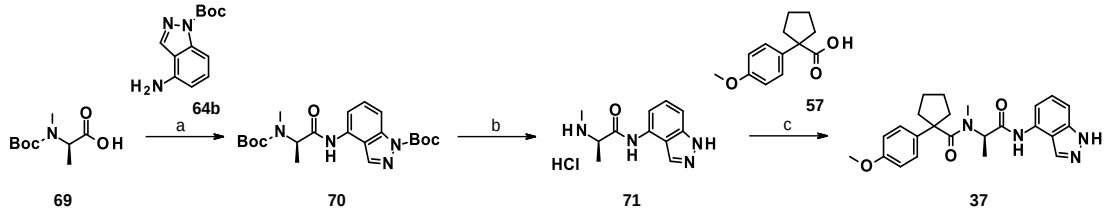
Scheme 2-1. Preparation of compounds **33–35**. (a) NaH, bis(2-bromoethyl)ether, DMF, 0 °C to rt, 74%; (b) NaH, DMF/THF, rt, 63%; (c) (i) 2 mol/L HCl/H_2O, MeOH, rt; (ii) DIPEA, benzyl chloroformate, DCM, 0 °C, 98%; (d) i. 9-BBN, THF, reflux; ii. K_2CO_3, **50** or **51**, Pd(dppf)Cl_2-DCM adduct, DMF, H_2O, 65 °C, 73–85%; (e) Pd/C, H_2, EtOH, rt; (f) carboxylic acid, DIPEA, HATU, DMF, rt, 36–82% (two steps); (g) 4 mol/L HCl/1,4-dioxane, DCM, rt, 79–82%; (h) TFA, DCM, rt, 28%; (i) chiral separation (CHIRALPAK IA).

化合物 **36** の合成法を **Scheme 2-2** に示す。市販の 2-メチルアニリン **61** から、ジアゾ化を経由した分子内環化反応によりインダゾール環を構築した後、インダゾールの窒素原子上への保護基導入とニトロ基の還元を経て、中間体 **64a** 及び **64b** を得た。次に、市販のカルボン酸 **56** をアミン **65** と縮合した後、エチルエステルの加水分解によって化合物 **67** を得た。その後、**64a** との縮合と、続く Cbz 基の除去によって目的の化合物 **36** を得た。



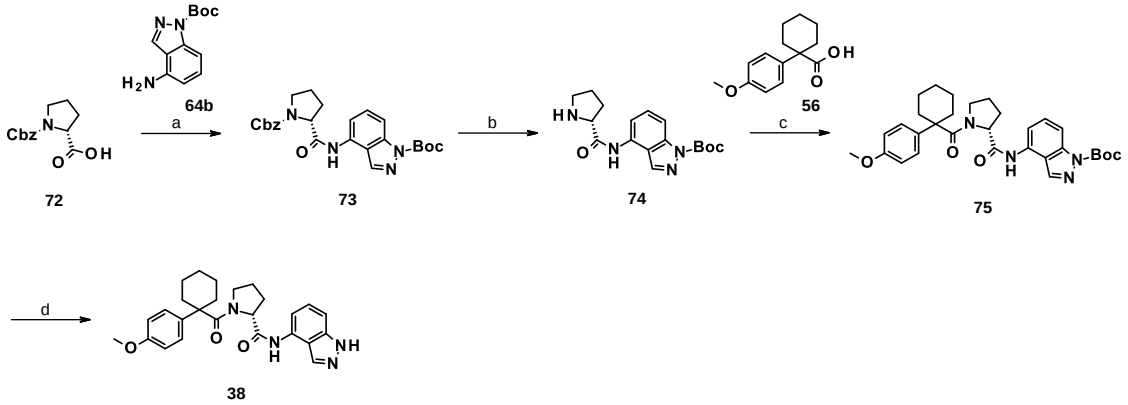
Scheme 2-2. (a) $NaNO_2$, AcOH, H_2O, rt, 79%; (b) DBU, benzyl chloroformate, DMF, rt, 77%; (c) TEA, Boc_2O, DCM, rt, 100%; (d) Zn, NH_4Cl, MeOH, H_2O, rt, 93–94%; (e) DIPEA, HATU, DMF, rt, 54%; (f) LiOH, MeOH, H_2O, rt, 93%; (g) DIPEA, HATU, DMF, rt, 55%; (h) LiOH, MeOH, H_2O, rt, 23%.

化合物 **37** の合成法を **Scheme 2-3** に示す。市販のサルコシン誘導体 **69** と **64b** を縮合した後、Boc 基を除去し、その後カルボン酸 **57** と縮合することで目的とする化合物 **37** を得た。



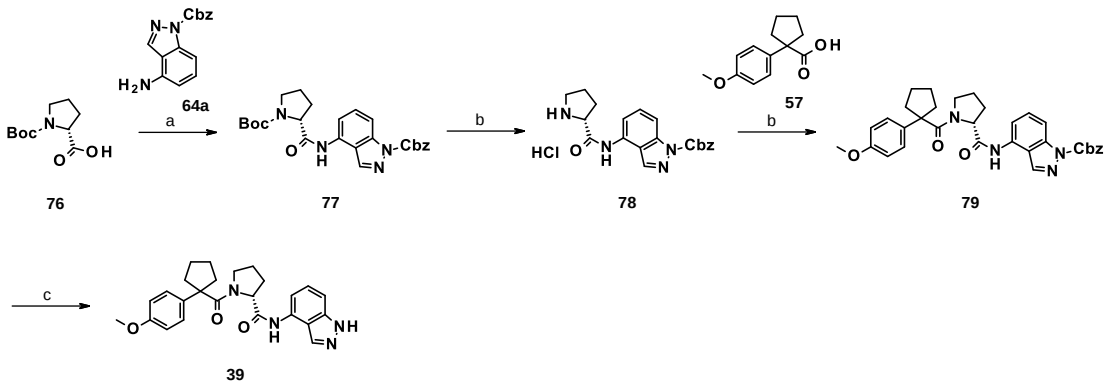
Scheme 2-3. (a) DIPEA, COMU, DMF, rt, 50%; (b) 4 mol/L HCl/1,4-dioxane, DCM, rt; (c) DIPEA, COMU, DMF, rt, 54% (two steps).

化合物 **38** の合成法を **Scheme 2-4** に示す。市販のプロリン誘導体 **72** を出発原料として、**64b** との縮合と Cbz 基の除去によって化合物 **74** を得た後、カルボン酸 **56** との縮合と続く Boc 基の除去によって目的とする化合物 **38** を得た。



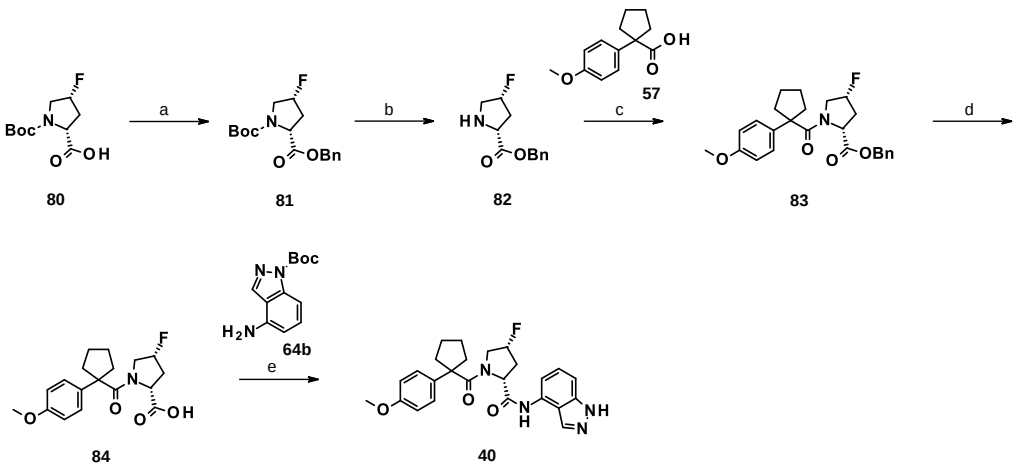
Scheme 2-4. (a) DIPEA, COMU, DMF, rt, 73%; (b) Pd/C, H_2, EtOH, rt; (c) DIPEA, COMU, DMF, rt, 50%; (d) 4 mol/L HCl/1,4-dioxane, DCM, rt, 70% (two steps).

化合物 **39** の合成法を **Scheme 2-5** に示す。市販のプロリン誘導体 **76** と **64a** を縮合し、Boc 基を除去することでアミン **78** を得た。続いてカルボン酸 **57** と縮合した後、Cbz 基を除去することで目的とする化合物 **39** を得た。



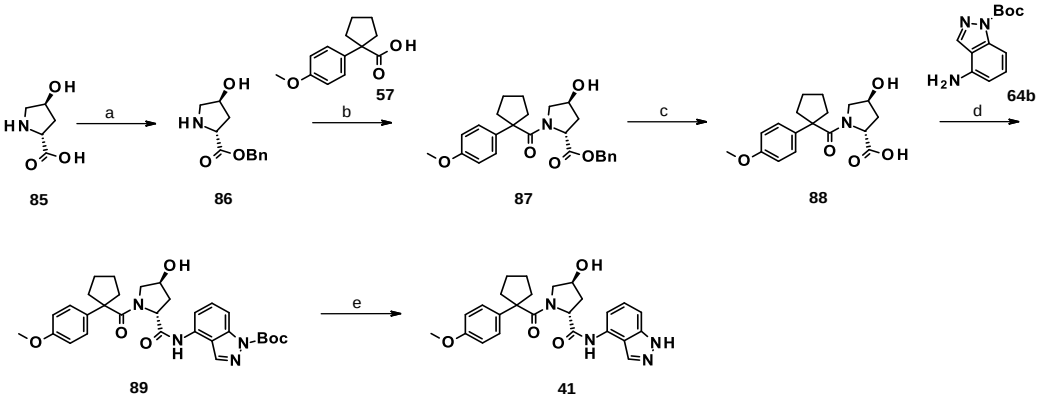
Scheme 2-5. (a) DIPEA, HATU, DMF, rt, 65%; (b) (i) 4 mol/L HCl/1,4-dioxane, DCM, rt; (ii) $SOCl_2$, DMF, DCM, 40 °C, (iii) DIPEA, DCM, rt; (c) K_2CO_3, MeOH, THF, rt, 75% (two steps).

化合物 **40** の合成法を **Scheme 2-6** に示す。市販のプロリン誘導体 **80** をベンジルエステル **81** へと誘導後、Boc 基を脱保護することでアミン **82** を得た。続いて、カルボン酸 **57** との縮合、ベンジル基の除去によってカルボン酸 **84** を得た後、**64b** との縮合と続く Boc 基の脱保護によって目的とする化合物 **40** を得た。



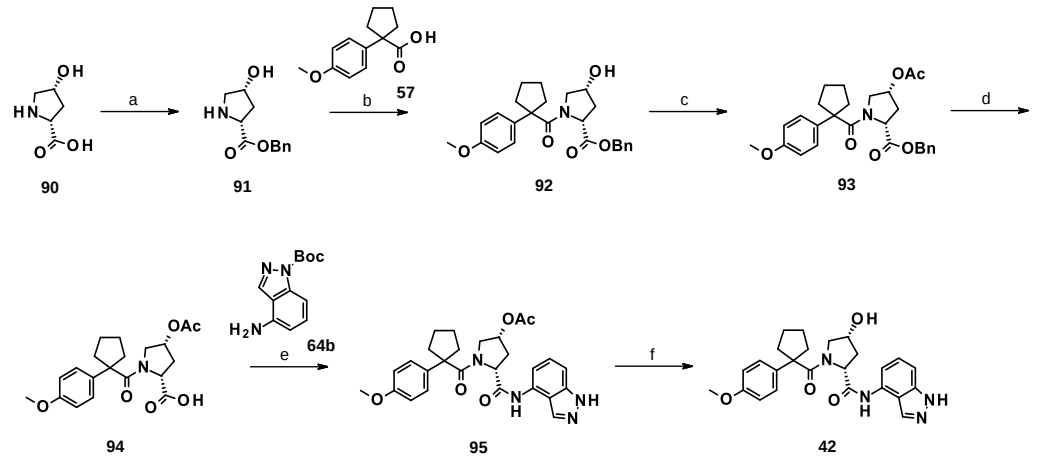
Scheme 2-6. (a) (i) Cs_2CO_3, MeOH, H_2O, 0 °C; (ii) BnBr, DMF, rt, 91%; (b) 4 mol/L HCl/1,4-dioxane, rt, 98%; (c) DIPEA, COMU, DMF, rt, 97%; (d) Pd/C, H_2, EtOH, rt, 91%; (e) (i) DIPEA, COMU, DMF, rt; (ii) 4 mol/L HCl/1,4-dioxane, rt, 88%.

化合物 **41** の合成法を **Scheme 2-7** に示す。市販のプロリン誘導体 **85** を酸性条件下ベンジルエステル **86** へと誘導後、カルボン酸 **57** と縮合して化合物 **87** を得た。続いてベンジルエステルを加水素分解した後、**64b** との縮合と Boc 基の脱保護を経て目的の化合物 **41** を得た。



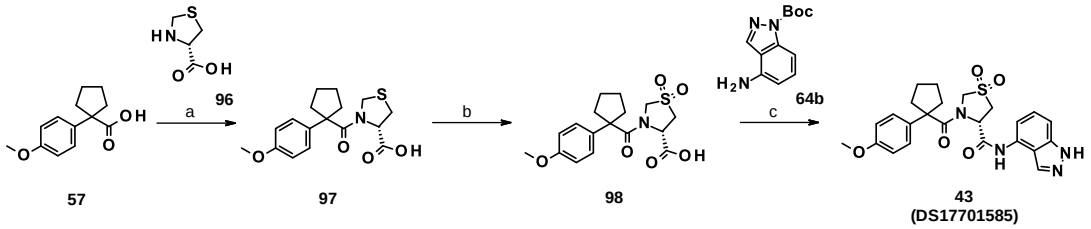
Scheme 2-7. (a) BnOH, PTSA, toluene, 120 °C, 61%; (b) DIPEA, COMU, DMF, rt, 80%; (c) Pd/C, H_2, EtOH, rt, 100%; (d) DIPEA, COMU, DMF, rt, 76%; (e) 4 mol/L HCl/1,4-dioxane, DCM, rt, 88%.

化合物 **42** の合成法を **Scheme 2-8** に示す。**Scheme 2-7** と同様の条件を用いて市販のプロリン誘導体 **50** からベンジルエステル **91** へと誘導後、カルボン酸 **57** と縮合して化合物 **92** を得た。続いてヒドロキシ基をアセチル基で保護し、ベンジルエステルを加水素分解した後、**64b** との縮合と続く Boc 基の除去によって目的とする化合物 **42** を得た。



Scheme 2-8. (a) BnOH, PTSA, benzene, reflux, 63%; (b) DIPEA, COMU, DMF, rt, 92%; (c) Ac_2O, pyridine, DCM, rt, 100%; (d) Pd/C, H_2, EtOH, rt, 100%; (e) (i) DIPEA, COMU, DMF, rt; (ii) 4 mol/L HCl/1,4-dioxane, rt, 92%; (f) Na_2CO_3, MeOH, H_2O, rt, 89%.

化合物 **43** の合成法を **Scheme 2-9** に示す。カルボン酸 **57** を酸クロリドへと誘導後、アミン **96** と縮合することでカルボン酸 **97** を得た。続いて、過酸化水素を作用させることでスルホン **98** とした後、**64b** との縮合と続く Boc 基の脱保護によって目的の化合物 **43** を得た。



Scheme 2-9. (a) (i) $SOCl_2$, DMF, toluene, 60 °C; (ii) **96**, $NaHCO_3$, THF, H_2O, rt, 80%; (b) H_2O_2, AcOH, 50 °C, 74%; (c) (i) $POCl_3$, *N*-methylmorpholine, DCM, 0 °C; (ii) 4 mol/L HCl/1,4-dioxane, DCM, rt, 77%.

第八節　小括

本章では、自社化合物ライブラリーを用いたHTSで見出されたヒット化合物からスキャフォールドホッピングとSBDDアプローチにより、*in vivo*において薬効を示す化合物を創製した。

まず、EP300酵素阻害活性を指標として自社化合物ライブラリーの約46万化合物について、HTSを実施することで、1,4-オキサゼパン骨格を有するヒット化合物を得た。次に、初期の誘導体展開において、テトラヒドロピラン環をシクロアルキル環に変換することで、EP300 HAT阻害活性及び細胞内でのH3K27アセチル化阻害活性が向上した化合物 **(+)-35** を見出した。

化合物 **(+)-35** は強力な *in vitro* 活性を有する一方で、経口投与による *in vivo* 薬理評価においては血中暴露を示さず、有意な薬理活性が認められなかったため、続いて *in vivo* 試験において活性を示す化合物の取得を目指し、オキサゼパン環の合成展開に着手した。しかし、オキサゼパン環上の置換基変換や環サイズの調節では活性を保持できず、オキサゼパン環周辺での誘導体展開では阻害活性を維持する化合物の取得が困難であった。そこで、オキサゼパン骨格の脱却を目指したアミノ酸ベースのスキャフォールドホッピングを実施したところ、ヒット化合物と同程度の *in vitro* 活性を有する化合物として、プロリン誘導体 **39** を見出した。さらに、**(+)-35** と **39** の共結晶構造を基にしたSBDDアプローチから化合物 **39** のプロリン環4位への置換基導入を計画し、フッ素原子を導入した4*R*-フルオロ-D-プロリン **40** はフッ素原子の立体電子効果による活性配座の制御によりEP300 HAT阻害活性が **39** から数倍向上することが示された。また、プロリン環にスルホニル基を導入した化合物 **43** (DS17701585)は *in vitro* 活性と代謝安定性を両立する結果となった。

DS17701585 を用いて、50 mg/kg、200 mg/kgの2用量にてLK2細胞株を移植したマウスモデルによる *in vivo* 薬効試験を実施した結果、用量依存性を持ってSOX2 mRNAの発現抑制活性が認められた。**DS17701585** はEP300/CBP HATファミリー選択的な阻害活性を有しており、EP300/CBP 阻害活性に基づく *in vivo* 活性であることが示唆された。

第三章　1 日 1 回の経口投与で去勢抵抗性前立腺がんモデルに抗腫瘍効果を示す DS-9300 の獲得

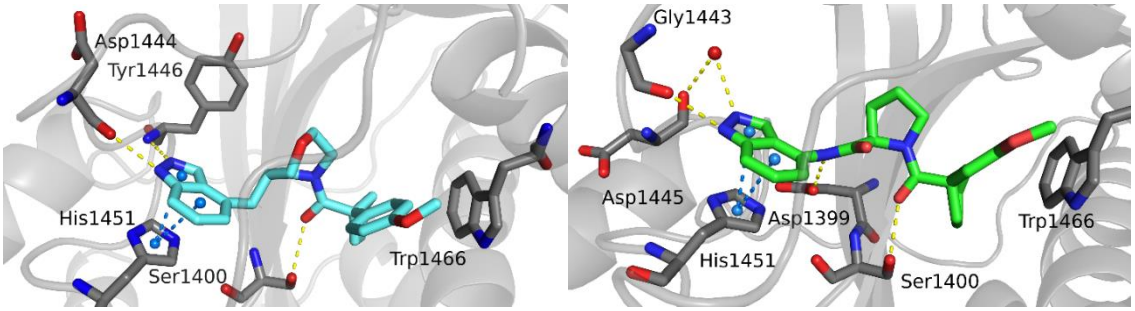
第一節　EP300 HAT 阻害活性と複合体構造情報の考察

前章では、オキサゼパン環のスキャフォールドホッピングと、続く SBDD アプローチによるプロリン環の修飾によって **DS17701585** を取得し、*in vitro* 活性と代謝安定性の 2 点を指標とした誘導体展開が *in vivo* 活性発現に有用であることを示した。本章では、更なる *in vivo* 活性向上と、臨床で適用可能なプロファイルを持った化合物の取得を目指し、化合物 **39** を起点として *in vitro* 活性と代謝安定性を指標とした誘導体展開を実施することとした。

第二章では、化合物 **(+)-35** と **39** の共結晶構造の比較から、プロリン環周辺の構造の違いに着目して誘導体展開を実施した。本章では D 環部周辺に着目して相互作用を比較すると、**(+)-35** の結合様式は第二章で指摘したように、インダゾール環が His1451 と π-π 相互作用を形成しており、N_1H 基は Asp1444 の主鎖カルボニル基と、2 位の窒素原子は Tyr1446 の主鎖 NH 基とそれぞれ水素結合を形成していることが観察された（**Figure 3-1A**）。化合物 **39** においては、インダゾール環と His1451 との π-π 相互作用は共通して観測できる一方、インダゾール環の N_1H 基は Gly1443 の主鎖カルボニル基と、2 位の窒素原子は水分子を介して Asp1445 の主鎖カルボニル基と水素結合を形成しており、**(+)-35** とは異なる結合様式を持つことが明らかとなった（**Figure 3-1B**）。

ここで、両化合物の EP300 HAT 阻害活性と結合様式の比較を **Figure 3-2** に示す。**(+)-35** は IC_{50} 値で 50 nM と強力な EP300 HAT 阻害活性を有している一方で、化合物 **39** は**(+)-35** と比較して数倍阻害活性が減弱している。このことから、**(+)-35** と Asp1444 及び Tyr1446 との相互作用が *in vitro* 活性に重要な役割を果たしていることが示唆された。そこで、プロリン誘導体 **39** に対して**(+)-35** で見られるような D 環部の相互作用を付加することができれば、中程度に留まる **39** の EP300 HAT 阻害活性をさらに向上できると考え、インダゾール環の変換を実施することとした。

(A) (B)



(C)

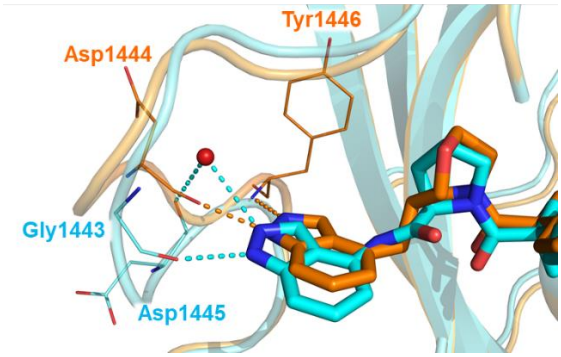


Figure 3-1. (A) Detailed interactions of **(+)-35** bound to EP300 (PDB ID: 7VHY). H-bonding interactions (yellow) and π-π stacking interactions (blue) are shown as dashed lines. (B) Detailed interactions of **39** bound to EP300 (PDB ID: 7VHZ). H-bonding interactions (yellow) and π-π stacking interactions (blue) are shown as dashed lines. (C) Overlay of crystal structures of **(+)-35** and **39** bound to EP300. **(+)-35** is orange, and **39** is cyan.

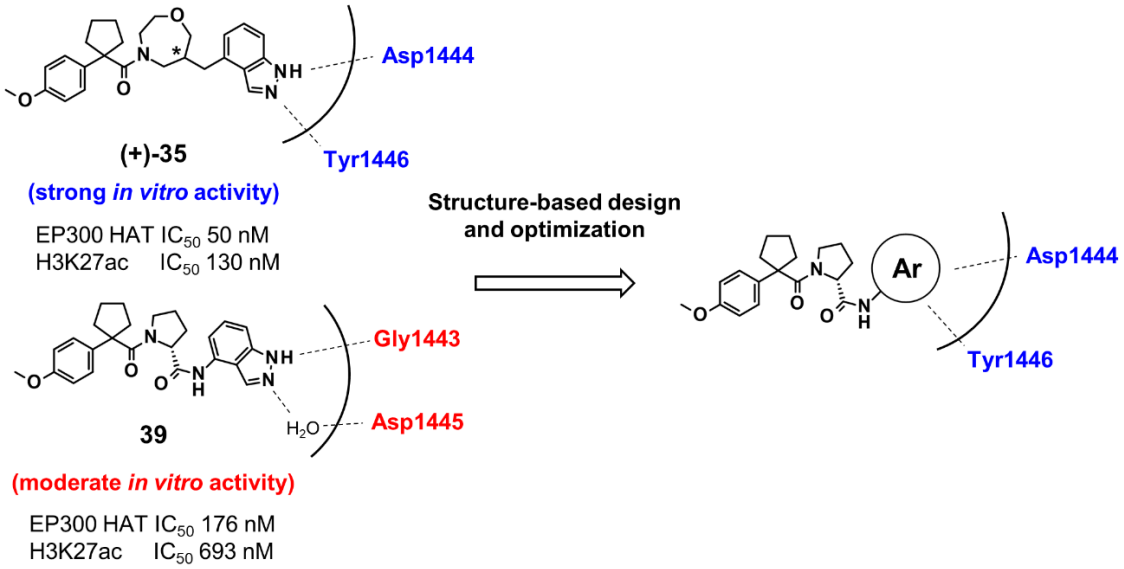
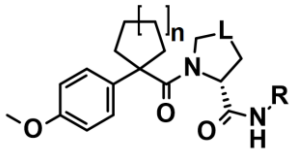


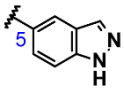
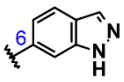
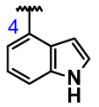
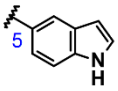
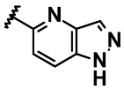
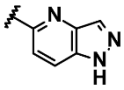
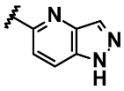
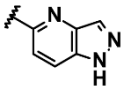
Figure 3-2. Design and modification strategy of novel EP300 inhibitor.

第二節　インダゾール環の構造変換

まず、化合物 **39** の B 環部をシクロペンタン環もしくはシクロヘキサン環に固定して、インダゾール環部分の変換を実施した（**Table 3-1**）。中心骨格との接続位置をインダゾール 5 位とした化合物 **99** は、インダゾール 4 位で接続した **39** と同等の EP300 HAT 阻害活性を維持し、6 位で接続した **100** は阻害活性が減弱する結果となった。次に、インダゾール環を 2 位の窒素原子を炭素原子に置換した **101** は化合物 **39** と比較して 3 倍阻害活性が向上し、中心骨格との接続位置をインドール 5 位とした **102** は阻害活性を保持した。さらに、インダゾール 4 位の炭素原子を窒素原子に置き換えた **103** は、化合物 **99** と比較して EP300 HAT 阻害活性が 10 倍向上した。このことから、4 位の窒素原子が新たに相互作用を獲得し、EP300 HAT 阻害活性が向上したことが示唆された。続いて、前章で実施したプロリン環上への置換基導入で得られた構造活性相関情報を活用し、プロリン環にヘテロ原子を導入した骨格を組み合わせた化合物の合成、評価を検討した。B 環部をシクロヘキサン環として、プロリン環にフッ素原子 **104**、ヒドロキシ基 **105**、スルホニル基 **106** を導入したところ、EP300 HAT 阻害活性の向上は認められなかったが、化合物 **104** 及び **106** は化合物 **99** と比較して H3K27 アセチル化阻害活性が 2 倍向上した。

Table 3-1. SAR of the optimization of the D-ring moiety.



Compound	n	L	R	EP300 HAT IC_{50} (nM)	H3K27ac IC_{50} (nM)
39	1	-CH_2-		176	693
99	1	-CH_2-		254	916
100	1	-CH_2-		500	2786
101	1	-CH_2-		61	304
102	1	-CH_2-		213	709
103	1	-CH_2-		24	52
104	2			24	21
105	2			50	106
106	2			22	27

第三節　化合物 104 と EP300 の共結晶構造解析

新たに見出された新規アザインダゾール誘導体の結合様式を確認するために、化合物 **104** と EP300 の X 線共結晶構造を決定した（**Figure 3-3**）。化合物 **104** の中心骨格の結合様式は、化合物 **39**/EP300 二者複合体における化合物 **39** の結合様式（**Figure 3-1B**）を保持していた。一方で D 環部の相互作用は、アザインダゾール 4 位の窒素原子が水を介して Tyr1446 の主鎖 NH と水素結合を形成しており、予想通り、新たな水素結合を獲得していることが明らかとなった。また、アザインダゾール環は His1451 と π-π 相互作用を形成しており、アザインダゾール 3 位の CH 基は Asp1444 の主鎖カルボニル基と非古典的な水素結合を形成していた。さらに、N_1H 基は新たに水を介して Pro1452 の主鎖カルボニル基と水素結合を形成していることが観察された。

以上より、新たに見出されたアザインダゾール誘導体は Asp1444 及び Tyr1446 との相互作用を獲得していることが明らかとなり、第一節で示した化合物 **39** に対して**(+)-35**/EP300 二者複合体で観察される相互作用を付加することで活性が向上するという仮説を実証する結果となった。

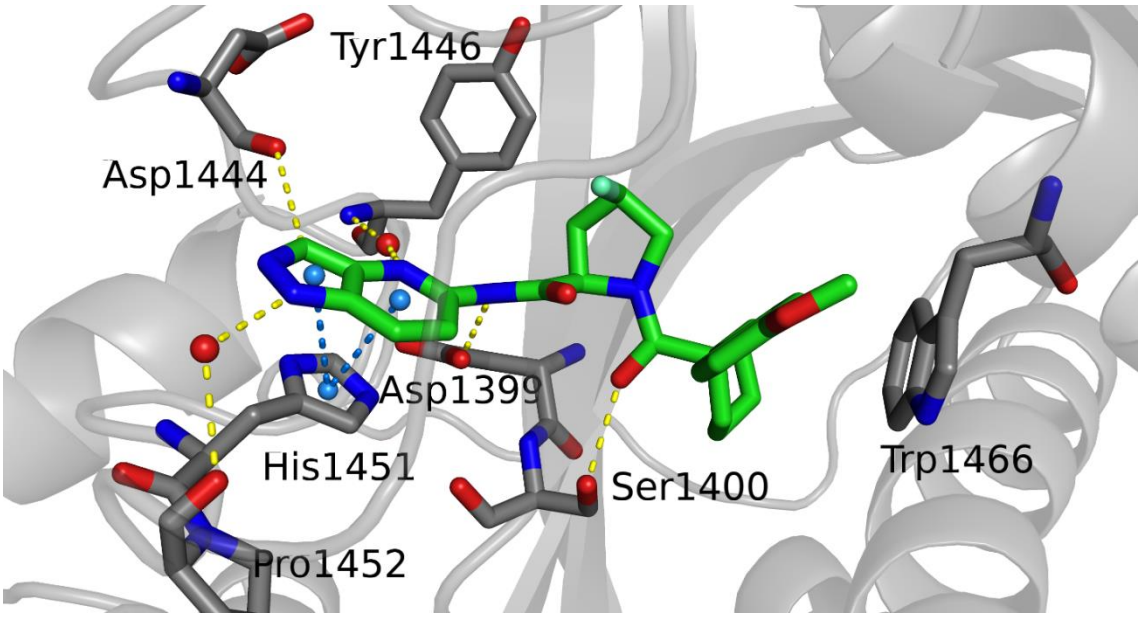


Figure 3-3. Crystal structure of compound **104** bound to EP300 (PDB ID: 8GZC). H-bonding interactions (yellow) and π-π stacking interactions (blue) are shown as dashed lines.

第四節　化合物 104 の構造最適化研究

次に、*in vivo* 試験実施に適した薬物動態プロファイルを有する化合物の取得を目指し、代謝安定性の改善と更なる構造最適化を実施することとした。

細胞内での H3K27 アセチル化阻害活性が最も良好な化合物 **104** の ADME プロファイルを確認すると、肝ミクロソーム中の代謝安定性は低値に留まる結果であった（**Figure 3-4**）。この要因としては、A 環部フェニル基上のメトキシ基が CYP 代謝による脱メチル化や、フェニル基の高い電子密度に起因する酸化代謝及び、脂溶性領域であるシクロヘキサン環の酸化代謝が想定された。そこで、分子全体の脂溶性低減、推定代謝部位のブロック、電子豊富な芳香環の電子密度低減に焦点を当てて誘導体展開を実施した。

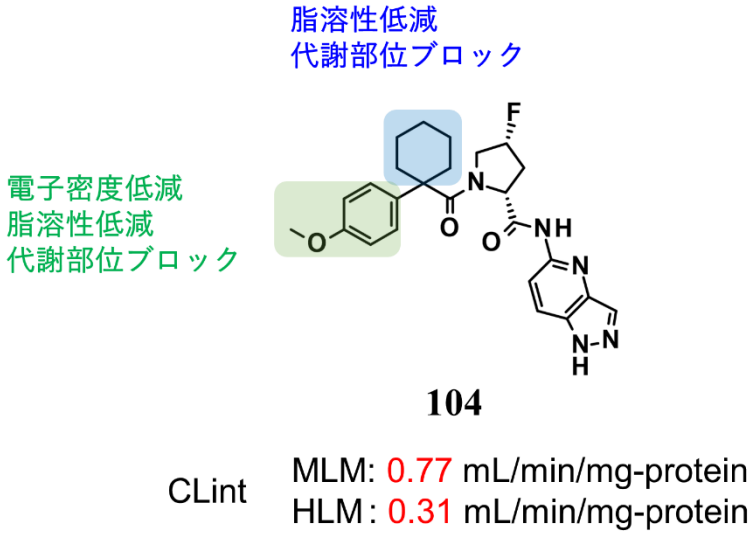
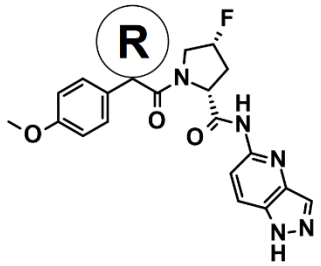


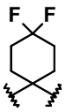
Figure 3-4. Optimization strategies for improving metabolic stability.

まず、化合物 **104** の B 環部シクロヘキサン部位の変換を検討した（**Table 3-2**）。シクロヘキサン環をテトラヒドロピラン環及びジメチル基に変換した化合物 **107** 及び **108** は、EP300 HAT 阻害活性を保持し、代謝安定性も大幅に向上する結果となった。一方で、脂溶性が大幅に低減した影響により、細胞内での EP300 HAT 阻害の指標である、H3K27 アセチル化阻害活性は低下した。B 環部のシクロアルカン構造の代謝部位をブロックするように設計したジフルオロシクロブチル **109**、ジフルオロシクロヘキシル **110**、ジメチルシクロブチル **111** は *in vitro*

活性を保持したまま、代謝安定性を改善した。

Table 3-2. Optimization of the B-ring.

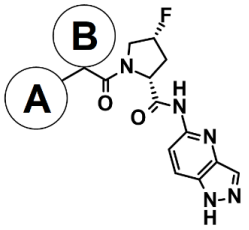


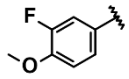
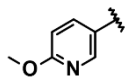
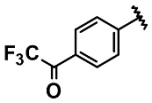
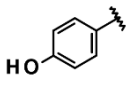
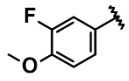
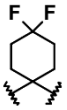
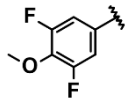
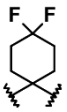
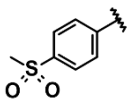
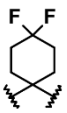
Cmpd	R	EP300 HAT IC_{50} (nM)	H3K27ac IC_{50} (nM)	Log D	CL_{int} (mL/min/mg-protein)	
					Mouse liver microsome (MLM)	Human liver microsome (HLM)
104		24	21	3.1	0.77	0.31
107		31	101	1.6	0.05	0.01
108		13	77	1.9	0.38	0.04
109		24	20	2.1	0.12	0.04
110		28	54	3.0	0.05	0.05
111		48	53	3.1	0.24	0.10

続いて、A 環部の構造最適化を実施した（**Table 3-3**）。電子求引性基を導入したフッ素体 **112** 及びトリフルオロメチルカルボニル体 **114**、電子密度を低下させたピリジン **113**、脱メチル体 **115** をそれぞれ設計・合成したところ、いずれの化合物も **104** と同等の EP300 HAT 阻害活性及び H3K27 アセチル化阻害活性を保持し、肝ミクロソーム中代謝安定性も改善傾向であった。続いて B 環部に **Table 3-1** の検討で代謝安定性の改善に寄与したジフルオロシクロヘキサン環を導入

し、さらに A 環部の置換基変換を実施した。フッ素基を導入した **116** は **112** と比較して想定通り代謝安定性を改善した。さらにフッ素基を導入して A 環の電子密度を下げると、代謝安定性は低下し、*in vitro* 活性も減弱した。化合物 **110** のメトキシ基をメタンスルホニル基に変換した **118** は、阻害活性はわずかに減弱したが、脂溶性が低減し、肝ミクロソーム中代謝安定性は大幅に改善した。

Table 3-3. Optimization of the A-ring.



Cmpd	A ring	B ring	EP300 HAT IC_{50} (nM)	H3K27ac IC_{50} (nM)	Log D	CLint (mL/min/mg-protein)[a]	
						MLM	HLM
112			14	16	3.1	0.40	0.23
113			34	15	2.3	0.16	0.11
114			56	21	3.4	0.38	0.15
115			12	13	2.7	NT[b]	0.14
116			36	57	3.0	NT	0.04
117			76	142	3.2	NT	0.06
118			55	95	1.6	NT	0.00

[a]Intrinsic clearance of tested compounds (1.0 μM) with mouse or human liver microsomes. [b]NT = not tested.

第五節　有望化合物の *in vivo* 薬物動態プロファイル

これまでの構造最適化検討において得られた有望な化合物 **104**、**109**、**110**、**112**、**115**、**118** について、BALB/c マウスを用いた *in vivo* 薬物動態プロファイリングを実施した（**Table 3-4**）。化合物 **109**、**115**、**118** は、**104** と比較して代謝安定性が改善した化合物であったが、経口投与時における血漿中薬物濃度は減少した。一方で、化合物 **110** 及び **112** は、化合物 **104** と同等以上の暴露を示し、特に化合物 **110** は 8 倍以上の血漿中薬物濃度を示した。また、これら 2 化合物について静脈内投与時における分布容積及びクリアランスを評価すると、化合物 **110** は化合物 **104** と比較していずれのプロファイルも大きく改善しており、バイオアベイラビリティも 3 倍程度改善していた。以上より、化合物 **110** は *in vitro* 活性及び動態の点において望ましいプロファイルを有することから、開発候補化合物に資する化合物であると考え、DS-9300 と命名し、更に詳細な評価を進めることとした。

Table 3-4. *In vivo* PK properties for compound **(+)-35**, **39**, **104**, **109**, **110**, **112**, **115**, **118** in BALB/c mice.[a]

Compound	PO[b]			IV		
	AUC (μM·h)	C_{max} (μM)	$T_{1/2}$ (h)	Vd_{ss} (L/kg)	CL (mL/min/kg)	*F*%
(+)-35[c]	0.10	0.08	2.0	1.76	87	2.2
39	0.42	0.32	0.60	NT	NT	NT
104[d]	15.0	10.8	1.4	0.29	5.3	21
109	2.29	1.61	2.1	NT	NT	NT
110[e]	130	34.0	2.4	0.14	1.6	62
112[e]	16.8	12.7	1.2	0.15	6.3	31
115	0.87	0.34	2.8	NT	NT	NT
118	0.23	0.09	1.5	NT	NT	NT

[a]Values represent the average of two or three independent experiments. [b]10 mg/kg, 0.5% methyl cellulose suspension. [c]IV = 2 mg/kg, DMA/Tween80/saline = 1/1/8. [d]IV = 1 mg/kg; DMSO/10% SBE-7-βCD=1/9). [e]IV = 1 mg/kg; DMSO/Tween80/saline = 1/1/8.

第六節　DS-9300 の HAT 選択性評価

DS-9300 の HAT ファミリーに対する選択性を評価した（**Table 3-5**）。DS-9300 は、EP300/CBP ファミリーを構成する CBP に対して、EP300 と同等の阻害活性を示した（IC_{50} = 22 nM）。その一方で、他の HAT ファミリーには阻害活性を示さず、2000 倍以上という高い選択性を有する化合物であることが示された。

Table 3-5. Selectivity assessment of DS-9300 against HAT family members.

HATs	CBP	TIP60	MYST2	MYST4	PCAF	GCN5
IC_{50} (nM)	22	>50000	>50000	>50000	>50000	>50000

第七節　前立腺がん細胞株における DS-9300 の *in vitro* 評価

EP300 及び CBP は、アンドロゲン受容体（Androgen Receptor; AR）の転写コアクチベーターとして知られている。また、前立腺がんにおいては、EP300/CBP の HAT 活性が AR 活性を増強し、AR 応答性遺伝子の発現を誘導することが報告されている[42]。近年、AR 陽性前立腺がんに対して EP300/CBP 阻害剤が感受性を有することが報告されているため[31]、種々の前立腺がん細胞株に対する DS-9300 の細胞増殖抑制活性評価を実施した（**Table 3-6**）。AR 陽性前立腺がん細胞株である VCaP、22Rv1 及び LNCaP に対しては、それぞれ 0.6 nM、6.5 nM、3.4 nM の GI_{50} 値を示し、強力な増殖抑制活性を示した。一方で、AR 陰性の PC3 細胞株に対しては AR 陽性細胞株と比較して増殖阻害活性は低く、44–478 倍の選択性を示し、AR 陽性細胞株に選択的な増殖阻害活性を示すことが明らかとなった。

Table 3-6. Antiproliferative activity of DS-9300 against VCaP, 22Rv1, LNCaP, and PC3 cells.[a]

Cell Lines	VCaP	22Rv1	LNCaP	PC3
GI_{50} (nM)	0.6	6.5	3.4	287.2

[a]Compounds were incubated for 7 days.

次に、細胞内での H3K18 及び H3K27 のアセチル化と AR 制御タンパク質である前立腺特異抗原（Prostate-Specific Antigen; PSA）の発現に対する DS-9300 の阻害効果を評価した（**Figure 3-5**）。AR 陽性前立腺がん細胞株である VCaP 細胞株と LNCaP 細胞株に対して、DS-9300 を 72 時間作用させたところ、いずれの細胞株においても低濃度から用量依存的に H3K18 及び H3K27 のアセチル化と PSA の発現が抑制された。このことから、DS-9300 は前立腺がん細胞株における細胞増殖及び関連タンパク質の発現を効果的に制御できることが示唆された。

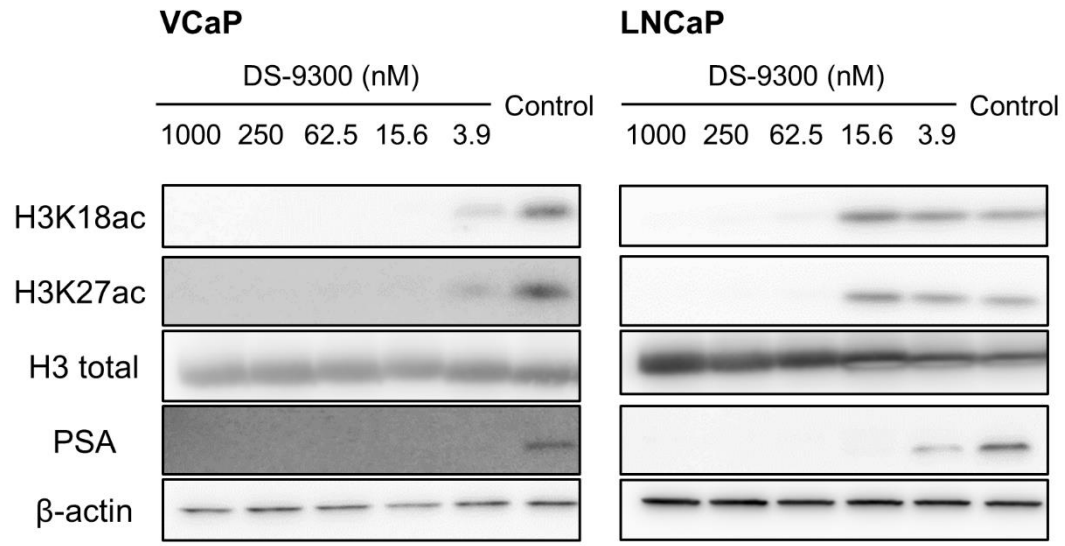


Figure 3-5. Representative western blotting showing acetylated H3K18, H3K27, total H3, and PSA levels in VCaP and LNCaP cells exposed to DS-9300 for 72 h.

第八節　DS-9300 の *in vivo* 薬効評価

続いて *in vivo* における DS-9300 の薬効を評価するために、抗腫瘍試験を実施することとした。前立腺がんはアンドロゲンシグナルに依存して増殖する腫瘍であり、性腺刺激ホルモン放出ホルモン（gonadotropin releasing hormone; GnRH）アゴニストや抗アンドロゲン薬などの内分泌療法によってアンドロゲンの分泌やシグナル伝達を阻害する治療が第一選択となっている。しかし、大部分は内分泌療法に抵抗性を示すようになり、去勢抵抗性前立腺がん（castration-resistant prostate cancer; CRPC）と呼ばれる病態に移行する [43]。去勢抵抗性を獲得する代表的な機序の一つとして、AR の遺伝子変異や、増幅、スプライシングバリアントの発現、などの AR の変化によるシグナル経路の活性化が挙げられる [44]。EP300/CBP は AR 活性を制御する転写コアクチベーターであり、実際に DS-9300 は *in vitro* 評価系において、AR 陽性前立腺がん細胞株に対する細胞増殖阻害活性を有しており、AR によって制御される PSA の発現を抑制していることを示した。そこで、CRPC の病態を模した、去勢雄マウスで樹立した VCaP 細胞 xenograft モデルで *in vivo* 薬効試験を実施した。

DS-9300 を 1 日 1 回経口投与し、投与後 32 日後までの腫瘍径と体重変化を対照群と比較したところ、0.3、1、3 mg/kg で用量依存的な腫瘍増殖抑制が確認され、特に最高用量の 3 mg/kg では、腫瘍増殖が完全に抑制されていた（**Figure 3-6**）。この時、いずれの投与群においても有意な体重減少は認められず、化合物の高い安全性が示唆された。また、DS-9300 投与群における血中 PSA 量を測定したところ、抗腫瘍効果と相関して用量依存的に血中 PSA 量を低下させることが示された（**Figure 3-7**）。以上より、DS-9300 は *in vivo* 評価系において AR 制御タンパク質の発現を抑制し、それによって去勢抵抗性前立腺がんに対する抗腫瘍効果を示すことが明らかとなった。そして、DS-9300 は去勢抵抗性前立腺がんモデルにおいて 1 日 1 回の投与によって顕著な抗腫瘍効果を示した最初の経口 EP300/CBP HAT 選択的阻害薬となった。

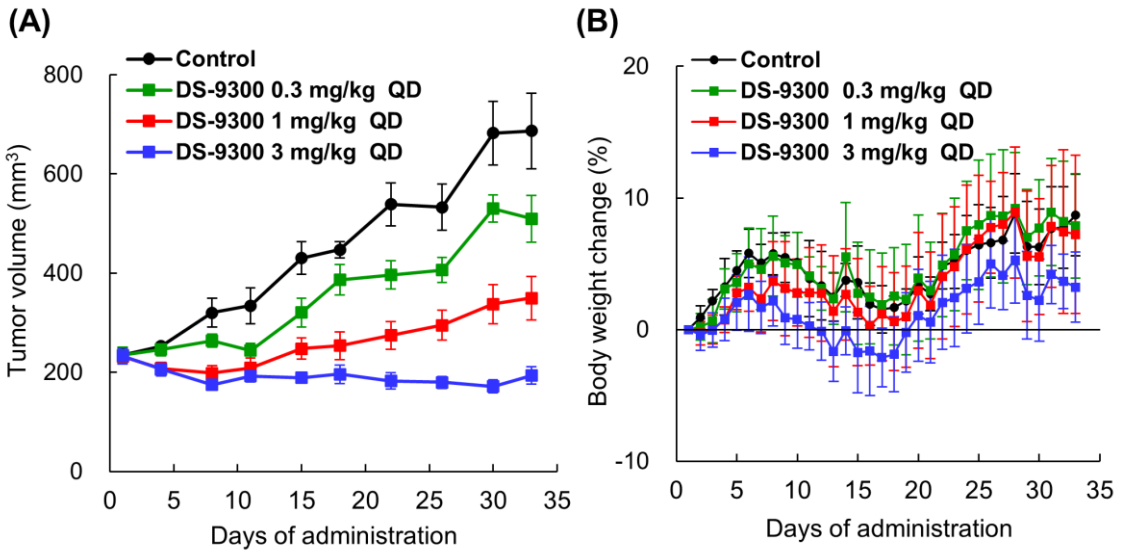


Figure 3-6. Antitumor activities of DS-9300 against xenografted VCaP tumors in castrated mice. (A) Tumor growth curves of four groups: vehicle control, DS-9300 (0.3 mg/kg, QD), DS-9300 (1 mg/kg, QD), and DS-9300 (3 mg/kg, QD). The tumor volumes are plotted as the mean ± SEM. (B) Effects on body weight changes of the mice during treatment with DS-9300.

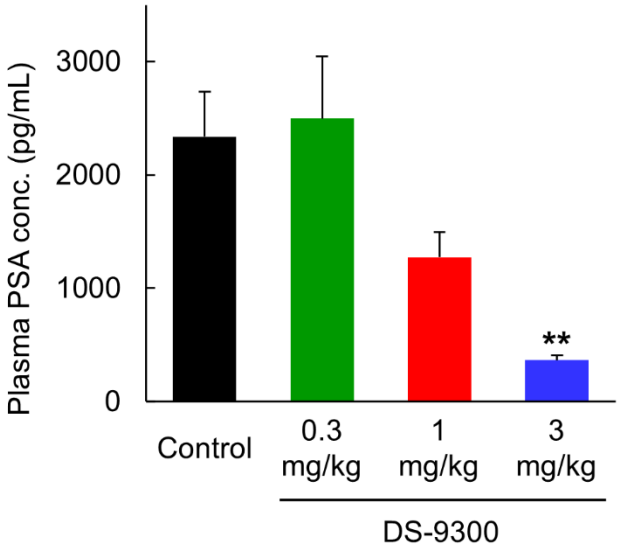
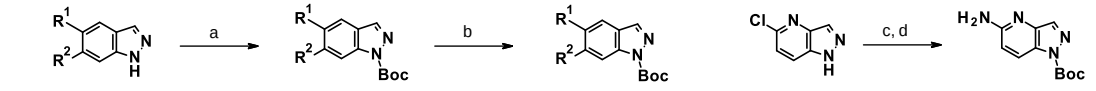


Figure 3-7. The effect of DS-9300 on plasma PSA levels in the subcutaneous VCaP xenograft model in castrated mice at 6 h after the final dosing of repeated administration (n = 5). Data are shown as means ± SEM. **p < 0.01 vs. control.

第九節　評価化合物の合成

D 環部のインダゾール及びアザインダゾール中間体の合成法を **Scheme 3-1** に示す。ニトロインダゾール **119a** 及び **119b** を Boc 基で保護した後、ニトロ基を還元することで、中間体 **121a** 及び **121b** を得た。また、中間体 **123** は 5-クロロ-4-アザインダゾールを出発原料として、Pd 触媒を用いたアミノ化と、続くインダゾール窒素原子上への Boc 基導入によって得た。

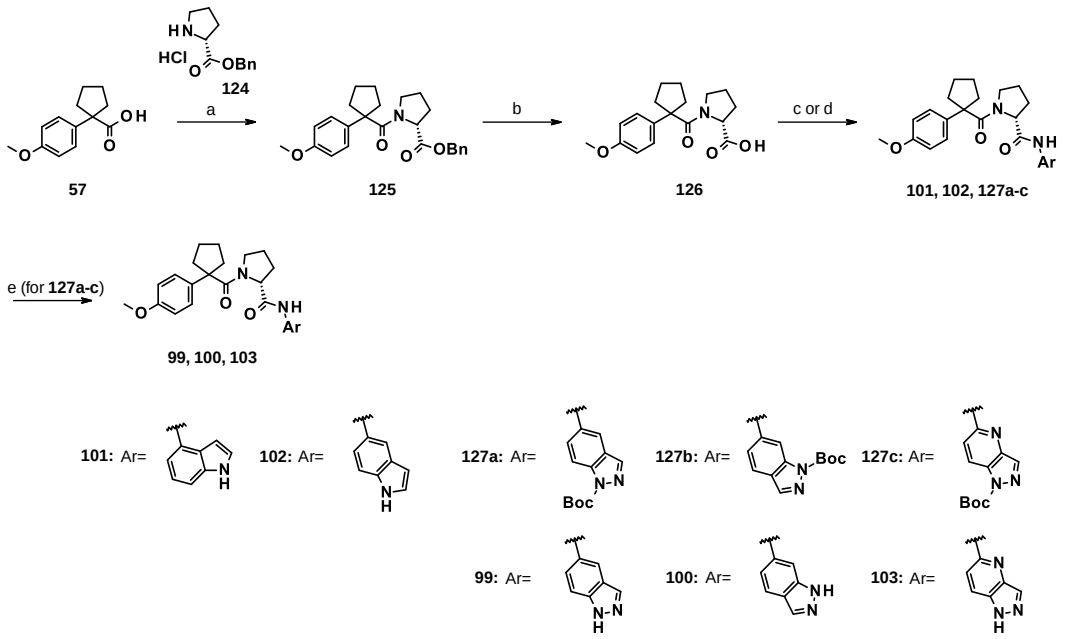


119a $R_1 = NO_2$, $R_2 = H$　**120a** $R_1 = NO_2$, $R_2 = H$　**121a** $R_1 = NH_2$, $R_2 = H$　**122**　**123**
119b $R_1 = H$, $R_2 = NO_2$　**120b** $R_1 = H$, $R_2 = NO_2$　**121b** $R_1 = H$, $R_2 = NH_2$

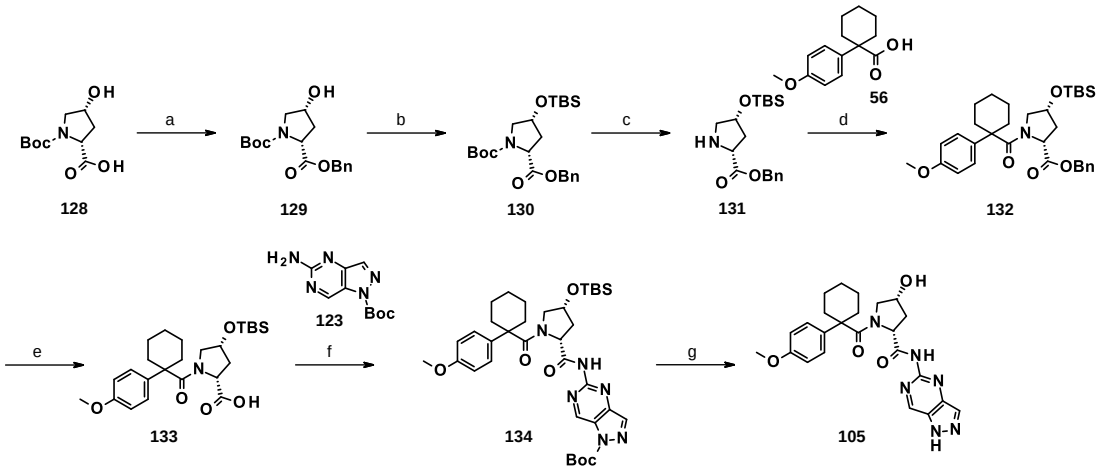
Scheme 3-1. (a) Boc_2O, DIPEA, DMAP, THF, rt, 89–90%; (b) Zn, NH_4Cl, MeOH/H_2O, rt, 72–86%; (c) LHMDS, $Pd_2(dba)_3$, XPhos, THF, reflux; (d) Boc_2O, TBAF, 0 °C, 76% (two steps).

次に、化合物 **99–103** の合成法を **Scheme 3-2** に示す。市販のカルボン酸 **57** を酸クロリドへと誘導後、アミン **124** と縮合することでエステル **125** を得た。続いてベンジルエステルを加水素分解した後、対応する芳香族アミンと縮合することで化合物 **101**、**102** 及び **127a–c** を得た。**127a–c** は酸性条件下で Boc 基を脱保護することで目的とする化合物 **99**、**100** 及び **103** へと導いた。

化合物 **105** の合成法を **Scheme 3-3** に示す。市販のプロリン誘導体 **128** をベンジルエステル **129** へと誘導した後、二級アルコールを TBS 基により保護、続いて Boc 基を脱保護することでアミン **131** を得た。化合物 **105** は、アミン **131** をカルボン酸 **56** と縮合後、ベンジルエステルの加水素分解と続くアミン **123** との縮合、Boc 基の脱保護によって得た。

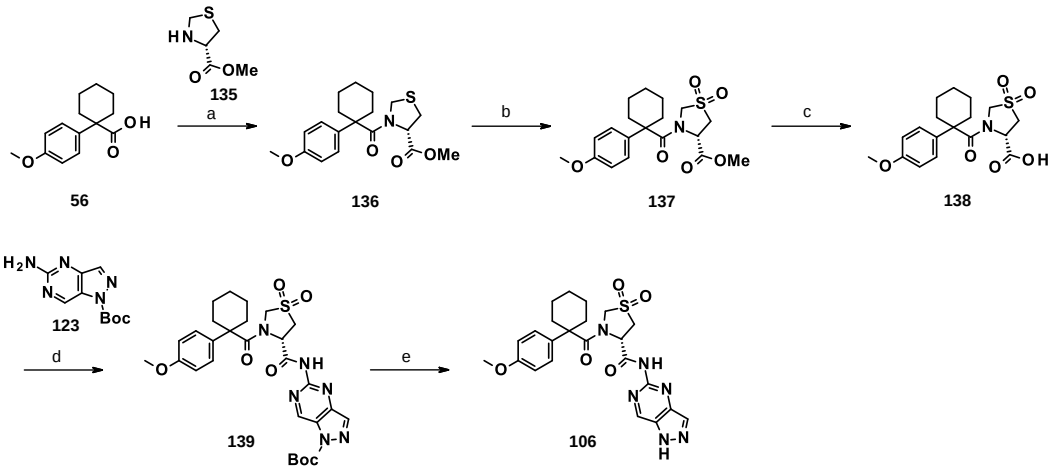


Scheme 3-2. (a) (i) $SOCl_2$, DMF, toluene, reflux; (ii) DIPEA, DCM, rt, 90%; (b) H_2, Pd/C, EtOH, rt, 82%; (c) DIPEA, COMU, amine, DMF, rt, 81%; (d) $POCl_3$, pyridine, amine, 0 °C to rt, 83%; (e) 4 mol/L HCl/1,4-dioxane, rt, 25–52% (two steps).



Scheme 3-3. (a) Cs_2CO_3, MeOH/H_2O, BnBr, DMF, rt, 97%; (b) TBSCl, imidazole, DMF, rt; (c) TFA, DCM, rt, 55% (two steps); (d) COMU, DIPEA, DMF, rt, 85%; (e) H_2, Pd/C, EtOH, rt, 92%; (f) $POCl_3$, pyridine, rt, 20%; (g) TFA, DCM, rt, 92%.

化合物 **106** の合成法を **Scheme 3-4** に示す。市販のカルボン酸 **56** を酸クロリドへと誘導後、アミン **135** との縮合、*m*-CPBA を用いた酸化によってスルホン **137** を得た。**137** はメチルエステルを加水分解した後、酸クロリドへと変換し、アミン **123** との縮合、Boc 基の脱保護を経て化合物 **106** へと導いた。

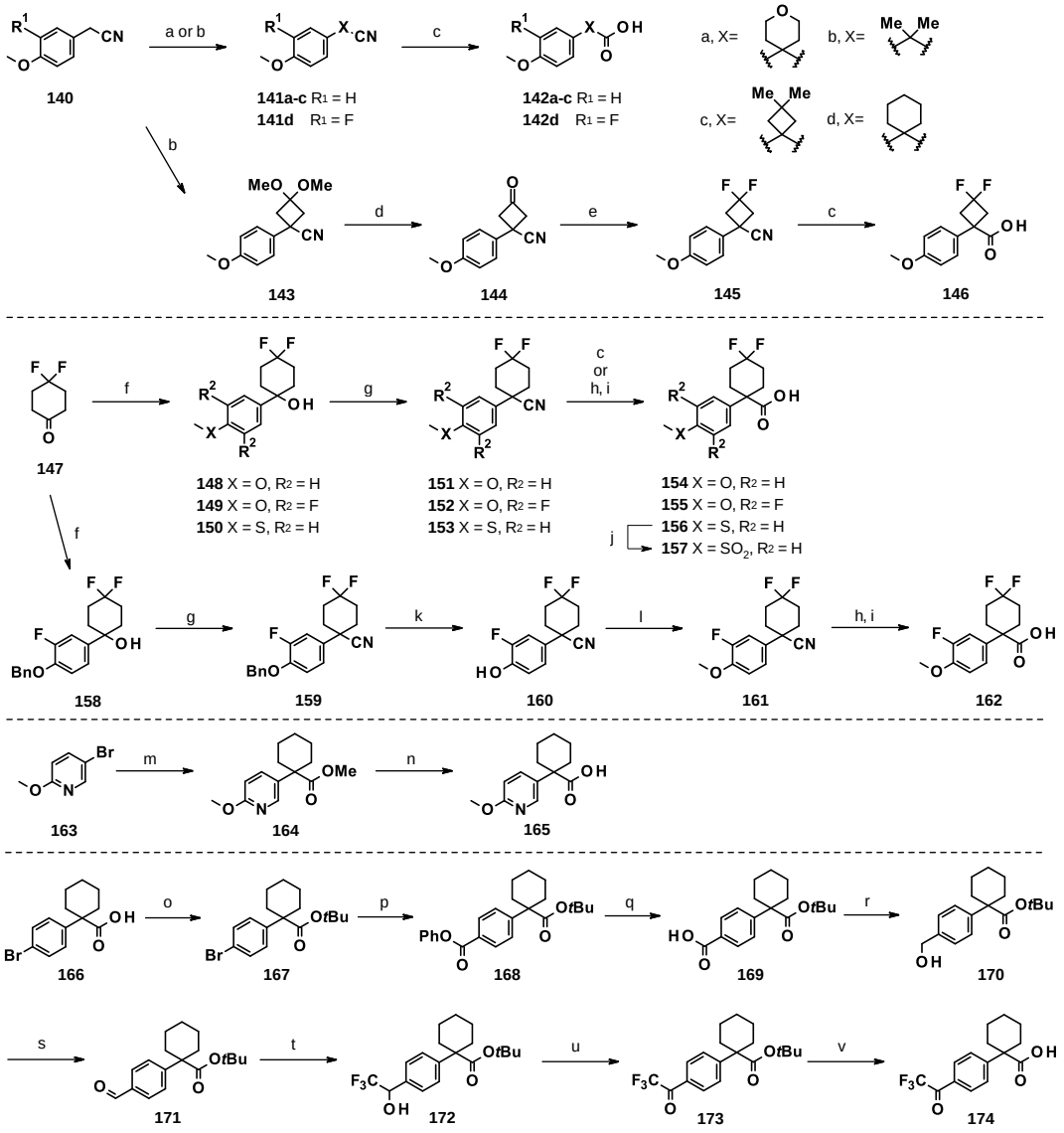


Scheme 3-4. (a) (i) oxalyl chloride, DMF, DCE, rt to 75 °C; (ii) **135**, DMAP, pyridine, DCE, 75 °C, 57%; (b) *m*-CPBA, DCM, rt, 53%; (c) LiOH, THF, H_2O, rt, 93%; (d) $POCl_3$, pyridine, 0 °C to rt; (e) TFA, DCM, rt, 32% (two steps).

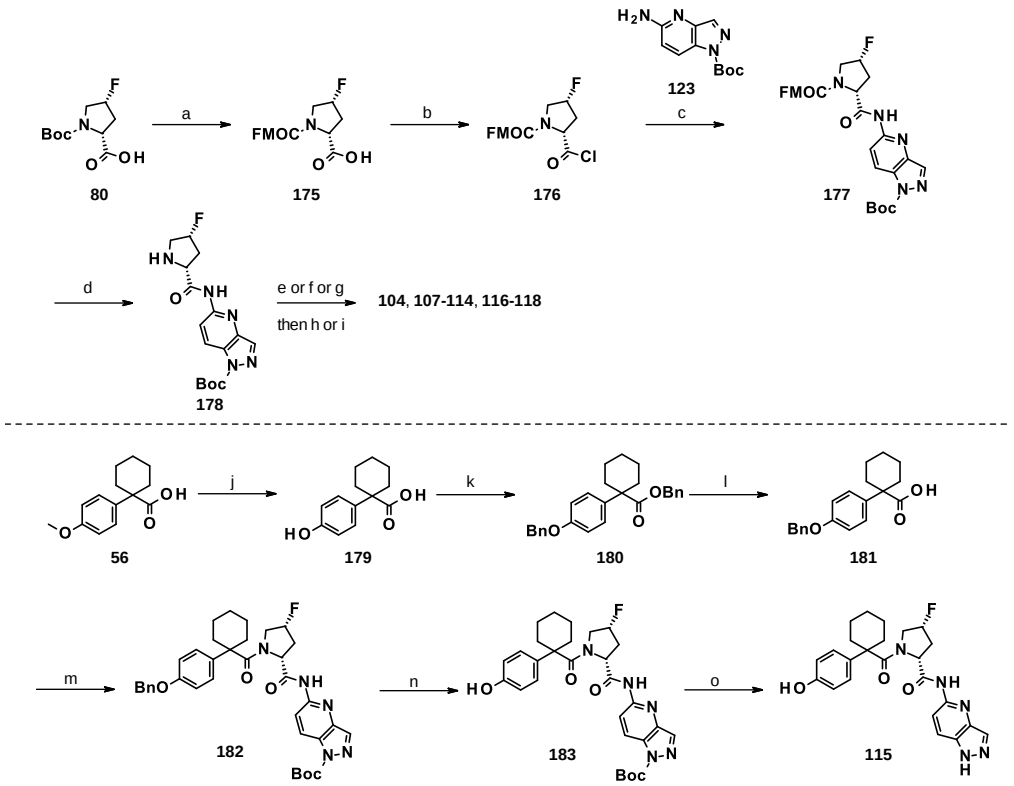
次に、種々のカルボン酸中間体の合成法を **Scheme 3-5** に示す。中間体 **142a–d** は、市販のフェニルアセトニトリル **140** に対応するハロゲン化アルキルを作用させた後、ニトリルを塩基性条件下加水分解することで得た。中間体 **146** は、ニトリル **140** のアルキル化と、アセタールの脱保護を経てケトン **144** へと導いた後、DAST を用いた脱酸素的フッ素化と、続くニトリルの加水分解によって得た。中間体 **154**、**155**、及び **156** は、市販のシクロヘキサノン **147** に対して対応する Grignard 試薬を作用させた後、触媒量の臭化インジウム存在下 TMSCN を作用させ [45]、生じたニトリルを加水分解、あるいは DIBALH を用いたアルデヒドへの還元と続く Pinnick 酸化によって得た。中間体 **157** は、過酸化水素を用いたスルフィド **156** の酸化によって得た。中間体 **162** は、**147** から中間体 **154** と同様の方法でニトリル **159** へと導いた後、ベンジルエーテルの加水素分解とメチル化によって **161** とし、中間体 **155** と同様の方法でカルボン酸へと誘導することで得た。中間体 **165** は、パラジウム触媒によるブロモピリジン **163** とシクロヘキサンカルボン酸メチルのリチウムエノラートとのクロスカップリング反応 [46] と、続くメチルエステルの加水分解によって得た。中間体 **174** は、市販のカルボン酸 **166** から 8 工程を経て合成した。すなわち、カルボン酸 **166** を酸クロリドへと誘導し、カリウム *tert*-ブトキシドを作用させることで *tert*-ブチルエステル **167** を得た。続いて、ギ酸フェニルを用いたパラジウム触媒によるカルボニル化反応

[47] によりフェニルエステル **168** を得た後、加水分解して得られたカルボン酸とクロロギ酸イソブチルを縮合して活性エステルとし、続いて水素化ホウ素ナトリウムによって還元することでアルコール **170** を得た。次に、化合物 **170** をアルデヒドへと酸化した後、$TMSCF_3$ を用いたトリフルオロメチル基の導入、二酸化マンガンを用いた酸化と、*t*Bu エステルの脱保護を経て、中間体 **174** を得た。

化合物 **104** 及び **107**–**118** の合成法を **Scheme 3-6** に示す。市販のプロリン誘導体 **80** の Boc 基を Fmoc 基へと架け替えた後、酸クロリド **176** へと導き、アミン **123** と縮合することで化合物 **177** を得た。次に、Fmoc 基を脱保護した後、対応するカルボン酸と縮合し、Boc 基を脱保護することで化合物 **104**、**107**–**114**、及び **116**–**118** を得た。化合物 **115** は、市販のカルボン酸 **56** から 6 工程を経て合成した。すなわち、カルボン酸 **56** の脱メチル化、ジベンジル化と続く加水分解によってカルボン酸 **181** を得た。次に、酸クロリドへと導いた後、アミン **178** との縮合と、ベンジル基及び Boc 基を脱保護することで化合物 **115** を得た。



Scheme 3-5. (a) NaH, 2-bromo-1-(2-bromoethoxy)ethane (for **50a**) or MeI (for **50b**) or 1,5-dibromopentane (for **50d**), DMF, rt; (b) NaH, 1,3-dibromo-2,2-dimethylpropane (for **50c**) or 1,3-dibromo-2,2-dimethoxypropane (for **52**), DMSO, Et_2O, rt, 99%; (c) KOH, ethylene glycol, reflux, 23–88%; (d) 6 mol/L HCl/H_2O, acetone, 60 °C, 75%; (e) DAST, DCM, rt, 83%; (f) RMgBr, THF, 0 °C to rt, 62–75%; (g) $InBr_3$, TMSCN, DCM, rt, 37–96%; (h) DIBALH, toluene, rt; (i) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*BuOH/H_2O, rt, 57–59% (two steps); (j) H_2O_2, AcOH, 50 °C, 47%; (k) Pd/C, EtOH, rt, 99%; (l) MeI, K_2CO_3, acetone, rt; (m) *n*BuLi, $Pd_2(dba)_3$, TTBP·HBF_4, dicyclohexylamine, methyl cyclohexanecarboxylate, toluene, rt, 40%; (n) NaOH, THF/MeOH, 50 °C, 78%; (o) (i) $SOCl_2$, DMF, DCM, 40 °C; (ii) KO*t*Bu, THF, rt, 78%; (p) phenyl formate, $Pd(OAc)_2$, TEA, TTBP·HBF_4, MeCN, reflux, 71%; (q) NaOH, THF/MeOH, rt, 99%; (r) (i) TEA, isobutyl chloroformate, THF, 0 °C; (ii) $NaBH_4$, H_2O, 0 °C to rt, 90%; (s) DMP, DCM, rt, 67%; (t) $TMSCF_3$, K_2CO_3, TBAF, DMF, rt, 96%; (u) MnO_2, DCM, rt, 91%; (v) TFA, DCM, rt, 88%.



Scheme 3-6. (a) (i) 4 mol/L HCl/1,4-dioxane, rt; (ii) $NaHCO_3$, FmocCl, 1,4-dioxane/H_2O, rt, 88%; (b) $SOCl_2$, DMF, DCM, rt to 40 °C; (c) DIPEA, DCM, rt, 66%; (d) piperidine, DMF, 0 °C to rt, 92%; (e) (i) carboxylic acid, $SOCl_2$, DMF, DCM, 40 °C; (ii) DIPEA, DMF, rt; (f) (i) carboxylic acid, $(COCl)_2$, DMF, DCM, rt; (ii) DIPEA, DMF, rt; (g) COMU, DIPEA, DMF, rt; (h) TFA, DCM, rt, 5.5–81% (two steps); (i) K_2CO_3, THF/MeOH, rt, 44–68% for 2 steps; (j) BBr_3, DCM, −78 °C to rt, 98%; (k) BnBr, K_2CO_3, DMF, rt, 68%; (l) KOH, ethylene glycol, reflux, 97%; (m) (i) $SOCl_2$, DMF, DCM, 40 °C; (ii) **178**, DIPEA, DCM, 0 °C to rt, 95%; (n) H_2, Pd/C, EtOAc, rt, 95%; (o) TFA, DCM, rt, 98%.

第十節　小括

本章では、前章で得られたリード化合物 **(+)-35** と **39** のインダゾール環部周辺の相互作用の違いに着目し、プロリン誘導体 **39** のインダゾール環を最適化することで更なる EP300 HAT 阻害活性の向上が図れると考え、誘導体展開を実施した。その結果、アザインダゾール骨格に変換した化合物 **104** において大幅な阻害活性の向上が認められた。化合物 **104** と EP300 HAT ドメインの X 線共結晶構造より、アザインダゾール骨格を導入することでオキサゼパン誘導体 **(+)-35** と同様の結合様式をとることが示唆され、化合物 **39** に対して **(+)-35**/EP300 二者複合体で観察される相互作用を付加することで阻害活性が向上するという仮説を実証する結果となった。

続いて薬物動態改善を指向した誘導体展開を実施した結果、シクロヘキサン環上の代謝部位への置換基導入や脂溶性を低減した化合物群において代謝安定性の向上が認められた。中でも、化合物 **104** のシクロヘキサン環をジフルオロシクロヘキサン環とした化合物 **110**（DS-9300）は良好な *in vitro* 阻害活性を維持し、マウスにおいて高い経口吸収性を示した。

前立腺がん細胞を用いた DS-9300 の *in vitro* 評価を実施したところ、AR 陽性細胞株に対して選択的かつ強力な細胞増殖阻害活性を示し、細胞内において用量依存的な H3K18 及び H3K27 アセチル化の阻害、並びに AR 制御タンパク質である PSA の発現抑制が認められた。さらに、去勢雄マウスで樹立した VCaP 細胞 xenograft マウスを用いた *in vivo* 薬効評価において、DS-9300 は用量依存的な腫瘍増殖抑制作用と血中 PSA 量の抑制効果を示すことを確認した。DS-9300 は去勢抵抗性前立腺がんモデルにおいて 1 日 1 回の投与で顕著な抗腫瘍効果を示した最初の経口 EP300/CBP HAT 阻害薬であり、有用な去勢抵抗性前立腺がん治療薬となりうる可能性を示した。

総論

本論文では、新規創薬標的候補としての可能性を有する EP300/CBP ヒストンアセチルトランスフェラーゼに対して、バーチャルスクリーニング及びハイスループットスクリーニングという 2 つの創薬手法を用いて新規 EP300/CBP HAT 阻害薬の獲得を目指した。そして、HTS によって得られたヒット化合物からスキャフォールドホッピング、X 線共結晶構造情報を基にしたアプローチ、及び薬物動態改善を指向した構造最適化を経て、去勢抵抗性前立腺がんモデルにおいて 1 日 1 回の経口投与で顕著な抗腫瘍効果を示す DS-9300 を創出した。

第一章では、既に報告されている EP300 HAT ドメインと Lys-CoA との共結晶構造を鋳型として、市販のライブラリー400 万化合物についてバーチャルスクリーニングを実施することで、ピラゾロン骨格を有するヒット化合物 **1** を得た。次に、初期の誘導体展開においてエキソメチレン構造を脱却しつつ、ヒット化合物と同等以上の EP300 HAT 阻害活性を有する化合物 **2f** を得た。さらに、安息香酸部位の誘導体展開によって、細胞増殖阻害活性が大幅に向上した 4-ピリドン-3-カルボン酸誘導体 **3c**–**3f** を取得した。このことから、4-ピリドン-3-カルボン酸骨格が、安息香酸のバイオアイソスターとなり得ることを示した。

また、各種 HAT ファミリーに対する選択性評価から、化合物 **3c** 及び **3f** が **C646** と比較して高い EP300/CBP 選択性を示すことが明らかとなり、**3c** 及び **3f** が **C646** よりも優れた EP300/CBP HAT 阻害研究のツール化合物となり得ることを示した。

第二章では、EP300 HAT 阻害活性を指標として、社内ライブラリー化合物の約 46 万化合物についてハイスループットスクリーニングを実施することで、化合物 **33** に代表される 1,4-オキサゼパン誘導体をヒット化合物として得た。次に、テトラヒドロピラン骨格の変換と光学分割によって、EP300 HAT 阻害活性が二桁 nM の IC_{50} 値を示す化合物 **(+)-35** を見出した。化合物 **(+)-35** は強力な *in vitro* 活性を示す一方で、*in vivo* 評価においては薬理活性が認められなかった。アミノ酸ベースのスキャフォールドホッピングを実施したところ、オキサゼパン骨格を脱却して、かつヒット化合物と同程度の *in vitro* 活性を有するプロリン誘導体 **39** を獲得した。さらに、共結晶構造を基にした誘導体展開では化合物 **39** のプロ

リン 4 位への置換基導入によって阻害活性と代謝安定性を両立させた化合物 **43** (DS17701585)を獲得した。化合物 **43** は、*in vivo* 薬効評価においても用量依存的な SOX2 mRNA 発現抑制活性を示し、*in vitro* 活性向上と代謝安定性改善を指標とした誘導体展開が EP300/CBP 阻害による *in vivo* 活性発現に有用であることを示した。

第三章では、前章で獲得したリード化合物 **(+)-35** 及び **39** のインダゾール環部周辺の EP300 との相互作用の違いに着目して誘導体展開を実施した。その結果、10 倍程度 EP300 阻害活性が向上した化合物として、インダゾール環をアザインダゾール環に変換した化合物 **104** を見出した。続いて、薬物動態改善を目的に代謝部位への置換基導入や、脂溶性低減、及び芳香環上の電子密度低減を目指した誘導体展開を実施した結果、化合物 **104** のシクロヘキサン環をジフルオロシクロヘキサン環へと変換した化合物 **110**（DS-9300）が良好な *in vitro* 活性を保持し、かつマウスにおいて良好な経口吸収性を示す化合物として見出された。DS-9300 は AR 陽性前立腺がん細胞に対して選択的かつ強力な増殖阻害活性を示し、去勢抵抗性前立腺がんモデルマウスを用いた *in vivo* 評価において、用量依存的な抗腫瘍効果と血中 PSA 量の抑制効果を示した。

EP300/CBP HAT 阻害による前立腺がんモデルマウスへの薬理作用は既に報告されているが、1 日 1 回の経口投与で完全な腫瘍の増殖阻害を示した例はこれが初めてであり、DS-9300（**110**）によって実臨床においても容認できる投与形態で去勢抵抗性前立腺がんの増殖抑制が見られたことは、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 EP300/CBP HAT 阻害薬の研究開発につながることを強く願っている。

実験項

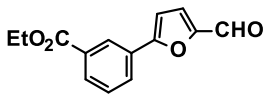
1. Chemistry

General

Unless otherwise noted, commercial reagents and solvents were obtained from commercial suppliers and used without further purification. Normal-phase column chromatography was performed on silica gel (SiO_2) or amino-silica gel using prepackaged cartridges. Analytical TLC was performed on Merck pre-coated TLC glass sheets with silica gel 60 F_{254}. ^1H NMR spectra were recorded at 400 MHz, and chemical shifts are given in ppm from TMS as an internal standard. ESI/APCI mass spectra were recorded on Agilent Infinity 1260 series LC/MS. Conditions [column: Develosil Combi-RP-5 2.0 mm × 50 mm, gradient elution: 0.1% HCO_2H-H_2O / 0.1% HCO_2H-MeCN = 98/2 – 0/100 (v/v), flow rate: 1.2 mL/min, UV detection: 254 nm, column temperature: 40 °C, ionization: APCI/ESI]. Purities of all tested compounds were confirmed to be >95% by HPLC analysis. High resolution mass spectra (HRMS) were obtained on an LC/MS system composed of the Waters Xevo Q-Tof MS system and the Acuity UPLC system. 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.

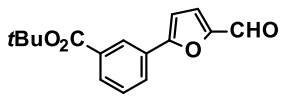
第一章

Ethyl 3-(5-formylfuran-2-yl)benzoate (5a)



To a solution of ethyl 3-bromobenzoate (2.00 g, 8.73 mmol) in 1,4-dioxane (20 mL), ethanol (10 mL) and water (5.0 mL), 5-formyl-2-furanylboronic acid (**4a**, 1.28 g, 9.15 mmol) and $NaHCO_3$ (2.20 g, 26.2 mmol) and $PdCl_2(PPh_3)_2$ (613 mg, 0.873 mmol) were added. The mixture was stirred at 70 °C for 3.5 h under a N_2 atmosphere. The reaction solution was diluted with ethyl acetate, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **5a** (1.97 g, 8.08 mmol, 92% yield) as a pale-yellow solid. ^1H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7.1 Hz), 4.43 (2H, q, J = 7.1 Hz), 6.94 (1H, d, J = 3.6 Hz), 7.35 (1H, d, J = 3.6 Hz), 7.54 (1H, t, J = 7.9 Hz), 8.03 (1H, d, J = 7.9 Hz), 8.07 (1H, d, J = 7.9 Hz), 8.46 (1H, s), 9.69 (1H, s).

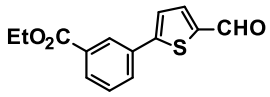
***tert*-Butyl 3-(5-formylfuran-2-yl)benzoate (5b)**



The reaction was carried out according to the procedure for **5a** with *tert*-butyl 3-bromobenzoate instead of ethyl 3-bromobenzoate to give

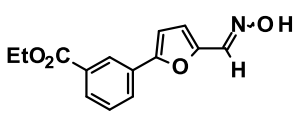
compound **5b** (470 mg, 1.73 mmol, 91% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.63 (9H, s), 6.92 (1H, d, *J* = 3.6 Hz), 7.34 (1H, d, *J* = 3.6 Hz), 7.51 (1H, t, *J* = 7.9 Hz), 8.01 (2H, dd, *J* = 7.9, 1.8 Hz), 8.38 (1H, d, *J* = 1.8 Hz), 9.69 (1H, s).

***tert*-Butyl 3-(5-formylthiophen-2-yl)benzoate (5c)**



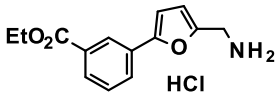
The reaction was carried out according to the procedure for **5a** with 5-formyl-2-thiopheneboronic acid (**4b**) instead of **4a** to give compound **5c** (243 mg, 0.934 mmol, 71% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.43 (3H, t, *J* = 7.0 Hz), 4.43 (2H, q, *J* = 7.3 Hz), 7.49 (1H, d, *J* = 4.2 Hz), 7.52 (1H, dd, *J* = 7.9, 7.9 Hz), 7.77 (1H, d, *J* = 3.6 Hz), 7.85 (1H, d, *J* = 7.9 Hz), 8.07 (1H, d, *J* = 7.9 Hz), 8.35 (1H, dd, *J* = 1.8, 1.8 Hz), 9.92 (1H, s).

Ethyl 3-{5-[(hydroxyimino)methyl]furan-2-yl}benzoate (6)



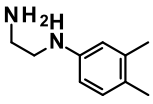
To a solution of **5a** (500 mg, 2.05 mmol) in ethanol (10 mL), hydroxylammonium chloride (156 mg, 2.24 mmol) and sodium acetate (184 mg, 2.24 mmol) were added. The mixture was stirred at 70 °C for 1 h. The reaction solution was diluted with ethyl acetate, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure to obtain compound **6** (513 mg, 1.98 mmol, 97% yield) as an *E*/*Z*-mixture. MS (ESI/APCI) *m/z*: 260 $(M + H)^+$.

Ethyl 3-[5-(aminomethyl)furan-2-yl]benzoate-hydrogen chloride (7)



To a solution of **6** (513 mg, 1.98 mmol) in ethanol (30 mL), 10% palladium-carbon (100 mg) and 1 mol/L aqueous HCl solution (3.0 mL, 3.00 mmol) were added, and the mixture was stirred at rt for 1.5 h under hydrogen atmosphere. The reaction solution was filtered through celite using ethanol. The solvent was distilled off under reduced pressure, and the residue obtained was formed into a slurry with ethyl acetate, and then filtered off to obtain compound **7** (400 mg, 1.42 mmol, 72% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.35 (3H, t, *J* = 7.3 Hz), 4.18 (2H, s), 4.36 (2H, q, *J* = 7.3 Hz), 6.70 (1H, d, *J* = 3.0 Hz), 7.12 (1H, d, *J* = 3.0 Hz), 7.61 (1H, t, *J* = 7.9 Hz), 7.90 (1H, d, *J* = 7.9 Hz), 8.03 (1H, d, *J* = 7.9 Hz), 8.30 (1H, s), 8.59 (3H, brs). MS (ESI/APCI) *m/z*: 246 $(M + H)^+$.

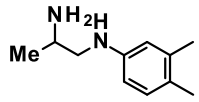
***N*^{1}-(3,4-Dimethylphenyl)ethane-1,2-diamine (10a)**



To a solution of 3,4-dimethylaniline (**8**, 570 mg, 4.70 mmol) and sodium triacetoxyborohydride (3.32 g, 15.7 mmol) in DCM (20 mL), a solution of *N*-Boc-2-aminoacetaldehyde (**9a**, 500 mg, 3.14 mmol) in DCM (10 mL) was added at 0 °C.

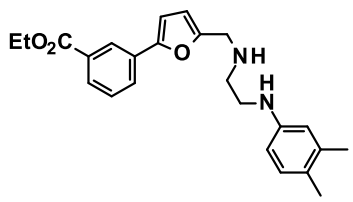
The mixture was stirred at rt for 19 h. A saturated aqueous $NaHCO_3$ solution was then added, and the mixture was stirred and then extracted with DCM. The organic layer obtained was washed with saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was dissolved in DCM (5.0 mL). To the solution, trifluoroacetic acid (5.0 mL) was added at 0 °C, and the mixture was stirred at rt for 2.5 h. The solvent was distilled off under reduced pressure, and the residue obtained was subjected to amino silica gel column chromatography (hexane/ethyl acetate) to obtain compound **10a** (403 mg, 2.45 mmol, 78% yield) as a brown solid. ^{1}H NMR ($CDCl_3$) δ: 2.15 (3H, s), 2.19 (3H, s), 2.93 (2H, t, J = 6.0 Hz), 3.16 (2H, t, J = 6.0 Hz), 6.42 (1H, dd, J = 7.9, 2.4 Hz), 6.48 (1H, d, J = 2.4 Hz), 6.94 (1H, d, J = 7.9 Hz). MS (ESI/APCI) *m/z*: 165 $(M + H)^+$.

N^1-(3,4-Dimethylphenyl)propane-1,2-diamine (10b)



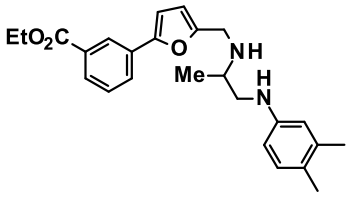
The reaction was carried out according to the procedure for **10a** with *tert*-butyl *N*-(1-methyl-2-oxo-ethyl)carbamate (**9b**) instead of **9a** to give the title compound **10b** (369 mg, 2.07 mmol, 71% yield) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.14 (3H, d, J = 6.0 Hz), 2.15 (3H, s), 2.19 (3H, s), 2.82–2.87 (1H, m), 3.09–3.17 (2H, m), 6.41 (1H, dd, J = 7.9, 2.4 Hz), 6.47 (1H, d, J = 2.4 Hz), 6.93 (1H, d, J = 7.9 Hz). MS (ESI/APCI) *m/z*: 179 $(M + H)^+$.

Ethyl 3-[5-({[2-(3,4-dimethylanilino)ethyl]amino}methyl)furan-2-yl]benzoate (11a)



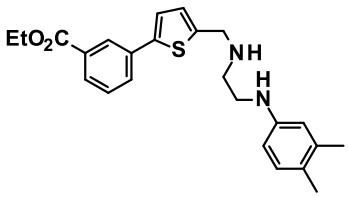
To a solution of **10a** (220 mg, 1.34 mmol) and sodium triacetoxyborohydride (868 mg, 4.10 mmol) in DCM (10 mL), a solution of **5a** (200 mg, 0.819 mmol) in DCM (5.0 mL) was added at 0 °C. The mixture was stirred at rt for 64 h. A saturated aqueous $NaHCO_3$ solution was then added, and the mixture was stirred and then extracted with DCM. The organic layer obtained was washed with saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **11a** (116 mg, 0.296 mmol, 36% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.42 (3H, t, J = 7.3 Hz), 2.14 (3H, s), 2.18 (3H, s), 2.93 (2H, t, J = 5.4 Hz), 3.22 (2H, t, J = 5.4 Hz), 3.87 (2H, s), 4.41 (2H, q, J = 7.1 Hz), 6.28 (1H, d, J = 3.0 Hz), 6.42 (1H, dd, J = 7.9, 2.1 Hz), 6.47 (1H, d, J = 2.1 Hz), 6.66 (1H, d, J = 3.0 Hz), 6.93 (1H, d, J = 7.9 Hz), 7.44 (1H, t, J = 7.9 Hz), 7.82 (1H, d, J = 7.9 Hz), 7.91 (1H, d, J = 7.9 Hz), 8.29 (1H, brs). MS (ESI/APCI) *m/z*: 393 $(M + H)^+$.

Ethyl 3-[5-({[1-(3,4-dimethylanilino)propan-2-yl]amino}methyl)furan-2-yl]benzoate (11b)



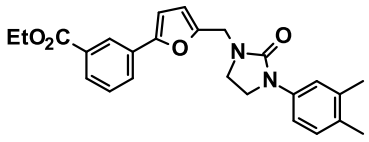
The reaction was carried out according to the procedure for **11a** with **10b** instead of **10a** to give the title compound **11b** (132 mg, 0.325 mmol, 40% yield) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.17 (3H, d, *J* = 5.4 Hz), 1.42 (3H, t, *J* = 7.0 Hz), 2.12 (3H, s), 2.15 (3H, s), 2.97–3.03 (2H, m), 3.10–3.16 (1H, m), 3.83 (1H, d, *J* = 14.5 Hz), 3.93 (1H, d, *J* = 14.5 Hz), 4.41 (2H, q, *J* = 7.0 Hz), 6.27 (1H, d, *J* = 3.0 Hz), 6.39 (1H, dd, *J* = 7.9, 1.8 Hz), 6.44 (1H, d, *J* = 1.8 Hz), 6.66 (1H, d, *J* = 3.0 Hz), 6.90 (1H, d, *J* = 7.9 Hz), 7.43 (1H, t, *J* = 7.9 Hz), 7.80 (1H, d, *J* = 7.9 Hz), 7.91 (1H, d, *J* = 7.9 Hz), 8.28 (1H, s). MS (ESI/APCI) *m/z*: 407 $(M + H)^+$.

Ethyl 3-[5-({[2-(3,4-dimethylanilino)ethyl]amino}methyl)thiophen-2-yl]benzoate (11c)



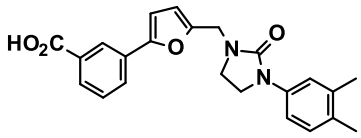
The reaction was carried out according to the procedure for **11a** with **5c** instead of **5a** to give the title compound **11c** (141 mg, 0.345 mmol, 37% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.42 (3H, t, *J* = 7.1 Hz), 2.15 (3H, s), 2.19 (3H, s), 2.95 (2H, t, *J* = 5.7 Hz), 3.23 (2H, t, *J* = 5.7 Hz), 4.01 (2H, s), 4.41 (2H, q, *J* = 7.1 Hz), 6.43 (1H, dd, *J* = 8.2, 2.7 Hz), 6.49 (1H, d, *J* = 2.7 Hz), 6.90 (1H, d, *J* = 3.6 Hz), 6.94 (1H, d, *J* = 7.9 Hz), 7.22 (1H, d, *J* = 3.6 Hz), 7.43 (1H, t, *J* = 7.9 Hz), 7.74 (1H, d, *J* = 7.9 Hz), 7.93 (1H, d, *J* = 7.9 Hz), 8.25 (1H, s). MS (ESI/APCI) *m/z*: 409 $(M + H)^+$.

Ethyl 3-(5-{[3-(3,4-dimethylphenyl)-2-oxoimidazolidin-1-yl]methyl}furan-2-yl)benzoate (S1)



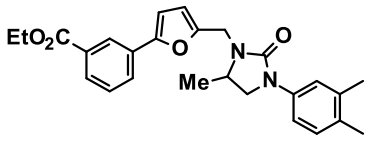
To a solution of **11a** (116 mg, 0.296 mmol) in 1,2-dichloroethane (5.0 mL), triethylamine (0.0614 mL, 0.443 mmol) and triphosgene (35.1 mg, 0.118 mmol) in 1,2-dichloroethene (1.0 mL) were added. The mixture was stirred at 70 °C for 3.5 h. A saturated aqueous $NaHCO_3$ solution was then added, and the mixture was stirred and then extracted with ethyl acetate, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **S1** (73.0 mg, 0.174 mmol, 59% yield) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, t, *J* = 7.0 Hz), 2.22 (3H, s), 2.26 (3H, s), 3.52 (2H, t, *J* = 7.9 Hz), 3.79 (2H, t, *J* = 7.9 Hz), 4.41 (2H, q, *J* = 7.1 Hz), 4.53 (2H, s), 6.40 (1H, d, *J* = 3.6 Hz), 6.69 (1H, d, *J* = 3.0 Hz), 7.08 (1H, d, *J* = 7.9 Hz), 7.23 (1H, dd, *J* = 7.9, 2.1 Hz), 7.41–7.46 (2H, m), 7.83 (1H, d, *J* = 7.9 Hz), 7.92 (1H, d, *J* = 7.9 Hz), 8.29 (1H, brs). MS (ESI/APCI) *m/z*: 419 $(M + H)^+$.

3-(5-{[3-(3,4-Dimethylphenyl)-2-oxoimidazolidin-1-yl]methyl}furan-2-yl)benzoic acid (2a)



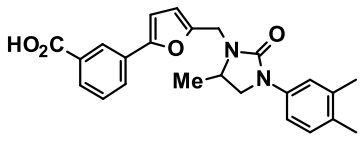
To a solution of **S1** (38.3 mg, 0.0981 mmol) in ethanol (5.0 mL), 5 mol/L aqueous NaOH solution (0.100 mL, 0.500 mmol) was added. The mixture was stirred at 70 °C for 2 h. The temperature was lowered to rt, 5 mol/L aqueous NaOH solution (0.100 mL, 0.500 mmol) was added and stirred at 70 °C for 45 min. The temperature was lowered to rt and Dowex 50W-X8 ion exchange resin (H^+-form) was added. Dowex was filtered off and the residue obtained by concentration was formed into a slurry with ethyl acetate, and then filtered off to obtain the title compound **2a** (38.3 mg, 0.0981 mmol, 56% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 2.13 (3H, s), 2.17 (3H, s), 3.43 (2H, t, J = 7.9 Hz), 3.75 (2H, t, J = 7.9 Hz), 4.43 (2H, s), 6.50 (1H, d, J = 3.0 Hz), 7.01–7.05 (2H, m), 7.27 (1H, dd, J = 7.9, 2.1 Hz), 7.32 (1H, s), 7.52 (1H, t, J = 7.9 Hz), 7.81 (1H, d, J = 7.3 Hz), 7.89 (1H, d, J = 7.9 Hz), 8.17 (1H, s), 13.12 (1H, brs). MS (ESI/APCI) *m/z*: 391 (M + H)$^+$. HRMS (ESI) *m/z*: calcd for $C_{23}H_{23}N_2O_4$ (M + H)$^+$ 391.1652. Found: 391.1648.

Ethyl 3-(5-{[3-(3,4-dimethylphenyl)-5-methyl-2-oxoimidazolidin-1-yl]methyl}furan-2-yl)benzoate (S2)



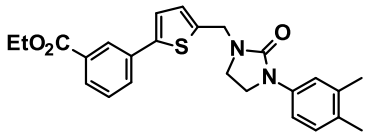
The reaction was carried out according to the procedure for **S1** with **11b** instead of **11a** to give the title compound **S2** (120 mg, 0.277 mmol, 85% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.39–1.43 (6H, m), 2.21 (3H, s), 2.26 (3H, s), 3.36 (1H, t, J = 7.9 Hz), 3.72–3.81 (1H, m), 3.89 (1H, t, J = 8.5 Hz), 4.28 (1H, d, J = 15.7 Hz), 4.40 (2H, q, J = 7.3 Hz), 4.82 (1H, d, J = 15.7 Hz), 6.40 (1H, d, J = 3.0 Hz), 6.68 (1H, d, J = 3.0 Hz), 7.08 (1H, d, J = 8.5 Hz), 7.23 (1H, dd, J = 8.2, 2.1 Hz), 7.40–7.46 (2H, m), 7.82 (1H, d, J = 7.9 Hz), 7.92 (1H, d, J = 7.9 Hz), 8.28 (1H, s). MS (ESI/APCI) *m/z*: 433 (M + H)$^+$.

3-(5-{[3-(3,4-Dimethylphenyl)-5-methyl-2-oxoimidazolidin-1-yl]methyl}furan-2-yl)benzoic acid (2e)



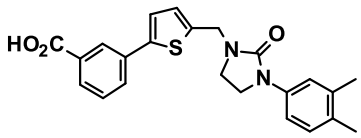
The reaction was carried out according to the procedure for **2a** with **S2** instead of **S1** to give the title compound **2e** (64.4 mg, 0.159 mmol, 57% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.31 (3H, d, J = 6.0 Hz), 2.16 (3H, s), 2.20 (3H, s), 3.31–3.35 (1H, m), 3.63–3.72 (1H, m), 3.93 (1H, t, J = 8.8 Hz), 4.33 (1H, d, J = 15.7 Hz), 4.62 (1H, d, J = 15.7 Hz), 6.52 (1H, d, J = 3.0 Hz), 7.03 (1H, d, J = 3.0 Hz), 7.07 (1H, d, J = 8.2 Hz), 7.29 (1H, dd, J = 8.2, 2.1 Hz), 7.34 (1H, brs), 7.55 (1H, t, J = 7.9 Hz), 7.84 (1H, d, J = 7.9 Hz), 7.90 (1H, d, J = 7.9 Hz), 8.20 (1H, s), 13.13 (1H, brs). MS (ESI/APCI) *m/z*: 405 (M + H)$^+$. HRMS (ESI) *m/z*: calcd for $C_{24}H_{25}N_2O_4$ (M + H)$^+$ 405.1809. Found: 405.1814.

Ethyl 3-(5-{[3-(3,4-dimethylphenyl)-2-oxoimidazolidin-1-yl]methyl}thiophen-2-yl)benzoate (S3)



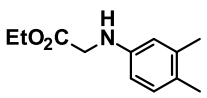
The reaction was carried out according to the procedure for **S1** with **11c** instead of **11a** to give the title compound **S2** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, *J* = 7.3 Hz), 2.22 (3H, s), 2.27 (3H, s), 3.46 (2H, t, *J* = 7.9 Hz), 3.80 (2H, t, *J* = 7.9 Hz), 4.40 (2H, q, *J* = 7.3 Hz), 4.64 (2H, s), 7.00 (1H, d, *J* = 3.6 Hz), 7.09 (1H, d, *J* = 7.9 Hz), 7.21–7.26 (2H, m), 7.43 (2H, dd, *J* = 7.9, 7.9 Hz), 7.74 (1H, d, *J* = 8.5 Hz), 7.94 (1H, d, *J* = 7.9 Hz), 8.24 (1H, s). MS (ESI/APCI) *m/z*: 435 $(M + H)^+$.

3-(5-{[3-(3,4-Dimethylphenyl)-2-oxoimidazolidin-1-yl]methyl}thiophen-2-yl)benzoic acid (2h)



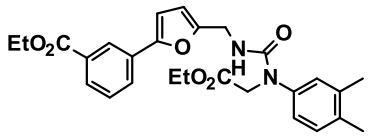
The reaction was carried out according to the procedure for **2a** with **S3** instead of **S1** to give the title compound **2h** (68.0 mg, 0.167 mmol, 49% yield for 2 steps) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 2.16 (3H, s), 2.20 (3H, s), 3.41 (2H, t, *J* = 7.9 Hz), 3.77 (2H, t, *J* = 7.9 Hz), 4.56 (2H, s), 7.05–7.09 (2H, m), 7.29 (1H, dd, *J* = 8.2, 2.1 Hz), 7.36 (1H, brs), 7.48 (1H, d, *J* = 3.6 Hz), 7.52 (1H, t, *J* = 7.9 Hz), 7.84 (1H, d, *J* = 7.9 Hz), 7.88 (1H, d, *J* = 7.9 Hz), 8.09 (1H, s), 13.15 (1H, brs). MS (ESI/APCI) *m/z*: 407 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{23}H_{23}N_2O_3S$ $(M + H)^+$ 407.1424. Found: 407.1427.

Ethyl *N*-(3,4-dimethylphenyl)glycinate (12)



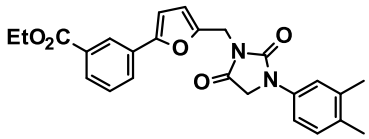
To a solution of **8** (904 mg, 7.46 mmol) and sodium triacetoxyborohydride (3.16 g, 14.9 mmol) in DCM (40 mL), a solution of 50% solution ethyl glyoxalate in toluene (1.48 mL, 7.44 mmol) in DCM (10 mL) was added portionwise at 0 °C. The mixture was stirred at rt for 18 h. A saturated aqueous $NaHCO_3$ solution was then added, and the mixture was stirred and then extracted with DCM, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **12** (1.10 g, 5.31 mmol, 71% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.29 (3H, t, *J* = 7.1 Hz), 2.15 (3H, s), 2.20 (3H, s), 3.87 (2H, s), 4.10 (1H, brs), 4.23 (2H, q, *J* = 7.1 Hz), 6.38 (1H, dd, *J* = 7.9, 1.8 Hz), 6.45 (1H, d, *J* = 1.8 Hz), 6.95 (1H, d, *J* = 7.9 Hz). MS (ESI/APCI) *m/z*: 208 $(M + H)^+$.

Ethyl 3-[5-({[(3,4-dimethylphenyl)(2-ethoxy-2-oxoethyl)carbamoyl]amino}methyl)furan-2-yl]benzoate (13)



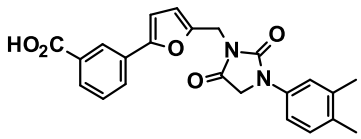
To a solution of **12** (80.9 mg, 0.390 mmol) in 1,2-dichloroethane (4.0 mL), triethylamine (0.147 mL, 1.06 mmol) and triphosgene (42.1 mg, 0.142 mmol) in 1,2-dichloroethane (1.0 mL) was added at 0 °C. The mixture was stirred at rt for 2 h. To the reaction mixture, a solution of **7** (100 mg, 0.355 mmol) was added, and the mixture was stirred at 70 °C for 1 h. A saturated aqueous $NaHCO_3$ solution was then added, and the mixture was stirred and then extracted with ethyl acetate. The organic layer obtained was washed with saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **13** (119 mg, 0.249 mmol, 70% yield) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, *J* = 7.0 Hz), 1.42 (3H, t, *J* = 7.0 Hz), 2.23 (3H, s), 2.25 (3H, s), 4.20 (2H, q, *J* = 7.0 Hz), 4.34 (2H, s), 4.38–4.45 (4H, m), 4.84 (1H, t, *J* = 5.4 Hz), 6.28 (1H, d, *J* = 3.0 Hz), 6.64 (1H, d, *J* = 3.0 Hz), 7.12–7.17 (3H, m), 7.43 (1H, t, *J* = 7.9 Hz), 7.77 (1H, d, *J* = 7.9 Hz), 7.90 (1H, d, *J* = 7.9 Hz), 8.24 (1H, s). MS (ESI/APCI) *m/z*: 479 $(M + H)^+$.

Ethyl 3-(5-{[3-(3,4-dimethylphenyl)-2,5-dioxoimidazolidin-1-yl]methyl}furan-2-yl)benzoate (S4)



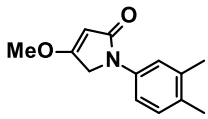
To a solution of **13** (106 mg 0.222 mmol) in DMSO (5.0 mL), *N*,*N*-diisopropylethylamine (0.0565 mL, 0.332 mmol) was added. The mixture was stirred at 120 °C for 20 h. The reaction solution was diluted with ethyl acetate, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure to obtain the title compound **S4** (79.6 mg, 0.184 mmol, 83% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, *J* = 7.1 Hz), 2.24 (3H, s), 2.28 (3H, s), 4.32 (2H, s), 4.40 (2H, q, *J* = 7.1 Hz), 4.84 (2H, s), 6.51 (1H, d, *J* = 3.6 Hz), 6.68 (1H, d, *J* = 3.6 Hz), 7.13 (1H, d, *J* = 7.9 Hz), 7.24 (1H, dd, *J* = 7.9, 1.8 Hz), 7.37 (1H, d, *J* = 1.8 Hz), 7.44 (1H, t, *J* = 7.9 Hz), 7.85 (1H, d, *J* = 7.9 Hz), 7.92 (1H, d, *J* = 7.9 Hz), 8.27 (1H, s). MS (ESI/APCI) *m/z*: 433 $(M + H)^+$.

3-(5-{[3-(3,4-Dimethylphenyl)-2,5-dioxoimidazolidin-1-yl]methyl}furan-2-yl)benzoic acid (2c)



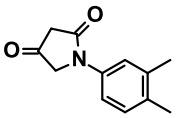
The reaction was carried out according to the procedure for **2a** with **S4** instead of **S1** to give the title compound **2c** (10.6 mg, 0.0262 mmol, 14% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 2.19 (3H, s), 2.22 (3H, s), 4.54 (2H, s), 4.73 (2H, s), 6.54 (1H, d, *J* = 3.6 Hz), 7.02 (1H, d, *J* = 3.6 Hz), 7.14 (1H, d, *J* = 8.5 Hz), 7.37 (1H, dd, *J* = 7.9, 1.8 Hz), 7.41 (1H, d, *J* = 1.8 Hz), 7.55 (1H, t, *J* = 7.9 Hz), 7.84 (1H, d, *J* = 7.9 Hz), 7.90 (1H, d, *J* = 7.9 Hz), 8.19 (1H, s), 13.11 (1H, brs). MS (ESI/APCI) *m/z*: 405 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{23}H_{21}N_2O_5$ $(M + H)^+$ 405.1445. Found: 405.1447.

1-(3,4-Dimethylphenyl)-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (14)



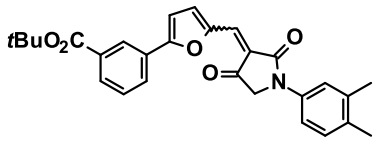
To a solution of **8** (1.78 mL, 12.4 mmol) in acetonitrile (40 mL), potassium acetate (1.26 g, 12.4 mmol) and a solution of methyl 4-chloro-3-methoxy-2(*E*)-butenoate (2.15 g, 12.4 mmol) in acetonitrile (20 mL) were added. The mixture was heated to reflux for 12 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **14** (1.45 g, 6.67 mmol, 54% yield) as an orange solid. ^{1}H NMR ($CDCl_3$) δ: 2.22 (3H, s), 2.26 (3H, s), 3.85 (3H, s), 4.26 (2H, s), 5.18 (1H, s), 7.09 (1H, d, *J* = 8.2 Hz), 7.30 (1H, dd, *J* = 8.2, 2.4 Hz), 7.43 (1H, d, *J* = 2.4 Hz). MS (ESI/APCI) *m/z*: 218 $(M + H)^+$.

1-(3,4-Dimethylphenyl)pyrrolidine-2,4-dione (15)



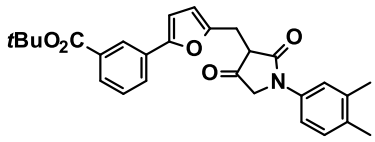
To a solution of **14** (1.00 g, 4.60 mmol) in 1,4-dioxane (20 mL), concentrated hydrochloric acid (20 mL) was added. The mixture was stirred at rt for 1 h. The volatile materials were removed under reduced pressure. The residue was diluted with water, and the precipitated solid was collected by filtration and dried to obtain the title compound **15** (846 mg, 4.16 mmol, 90% yield) as mixture of keto-enol tautomers: ^{1}H NMR ($CDCl_3$) δ: 2.22–2.14 (6H, m), 4.30 (2H, s), 4.93 (1H, s), 7.03–7.13 (1H, m), 7.37–7.45 (2H, m), 11.75 (1H, s). MS (ESI/APCI) *m/z*: 204 $(M + H)^+$.

***tert*-Butyl 3-(5-{[1-(3,4-dimethylphenyl)-2,4-dioxopyrrolidin-3-ylidene]methyl}furan-2-yl)benzoate (16)**



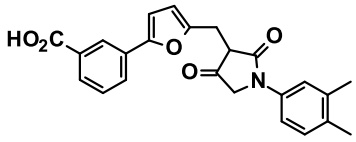
To a suspension of **15** (328 mg, 1.62 mml) and **5b** (440 mg, 1.62 mmol) in toluene (30 mL), diethylamine (1 drop) was added. The mixture was stirred at rt overnight. The reaction mixture was concentrated *in vacuo* and ethanol was added to the residue. The precipitated solid was collected by filtration and dried to obtain the title compound **16** (336 mg, 0.734 mmol, 65% yield) as an orange solid. The present compound was used for the next reaction without further purification. MS (ESI/APCI) *m/z*: 458 $(M + H)^+$.

***tert*-Butyl 3-(5-{[1-(3,4-dimethylphenyl)-2,4-dioxopyrrolidin-3-yl]methyl}furan-2-yl)benzoate (S5)**



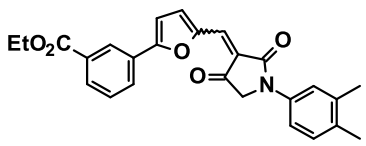
To a solution of **16** (233 mg, 0.509 mmol) in toluene (10 mL) and ethanol (10 mL), diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (149 mg, 0.450 mmol) was added. The mixture was stirred at room temperature for 4 h. The solvent was removed by concentration under reduced pressure, and the residue was purified by silica gel column chromatography (chloroform/methanol) to obtain the title compound **S5** (100 mg, 0.218 mmol, 43% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.55 (9H, s), 2.15 (3H, s), 2.19 (3H, s), 3.56 (2H, s), 4.32 (2H, s), 6.16 (1H, d, *J* = 3.0 Hz), 6.91 (1H, d, *J* = 3.0 Hz), 7.05 (1H, d, *J* = 8.5 Hz), 7.39 (1H, d, *J* = 8.5 Hz), 7.48–7.52 (2H, m), 7.75 (1H, d, *J* = 7.9 Hz), 7.86 (1H, d, *J* = 7.9 Hz), 8.11 (1H, s), 11.47 (1H, s). MS (ESI/APCI) *m/z*: 404 (M - ^{t}Bu + H)$^+$.

3-(5-{[1-(3,4-dimethylphenyl)-2,4-dioxopyrrolidin-3-yl]methyl}furan-2-yl)benzoic acid (2b)



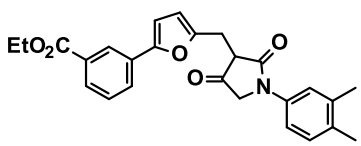
To a solution of **S5** (69.0 mg, 0.150 mmol) in DCM (4.0 mL), trifluoroacetic acid (4.0 mL) was added. The mixture was stirred at 100 °C for 2 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with hexane/diethyl ether and the precipitated solid was collected by filtration and dried to obtain the title compound **2b** (53.0 mg, 0.131 mmol, 88% yield) as a brown solid. ^{1}H NMR (DMSO-d_6) δ: 2.15 (3H, s), 2.19 (3H, s), 3.56 (2H, s), 4.32 (2H, s), 6.15 (1H, d, *J* = 3.6 Hz), 6.92 (1H, d, *J* = 3.6 Hz), 7.05 (1H, d, *J* = 8.5 Hz), 7.38 (1H, dd, *J* = 8.5, 2.1 Hz), 7.46 (1H, d, *J* = 2.1 Hz), 7.51 (1H, dd, *J* = 7.6, 7.6 Hz), 7.79 (1H, d, *J* = 7.9 Hz), 7.87 (1H, d, *J* = 7.9 Hz), 8.16 (1H, s), 11.48 (1H, s), 13.11 (1H, brs). MS (ESI/APCI) *m/z*: 404 (M + H)$^+$. HRMS (ESI) *m/z*: calcd for $C_{24}H_{22}NO_5$ (M + H)$^+$ 404.1492. Found: 404.1503.

Ethyl 3-(5-{[1-(3,4-dimethylphenyl)-2,4-dioxopyrrolidin-3-ylidene]methyl}furan-2-yl)benzoate (17)



The reaction was carried out according to the procedure for **16** with **5a** instead of **5b** to give the title compound **17** (256 mg, 0.596 mmol, 73% yield) as an orange solid. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, t, J = 7.2 Hz), 2.27 (3H, s), 2.32 (3H, s), 4.27 (1.83H, s), 4.32 (0.17H, s), 4.45 (2H, q, *J* = 7.2 Hz), 7.08 (1H, d, *J* = 3.6 Hz), 7.18 (1H, d, *J* = 8.5 Hz), 7.50–7.58 (3H, m), 7.78 (1H, s), 8.06–8.09 (2H, m), 8.49 (1H, s), 8.61 (1H, s). MS (ESI/APCI) *m/z*: 430 (M + H)$^+$.

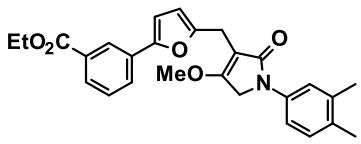
Ethyl 3-(5-{[1-(3,4-dimethylphenyl)-2,4-dioxopyrrolidin-3-yl]methyl}furan-2-yl)benzoate (S6)



The reaction was carried out according to the procedure for **S5** with **17** instead of **16** to give the title compound **S6** (250 mg, 0.579 mmol, 96% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.42

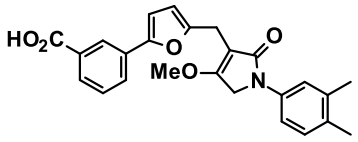
(3H, t, *J* = 7.2 Hz), 2.22 (3H, s), 2.26 (3H, s), 3.74 (2H, s), 4.23 (2H, s), 4.41 (2H, q, *J* = 7.2 Hz), 6.31 (1H, d, *J* = 3.0 Hz), 6.69 (1H, d, *J* = 3.0 Hz), 7.00 (1H, s), 7.09 (1H, d, *J* = 8.5 Hz), 7.30–7.34 (1H, m), 7.46–7.51 (2H, m), 7.78 (1H, d, *J* = 7.9 Hz), 7.94 (1H, d, *J* = 7.9 Hz), 8.26 (1H, s). MS (ESI/APCI) *m/z*: 432 $(M + H)^+$.

Ethyl 3-(5-{[1-(3,4-dimethylphenyl)-4-methoxy-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}furan-2-yl)-benzoate (S7)



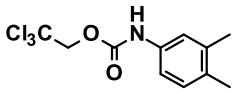
To a solution of **S6** (250 mg, 0.579 mmol) in toluene (30 mL) and methanol (6 mL), 2 mol/L (trimethylsilyl)diazomethane in hexane (0.637 mL, 1.27 mmol) was added. The mixture was stirred at rt for 3 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **S7** (226 mg, 0.507 mmol, 88% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, *J* = 7.2 Hz), 2.22 (3H, s), 2.26 (3H, s), 3.79 (2H, s), 4.08 (3H, s), 4.30 (2H, s), 4.40 (2H, q, *J* = 7.2 Hz), 6.20 (1H, d, *J* = 3.0 Hz), 6.63 (1H, d, *J* = 3.0 Hz), 7.09 (1H, d, *J* = 8.4 Hz), 7.33 (1H, dd, *J* = 8.4, 2.0 Hz), 7.42 (1H, t, *J* = 7.9 Hz), 7.53 (1H, d, *J* = 2.0 Hz), 7.79 (1H, d, *J* = 7.9 Hz), 7.88 (1H, d, *J* = 7.9 Hz), 8.27 (1H, s). MS (ESI/APCI) *m/z*: 446 $(M + H)^+$.

3-(5-{[1-(3,4-Dimethylphenyl)-4-methoxy-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}furan-2-yl)benzoic acid (2f)



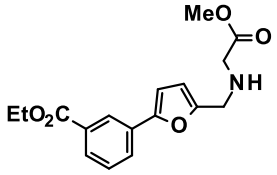
To a solution of **S7** (226 mg, 0.507 mmol) in THF (8.0 mL) and methanol (4.0 mL), 1 mol/L aqueous NaOH solution (4.0 mL) was added. The mixture was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was diluted with water. 1 mol/L aqueous HCl solution (4.0 mL) was added to the above solution and the mixture was extracted with ethyl acetate. The organic layer obtained was washed with saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was diluted with ethyl acetate, and the precipitated solid was collected by filtration and dried to obtain the title compound **2f** (192 mg, 0.460 mmol, 91% yield) as a pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ: 2.16 (3H, s), 2.20 (3H, s), 3.57 (2H, s), 3.98 (3H, s), 4.65 (2H, s), 6.19 (1H, d, *J* = 3.3 Hz), 6.92 (1H, d, *J* = 3.3 Hz), 7.08 (1H, d, *J* = 7.9 Hz), 7.45–7.53 (3H, m), 7.80 (1H, d, *J* = 7.9 Hz), 7.86 (1H, d, *J* = 7.9 Hz), 8.16 (1H, s), 13.10 (1H, brs). MS (ESI/APCI) *m/z*: 418 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{25}H_{24}NO_5$ $(M + H)^+$ 418.1649. Found: 418.1672.

2,2,2-Trichloroethyl (3,4-dimethylphenyl)carbamate (18)



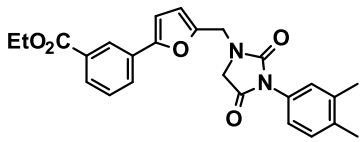
To a solution of **8** (500 mg, 4.13 mmol) and triethylamine (0.744 mL, 5.36 mmol) in DCM (10 mL), 2,2,2-trichloroethyl chloroformate (0.609 mL, 4.54 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 2 h. A saturated aqueous $NaHCO_3$ solution was then added, and the mixture was stirred and then extracted with ethyl acetate, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **18** (625 mg, 2.11 mmol, 51% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.23 (3H, s), 2.25 (3H, s), 4.82 (2H, s), 6.76 (1H, s), 7.09 (1H, d, *J* = 7.9 Hz), 7.14 (1H, d, *J* = 7.9 Hz), 7.21 (1H, s).

Ethyl 3-(5-{[(2-methoxy-2-oxoethyl)amino]methyl}furan-2-yl)benzoate (19)



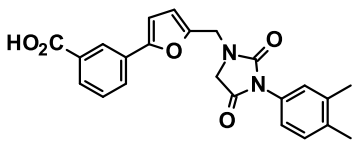
The reaction was carried out according to the procedure for **11a** with glycine methyl ester hydrochloride instead of **10a** to give the title compound **19** (172 mg, 0.542 mmol, 44% yield) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.42 (3H, t, *J* = 7.3 Hz), 3.50 (2H, s), 3.72 (3H, s), 3.89 (2H, s), 4.41 (2H, q, *J* = 7.3 Hz), 6.31 (1H, d, *J* = 3.0 Hz), 6.67 (1H, d, *J* = 3.0 Hz), 7.44 (1H, t, *J* = 7.9 Hz), 7.83 (1H, d, *J* = 7.9 Hz), 7.92 (1H, d, *J* = 7.9 Hz), 8.30 (1H, s).

Ethyl 3-(5-{[3-(3,4-dimethylphenyl)-2,4-dioxoimidazolidin-1-yl]methyl}furan-2-yl)benzoate (20)



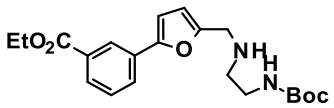
To a solution of **18** (168 mg, 0.566 mmol) and **19** (172 mg, 0.542 mmol) in toluene (5.0 mL), *N*,*N*-diisopropylethylamine (0.120 mL, 0.706 mmol) was added. The mixture was heated to reflux for 4 h. The temperature was lowered to rt, DMSO (2.0 mL) was added and stirred at 120 °C for 8.5 h. The temperature was lowered to rt, **18** (160 mg, 0.539 mmol) and *N*,*N*-diisopropylethylamine (0.0920 mL, 0.541 mmol) were further added and stirred at 120 °C for 2 h. The reaction solution was diluted with ethyl acetate, sequentially washed with water and saturated brine, and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the title compound **20** (173 mg, 0.400 mmol, 74% yield) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.42 (3H, t, *J* = 7.1 Hz), 2.27 (3H, s), 2.28 (3H, s), 4.10 (2H, s), 4.42 (2H, q, *J* = 7.1 Hz), 4.72 (2H, s), 6.46 (1H, d, *J* = 3.6 Hz), 6.72 (1H, d, *J* = 3.6 Hz), 7.11 (1H, dd, *J* = 7.9, 2.1 Hz), 7.15 (1H, brs), 7.22 (1H, d, *J* = 7.9 Hz), 7.48 (1H, t, *J* = 7.9 Hz), 7.84 (1H, d, *J* = 7.3 Hz), 7.96 (1H, d, *J* = 7.9 Hz), 8.30 (1H, s). MS (ESI/APCI) *m/z*: 433 $(M + H)^+$.

3-(5-{[3-(3,4-Dimethylphenyl)-2,4-dioxoimidazolidin-1-yl]methyl}furan-2-yl)benzoic acid (2d)



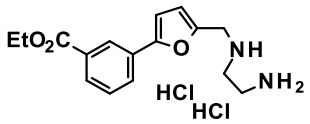
The reaction was carried out according to the procedure for **2a** with **S7** instead of **S1** to give the title compound **2d** (61.8 mg, 0.153 mmol, 38% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 2.24 (3H, s), 2.25 (3H, s), 4.16 (2H, s), 4.66 (2H, s), 6.62 (1H, d, J = 3.6 Hz), 7.07–7.12 (3H, m), 7.23 (1H, d, J = 7.9 Hz), 7.56 (1H, dd, J = 7.9, 7.9 Hz), 7.86 (1H, d, J = 7.9 Hz), 7.96 (1H, d, J = 7.9 Hz), 8.22 (1H, s), 13.18 (1H, brs). MS (ESI/APCI) *m/z*: 405 (M + H)$^+$. HRMS (ESI) *m/z*: calcd for $C_{23}H_{21}N_2O_5$ (M + H)$^+$ 405.1445. Found: 405.1416.

Ethyl 3-{5-[({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)methyl]furan-2-yl}benzoate (22)



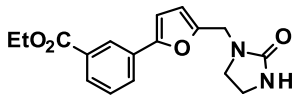
The reaction was carried out according to the procedure for **11a** with *tert*-butyl (2-aminoethyl)carbamate (**21**) instead of **10a** to give the title compound **22** (1.05 g, 2.53 mmol, 62% yield) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.39–1.47 (12H, m), 2.79 (2H, t, J = 6.0 Hz), 3.22–3.28 (2H, m), 3.85 (2H, s), 4.41 (2H, q, J = 7.3 Hz), 4.96 (1H, brs), 6.28 (1H, d, J = 3.6 Hz), 6.67 (1H, d, J = 3.0 Hz), 7.44 (1H, dd, J = 7.9, 7.9 Hz), 7.81–7.84 (1H, m), 7.90–7.93 (1H, m), 8.28–8.30 (1H, m). MS (ESI/APCI) *m/z*: 389 (M + H)$^+$.

Ethyl 3-(5-{[(2-aminoethyl)amino]methyl}furan-2-yl)benzoate-hydrogen chloride (1/2) (23)



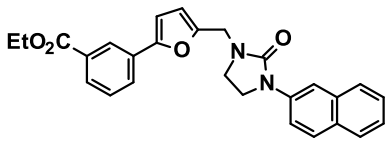
Hydrogen chloride (4 mol/L, 1,4-dioxane solution, 8 mL) was added to **22** (1.05 g, 2.53 mmol) in 1,4-dioxane (40 mL) at 0 °C, and the mixture was stirred at rt for 0.5 h, and then concentrated under reduced pressure. Then, the resultant sample was slurried with diethyl ether, and then filtered to obtain the title compound **23** (829 mg, 2.29 mmol, 91% yield) as a white solid. The present compound was used for the next reaction without further purification. MS (ESI/APCI) *m/z*: 289 (M + H)$^+$.

Ethyl 3-{5-[(2-oxoimidazolidin-1-yl)methyl]furan-2-yl}benzoate (24)



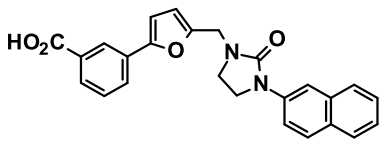
To a suspension of **23** (829 mg, 2.29 mmol) in THF (40 mL) were added 1,1'-carbonyldiimidazole (409 mg, 2.52 mmol) and triethylamine (0.795 mL, 5.74 mmol), and stirred at 70 °C for 1 h. Then, the mixture was concentrated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (ethyl acetate/methanol) to obtain compound **24** (649 mg, 2.06 mmol, 90% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.42 (3H, t, J = 7.0 Hz), 3.40–3.53 (4H, m), 4.38–4.50 (5H, m), 6.36 (1H, d, J = 3.0 Hz), 6.68 (1H, d, J = 3.6 Hz), 7.45 (1H, dd, J = 7.9, 7.9 Hz), 7.81–7.85 (1H, m), 7.90–7.94 (1H, m), 8.27–8.30 (1H, m). MS (ESI/APCI) *m/z*: 315 (M + H)$^+$.

Ethyl 3-(5-{[3-(naphthalen-2-yl)-2-oxoimidazolidin-1-yl]methyl}furan-2-yl)benzoate (S9)



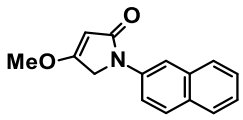
To a solution of **24** (100 mg, 0.318 mmol) in 1,4-dioxane (3.0 mL) were added 2-bromonaphthalene (72.4 mg, 0.350 mmol), copper (I) iodide (60.5 mg, 0.318 mmol), potassium carbonate (87.9 mg, 0.636 mmol), *trans*-1,2-cyclohexanediamine (0.115 mL, 0.957 mmol), and stirred at 110 °C for 4.5 h. Then, the reaction solution was filtered through celite using ethyl acetate, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **S9** (98.9 mg, 0.225 mmol, 71% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7.3 Hz), 3.58–3.64 (2H, m), 3.93–3.99 (2H, m), 4.41 (2H, q, J = 7.3 Hz), 4.59 (2H, s), 6.44 (1H, d, J = 3.0 Hz), 6.71 (1H, d, J = 3.6 Hz), 7.34–7.48 (3H, m), 7.65 (1H, d, J = 2.4 Hz), 7.74–7.87 (4H, m), 7.91–7.95 (1H, m), 8.14 (1H, dd, J = 8.8, 2.1 Hz), 8.29–8.32 (1H, m).

3-(5-{[3-(Naphthalen-2-yl)-2-oxoimidazolidin-1-yl]methyl}furan-2-yl)benzoic acid (2g)



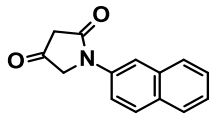
The reaction was carried out according to the procedure for **2a** with **S9** instead of **S1** to give the title compound **2g** (72.6 mg, 0.176 mmol, 78% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.55 (2H, t, J = 7.9 Hz), 3.96 (2H, t, J = 7.9 Hz), 4.53 (2H, s), 6.57 (1H, d, J = 3.0 Hz), 7.06 (1H, d, J = 3.0 Hz), 7.38 (1H, t, J = 7.9 Hz), 7.46 (1H, t, J = 7.9 Hz), 7.55 (1H, t, J = 7.9 Hz), 7.73 (1H, d, J = 2.1 Hz), 7.82–7.89 (4H, m), 7.94 (1H, d, J = 7.9 Hz), 8.18 (1H, dd, J = 9.1, 2.1 Hz), 8.22 (1H, s), 13.16 (1H, s). MS (ESI/APCI) *m/z*: 413 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{25}H_{21}N_2O_4$ $(M + H)^+$ 413.1496. Found: 413.1494.

4-Methoxy-1-(naphthalen-2-yl)-1,5-dihydro-2*H*-pyrrol-2-one (26)



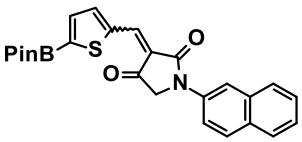
To a suspension of 4-methoxy-3-pyrroline-2-one (**25**, 2.53 g, 22.1 mmol), 2-bromonaphthalene (5.20 g, 24.4 mmol), copper (I) iodide (426 mg, 2.21 mmol) and potassium carbonate (4.92 g, 35.4 mmol) in toluene (50 mL), *N*,*N*-dimethylethylenediamine (0.491 mL, 4.43 mmol) was added. The mixture was stirred at 100 °C for 8 h. The reaction solution was filtered through celite using DCM, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate) to obtain compound **26** (3.51 g, 14.7 mmol, 66% yield) as a pale-yellow solid: ^{1}H NMR ($CDCl_3$) δ: 3.89 (3H, s), 4.42 (2H, s), 5.24 (1H, s), 7.38–7.45 (2H, m), 7.79–7.83 (3H, m), 7.89 (1H, s), 8.02 (1H, dd, J = 8.8, 2.1 Hz). MS (ESI/APCI) *m/z*: 240 $(M + H)^+$.

1-(Naphthalen-2-yl)pyrrolidine-2,4-dione (27)



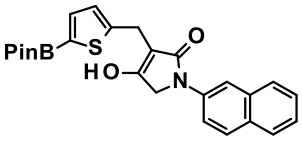
To a solution of **26** (3.51 g, 14.7 mmol) in 1,4-dioxane (10 mL), concentrated hydrochloric acid (10 mL) was added. The mixture was stirred at rt overnight. The reaction mixture was diluted with water, and the precipitated solid was collected by filtration and dried to obtain the title compound **27** (3.17 g, 14.1 mmol, 96% yield) as a pale-orange solid. ^{1}H NMR ($CDCl_3$) δ: 3.38 (2H, s), 4.46 (2H, s), 7.41–7.54 (2H, m), 7.76–8.00 (5H, m). MS (ESI/APCI) *m/z*: 226 $(M + H)^+$.

1-(Naphthalen-2-yl)-3-{[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl]methylidene}pyrrolidine-2,4-dione (S10)



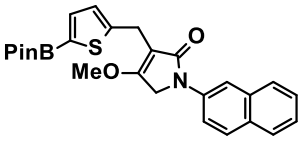
The reaction was carried out according to the procedure for **16** with **27** and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-carboxaldehyde instead of **5b** and **15** to give the title compound **S10** as an orange solid. The present compound was used for the next reaction without further purification.

4-Hydroxy-1-(naphthalen-2-yl)-3-{[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl]methyl}-1,5-dihydro-2H-pyrrol-2-one (S11)



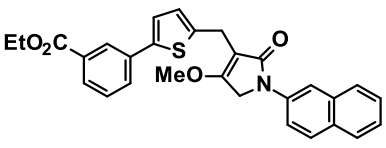
The reaction was carried out according to the procedure for **S5** with **S10** instead of **16** to give the title compound **S11** (600 mg, 1.34 mmol, 64% yield for 2 steps) as a white solid. MS (ESI/APCI) *m/z*: 448 $(M + H)^+$.

4-Methoxy-1-(naphthalen-2-yl)-3-{[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl]methyl}-1,5-dihydro-2H-pyrrol-2-one (28)



The reaction was carried out according to the procedure for **S7** with **S11** instead of **S6** to give the title compound **28** (346 mg, 0.750 mmol, 59% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.31 (12H, s), 3.94 (2H, s), 4.02 (3H, s), 4.42 (2H, s), 7.04 (1H, d, J = 3.6 Hz), 7.35–7.49 (3H, m), 7.77 (2H, d, J = 8.5 Hz), 7.81 (1H, d, J = 9.1 Hz), 7.95 (1H, d, J = 2.4 Hz), 8.04 (1H, dd, J = 9.1, 2.4 Hz). MS (ESI/APCI) *m/z*: 448 $(M + H)^+$.

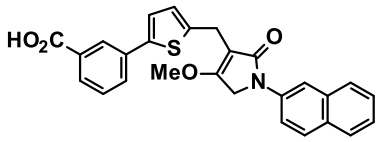
Ethyl 3-(5-{[4-methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1H-pyrrol-3-yl]methyl}thiophen-2-yl)benzoate (S12)



To a solution of **28** (100 mg, 0.217 mmol) and ethyl 3-bromobenzoate (52.8 mg, 0.219 mmol) in 1,4-dioxane (2.55 mL) and water (0.5 mL) were added palladium acetate (5.0 mg, 0.022 mmol), SPhos (18.3 mg, 0.0432 mmol), and potassium

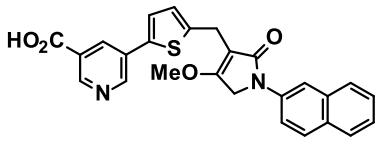
phosphate (318 mg, 1.50 mmol). After a N_2 purge, the mixture was stirred at 60 °C for 1 h. The solvent was removed by concentration under reduced pressure, and the residue was diluted with water. The precipitate solid was collected by filtration and formed into a slurry with diethyl ether/ethyl acetate, and filtered off to obtain the title compound **S12** as a white solid. The present compound was used for the next reaction without further purification. MS (ESI/APCI) *m/z*: 484 $(M + H)^+$.

3-(5-{[4-Methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)benzoic acid (3a)



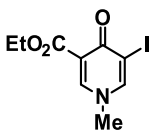
To a solution of crude compound **S12** in THF (2.0 mL) and MeOH (2.0 mL) was added 1 mol/L aqueous NaOH solution (1.0 mL). The mixture was stirred at rt for 18 h. The reaction mixture was concentrated *in vacuo*, and the residue was diluted with water. 1 mol/L aqueous HCl solution (1.0 mL) was added to the above solution, and the precipitated solid was collected by filtration and dried to obtain the title compound **3a** (28.3 mg, 0.0621 mmol, 29% yield for 2 steps) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 3.71 (2H, s), 4.03 (3H, s), 4.85 (2H, s), 6.92 (1H, d, J = 3.6 Hz), 7.39–7.40 (2H, m), 7.46–7.52 (2H, m), 7.80–7.85 (4H, m), 7.91 (1H, d, J = 8.5 Hz), 8.05 (1H, s), 8.10 (1H, s), 8.15 (1H, dd, J = 9.1, 1.8 Hz), 13.11 (1H, brs). MS (ESI/APCI) *m/z*: 456 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{27}H_{22}NO_4S$ $(M + H)^+$ 456.1264. Found: 456.1276.

5-(5-{[4-Methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)pyridine-3-carboxylic acid (3b)



The reaction was carried out according to the procedure for **S12** with 5-bromopyridine-3-carboxylic acid instead of ethyl 3-bromobenzoate to give the title compound **3b** (109 mg, 0.239 mmol, 92% yield) as a brown solid. ^{1}H NMR (DMSO-d_6) δ: 3.73 (2H, s), 4.03 (3H, s), 4.86 (2H, s), 6.98 (1H, d, J = 3.6 Hz), 7.39–7.47 (2H, m), 7.56 (1H, d, J = 3.6 Hz), 7.81–7.92 (3H, m), 8.14–8.24 (3H, m), 8.91 (1H, d, J = 1.8 Hz), 9.04 (1H, d, J = 2.4 Hz). MS (ESI/APCI) *m/z*: 457 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{27}H_{21}N_2O_4S$ $(M + H)^+$ 457.1217. Found: 457.1212.

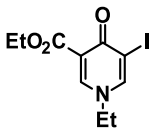
Ethyl 5-iodo-1-methyl-4-oxo-1,4-dihydropyridine-3-carboxylate (30c)



To a solution of ethyl 4-hydroxy-5-iodopyridine-3-carboxylate (**29**, 1.30 g, 4.44 mmol) and cesium carbonate (2.89 g, 8.87 mmol) in DMF (15 mL) was added iodomethane (0.552 mL, 8.87 mmol). The mixture was stirred at rt for 2 h. The solvent was removed by concentration under reduced pressure, and the residue was purified by silica gel column chromatography (chloroform/methanol) to obtain a slurry with a diethyl

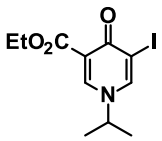
ether. The slurry was then filtered to obtain the title compound **30c** (1.35 g, 4.40 mmol, 90% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.24 (3H, t, J = 7.0 Hz), 3.69 (3H, s), 4.18 (2H, q, J = 7.0 Hz), 8.29 (1H, d, J = 2.4 Hz), 8.34 (1H, d, J = 2.4 Hz). MS (ESI/APCI) *m/z*: 308 $(M + H)^+$.

Ethyl 1-ethyl-5-iodo-4-oxo-1,4-dihydropyridine-3-carboxylate (30d)



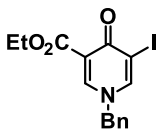
The reaction was carried out according to the procedure for **30c** with iodoethane instead of iodomethane to give the title compound **30d** (978 mg, 3.05 mmol, 89% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.24 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 3.98 (2H, q, J = 7.1 Hz), 4.18 (2H, q, J = 7.1 Hz), 8.35 (1H, d, J = 2.4 Hz), 8.43 (1H, d, J = 2.4 Hz). MS (ESI/APCI) *m/z*: 322 $(M + H)^+$.

Ethyl 5-iodo-4-oxo-1-(propan-2-yl)-1,4-dihydropyridine-3-carboxylate (30e)



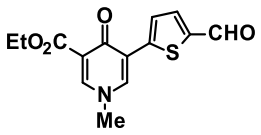
The reaction was carried out according to the procedure for **30c** with 2-iodopropane instead of iodomethane to give the title compound (572 mg, 1.71 mmol, 50% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.24 (3H, t, J = 7.1 Hz), 1.38 (6H, d, J = 6.7 Hz), 4.19 (2H, q, J = 7.1 Hz), 4.36–4.43 (1H, m), 8.34 (1H, d, J = 2.4 Hz), 8.47 (1H, d, J = 2.4 Hz). MS (ESI/APCI) *m/z*: 336 $(M + H)^+$.

Ethyl 1-benzyl-5-iodo-4-oxo-1,4-dihydropyridine-3-carboxylate (30f)



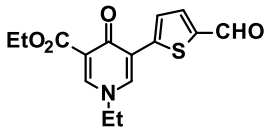
The reaction was carried out according to the procedure for **30c** with benzyl bromide instead of iodomethane to give the title compound (1.06 g, 2.77 mmol, 81% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.22 (3H, t, J = 7.1 Hz), 4.18 (2H, q, J = 7.1 Hz), 5.19 (2H, s), 7.34–7.43 (5H, m), 8.46–8.49 (2H, m). MS (ESI/APCI) *m/z*: 384 $(M + H)^+$.

Ethyl 5-(5-formylthiophen-2-yl)-1-methyl-4-oxo-1,4-dihydropyridine-3-carboxylate (31c)



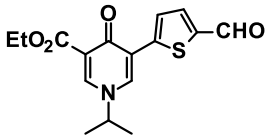
The reaction was carried out according to the procedure for **S12** with **30c** and 5-(6-methyl-4,8-dioxo-1,3,6,2-dioxazaborocan-2-yl)thiophene-2-carbaldehyde instead of ethyl 3-bromobenzoate and **28** to give the title compound **31c** (85.4 mg, 0.293 mmol, 60% yield) as a brown solid. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 3.86 (3H, s), 4.41 (2H, q, J = 7.1 Hz), 7.64 (1H, d, J = 3.6 Hz), 7.73 (1H, d, J = 3.6 Hz), 7.87 (1H, d, J = 1.8 Hz), 8.15 (1H, d, J = 1.8 Hz), 9.92 (1H, s). MS (ESI/APCI) *m/z*: 292 $(M + H)^+$.

Ethyl 1-ethyl-5-(5-formylthiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (31d)



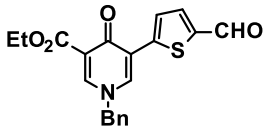
The reaction was carried out according to the procedure for **31c** with **30d** instead of **30c** to give the title compound **31d** (133 mg, 0.436 mmol, 28% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, *J* = 7.1 Hz), 1.58 (6H, t, *J* = 7.1 Hz), 4.05 (2H, q, *J* = 7.1 Hz), 4.41 (2H, q, *J* = 7.1 Hz), 7.62 (1H, d, *J* = 4.2 Hz), 7.71 (1H, d, *J* = 4.2 Hz), 7.91 (1H, d, *J* = 2.4 Hz), 8.17 (1H, d, *J* = 2.4 Hz), 9.91 (1H, s). MS (ESI/APCI) *m/z*: 306 $(M + H)^+$.

Ethyl 5-(5-formylthiophen-2-yl)-4-oxo-1-(propan-2-yl)-1,4-dihydropyridine-3-carboxylate (31e)



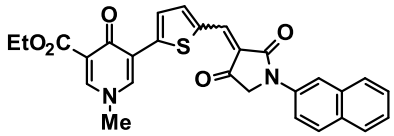
The reaction was carried out according to the procedure for **31c** with **30e** instead of **30c** to give the title compound **31e** (239 mg, 0.748 mmol, 46% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, *J* = 7.0 Hz), 1.60 (6H, d, *J* = 7.3 Hz), 4.26–4.33 (1H, m), 4.42 (2H, q, *J* = 7.0 Hz), 7.65 (1H, d, *J* = 4.2 Hz), 7.72 (1H, d, *J* = 4.2 Hz), 7.97 (1H, d, *J* = 2.4 Hz), 8.24 (1H, d, *J* = 2.4 Hz), 9.91 (1H, s). MS (ESI/APCI) *m/z*: 320 $(M + H)^+$.

Ethyl 1-benzyl-5-(5-formylthiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (31f)



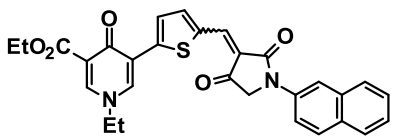
The reaction was carried out according to the procedure for **31c** with **30f** instead of **30c** to give the title compound **31f** (814 mg, 2.22 mmol, 85% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H, t, *J* = 7.2 Hz), 4.39 (2H, q, *J* = 7.2 Hz), 5.14 (2H, s), 7.28 (1H, d, *J* = 2.4 Hz), 7.38–7.43 (4H, m), 7.53 (1H, d, *J* = 4.2 Hz), 7.67 (1H, d, *J* = 4.2 Hz), 7.90 (1H, d, *J* = 2.4 Hz), 8.22 (1H, d, *J* = 2.4 Hz), 9.90 (1H, s). MS (ESI/APCI) *m/z*: 368 $(M + H)^+$.

Ethyl 1-methyl-5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-ylidene]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (32c)



The reaction was carried out according to the procedure for **S10** with **31c** instead of 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene-2-carboxaldehyde to give the title compound **32c** (177 mg, 0.355 mmol, 61% yield) as an orange solid. The present compound was used for the next reaction without further purification.

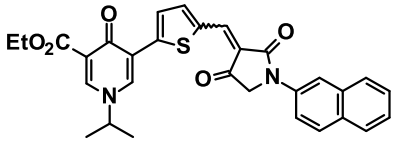
Ethyl 1-ethyl-5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-ylidene]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (32d)



The reaction was carried out according to the procedure for **32c** with **31d** instead of **31c** to give the title compound **32d** as a red solid. The present compound was used for the next reaction

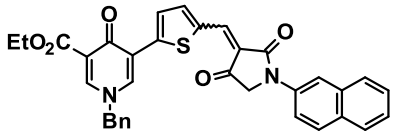
without further purification. MS (ESI/APCI) *m/z*: 513 $(M + H)^+$.

Ethyl 5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-ylidene]methyl}thiophen-2-yl)-4-oxo-1-(propan-2-yl)-1,4-dihydropyridine-3-carboxylate (32e)



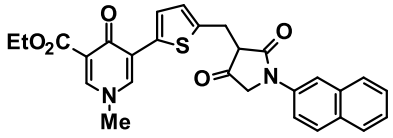
The reaction was carried out according to the procedure for **32c** with **31e** instead of **31c** to give the title compound **32e** as a red solid. The present compound was used for the next reaction without further purification.

Ethyl 1-benzyl-5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-ylidene]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (32f)



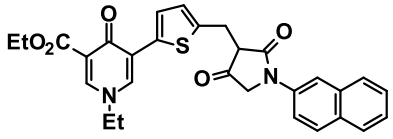
The reaction was carried out according to the procedure for **32c** with **31f** instead of **31c** to give the title compound **32f** as a red solid. The present compound was used for the next reaction without further purification. MS (ESI/APCI) *m/z*: 575 $(M + H)^+$.

Ethyl 1-methyl-5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-yl]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (S13)



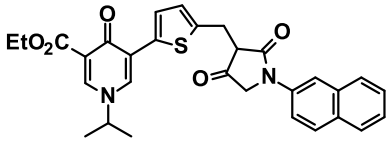
The reaction was carried out according to the procedure for **S5** with **32c** instead of **16** to give the title compound **S13** as a brown solid. The present compound was used for the next reaction without further purification.

Ethyl 1-ethyl-5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-yl]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (S14)



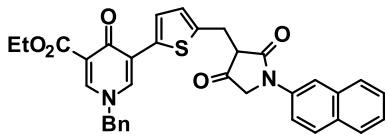
The reaction was carried out according to the procedure for **S5** with **32d** instead of **16** to give the title compound **S14** as a brown solid. The present compound was used for the next reaction without further purification.

Ethyl 5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-yl]methyl}thiophen-2-yl)-4-oxo-1-(propan-2-yl)-1,4-dihydropyridine-3-carboxylate (S15)



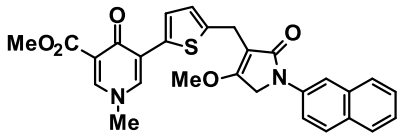
The reaction was carried out according to the procedure for **S5** with **32e** instead of **16** to give the title compound **S15** as an orange solid. The present compound was used for the next reaction without further purification.

Ethyl 1-benzyl-5-(5-{[1-(naphthalen-2-yl)-2,4-dioxopyrrolidin-3-yl]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (S16)



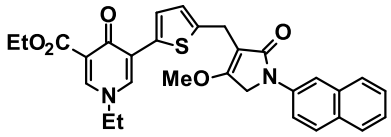
The reaction was carried out according to the procedure for **S5** with **32f** instead of **16** to give the title compound **S16** as a brown solid. The present compound was used for the next reaction without further purification.

Methyl 5-(5-{[4-methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-1-methyl-4-oxo-1,4-dihydropyridine-3-carboxylate (S17)



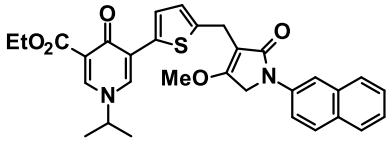
The reaction was carried out according to the procedure for **S7** with **S13** instead of **S6** to give the title compound **S17** (71.0 mg, 0.142 mmol, 83% yield from **32c**) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 3.76 (3H, s), 3.91–3.92 (5H, m), 4.05 (3H, s), 4.43 (2H, s), 6.93 (1H, d, J = 3.6 Hz), 7.36–7.39 (2H, m), 7.42–7.47 (1H, m), 7.68 (1H, d, *J* = 2.4 Hz), 7.77–7.81 (3H, m), 7.95 (1H, d, *J* = 2.4 Hz), 8.03 (1H, dd, *J* = 9.1, 2.4 Hz), 8.08 (1H, d, *J* = 2.4 Hz). MS (ESI/APCI) *m/z*: 501 $(M + H)^+$.

Ethyl 1-ethyl-5-(5-{[4-methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (S18)



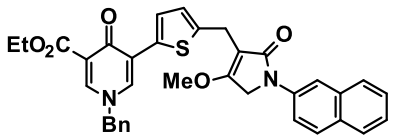
The reaction was carried out according to the procedure for **S7** with **S14** instead of **S6** to give the title compound **S18** (120 mg, 0.227 mmol, 53% yield from **31d**) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.38 (3H, t, *J* = 7.0 Hz), 1.50 (3H, t, *J* = 7.3 Hz), 3.92–3.94 (4H, m), 4.04 (3H, s), 4.37–4.41 (4H, m), 6.92–6.93 (1H, m), 7.38–7.43 (3H, m), 7.69–7.82 (4H, m), 7.95–8.12 (3H, m). MS (ESI/APCI) *m/z*: 529 $(M + H)^+$.

Ethyl 5-(5-{[4-methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-4-oxo-1-(propan-2-yl)-1,4-dihydropyridine-3-carboxylate (S19)



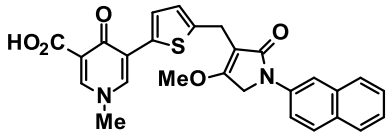
The reaction was carried out according to the procedure for **S7** with **S15** instead of **S6** to give the title compound **S19** (239 mg, 0.440 mmol, 60% yield from **31e**) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H, t, *J* = 7.3 Hz), 1.52 (6H, d, *J* = 6.7 Hz), 3.90 (2H, s), 4.04 (3H, s), 4.15–4.22 (1H, m), 4.36–4.41 (4H, m), 6.94 (1H, d, *J* = 2.4 Hz), 7.35–7.46 (3H, m), 7.75–7.82 (4H, m), 7.95 (1H, s), 8.03 (1H, dd, *J* = 8.8, 2.4 Hz), 8.16 (1H, d, *J* = 2.4 Hz).

Ethyl 1-benzyl-5-(5-{[4-methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylate (S20)



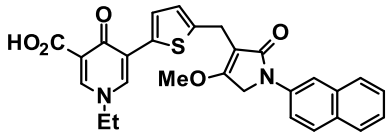
The reaction was carried out according to the procedure for **S7** with **S16** instead of **S6** to give the title compound **S20** (76.0 mg, 0.129 mmol, 18% yield from **31f**) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ: 1.37 (3H, t, *J* = 7.3 Hz), 3.89 (2H, s), 4.03 (3H, s), 4.34–4.41 (4H, m), 5.03 (2H, s), 6.90 (1H, d, *J* = 3.6 Hz), 7.19–7.21 (2H, m), 7.29–7.45 (6H, m), 7.69 (1H, d, *J* = 1.8 Hz), 7.75–7.81 (3H, m), 7.94–8.03 (2H, m), 8.14 (1H, d, *J* = 1.8 Hz). MS (ESI/APCI) *m/z*: 591 $(M + H)^+$.

5-(5-{[4-Methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-1-methyl-4-oxo-1,4-dihydropyridine-3-carboxylic acid (3c)



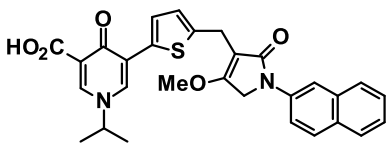
The reaction was carried out according to the procedure for **3a** with **S17** instead of **S12** to give the title compound **3c** (53.0 mg, 0.109 mmol, 77% yield) as a pale-yellow solid: ^{1}H NMR (DMSO-d_6) δ: 3.71 (2H, s), 3.95 (3H, s), 4.02 (3H, s), 4.84 (2H, s), 6.93 (1H, d, *J* = 3.9 Hz), 7.39 (1H, t, *J* = 7.0 Hz), 7.47 (1H, t, *J* = 7.0 Hz), 7.58 (1H, d, *J* = 3.9 Hz), 7.81 (1H, d, *J* = 8.5 Hz), 7.84 (1H, d, *J* = 8.5 Hz), 7.90 (1H, d, *J* = 9.1 Hz), 8.09 (1H, d, *J* = 2.4 Hz), 8.14 (1H, dd, *J* = 9.1, 2.4 Hz), 8.64 (1H, d, *J* = 1.8 Hz), 8.69 (1H, d, *J* = 1.8 Hz). MS (ESI/APCI) *m/z*: 487 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{27}H_{21}N_2O_5S$ $(M - H)^-$ 485.1177. Found: 485.1171.

1-Ethyl-5-(5-{[4-methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylic acid (3d)



The reaction was carried out according to the procedure for **3a** with **S18** instead of **S12** to give the title compound **3d** (82.0 mg, 0.164 mmol, 75% yield) as a pale-yellow solid: ^{1}H NMR (DMSO-d_6) δ: 1.41 (3H, t, *J* = 7.3 Hz), 3.72 (2H, s), 4.02 (3H, s), 4.25 (2H, q, *J* = 7.3 Hz), 4.84 (2H, s), 6.94 (1H, d, *J* = 3.6 Hz), 7.37–7.49 (2H, m), 7.61 (1H, d, *J* = 3.6 Hz), 7.81–7.92 (3H, m), 8.10–8.16 (2H, m), 8.72–8.76 (2H, m). MS (ESI/APCI) *m/z*: 501 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{28}H_{25}N_2O_5S$ $(M + H)^+$ 501.1479. Found: 501.1489.

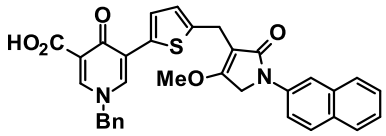
5-(5-{[4-Methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-4-oxo-1-(propan-2-yl)-1,4-dihydropyridine-3-carboxylic acid (3e)



The reaction was carried out according to the procedure for **3a** with **S19** instead of **S12** to give the title compound **3e** (157 mg, 0.305 mmol, 72% yield) as a pale-yellow solid: ^{1}H NMR

(DMSO-d_6) δ: 1.52 (6H, d, J = 6.7 Hz), 3.73 (2H, s), 4.03 (3H, s), 4.66–4.73 (1H, m), 4.86 (2H, s), 6.96 (1H, d, J = 4.3 Hz), 7.38–7.51 (2H, m), 7.70 (1H, d, J = 4.3 Hz), 7.82–7.93 (3H, m), 8.10–8.17 (2H, m), 8.71 (1H, d, J = 2.4 Hz), 8.75 (1H, d, J = 2.4 Hz). MS (ESI/APCI) *m/z*: 515 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{29}H_{27}N_2O_5S$ $(M + H)^+$ 515.1635. Found: 515.1668.

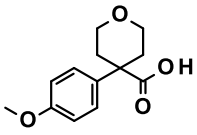
1-Benzyl-5-(5-{[4-methoxy-1-(naphthalen-2-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl]methyl}thiophen-2-yl)-4-oxo-1,4-dihydropyridine-3-carboxylic acid (3f)



The reaction was carried out according to the procedure for **3a** with **S20** instead of **S12** to give the title compound **3f** (37.0 mg, 0.0658 mmol, 55% yield) as a pale-yellow solid: ^{1}H NMR (DMSO-d_6) δ: 3.71 (2H, s), 4.01 (3H, s), 4.83 (2H, s), 5.46 (2H, s), 6.94 (1H, d, J = 3.6 Hz), 7.34–7.49 (7H, m), 7.60 (1H, d, J = 3.6 Hz), 7.80–7.91 (3H, m), 8.09–8.15 (2H, m), 8.80 (1H, s), 8.87 (1H, s). MS (ESI/APCI) *m/z*: 563 $(M + H)^+$. HRMS (ESI) *m/z*: calcd for $C_{33}H_{27}N_2O_5S$ $(M + H)^+$ 563.1635. Found: 563.1648.

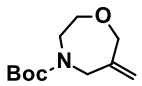
第二章

4-(4-Methoxyphenyl)tetrahydro-2*H*-pyran-4-carboxylic acid (45)



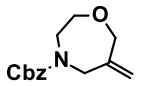
Under a N_2 atmosphere, a suspension of sodium hydride (purity >55%, 7.51 g, 172 mmol) in DMF (150 mL) was ice-cooled, a solution of 4-methoxyphenylacetonitrile (**44**, 10.5 mL, 78.1 mmol) and bis(2-bromoethyl)ether (20.2 g, 86.0 mmol) in DMF (50 mL) was added dropwise over a period of 30 min, stirred under ice-cooling for 1 h and at rt for 4 h. After ice-cooling again, water was added to the reaction solution, and the mixture was extracted with Et_2O three times, and the organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The organic layer was filtered and concentrated under reduced pressure to obtain crude 4-(4-methoxyphenyl)tetrahydro-2*H*-pyran-4-carbonitrile (22.4 g) as an oil. To this crude product (22.4 g), ethylene glycol (100 mL) and potassium hydroxide (13.01 g, 234 mmol) were added and stirred for 8 h under heated reflux. After allowing to cool to rt, water was added to the reaction solution, and the mixture was washed with Et_2O twice. The aqueous layer was acidified by adding 1 mol/L hydrochloric acid and stirred at rt overnight. The precipitated solid was collected by filtration and purified by silica gel column chromatography (chloroform/methanol) to obtain **45** (13.6 g, 57.6 mmol, 74% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.93–2.00 (2H, m), 2.47–2.53 (2H, m), 3.58–3.64 (2H, m), 3.80 (3H, s), 3.89–3.94 (2H, m), 6.89 (2H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.5 Hz).

***tert*-Butyl 6-methylidene-1,4-oxazepane-4-carboxylate (48)**



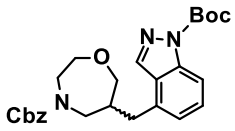
To a suspension of sodium hydride (purity >55%, 11.4 g, 261 mmol) in DMF (150 mL) was added 3-chloro-2-(chloromethyl)prop-1-ene (**47**, 12.7 mL, 120 mmol) at 0 °C. After the mixture was stirred for 10 min, 2-(*tert*-butoxycarbonylamino)ethan-1-ol (**46**, 19.3 g, 120 mmol) in THF (150 mL) was added dropwise over 1.5 h to the reaction mixture. The mixture was stirred at rt for 2.5 h. The reaction mixture was diluted with water and then extracted with Et_2O three times. The organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The organic layer was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain **48** (16.0 g, 75.0 mmol, 63% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (9H, s), 3.48–3.54 (2H, m), 3.69–3.76 (2H, m), 4.06–4.19 (4H, m), 4.95–5.06 (2H, m).

Benzyl 6-methylidene-1,4-oxazepane-4-carboxylate (49)



To **45** (5.00 g, 23.4 mmol) was added HCl (2 mol/L, MeOH solution, 100 mL) at rt. After stirring at the same temperature overnight, HCl (2 mol/L, 1,4-dioxane solution, 20 mL) was added and stirred at rt for 3 h. The reaction solution was concentrated and DIPEA (10.2 mL, 58.6 mmol) and benzyl chloroformate (3.67 mL, 25.8 mmol) were added to the suspension of the residue in DCM (80 mL) under ice-cooling and stirred at the same temperature for 30 min. To the reaction solution, 1 mol/L hydrochloric acid was added, and the mixture was extracted with DCM three times. The organic layer was dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain **49** (5.70 g, 23.0 mmol, 98% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.56–3.62 (2H, m), 3.69–3.77 (2H, m), 4.16–4.21 (4H, m), 4.99–5.10 (2H, m), 5.15 (2H, s), 7.29–7.38 (5H, m).

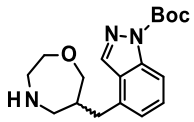
***tert*-Butyl 4-({4-[(benzyloxy)carbonyl]-1,4-oxazepan-6-yl}methyl)-1*H*-indazole-1-carboxylate (52)**



Under a N_2 atmosphere, 9-BBN (0.50 mol/L, THF solution, 46 mL) was added to **49** (5.63 g, 22.8 mmol) and stirred under reflux for 3 h. The reaction solution was allowed to cool to rt and DMF (92 mL), water (9.2 mL), potassium carbonate (3.93 g, 28.5 mmol), *tert*-butyl 4-bromo-1*H*-indazole-1-carboxylate (**50**, 5.64 g, 19.0 mmol) and Pd(dppf)Cl_2 (0.775 g, 0.949 mmol) were added thereto and stirred at 65 °C for 4 h. After the reaction solution was allowed to cool to rt, water was added thereto at 0 °C, the mixture was extracted with ethyl acetate three times, and the organic layer obtained was washed with saturated brine and dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was subjected to silica gel column chromatography (hexane/ethyl acetate) then to amino silica gel column chromatography (hexane/ethyl

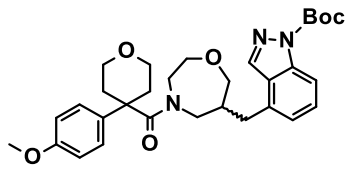
acetate) to obtain **52** (7.53 g, 16.2 mmol, 85% yield) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: 1.66 (9H, s), 2.27–2.33 (1H, m), 2.81–2.93 (2H, m), 3.27 (1H, dd, J = 14.2, 8.2 Hz), 3.44 (2H, dd, J = 12.4, 7.0 Hz), 3.61–3.71 (5H, m), 5.02 (2H, s), 7.14–7.33 (6H, m), 7.45 (1H, t, J = 7.9 Hz), 7.92 (1H, d, J = 8.5 Hz), 8.41 (1H, s).

***tert*-Butyl 4-[(1,4-oxazepan-6-yl)methyl]-1*H*-indazole-1-carboxylate (54)**



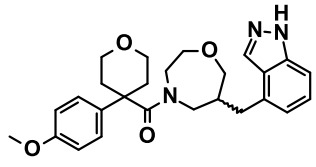
To a solution of **52** (300 mg, 0.644 mmol) in ethanol (10 mL) was added 10%-palladium carbon (100 mg) and stirred at rt for 2 h under a hydrogen atmosphere. After a N_2 purge, the reaction solution was filtered, and the filtrate was concentrated under reduced pressure to obtain the title compound as an oil. The present compound was used for the next reaction without further purification. MS (ESI/APCI) *m/z*: calcd for $C_{18}H_{26}N_3O_3$ $(M + H)^+$ 332.2, Found: 332.2.

***tert*-Butyl 4-({4-[4-(4-methoxyphenyl)oxane-4-carbonyl]-1,4-oxazepan-6-yl}methyl)-1*H*-indazole-1-carboxylate (58)**



To a mixture of **54**, **45** (0.198 g, 0.838 mmol), HATU (0.368 g, 0.967 mmol) in DMF (7 mL) was added DIPEA (0.225 mL, 1.29 mmol) and stirred at rt overnight. Then, aqueous saturated NH_4Cl solution was added, then the mixture was extracted with ethyl acetate. The organic layer obtained was washed with aqueous saturated $NaHCO_3$ and saturated brine and dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was subjected to silica gel column chromatography (hexane/ethyl acetate) then to amino silica gel column chromatography (hexane/ethyl acetate) to obtain **58** (0.224 g, 0.408 mmol, 63% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: 1.67 (9H, s), 1.72–1.81 (2H, m), 1.96–2.05 (2H, m), 2.17 (1H, d, J = 13.9 Hz), 2.59–2.66 (1H, m), 2.70–2.75 (1H, m), 2.85–2.91 (1H, m), 3.11–3.18 (1H, m), 3.28–3.46 (4H, m), 3.55–3.72 (9H, m), 6.82 (2H, d, J = 7.3 Hz), 6.99–7.07 (3H, m), 7.47–7.51 (1H, m), 7.96 (1H, d, J = 8.5 Hz), 8.33 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{31}H_{40}N_3O_6$ $(M + H)^+$ 550.3, Found: 550.3.

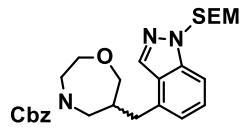
{6-[(1*H*-Indazol-4-yl)methyl]-1,4-oxazepan-4-yl}[4-(4-methoxyphenyl)oxan-4-yl]methanone (33)



To a solution of **58** (217 mg, 0.395 mmol) in DCM (0.5 mL) was added HCl (4 mol/L, 1,4-dioxane solution, 5 mL) and stirred at rt for 4 h. After the reaction solution was concentrated, the residue was basified by adding aqueous saturated $NaHCO_3$ and extracted with ethyl acetate. The organic layer obtained was washed with aqueous saturated $NaHCO_3$ and saturated brine and dried

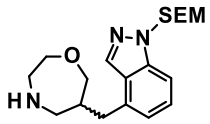
over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was suspended in ethyl acetate, collected by filtration and dried to obtain **33** (141 mg, 0.314 mmol, 79% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: 1.67–1.80 (2H, m), 1.97–2.06 (2H, m), 2.16–2.20 (1H, m), 2.54–2.69 (2H, m), 2.83–2.90 (1H, m), 3.13–3.19 (1H, m), 3.26–3.65 (10H, m), 3.73 (3H, s), 6.77 (1H, d, *J* = 6.7 Hz), 6.84 (2H, d, *J* = 8.5 Hz), 7.00 (2H, d, *J* = 8.5 Hz), 7.24 (1H, t, *J* = 7.9 Hz), 7.38 (1H, d, *J* = 7.9 Hz), 7.98 (1H, s), 12.79 (1H, s). HRMS (ESI) *m/z*: calcd for $C_{26}H_{32}N_3O_4$ $(M + H)^+$ 450.2387, Found: 450.2387.

Benzyl 6-[(1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-indazol-4-yl)methyl]-1,4-oxazepane-4-carboxylate (53)



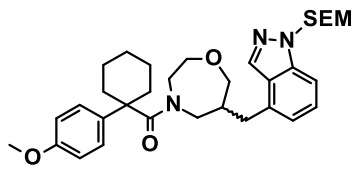
In a manner similar to that employed for the synthesis of **52**, **53** (2.2 g, 4.4 mmol, 73% yield) was obtained as a pale-yellow oil from **49** (1.66 g, 6.72 mmol) and 4-bromo-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-indazole[48] (**51**, 2 g, 6.11 mmol). ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: -0.11 (9H, s), 0.79 (2H, t, *J* = 7.9 Hz), 2.29–2.40 (1H, m), 2.79–2.87 (2H, m), 3.22–3.32 (1H, m), 3.36–3.49 (2H, m), 3.55 (2H, t, *J*= 7.9 Hz), 3.70-3.85 (5H, m), 5.14 (2H, s), 5.80 (2H, s), 7.04–7.09 (1H, m), 7.33–7.48 (6H, m), 7.58–7.63 (1H, m), 8.23 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{27}H_{38}N_3O_4Si$ $(M + H)^+$ 496.3, Found: 496.3.

4-[(1,4-Oxazepan-6-yl)methyl]-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-indazole (55)



To a solution of **53** (2.2 g, 4.4 mmol) in ethanol (50 mL) was added 10%-palladium carbon (1 g) and stirred at rt for 2 h under a hydrogen atmosphere. After a N_2 purge, the reaction solution was filtered and the filtrate was concentrated under reduced pressure to obtain **55** (1.62 g, 4.48 mmol) as a grey oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: -0.07 (9H, s), 0.86–0.91 (2H, m), 2.32–2.43 (1H, m), 2.73 (1H, dd, *J* = 13.3, 7.3 Hz), 2.89 (2H, d, *J* = 7.9 Hz), 2.93–2.99 (2H, m), 3.04 (1H, dd, *J* = 13.6, 5.1 Hz), 3.48 (1H, s), 3.52–3.63 (3H, m), 3.71–3.77 (2H, m), 3.88 (1H, dd, *J* = 12.4, 5.1 Hz), 5.80 (2H, s), 7.05 (1H, d, *J* = 7.3 Hz), 7.40 (1H, dd, *J* = 7.3, 8.5 Hz), 7.50 (1H, d, *J* = 8.5 Hz), 8.11–8.12 (1H, m). MS (ESI/APCI) *m/z*: calcd for $C_{19}H_{32}N_3O_2Si$ $(M + H)^+$ 362.2, Found: 362.2.

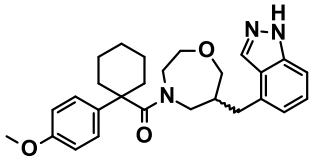
[1-(4-Methoxyphenyl)cyclohexyl]{6-[(1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-indazol-4-yl)methyl]-1,4-oxazepan-4-yl}methanone (59)



In a manner similar to that employed for the synthesis of **58**, **59** (0.144 g, 0.249 mmol, 36% yield) as a colorless oil from **55** (250 mg, 0.691 mmol) and 1-(4-methoxyphenyl)cyclohexane-1-carboxylic acid (**56**, 0.178 g, 0.761 mmol). ^{1}H NMR (400 MHz,

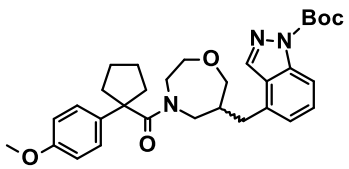
DMSO-d_6) δ: -0.10 (9H, s), 0.82 (2H, t, J = 7.9 Hz), 1.18–1.25 (1H, m), 1.42–1.54 (6H, m), 1.59–1.68 (1H, m), 2.01–2.08 (2H, m), 2.16–2.21 (1H, m), 2.53–2.60 (1H, m), 2.65–2.72 (1H, m), 2.81–2.88 (1H, m), 3.10–3.16 (1H, m), 3.28–3.37 (2H, m), 3.40–3.48 (2H, m), 3.54–3.60 (3H, m), 3.62–3.68 (1H, m), 3.72 (3H, s), 5.72 (2H, s), 6.80–6.85 (3H, m), 6.98 (2H, d, J = 8.5 Hz), 7.32 (1H, t, J = 7.9 Hz), 7.53 (1H, d, J = 8.5 Hz), 8.05 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{33}H_{48}N_3O_4Si$ $(M + H)^+$ 578.3, Found: 578.3.

{6-[(1*H*-Indazol-4-yl)methyl]-1,4-oxazepan-4-yl}[1-(4-methoxyphenyl)cyclohexyl]methanone (34)



To a solution of **59** (141 mg, 0.244 mmol) in DCM (5.0 mL), TFA (2.0 mL) was added at rt and stirred at the same temperature for 4 h. After the reaction solution was concentrated, the residue was azeotropically concentrated with DCM. MeOH (5.0 mL) and ammonia water (28%, 2.0 mL) were added thereto and stirred at rt for 1.5 h. Water was added, and then the mixture was extracted with DCM. The organic layer obtained was washed with saturated brine and dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was subjected to silica gel column chromatography (hexane/ethyl acetate) then to amino silica gel column chromatography (ethyl acetate /methanol) to obtain **34** (29.2 mg, 0.0652 mmol, 28% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: 1.13–1.31 (1H, m), 1.37–1.58 (6H, m), 1.59–1.73 (1H,m), 2.00–2.12 (2H, m), 2.15–2.24 (1H, m), 2.51–2.72 (2H, m), 2.77–2.90 (1H,m), 3.07–3.19 (1H,m), 3.26–3.35 (2H, m), 3.38–3.48 (2H, m), 3.53–3.69 (2H, m), 3.72 (3H, s), 6.73–6.84 (3H, m), 6.93–7.01 (2H, m), 7.20–7.26 (1H,m), 7.35–7.40 (1H, m), 7.98 (1H, s), 12.78 (1H, brs). ^{13}C NMR (101 MHz, DMSO-d_6, 100 °C) δ: 22.5, 22.6, 24.9, 33.7, 34.6, 37.3, 40.5, 49.5, 49.7, 51.3, 54.6, 68.5, 72.0, 107.6, 113.7, 119.4, 122.6, 125.2, 125.8, 131.4, 131.9, 137.0, 139.8, 157.3, 173.2. HRMS (ESI) *m/z*: calcd for $C_{27}H_{34}N_3O_3$ $(M + H)^+$ 448.2595, Found: 448.2602.

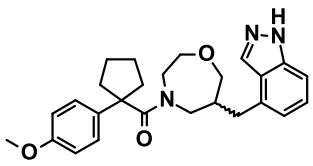
***tert*-Butyl 4-({4-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-1,4-oxazepan-6-yl}methyl)-1*H*-indazole-1-carboxylate (60)**



In a manner similar to that employed for the synthesis of **58**, **60** (2.65 g, 4.97 mmol, 82% yield) was obtained as a white solid from 1-(4-methoxyphenyl)cyclopentanecarboxylic acid (**57**, 1.73 g, 7.85 mmol) and **54** (2.00 g, 6.03 mmol). ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: 1.55–1.57 (4H, m), 1.67–1.71 (10H, m), 1.82–1.85 (1H, m), 1.97 (1H, brs), 2.18–2.29 (2H, m), 2.61–2.77 (2H, m), 2.91 (1H, brs), 3.13–3.19 (1H, m), 3.28–3.71 (9H, m), 6.78 (2H, d, J = 8.5 Hz), 6.95 (2H, d, J = 7.9 Hz), 7.06 (1H, d, J = 5.4 Hz), 7.48 (1H, td, J = 7.9, 2.6 Hz), 7.95 (1H, d, J = 8.5 Hz), 8.32 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{31}H_{40}N_3O_5$ $(M + H)^+$ 534.3,

Found: 534.3.

{6-[(1*H*-Indazol-4-yl)methyl]-1,4-oxazepan-4-yl}[1-(4-methoxyphenyl)cyclopentyl]methanone (35)



In a manner similar to that employed for the synthesis of **33**, **35** (2.11 g, 4.87 mmol, 98% yield) was obtained as a white solid from **60** (2.65 g, 4.97 mmol). ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ: 1.50–1.60 (4H, m), 1.72–1.86 (2H, m), 2.04–2.20 (2H, m), 2.25–2.33 (1H, m), 2.60–2.70 (2H, m), 2.93–2.87 (1H, m), 3.14–3.20 (1H, m), 3.27–3.45 (4H, m), 3.54–3.68 (2H, m), 3.72 (3H, s), 6,76–6.82 (3H, m), 6.98 (2H, d, J = 7.9 Hz), 7.22 (1H, t, J = 7.6 Hz), 7.37 (1H, d, J = 8.5 Hz), 7.97 (1H, s), 12.77 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{26}H_{32}N_3O_3$ $(M + H)^+$ 434.2, Found: 434.3.

{6-[(1*H*-Indazol-4-yl)methyl]-1,4-oxazepan-4-yl}[1-(4-methoxyphenyl)cyclopentyl]methanone ((-)-35) and {6-[(1*H*-indazol-4-yl)methyl]-1,4-oxazepan-4-yl}[1-(4-methoxyphenyl)cyclopentyl]methanone ((+)-35)

Chiral HPLC enantioseparation of **(±)-35** (600 mg) were performed on a shimadzu LC-20AD (UV detection at λ = 254 nm), equipped with a CHIRALPAK IA (250 mm × 20 mm) using hexane/ethanol (70/30) as the mobile phase with the flow rate of 20 mL/min. The separated **(-)-35** (281 mg, first peak, $[\alpha]_D^{20}$ -38.9°, c = 1.0, MeOH, ee > 99%) and **(+)-35** (282 mg, second peak, $[\alpha]_D^{20}$ +37.9°, c = 1.0, MeOH, ee > 99%) were collected and concentrated *in vacuo*.

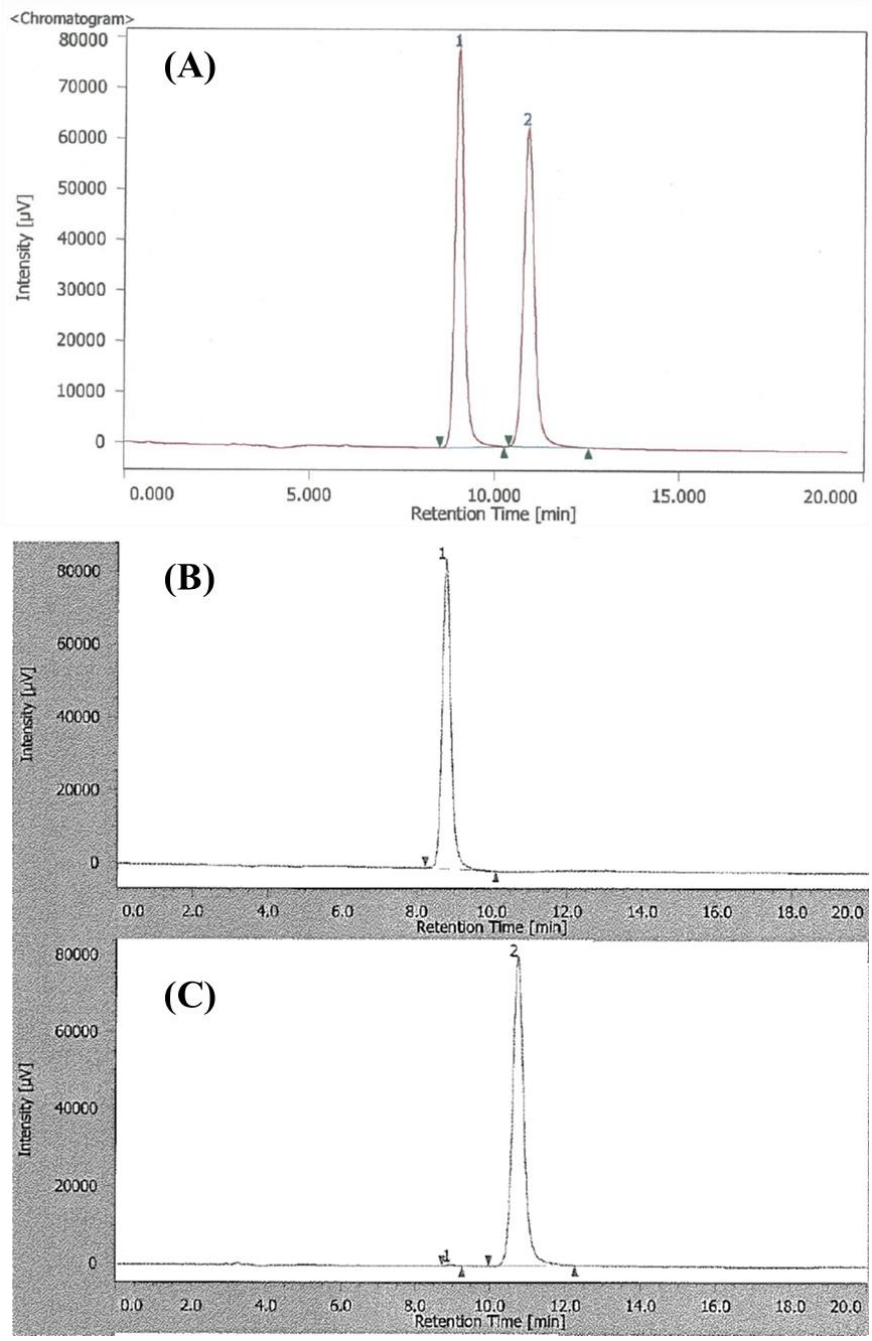
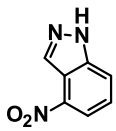


Figure S1. Chiral HPLC chromatograms for **(±)-35**, **(-)-35** and **(+)-35**. **Panel A**: **(±)-35** (purity >99%); **Panel B**: **(-)-35** (ee > 99%); **Panel C**: **(+)-35** (ee >99%).

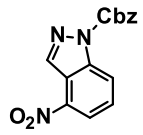
4-Nitro-1*H*-indazole (62)



To a solution of 2-methyl-3-nitroaniline (**61**, 2.27 g, 14.9 mmol) in AcOH (60.0 mL), a solution of sodium nitrite (1.13 g, 1.13 mmol) in water (5.00 mL) was added, and the mixture was stirred at rt for 2 h. Ice water was added to the reaction solution, the solid thus precipitated was filtered off and dried to obtain **62** (1.91 g, 11.7 mmol, 79% yield)

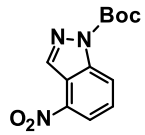
as an orange solid. MS (ESI/APCI) *m/z*: calcd for $C_7H_6N_3O_2$ $(M + H)^+$ 164.0, Found: 164.2.

Benzyl 4-nitro-1*H*-indazole-1-carboxylate (63a)



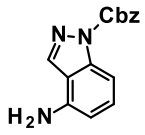
To a solution of **62** (1.91 g, 11.7 mmol) in DMF (60.0 mL), DBU (1.92 mL, 12.9 mmol) was added, the mixture was stirred at rt for 15 min, benzyl chloroformate (3.34 mL, 23.4 mmol) was added thereto, and the mixture was stirred at rt for 4 h. To the reaction solution, an aqueous NH_4Cl solution was added, and the mixture was extracted with ethyl acetate three times. The organic layer was washed with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was purified by silica gel column chromatography (hexane/ethyl acetate). After concentration under reduced pressure, the solid obtained was suspended in hexane, filtered off and dried to obtain **63a** (2.68 g, 9.02 mmol, 77% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.58 (2H, s), 7.39–7.45 (3H, m), 7.54–7.57 (2H, m), 7.70 (1H, t, *J* = 8.2 Hz), 8.28 (1H, d, *J* = 7.9 Hz), 8.66 (1H, d, *J* = 8.5 Hz), 8.85 (1H, s).

***tert*-Butyl 4-nitro-1*H*-indazole-1-carboxylate (63b)**



To a solution of **62** (10.0 g, 57.4 mmol) in DCM (200 mL), triethylamine (10.2 mL, 73.6 mmol) and Boc_2O (14.7 g, 67.4 mmol) were added, and the mixture was stirred at rt for 6 h. The mixture was separated into water and DCM, and the organic layer obtained was dried over anhydrous sodium sulfate. A residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) and then recrystallized (hexane/Et_2O) to obtain the **63b** (15.1 g, 57.4 mmol) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.75 (9H, s), 7.66–7.71 (1H, m), 8.27 (1H, d, *J* = 7.9 Hz), 8.64 (1H, d, *J* = 8.5 Hz), 8.83 (1H, s).

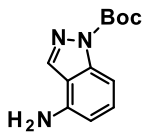
Benzyl 4-amino-1*H*-indazole-1-carboxylate (64a)



To a suspension of **63a** (1.00 g, 3.36 mmol) in MeOH (15.0 mL), zinc powder (1.54 g, 23.5 mmol) was added under ice cooling, the mixture was stirred at the same temperature for 10 min, a saturated aqueous NH_4Cl solution (15.0 mL) was then added, and the mixture was stirred at rt for 5.5 h. After the reaction solution was filtered through celite, the filtrate obtained was extracted with ethyl acetate three times. The organic layer was washed with saturated brine, and dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was purified by silica gel column chromatography (chloroform/ethyl acetate) to obtain **64a** (0.841 g, 3.15 mmol, 94% yield) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.47 (2H, s), 6.14 (2H, s), 6.42 (1H, dd, *J* = 6.7, 1.8 Hz), 7.19–7.25 (2H, m), 7.37–7.46 (3H, m), 7.51–7.54 (2H, m), 8.47 (1H, s). MS (ESI/APCI) *m/z*:

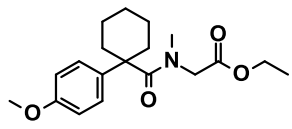
calcd for $C_{15}H_{14}N_3O_2$ $(M + H)^+$ 268.1, Found: 268.1.

***tert*-Butyl 4-amino-1*H*-indazole-1-carboxylate (64b)**



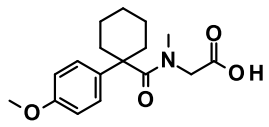
In a manner similar to that employed for the synthesis of **64a**, **64b** (12.4 g, 53.1 mmol, 93% yield) was obtained as a white solid from **63b** (15.0 g, 57.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72 (9H, s), 4.18 (2H, brs), 6.52 (1H, d, *J* = 7.3 Hz), 7.27–7.33 (1H, m), 7.54 (1H, d, *J* = 8.5 Hz), 8.12 (1H, s).

Ethyl *N*-[1-(4-methoxyphenyl)cyclohexane-1-carbonyl]-*N*-methylglycinate (66)



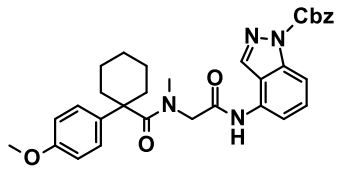
In a manner similar to that employed for the synthesis of **58**, **66** (0.272 g, 0.815 mmol, 54% yield) was obtained as a colorless oil from **56** (0.370 g, 1.50 mmol) and sarcosine ethyl ester hydrochloride (**65**, 0.261 g, 1.65 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.25–1.27 (4H, m), 1.65–1.74 (7H, m), 2.30–2.32 (2H, m), 2.63 (3H, s), 3.80 (3H, s), 3.98–4.00 (2H, m), 4.17–4.20 (2H, m), 6.88 (2H, d, *J* = 8.5 Hz), 7.23 (2H, d, *J* = 8.5 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 14.2, 23.5, 26.0, 34.6, 36.8, 50.6, 51.7, 55.2, 60.8, 114.2, 126.6, 137.9, 158.1, 169.4, 175.7. HRMS (ESI) *m/z*: calcd for $C_{19}H_{28}NO_4$ $(M + H)^+$ 334.2013, Found:334.2024.

***N*-[1-(4-Methoxyphenyl)cyclohexane-1-carbonyl]-*N*-methylglycine (67)**



A mixture of **66** (0.272 g, 0.815 mmol), 4 mol/L aqueous lithium hydroxide solution (3 mL) and MeOH (9 mL) was stirred at rt for 2 h. To the resultant, 1 mol/L hydrochloric acid was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine, and dried over anhydrous magnesium sulfate. Then the solvent was distilled off under reduced pressure, and the residue was dried to obtain **67** (0.231 g, 0.758 mmol, 93% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20–1.34 (1H, m), 1.61–1.73 (7H, m), 2.28–2.35 (2H, m), 2.64–2.72 (3H, m), 3.80 (3H, s), 3.97–4.05 (2H, m), 6.86–6.89 (2H, m), 7.19–7.23 (2H, m). MS (ESI/APCI) *m/z*: calcd for $C_{17}H_{24}NO_4$ $(M + H)^+$ 306.2, Found: 306.2.

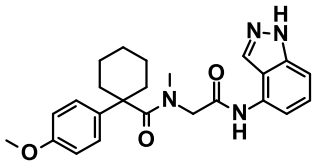
Benzyl 4-({*N*-[1-(4-methoxyphenyl)cyclohexane-1-carbonyl]-*N*-methylglycyl}amino)-1*H*-indazole-1-carboxylate (68)



In a manner similar to that employed for the synthesis of **58**, **68** (0.139 g, 0.250 mmol, 55% yield) was obtained as a pale-yellow oil from **67** (0.139 g, 0.455 mmol) and **64a** (0.152 g, 0.501 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.25–1.34 (1H, m), 1.65–1.75 (7H, m), 2.35–2.41 (2H, m), 2.75 (3H, s), 3.71 (3H, s), 4.09–4.14 (2H,m), 5.56 (2H, s), 6.71–6.75 (2H, m),

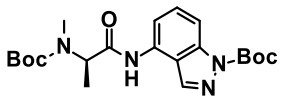
7.08–7.12 (2H, m), 7.36–7.45 (3H, m), 7.49–7.57 (3H, m), 7.88–7.99 (2H, m), 8.41 (1H, s), 9.89 (1H, s).

_N_-{2-[(1_H_-Indazol-4-yl)amino]-2-oxoethyl}-1-(4-methoxyphenyl)-_N_-methylcyclohexane-1-carboxamide (36)



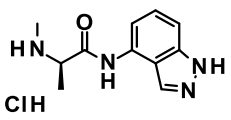
In a manner similar to that employed for the synthesis of **67**, **36** (0.024 g, 0.057 mmol, 23% yield) was obtained as a white solid from **68** (0.139 g, 0.250 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30–1.32 (1H, m), 1.69–1.72 (7H, m), 2.40–2.42 (2H, m), 2.77 (3H, s), 3.71 (3H, s), 4.13 (2H, s), 6.73 (2H, d, J = 8.2 Hz), 7.15 (2H, d, J = 8.2 Hz), 7.26–7.29 (1H, m), 7.37 (1H, t, J = 7.9 Hz), 7.81 (1H, d, J = 7.3 Hz), 8.24 (1H, s), 9.64 (1H, s), 10.20 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{24}H_{29}N_4O_3$ $(M + H)^+$ 421.2, Found: 421.2.

***tert*-Butyl 4-{[_N_-(*tert*-butoxycarbonyl)-_N_-methyl-D-alanyl]amino}-1_H_-indazole-1-carboxylate (70)**



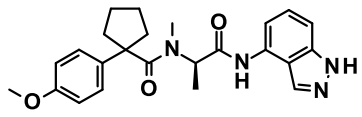
To a solution of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-alanine (**69**, 0.500 g, 2.46 mmol) in DMF (7 mL) were added COMU (1.16 g, 2.71 mmol) and DIPEA (0.558 mL, 3.20 mmol) at 0 °C, and the mixture was stirred at rt for 5 min. **64b** (0.632 g, 2.71 mmol) was then added, and the mixture was stirred at rt overnight. A saturated aqueous NH_4Cl solution was added to the reaction solution, and the mixture was extracted with ethyl acetate three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution, and with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was subjected to amino silica gel column chromatography (hexane/ethyl acetate) then to silica gel column chromatography (hexane/ethyl acetate) to obtain **70** (0.517 g, 1.23 mmol, 50% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47 (3H, d, J = 6.7 Hz), 1.51 (9H, s), 1.73 (9H, s), 2.86 (3H, s), 4.85–4.88 (1H, m), 7.47–7.50 (1H, m), 7.89 (1H, d, J = 7.9 Hz), 8.01 (1H, s), 8.27 (1H, s), 9.47 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 13.0, 28.1, 28.4, 30.0, 54.3, 81.5, 85.1, 110.0, 113.2, 117.6, 130.0, 131.2, 136.2, 140.4, 149.1, 157.9, 170.0. HRMS (ESI) *m/z*: calcd for $C_{21}H_{31}N_4O_5$ $(M + H)^+$ 419.2289, Found: 419.2292.

***tert*-Butyl 4-[(_N_-methyl-D-alanyl)amino]-1_H_-indazole-1-carboxylate—hydrogen chloride (71)**



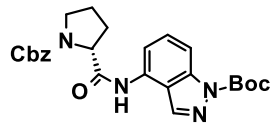
Hydrogen chloride (4 mol/L, 1,4-dioxane solution, 8 mL) was added to **70** (0.517 g, 1.23 mmol) in DCM (4 mL) at 0 °C, and the mixture was stirred at rt for 4 h, and then concentrated under reduced pressure to obtain **71** (0.330 g, 1.30 mmol) as a white solid. The present compound was used for the next reaction without further purification.

***N*-{(2*R*)-1-[(1*H*-Indazol-4-yl)amino]-1-oxopropan-2-yl}-1-(4-methoxyphenyl)-*N*-methylcyclopentane-1-carboxamide(37)**



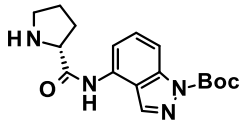
In a manner similar to that employed for the synthesis of **70**, **37** (0.0629 g, 0.150 mmol, 38% yield) was obtained as a white solid from **57** (0.0872 g, 0.393 mmol) and **71** (0.282 g, 1.05 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (3H, d, *J* = 6.7 Hz), 1.71–1.82 (4H, m), 2.00–2.02 (1H, m), 2.09–2.11 (1H, m), 2.39–2.51 (5H, m), 3.71 (3H, s), 5.38–5.40 (1H, m), 6.68 (2H, d, *J* = 7.9 Hz), 7.08 (2H, d, *J* = 7.9 Hz), 7.24–7.26 (1H, m), 7.35–7.37 (1H, m), 7.81–7.82 (1H, m), 8.20 (1H, s), 9.45 (1H, s), 10.15 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 12.7, 24.7, 25.1, 31.4, 37.7, 38.9, 54.0, 55.1, 58.2, 105.4, 110.6, 114.1, 115.8, 126.0, 127.9, 130.8, 132.0, 136.4, 140.9, 157.9, 169.5, 179.0. HRMS (ESI) *m/z*: calcd for $C_{24}H_{29}N_4O_3$ $(M + H)^+$ 421.2234, Found: 421.2238.

***tert*-Butyl 4-({1-[(benzyloxy)carbonyl]-D-prolyl}amino)-1*H*-indazole-1-carboxylate (73)**



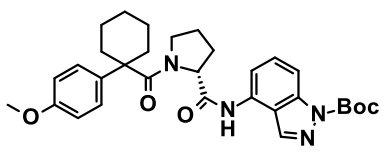
In a manner similar to that employed for the synthesis of **70**, **73** (4.06 g, 8.74 mmol, 73% yield) was obtained as a pale-yellow solid from **64b** (2.81 g, 12.0 mmol) and 1-[(benzyloxy)carbonyl]-D-proline (**72**, 3.00 g, 12.0 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.65 (9H, s), 1.85–2.08 (3H, m), 2.23–2.39 (1H, m), 3.44–3.59 (2H, m), 4.52–4.61 (1H, m), 4.97–5.14 (2H, m), 7.06–7.39 (5H, m), 7.55 (1H, td, *J* = 8.2, 2.8 Hz), 7.81 (2H, dd, *J* = 14.8, 8.2 Hz), 8.52 (1H, d, *J* = 32.0 Hz), 10.36 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{25}H_{29}N_4O_5$ $(M + H)^+$ 465.2, Found: 465.3.

***tert*-Butyl 4-(D-prolylamino)-1*H*-indazole-1-carboxylate (74)**



In a manner similar to that employed for the synthesis of **54**, **74** (2.05 g, 6.20 mmol, 80% yield) was obtained as a light brown solid from **73** (3.60 g, 7.75 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.73 (9H, s), 1.77–1.89 (2H, m), 2.07–2.15 (1H, m), 2.23–2.36 (2H, m), 3.03–3.09 (1H, m), 3.13–3.19 (1H, m), 3.97 (1H, s), 7.50 (1H, t, *J* = 8.2 Hz), 7.90 (1H, d, *J* = 8.5 Hz), 7.96 (1H, d, *J* = 7.9 Hz), 8.21 (1H, s), 10.35 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{17}H_{23}N_4O_3$ $(M + H)^+$ 331.2, Found: 331.2.

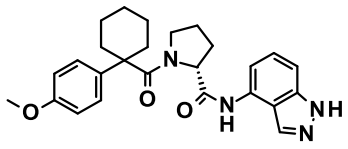
***tert*-Butyl 4-({1-[1-(4-methoxyphenyl)cyclohexane-1-carbonyl]-D-prolyl}amino)-1*H*-indazole-1-carboxylate (75)**



In a manner similar to that employed for the synthesis of **70**, **75** (0.913 g, 1.67 mmol, 98% yield) was obtained as a pale-yellow solid from **74** (0.677 g, 2.05 mmol) and **56** (0.400 g, 1.71 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24–1.89 (20H, m), 2.33–2.41

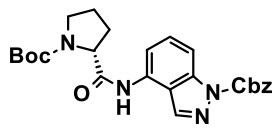
(3H, m), 2.99–3.02 (2H, m), 3.79 (3H, s), 4.98–5.01 (1H, m), 6.83 (2H, d, J = 9.2 Hz), 7.16 (2H, d, J = 9.2 Hz), 7.46 (1H, dd, J = 8.2, 8.2 Hz), 7.87 (1H, d, J = 7.9 Hz), 8.02 (1H, d, J = 7.9 Hz), 8.50 (1H, s), 10.53 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 23.3, 23.6, 25.4, 25.6, 28.2, 37.7, 48.0, 50.9, 55.2, 61.8, 85.1, 109.9, 113.3, 114.1, 117.7, 126.7, 130.0, 131.5, 136.3, 136.7, 140.3, 149.2, 158.1, 169.7, 177.6. HRMS (ESI) *m/z*: calcd for $C_{31}H_{39}N_4O_5$ $(M + H)^+$ 547.2915, Found: 547.2935.

_N_-1_H_-Indazol-4-yl-1-[1-(4-methoxyphenyl)cyclohexane-1-carbonyl]-D-prolinamide (38)



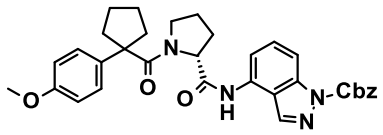
In a manner similar to that employed for the synthesis of **33**, **38** (0.520 g, 1.16 mmol, 70% yield) was obtained as a white solid from **75** (0.913 g, 1.67 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.25–1.77 (11H, m), 2.05–2.33 (3H, m), 3.01–3.06 (2H, m), 3.75 (3H, s), 4.67 (1H, t, J = 7.0 Hz), 6.90–6.93 (2H, m), 7.23–7.28 (4H, m), 7.63 (1H, d, J = 6.7 Hz), 8.30 (1H, s), 10.06 (1H, s), 13.06 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 22.7, 23.2, 25.4, 28.4, 37.2, 47.8, 50.0, 55.0, 61.5, 105.4, 110.1, 113.9, 115.8, 126.58, 126.64, 131.4, 132.2, 137.0, 140.9, 157.6, 171.4, 172.9. HRMS (ESI) *m/z*: calcd for $C_{26}H_{31}N_4O_3$ $(M + H)^+$ 447.2391, Found: 447.2397.

Benzyl 4-{[1-(*tert*-butoxycarbonyl)-D-prolyl]amino}-1_H_-indazole-1-carboxylate (77)



In a manner similar to that employed for the synthesis of **58**, **77** (0.302 g, 0.650 mmol, 65% yield) was obtained as a white solid from 1-(*tert*-butyoxycarbonyl)-D-proline (**76**, 0.216 g, 1.00 mmol) and **64a** (0.282 g, 1.05 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.35 (9H, s), 1.83–2.03 (3H, m), 2.25 (1H, s), 3.36–3.49 (2H, m), 4.41–4.44 (1H, m), 5.52 (2H, s), 7.36–7.45 (3H, m), 7.51–7.56 (3H, m), 7.71 (1H, d, J = 7.3 Hz), 7.86 (1H, d, J = 8.5 Hz), 8.51 (1H, s), 10.09 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{25}H_{29}N_4O_5$ $(M + H)^+$ 465.2, Found: 465.2.

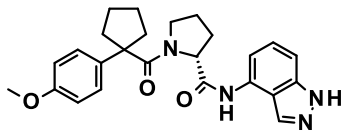
Benzyl 4-({1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolyl}amino)-1_H_-indazole-1-carboxylate (78)



To a suspension of **57** (0.168 g, 0.763 mmol) in DCM (5 mL), thionyl chloride (0.111 mL, 1.53 mmol) and DMF (0.01 mL) were added, and the mixture was stirred at 40 °C for 4 h. After allowing to cool to rt, the reaction solution was concentrated under reduced pressure to obtain the crude acid chloride as a light brown oil. Hydrogen chloride (4 mol/L, 1,4-dioxane solution, 5 mL) was added to **77** (0.295 g, 0.635 mmol), and the mixture was stirred at rt for 3 h, and then concentrated under reduced pressure to obtain the crude amine intermediate. The intermediate was dissolved in DCM (5 mL), and a solution of the acid chloride prepared previously in DCM (5 mL) was added. After ice-cooling, DIPEA (0.332 mL, 1.91 mmol)

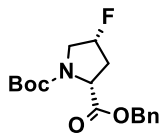
was added, and the mixture was stirred at rt overnight. To the reaction solution, 1 mol/L hydrochloric acid was added, and the mixture was extracted with DCM. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was purified by silica gel column chromatography (chloroform/methanol) to obtain **78** (0.353 g, 0.623 mmol) as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.55–1.66 (5H, m), 1.72–1.94 (4H, m), 2.07–2.13 (1H, m), 2.27–2.36 (2H, m), 2.99 (2H, t, J = 5.4 Hz), 3.74 (3H, s), 4.60 (1H, dd, J = 8.5, 5.4 Hz), 5.52 (2H, s), 6.90 (2H, d, J = 9.1 Hz), 7.19 (2H, d, J = 9.1 Hz), 7.38–7.48 (3H, m), 7.55–7.59 (3H, m), 7.82–7.88 (2H, m), 8.62 (1H, s), 10.36 (1H, s). MS (ESI/APCI) *m/z*: calcd for $C_{33}H_{35}N_4O_5$ $(M + H)^+$ 567.3, Found: 567.3.

_N_-1_H_-Indazol-4-yl-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolinamide (39)



To a solution of **79** (0.345 g, 0.609 mmol) in THF (1 mL), MeOH (5 mL) and potassium carbonate (0.252 g, 1.83 mmol) were added, and the mixture was stirred at rt for 2 h. To the reaction solution, 1 mol/L hydrochloric acid was added, and the mixture was extracted with ethyl acetate three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the residue obtained was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain **39** (0.197 g, 0.455 mmol, 75% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.68 (5H, m), 1.73–1.95 (4H, m), 2.05–2.11 (1H, m), 2.28–2.36 (2H, m), 2.97–3.00 (2H, m), 3.75 (3H, s), 4.64–4.68 (1H, m), 6.90 (2H, d, J = 8.5 Hz), 7.19–7.30 (4H, m), 7.66 (1H, d, J = 7.9 Hz), 8.29 (1H, s), 10.08 (1H, s), 13.07 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 24.65, 24.74, 25.0, 28.7, 36.9, 37.9, 47.5, 55.0, 57.6, 61.0, 105.4, 110.1, 113.9, 115.8, 126.6, 126.7, 131.3, 132.1, 136.5, 140.9, 157.4, 171.4, 173.7. HRMS (ESI) *m/z*: calcd for $C_{25}H_{29}N_4O_3$ $(M + H)^+$ 433.2234, Found: 433.2241.

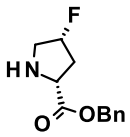
2-Benzyl 1-*tert*-butyl (2*R*,4*R*)-4-fluoropyrrolidine-1,2-dicarboxylate (81)



To a solution of (4*R*)-1-(*tert*-butoxycarbonyl)-4-fluoro-D-proline (**80**, 0.995 g, 4.23 mmol) in MeOH (18 mL), an aqueous solution (2 mL) of cesium carbonate (0.695 g, 2.13 mmol) cooled to 0 °C in advance was added at 0 °C and stirred at 0 °C for 5 min. The solvent was distilled off under reduced pressure and the residue was dried at 30 °C. To a solution of the solid obtained in DMF (44 mL), benzyl bromide (0.512 mL, 4.31 mmol) was added at 0 °C and stirred overnight. Ice was added to the reaction solution, and the mixture was extracted with ethyl acetate, and the organic layer was washed with water three times and with saturated brine, and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, and the residue obtained was purified by silica gel column chromatography (hexane/ethyl

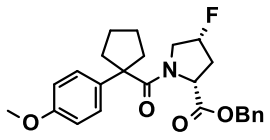
acetate) to obtain **81** (1.26 g, 3.90 mmol, 91% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 and 1.45 (9H, s), 2.24–2.55 (2H, m), 3.57–3.90 (2H, m), 4.46–4.61 (1H, m), 5.08–5.28 (3H, m), 7.34–7.38 (5H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 28.2, 28.3, 36.5, 36.8, 37.4, 37.6, 52.8, 53.0, 53.1, 53.3, 57.5, 57.8, 66.9, 80.4, 90.0, 90.2, 92.0, 93.0, 128.0, 128.1, 128.2, 128.3, 128.5, 135.7, 153.6, 153.9, 171.2, 171.5. HRMS (ESI) *m/z*: calcd for $C_{17}H_{23}FNO_4$ $(M + H)^+$ 324.1606, Found: 324.1619.

Benzyl (4*R*)-4-fluoro-D-prolinate (82)



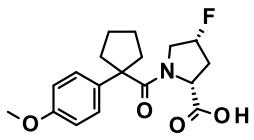
A mixture of **81** (1.26 g, 3.90 mmol) and HCl (4 mol/L, 1,4-dioxane solution, 20 mL) was stirred at rt for 4 h. After the solvent was distilled off under reduced pressure, a saturated aqueous $NaHCO_3$ solution was added. The mixture was extracted with a mixed solvent of $CHCl_3$/IPA (3/1), and the organic layer was dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure to obtain **82** (0.850 g, 3.81 mmol, 98% yield) as a beige oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17–2.40 (2H, m), 2.65 (1H, brs), 2.88–2.98 (1H, m), 3.37–3.46 (1H, m), 3.85–3.87 (1H, m), 5.12–5.19 (3H, m), 7.30–7.40 (5H, m).

Benzyl (4*R*)-4-fluoro-1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-prolinate (83)



In a manner similar to that employed for the synthesis of **70**, **83** (0.920 g, 2.16 mmol, 97% yield) was obtained as a colorless oil from **57** (0.498 g, 2.26 mmol) and **82** (0.500 g, 2.24 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.65–1.70 (4H, m), 1.83–1.86 (1H, m), 2.06–2.47 (5H, m), 2.97–3.05 (1H, m), 3.31–3.37 (1H, m), 3.77 (3H, s), 4.83–5.04 (2H, m), 5.14 (1H, d, J = 12.4 Hz), 5.22 (1H, d, J = 12.4 Hz), 6.77 (2H, d, J = 9.1 Hz), 7.14 (2H, d, J = 9.1 Hz), 7.31–7.39 (5H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 24.9, 25.0, 34.9 (J = 21.1 Hz), 36.2, 38.4, 53.3 (J = 25.9 Hz), 55.2, 58.7, 63.1, 67.1, 92.0 (J = 179.2 Hz), 114.2, 127.0, 128.2, 128.3, 128.5, 135.8, 136.0, 158.2, 171.0, 175.4. HRMS (ESI) *m/z*: calcd for $C_{25}H_{29}FNO_4$ $(M + H)^+$ 426.2075, Found: 426.2092.

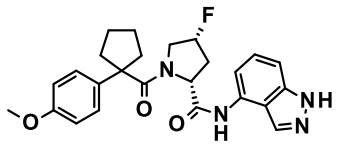
(4*R*)-4-Fluoro-1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-proline (84)



In a manner similar to that employed for the synthesis of **54**, **84** (0.660 g, 1.97 mmol, 91% yield) was obtained as a white solid from **83** (0.920 g, 2.16 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.67–1.79 (4H, m), 1.87–1.89 (1H, m), 2.03–2.18 (2H, m), 2.32–2.35 (1H, m), 2.46–2.49 (1H, m), 2.67–2.71 (1H, m), 2.96–3.05 (1H, m), 3.34–3.40 (1H, m), 3.80 (3H, s), 4.85–5.01 (2H, m), 6.86 (2H, d, J = 9.1 Hz), 7.15 (2H, d, J = 9.1 Hz). MS (ESI/APCI) *m/z*: calcd for $C_{18}H_{23}FNO_4$ $(M + H)^+$ 336.2, Found: 336.3.

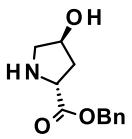
(4*R*)-4-Fluoro-*N*-1*H*-indazol-4-yl-1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-

prolinamide (40)



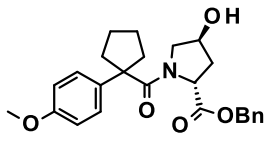
To a solution of **84** (0.657 g, 1.96 mmol) in DMF (10 mL), COMU (0.923 g, 2.15 mmol) and DIPEA (0.444 mL 2.55 mmol) were added at 0 °C, and the mixture was stirred at rt for 5 min. **64b** (0.594 g, 2.55 mmol) was then added at 0 °C, and the mixture was stirred at rt overnight. Ice was added to the reaction solution, and the mixture was extracted with ethyl acetate. The organic layer was washed with water three times, and with saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, and the residue obtained was purified by amino silica gel column chromatography (hexane/ethyl acetate) to obtain an intermediate. To a solution of the intermediate obtained in DCM (7 mL), HCl (4 mol/L, 1,4-dioxane solution, 10 mL) was added, and the mixture was stirred at 50 °C for 2 h. Ice was added at rt, and then the mixture was extracted with ethyl acetate. The organic layer was washed with water, and with saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, and the residue obtained was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain **40** (0.460 g, 1.02 mmol, 88% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72–1.80 (4H, m), 1.99–2.02 (1H, m), 2.06–2.18 (2H, m), 2.31–2.38 (1H, m), 2.49–2.56 (1H, m), 2.92–2.96 (1H, m), 3.19–3.28 (1H, m), 3.40–3.45 (1H, m), 3.77 (3H, s), 5.01–5.09 (2H, m), 6.82 (2H, d, *J* = 8.5 Hz), 7.14 (2H, d, *J* = 8.5 Hz), 7.24 (1H, d, *J* = 8.5 Hz), 7.36–7.38 (1H, m), 7.87–7.89 (1H, m), 8.17 (1H, s), 9.76 (1H, s), 10.19 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 24.5, 24.7, 32.3 (d, *J* = 23.1 Hz), 36.5, 37.9, 53.9 (d, *J* = 24.1 Hz), 55.2, 58.6, 61.4, 92.1 (d, *J* = 174.4 Hz), 105.4, 111.0, 114.4, 116.0, 126.5, 127.9, 130.8, 132.1, 134.8, 140.9, 158.3, 168.8, 177.9. HRMS (ESI) *m/z*: calcd for $C_{25}H_{28}FN_4O_3$ $(M + H)^+$ 451.2140, Found: 451.2163.

Benzyl (4*S*)-4-hydroxy-D-prolinate (86)



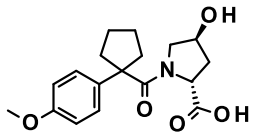
A mixture of (4*S*)-4-hydroxy-D-proline (**85**, 1.00 g, 7.63 mmol), benzyl alcohol (6 mL, 57.7 mmol), *p*-toluenesulfonic acid hydrate (1.48 g, 7.78 mmol) and toluene (6 mL) was stirred at 120 °C for 17 h. The solvent was distilled off under reduced pressure, and then a saturated aqueous $NaHCO_3$ solution was added to the residue obtained. The mixture was extracted with a mixed solvent of $CHCl_3$/IPA (3/1), and the organic layer was dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, and the residue obtained was purified by amino silica gel column chromatography (hexanes/ethyl acetate and ethyl acetate /methanol) to afford **86** (1.03 g, 4.66 mmol, 61% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.98–2.09 (1H, m), 2.13–2.22 (1H, m), 2.15–2.45 (2H, m), 2.89–2.98 (1H, m), 3.10–3.17 (1H, m), 3.98–4.08 (1H, m), 4.36–4.45 (1H, m), 5.16 (2H, s), 7.29–7.40 (5H, m).

Benzyl (4*S*)-4-hydroxy-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolinate (87)



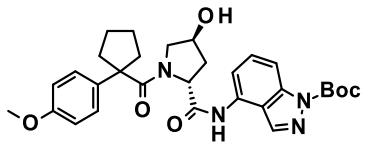
In a manner similar to that employed for the synthesis of **70**, **87** (1.18 g, 2.79 mmol, 80% yield) was obtained as a white solid from **57** (0.765 g, 3.47 mmol) and **86** (0.807 g, 3.65 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.23–1.29 (1H, m), 1.63–1.79 (4H, m), 1.81–1.94 (2H, m), 1.94–2.03 (1H, m), 2.11–2.21 (1H, m), 2.33–2.46 (2H, m), 3.00–3.06 (1H, m), 3.07–3.13 (1H, m), 3.77 (3H, s), 4.21–4.29 (1H, m), 4.66–4.73 (1H, m), 5.13 (1H, d, *J* = 12.1 Hz), 5.27 (1H, d, *J* = 12.1 Hz), 6.75–6.81 (2H, m), 7.11–7.18 (2H, m), 7.30–7.40 (5H, m).

(4*S*)-4-Hydroxy-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-proline (88)



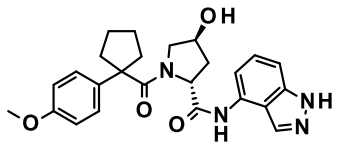
In a manner similar to that employed for the synthesis of **54**, **88** was obtained quantitatively as a white solid from **87** (0.400 g, 0.945 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.63–1.80 (4H, m), 1.91–2.06 (2H, m), 2.08–2.20 (1H, m), 2.20–2.31 (1H, m), 2.31–2.44 (2H, m), 2.95–3.03 (1H, m), 3.17–3.24 (1H, m), 3.75–3.81 (1H, m), 3.78 (3H, s), 4.22–4.30 (1H, m), 4.69–4.79 (1H, m), 6.85 (2H, d, *J* = 9.1 Hz), 7.16 (2H, d, *J* = 9.1 Hz).

***tert*-Butyl 4-({(4*S*)-4-hydroxy-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolyl}amino)-1*H*-indazole-1-carboxylate (89)**



In a manner similar to that employed for the synthesis of **70**, **89** (0.389 g, 0.709 mmol, 76% yield) was obtained as an orange solid from **64b** (0.282 g, 1.21 mmol) and **88** (0.310 g, 0.930 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.61–2.10 (8H, m), 1.74 (9H, s), 2.32–2.42 (1H, m), 2.46–2.57 (2H, m), 3.03–3.10 (1H, m), 3.23–3.31 (1H, m), 3.77 (3H, s), 4.29–4.37 (1H, m), 5.07–5.14 (1H, m), 6.80–6.86 (2H, m), 7.13–7.18 (2H, m), 7.28–7.36 (1H, m), 7.71–7.79 (1H, m), 7.98 (1H, d, *J* = 7.9 Hz), 8.50 (1H, s), 10.54 (1H, s).

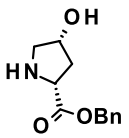
(4*S*)-4-Hydroxy-*N*-1*H*-indazol-4-yl-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolinamide (41)



In a manner similar to that employed for the synthesis of **33**, **41** (0.276 g, 0.616 mmol, 88% yield) was obtained as a white solid from **89** (0.385 g, 0.702 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70–1.74 (5H, m), 1.96–1.99 (2H, m), 2.09–2.13 (1H, m), 2.28–2.35 (1H, m), 2.58–2.65 (1H, m), 2.75–2.77 (1H, m), 3.06 (1H, dd, *J* = 11.8, 3.9 Hz), 3.29–3.32 (1H, m), 3.77 (3H, s), 4.32–4.35 (1H, m), 5.12–5.14 (1H, m), 6.82–6.83 (2H, m), 7.15–7.17 (2H, m), 7.22–7.24 (1H, m), 7.34–7.36 (1H, m), 7.86 (1H, d, *J* = 7.3 Hz), 8.36 (1H, s), 10.26 (1H, s), 10.52 (1H, brs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 24.7, 25.1, 35.2, 36.9, 38.5, 55.2, 55.5, 58.3, 60.3, 70.5, 105.7, 110.7, 114.1,

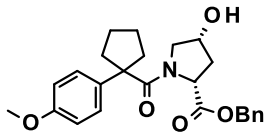
115.8, 126.7, 127.7, 130.8, 132.2, 135.9, 140.9, 158.1, 170.0, 177.6. HRMS (ESI) *m/z*: calcd for $C_{25}H_{29}N_4O_4$ $(M + H)^+$ 449.2183, Found: 449.2187.

Benzyl (4*R*)-4-hydroxy-D-prolinate (91)



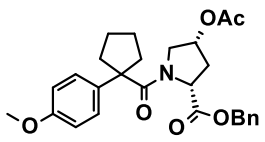
In a manner similar to that employed for the synthesis of **86** using benzene (9 mL) as a solvent, **91** (1.59 g, 7.19 mmol, 63% yield) was obtained as a colorless oil from (4*R*)-4-hydroxy-D-proline (**90**, 1.50 g, 11.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.51–2.21 (2H, m), 2.01–2.09 (1H, m), 2.21–2.33 (1H, m), 2.95–3.03 (1H, m), 3.09–3.16 (1H, m), 3.83–3.91 (1H, m), 4.30–4.40 (1H, m), 5.14–5.24 (2H, m), 7.33–7.45 (5H, m).

Benzyl (4*R*)-4-hydroxy-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolinate (92)



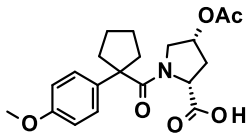
In a manner similar to that employed for the synthesis of **70**, **92** (1.24 g, 2.93 mmol, 92% yield) was obtained as a white solid from **57** (0.700 g, 3.18 mmol) and **91** (0.738 g, 3.34 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.63–1.80 (4H, m), 1.86–1.92 (1H, m), 1.92–2.03 (2H, m), 2.09–2.19 (1H, m), 2.29–2.46 (2H, m), 2.88–2.95 (1H, m), 3.25–3.33 (2H, m), 3.78 (3H, s), 4.05–4.14 (1H, m), 4.49–4.56 (1H, m), 5.19 (1H, d, J = 12.1 Hz), 5.33 (1H, d, J = 12.1 Hz), 6.79–6.85 (2H, m), 7.12–7.18 (2H, m), 7.31–7.43 (5H, m).

Benzyl (4*R*)-4-(acetyloxy)-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolinate (93)



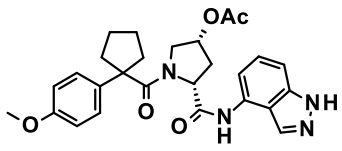
To a mixture of **92** (0.556 g, 1.31 mmol), pyridine (0.317 mL, 3.94 mmol) and DCM (5 mL), acetic anhydride (0.372 mL, 3.94 mmol) was added at 0 °C, and the mixture was stirred at rt for 4 h. After ethanol was added to the reaction solution, the solvent was distilled off under reduced pressure. 0.3 mol/L Hydrochloric acid was added to the residue obtained, and the mixture was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution, with water and with saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure to obtain **93** (0.610 g, 1.31 mmol, 100% yield) as a pink oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.58–2.11 (7H, m), 1.78 (3H, s), 2.27–2.43 (3H, m), 3.04–3.12 (1H, m), 3.14–3.21 (1H, m), 3.77–3.79 (3H, m), 4.72–4.79 (1H, m), 4.89–4.96 (1H, m), 5.12 (1H, d, J = 12.4 Hz), 5.27 (1H, d, J = 12.4 Hz), 6.75–6.80 (2H, m), 7.13–7.17 (2H, m), 7.29–7.42 (5H, m). MS (ESI/APCI) *m/z*: calcd for $C_{27}H_{32}NO_6$ $(M + H)^+$ 466.2, Found: 466.4.

(4*R*)-4-(Acetyloxy)-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-proline (94)



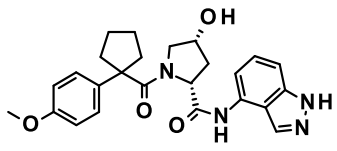
In a manner similar to that employed for the synthesis of **54**, **94** was obtained quantitatively as a colorless oil from **93** (0.610 g, 1.31 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.64–1.85 (4H, m), 1.86–2.00 (1H, m), 1.94 (3H, s), 2.02–2.15 (1H, m), 2.15–2.28 (1H, m), 2.28–2.49 (3H, m), 3.08–3.20 (2H, m), 3.80 (3H, s), 4.67–4.77 (1H, m), 4.90–4.98 (1H, m), 6.86 (2H, d, *J* = 8.5 Hz), 7.16 (2H, d, *J* = 8.5 Hz). MS (ESI/APCI) *m/z*: calcd for $C_{20}H_{26}NO_6$ $(M + H)^+$ 376.2, Found: 376.3.

(3*R*,5*R*)-5-[(1*H*-Indazol-4-yl)carbamoyl]-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]pyrrolidin-3-yl acetate (95)



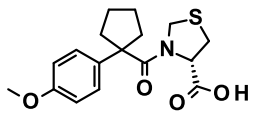
In a manner similar to that employed for the synthesis of **40**, **95** (1.24 g, 2.93 mmol, 92% yield) was obtained as a white solid from **94** (0.491 g, 1.31 mmol) and **64b** (0.397 g, 1.70 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.64–2.37 (8H, m), 1.95 (3H, s), 2.52–2.64 (1H, m), 2.78–2.88 (1H, m), 3.08–3.17 (1H, m), 3.27–3.37 (1H, m), 3.79 (3H, s), 4.89–4.98 (1H, m), 4.98–5.06 (1H, m), 6.81–6.87 (2H, m), 7.10–7.16 (2H, m), 7.22–7.26 (1H, m), 7.32–7.39 (1H, m), 7.83–7.91 (1H, m), 8.28 (1H, s), 10.00–10.77 (2H, m).

(4*R*)-4-Hydroxy-*N*-1*H*-indazol-4-yl-1-[1-(4-methoxyphenyl)cyclopentane-1-carbonyl]-D-prolinamide (42)



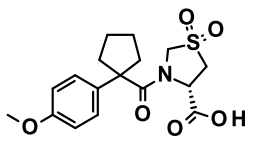
95 (0.153 g, 0.312 mmol) was dissolved in MeOH (4 mL) and water (1.5 mL), then sodium carbonate (0.066 g, 0.624 mmol) was added at rt, and the mixture was stirred at rt for 4 h. Water was added to the reaction solution, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate then ethyl acetate /methanol) to obtain **42** (0.124 g, 0.276 mmol, 89% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.59–1.81 (4H, m), 1.87–2.06 (2H, m), 2.11–2.22 (1H, m), 2.23–2.35 (1H, m), 2.40–2.49 (1H, m), 2.50–2.61 (1H, m), 2.92–3.00 (1H, m), 3.18–3.26 (1H, m), 3.78 (3H, s), 4.20–4.28 (1H, m), 4.61–4.66 (1H, m), 5.06–5.12 (1H, m), 6.79–6.84 (2H, m), 7.09–7.13 (2H, m), 7.29 (1H, d, *J* = 8.5 Hz), 7.38 (1H, dd, *J* = 8.5, 7.3 Hz), 7.78 (1H, d, *J* = 7.3 Hz), 8.34 (1H, s), 10.26–10.45 (2H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 24.8, 24.9, 35.1, 36.8, 38.0, 55.2, 57.4, 58.3, 62.0, 71.3, 106.3, 111.9, 114.2, 116.3, 126.6, 127.8, 130.3, 132.0, 135.3, 140.9, 158.1, 172.3, 177.8. HRMS (ESI) *m/z*: calcd for $C_{25}H_{29}N_4O_4$ $(M + H)^+$ 449.2183, Found: 449.2174.

(4*S*)-3-{[1-(4-Methoxyphenyl)cyclopentyl]carbonyl}-1,3-thiazolidine-4-carboxylic acid (97)



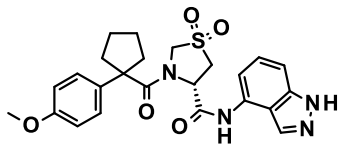
To a mixture of **57** (2.50 g, 11.3 mmol) and toluene (50.0 mL), thionyl chloride (2.60 mL, 34.0 mmol) and DMF (1 drop) were added at rt, and the mixture was heated and stirred at 60 °C for 1 h. The reaction solution was concentrated under reduced pressure, then the residue obtained was dissolved in THF (10.0 mL). Under ice-cooling, the above solution was added to a mixture of (4*S*)-1,3-thiazolidine-4-carboxylic acid (1.96 g, 14.8 mmol), a saturated aqueous $NaHCO_3$ solution (40.0 mL) and THF (30.0 mL), and the mixture was stirred for 22 h while gradually returning to rt. The reaction solution was diluted with water, and acidified by the addition of 6 mol/L hydrochloric acid under ice-cooling, and then the mixture was extracted with ethyl acetate. The organic layer obtained was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, then the residue obtained was suspended in Et_2O, and filtered to obtain **97** (3.04 g, 9.06 mmol, 80% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.51–1.74 (4H, m), 1.77–2.05 (2H, m), 2.21–2.35 (2H, m), 2.94 (1H, dd, J = 11.5, 4.8 Hz), 3.20–3.37 (1H, m), 3.74 (3H, s), 3.87–3.97 (1H, m), 4.05–4.17 (1H, m), 4.75–4.86 (1H, m), 6.90 (2H, d, J = 8.5 Hz), 7.16 (2H, d, J = 7.9 Hz), 12.80 (1H, brs). MS (ESI/APCI) *m/z*: calcd for $C_{17}H_{22}NO_4S$ $(M + H)^+$ 336.1, Found: 336.1.

(4*S*)-3-{[1-(4-Methoxyphenyl)cyclopentyl]carbonyl}-1,3-thiazolidine-4-carboxylic acid 1,1-dioxide (98)



To a suspension of **97** (3.04 g, 9.06 mmol) in AcOH (20.0 mL), hydrogen peroxide (34.5%, 3.73 mL) was added at rt, and the mixture was stirred for 1 h, and then heated and stirred at 50 °C for 8 h. The reaction solution was diluted with water, and then the precipitated solid was filtered to obtain **98** (2.47 g, 6.72 mmol, 74% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.55–1.72 (4H, m), 1.82–2.05 (2H, m), 2.17–2.36 (2H, m), 3.40–3.49 (1H, m), 3.71–3.91 (5H, m), 4.22–4.32 (1H, m), 5.05–5.15 (1H, m), 6.86–6.94 (2H, m), 7.13–7.21 (2H, m), 13.32 (1H, brs). MS (ESI/APCI) *m/z*: calcd for $C_{17}H_{20}NO_6S$ $(M - H)^-$ 366.1, Found: 366.2.

(4*S*)-*N*-(1*H*-Indazol-4-yl)-3-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-1,3-thiazolidine-4-carboxamide 1,1-dioxide (43, DS17701585)

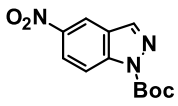


Phosphoryl chloride (0.504 mL, 5.45 mmol) was added to the mixture of **98** (1.67 g, 4.55 mmol), **64b** (1.38 g, 5.91 mmol), *N*-methylmorpholine (5.05 mL, 45.5 mmol) and DCM (50.0 mL) at 0 °C and then allowed to stand in a refrigerator overnight. A 10% aqueous citric acid solution was added to the reaction solution, and the mixture was extracted with DCM. The organic layer was washed with water and a saturated brine and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, and the residue obtained was

subjected to silica gel column chromatography (hexane/ethyl acetate) to obtain an intermediate (2.04 g). To a solution of the intermediate (0.200 g) obtained in DCM (2.5 mL), HCl (4 mol/L, 1,4-dioxane solution, 5 mL) was added at 0 °C, and the mixture was stirred at rt for 4 h. Ice was added at rt, and then the mixture was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, and the residue obtained was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain **43** (**DS17701585**, 0.168 g, 0.348 mmol, 77% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72–1.74 (2H, m), 1.80–1.86 (3H, m), 2.22–2.29 (1H, m), 2.37–2.44 (1H, m), 2.53–2.59 (1H, m), 3.32–3.35 (1H, m), 3.58–3.61 (1H, m), 3.74 (3H, s), 4.09–4.11 (1H, m), 4.46–4.49 (1H, m), 5.70–5.73 (1H, m), 6.75 (2H, d, *J* = 8.5 Hz), 7.00 (2H, d, *J* = 8.5 Hz), 7.32–7.43 (2H, m), 7.79 (1H, d, *J* = 7.9 Hz), 8.26 (1H, s), 9.63 (1H, s), 10.24 (1H, brs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 25.0, 25.2, 36.7, 39.1, 47.6, 55.2, 56.7, 58.4, 61.9, 106.6, 111.4, 114.9, 115.9, 126.2, 127.8, 129.8, 131.8, 134.3, 141.0, 158.6, 165.5, 178.0. HRMS (ESI) *m/z*: calcd for $C_{24}H_{27}N_4O_5S$ $(M + H)^+$ 483.1697, Found: 483.1707.

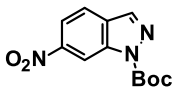
第三章

tert-Butyl 5-nitro-1*H*-indazole-1-carboxylate (120a).



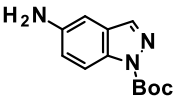
A solution of 5-nitroindazole (**119a**, 2.00 g, 12.3 mmol) in THF (30 mL) was allowed to cool to 0 °C, supplemented with Boc_2O (3.21 g, 14.7 mmol), DIPEA (3.84 mL, 22.1 mmol), and DMAP (150 mg, 1.23 mmol), and stirred at rt for 24 h. The solvent was distilled off under reduced pressure and the obtained residue was subjected to silica gel column chromatography (hexane/ethyl acetate) to obtain **120a** (2.86 g, 10.9 mmol, 89% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.75 (9H, s), 8.32–8.36 (2H, m), 8.43 (1H, dd, *J* = 9.4, 2.1 Hz), 8.70 (1H, d, *J* = 2.4 Hz).

tert-Butyl 6-nitro-1*H*-indazole-1-carboxylate (120b).



In a manner similar to that employed for the synthesis of **120a**, the title compound **120b** (2.89 g, 11.0 mmol, 90% yield) was obtained using **119b** (2.00 g, 12.3 mmol) and Boc_2O (3.21 g, 14.7 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.77 (9H, s), 7.88 (1H, d, *J* = 9.0 Hz), 8.21 (1H, dd, *J* = 9.0, 2.0 Hz), 8.31 (1H, s), 9.13 (1H, d, *J* = 2.0 Hz).

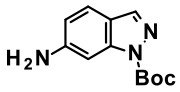
tert-Butyl 5-amino-1*H*-indazole-1-carboxylate (121a).



To a suspension of **120a** (2.86 g, 10.9 mmol) in methanol (50 mL) was added zinc powder (4.97 g, 76.0 mmol) under ice cooling. The mixture was stirred at the same

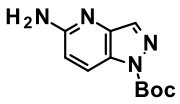
temperature for 10 min, supplemented with a saturated aqueous NH_4Cl solution (50 mL), and then stirred again at rt for 3 h. After the reaction solution was filtered through a Celite pad, the obtained filtrate was extracted with ethyl acetate and concentrated under reduced pressure. The obtained residue was then purified by silica gel column chromatography (chloroform/ethyl acetate) to obtain **121a** (2.18 g, 9.35 mmol, 86% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.71 (9H, s), 3.73 (2H, brs), 6.93–6.95 (2H, m), 7.96–7.99 (2H, m).

***tert*-Butyl 6-amino-1*H*-indazole-1-carboxylate (121b).**



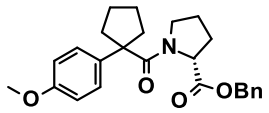
In a manner similar to that employed for the synthesis of **121a**, the title compound **121b** (1.83 g, 7.85 mmol, 72% yield) was obtained using **120b** (2.89 g, 11.0 mmol) and zinc powder (5.02 g, 76.8 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.71 (9H, s), 4.02 (2H, brs), 6.68 (1H, dd, J = 8.5, 1.8 Hz), 7.44 (1H, s), 7.47 (1H, d, J = 8.5 Hz), 7.97 (1H, s).

***tert*-Butyl 5-amino-1*H*-pyrazolo[4,3-*b*]pyridine-1-carboxylate (123).**



A mixture of 5-chloro-1*H*-pyrazolo[4,3-*b*]pyridine (**122**, 14.7 g, 92.9 mmol), $Pd_2(dba)_3$ (2.27 g, 1.86 mmol), XPhos (1.83 g, 3.71 mmol), LHMDS (1.09 mol/L, solution in THF, 200 mL, 218 mmol), and THF (100 mL) was stirred under reflux for 6 h. The reaction solution was allowed to cool to rt and then left to stand for 16 h. The reaction solution was then allowed to cool to 0 °C, supplemented with Boc_2O (22.4 g, 97.5 mmol) in small portions, and stirred at 0 °C for 40 min. The solution was then supplemented with TBAF (1 mol/L, solution in THF, 279 mL, 279 mmol) at 0 °C and the resultant mixture was stirred at the same temperature for 1 h. Water was added to this mixture at 0 °C, followed by extraction with ethyl acetate, and the obtained organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure and the obtained residue was subjected to silica gel column chromatography (hexane/ethyl acetate) to obtain a slurry with a diethyl ether/hexane mixed solvent. This slurry was then filtered to obtain **123** (16.6 g, 70.9 mmol, 76% yield) as a pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.63 (9H, s), 6.25 (2H, s), 6.73 (1H, d, J = 9.1 Hz), 8.02 (1H, d, J = 9.1 Hz), 8.09 (1H, s).

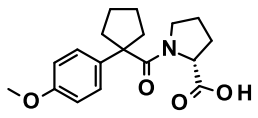
Benzyl 1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-prolinate (125).



To a mixture of 1-(4-methoxyphenyl)cyclopentane carboxylic acid (**57**, 8.20 g, 37.2 mmol) and toluene (100 mL) were added $SOCl_2$ (5.69 mL, 74.5 mmol) and DMF (1 drop) at rt, and this mixture was then heated under reflux for 45 min. The reaction solution was concentrated under reduced pressure and the obtained residue was dissolved in DCM (30 mL). The resultant solution was added, under ice cooling, in a dropwise manner to a mixture of D-proline benzyl ester hydrochloride (**124**,

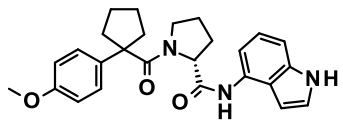
11.9 g, 48.4 mmol), DIPEA (26 mL), and DCM (70 mL), followed by stirring for 16 h while allowing the mixture to gradually return to rt. The reaction solution was washed with 1 mol/L aqueous HCl solution, a saturated aqueous $NaHCO_3$ solution, and saturated brine, and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure and the obtained residue was subjected to silica gel column chromatography (hexane/ethyl acetate) to obtain **125** (13.7 g, 33.6 mmol, 90% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.64–1.80 (7H, m), 1.95–2.11 (3H, m), 2.36–2.40 (2H, m), 2.96–2.98 (2H, m), 3.78 (3H, s), 4.53 (1H, dd, *J* = 8.8, 5.1 Hz), 5.13 (1H, d, *J* = 12.1 Hz), 5.25 (1H, d, *J* = 12.1 Hz), 6.76–6.79 (2H, m), 7.14–7.15 (2H, m), 7.33–7.37 (5H, m).

1-{[1-(4-Methoxyphenyl)cyclopentyl]carbonyl}-D-proline (126).



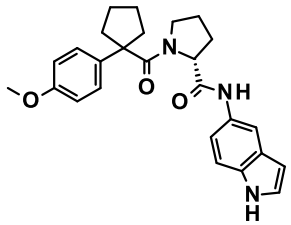
To a solution of **125** (13.7 g, 33.6 mmol) in ethanol (100 mL) was added 10% Pd/C (1.00 g), and the mixture was stirred at rt under a H_2 atmosphere for 3 h. The reaction solution was filtered through celite using DCM and methanol, and the solvent was distilled off under reduced pressure. The obtained residue was subjected to silica gel column chromatography (ethyl acetate/methanol) to obtain **126** (8.78 g, 27.7 mmol, 82% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.50–1.68 (7H, m), 1.85–2.00 (3H, m), 2.26–2.29 (2H, m), 2.90–2.91 (2H, m), 3.73 (3H, s), 4.22 (1H, dd, *J* = 9.1, 4.8 Hz), 6.87–6.88 (2H, m), 7.15–7.16 (2H, m), 12.29 (1H, brs).

***N*-1*H*-Indol-4-yl-1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-prolinamide (101).**



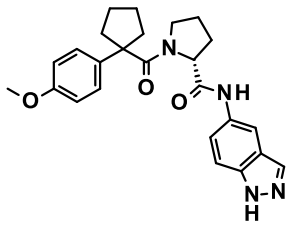
To a solution of **126** (40.0 mg, 0.126 mmol) in DMF (1.5 mL) were added COMU (59.4 mg, 0.139 mmol) and DIPEA (0.0329 mL, 0.189 mmol), and the mixture was then stirred at rt for 15 min. 1*H*-Indol-4-amine (20.0 mg, 0.151 mmol) was then added, followed by stirring at rt overnight. A saturated aqueous NH_4Cl solution was added to the reaction and the mixture was extracted with ethyl acetate three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution, and with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (chloroform/ethyl acetate) to obtain **101** (44.0 mg, 0.102 mmol, 81% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.64–2.00 (8H, m), 2.09–2.15 (1H, m), 2.30–2.36 (1H, m), 2.51–2.59 (2H, m), 2.94–3.05 (2H, m), 3.77 (3H, s), 4.97–5.03 (1H, m), 6.78–6.80 (3H, m), 7.11–7.21 (5H, m), 7.90–7.94 (1H, m), 8.29 (1H, s), 10.06 (1H, s). MS (ESI/APCI) *m/z*: 432.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{26}H_{30}N_3O_3$ $(M + H)^+$, 432.2282; found, 432.2302.

N-1*H*-Indol-5-yl-1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-prolinamide (102).



In a manner similar to that employed for the synthesis of **101**, the title compound **102** (45.0 mg, 0.104 mmol, 83% yield) was obtained using **126** (40.0 mg, 0.126 mmol) and 1*H*-indol-5-amine (20.0 mg, 0.151 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.58–1.91 (7H, m), 1.96–2.11 (2H, m), 2.33–2.48 (3H, m), 2.95–3.08 (2H, m), 3.78 (3H, s), 4.85–4.87 (1H, m), 6.50–6.52 (1H, m), 6.83 (2H, d, *J* = 9.1 Hz), 7.15 (2H, d, *J* = 9.1 Hz), 7.18–7.21 (1H, m), 7.24–7.26 (1H, m), 7.32 (1H, d, *J* = 8.5 Hz), 7.91 (1H, s), 8.20 (1H, s), 9.21 (1H, s). MS (ESI/APCI) *m/z*: 432.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{26}H_{30}N_3O_3$ $(M + H)^+$, 432.2282; found, 432.2305.

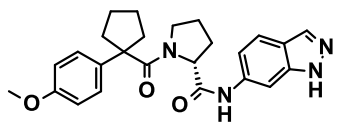
***N*-1*H*-Indazol-5-yl-1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-prolinamide (99).**



A solution of **126** (500 mg, 1.58 mmol) in DMF (20 mL) was cooled to 0 °C. Then, **121a** (441 mg, 1.89 mmol), COMU (810 mg, 1.89 mmol), and DIPEA (0.412 mL, 2.36 mmol) were added and the mixture was stirred at rt for 16 h. Next, water and saturated brine were added in this order and the mixture was extracted with a mixed solvent of ethyl acetate/hexane. The resultant sample was washed with saturated brine and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was subjected to silica gel column chromatography (hexane/ethyl acetate). The crude product **127a** was given as a pale-brown oil and could be used in the next step without further purification.

Next, 4 mol/L HCl (1,4-dioxane solution, 30 mL) was added at 0 °C to **127a** and the mixture was stirred at rt for 18 h. Toluene was added and the mixture was concentrated under reduced pressure. Then, the resultant sample was subjected to amino silica gel column chromatography (hexane/ethyl acetate). The obtained solid was slurried with ethyl acetate and hexane, and then filtered to obtain compound **99** (355 mg, 0.820 mmol, 52% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.44–2.02 (8H, m), 2.05–2.15 (1H, m), 2.31–2.52 (3H, m), 2.96–3.10 (2H, m), 3.79 (3H, s), 4.81–4.91 (1H, m), 6.83 (2H, d, *J* = 8.5 Hz), 7.14 (2H, d, *J* = 8.5 Hz), 7.36–7.44 (2H, m), 8.02 (1H, s), 8.12 (1H, s), 9.52 (1H, brs). MS (ESI/APCI) *m/z*: 433.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{29}N_4O_3$ $(M + H)^+$, 433.2234; found, 433.2253.

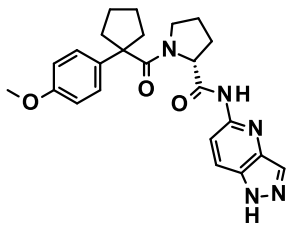
***N*-1*H*-Indazol-6-yl-1-{[1-(4-methoxyphenyl)cyclopentyl]carbonyl}-D-prolinamide (100).**



In a manner similar to that employed for the synthesis of **4**, the title compound **5** (11.0 mg, 0.0254 mmol, 27% yield) was obtained using **126** (30.0 mg, 0.0945 mmol) and **121b** (26.5 mg, 0.113 mmol). ^{1}H

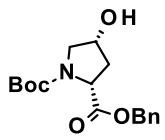
NMR (DMSO-d_6) δ: 1.50–1.79 (7H, m), 1.81–1.94 (2H, m), 2.01–2.11 (1H, m), 2.24–2.38 (2H, m), 2.94–3.01 (2H, m), 3.75 (3H, s), 4.43–4.49 (1H, m), 6.91 (2H, d, *J* = 8.6 Hz), 7.11 (1H, dd, *J* = 8.6, 1.2 Hz), 7.20 (2H, d, *J* = 8.6 Hz), 7.67 (1H, d, *J* = 8.6 Hz), 7.97 (1H, s), 8.16 (1H, s), 10.16 (1H, s), 12.89 (1H, s). MS (ESI/APCI) *m/z*: 433.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{29}N_4O_3$ $(M + H)^+$, 433.2234; found, 433.2235.

1-{[1-(4-Methoxyphenyl)cyclopentyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (103).



A solution of **126** (150 mg, 0.473 mmol) and **123** (166 mg, 0.709 mmol) in pyridine (4 mL) was cooled to 0 °C, supplemented with $POCl_3$ (0.130 mL, 1.42 mmol), and then stirred at rt overnight. The resultant mixture was diluted with ethyl acetate, washed with 10% aqueous citric acid solution three times, with a saturated aqueous $NaHCO_3$ solution, and with saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) and then by amino silica gel column chromatography (hexane/ethyl acetate) to obtain intermediate **127c** as a colorless oil. To this intermediate was added 4 mol/L HCl (1,4-dioxane solution, 8 mL), after which the mixture was stirred at rt for 4 h. The resultant mixture was then concentrated under reduced pressure, after which the obtained residue was subjected to amino silica gel column chromatography (hexane/ethyl acetate). The obtained solid was slurried with diethyl ether and hexane, followed by filtering to obtain compound **103** (51.0 mg, 0.118 mmol, 25% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.63–1.91 (8H, m), 1.97–2.15 (2H, m), 2.39–2.54 (2H, m), 3.06–3.15 (2H, m), 3.82 (3H, s), 4.63–4.72 (1H, m), 6.90 (2H, d, *J* = 8.5 Hz), 7.24 (2H, d, *J* = 8.5 Hz), 7.72 (1H, d, *J* = 9.1 Hz), 8.07 (1H, s), 8.14 (1H, d, *J* = 9.1 Hz), 8.77 (1H, s), 11.36 (1H, s). MS (ESI/APCI) *m/z*: 434.3 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{24}H_{28}N_5O_3$ $(M + H)^+$, 434.2187; found, 434.2189.

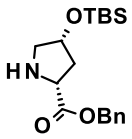
2-Benzyl 1-*tert*-butyl (2*R*,4*R*)-4-hydroxypyrrolidine-1,2-dicarboxylate (129).



A solution of (4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxy-D-proline (**128**, 2.03 g, 8.80 mmol) in methanol (30 mL) was cooled to 0 °C. Then, cesium carbonate (1.45 g, 4.40 mmol) in water (4 mL) was added and the mixture was stirred at rt for 5 min. The solvent was distilled off under reduced pressure, after which the residue was suspended in DMF (35 mL). Benzyl bromide was added at 0 °C and the mixture was stirred at rt overnight. Then, water was added and the mixture was extracted with ethyl acetate. The resultant sample was washed with saturated brine and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was subjected to silica gel

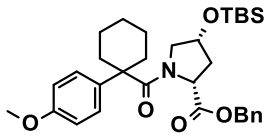
column chromatography (hexane/ethyl acetate) to obtain compound **129** (2.73 g, 8.49 mmol, 97% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, mixture of rotamers) δ: 1.34 and 1.47 (9H, 2s), 2.11 (1H, d, *J* = 14.5 Hz), 2.26–2.38 (1H, m), 3.19 and 3.24 (1H, 2d, *J* = 9.7 and 9.7 Hz), 3.50–3.71 (2H, m), 4.32–4.44 (2H, m), 5.12–5.38 (2H, m), 7.35–7.38 (5H, m).

Benzyl (4*R*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-D-prolinate (131).



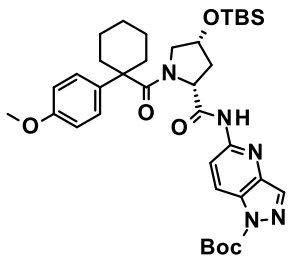
A solution of **129** (2.68 g, 8.34 mmol) in DMF (10 mL) was cooled to 0 °C. Then, imidazole (3.41 g, 50.0 mmol) and TBSCl (5.03 g, 33.4 mmol) were added and the mixture was stirred at rt overnight. The resultant mixture was diluted with ethyl acetate, washed with water three times and with saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain intermediate **130** as a colorless oil. To this intermediate were added DCM (8 mL) and TFA (16 mL) at 0 °C, after which the mixture was stirred at rt for 3 h. After the reaction solution was concentrated under reduced pressure, a saturated aqueous $NaHCO_3$ solution was added to the obtained residue. The mixture was extracted with diethyl ether and the organic layer was dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was subjected to amino silica gel column chromatography (hexane/ethyl acetate) and then by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **131** (1.53 g, 4.56 mmol, 55% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.01 (3H, s), 0.03 (3H, s), 0.84 (9H, s), 2.00–2.05 (1H, m), 2.17–2.24 (1H, m), 2.48 (1H, brs), 2.84–2.87 (1H, m), 2.97–3.03 (1H, m), 3.78–3.80 (1H, m), 4.26–4.29 (1H, m), 5.11–5.19 (2H, m), 7.30–7.39 (5H, m).

Benzyl (4*R*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-1-{[1-(4-methoxyphenyl)cyclohexyl]carbonyl}-D-prolinate (132).



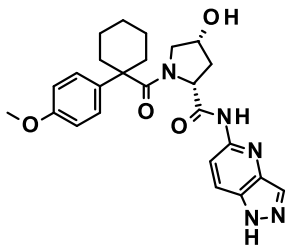
In a manner similar to that employed for the synthesis of **101**, the title compound **132** (841 mg, 1.52 mmol, 85% yield) was obtained using **56** (440 mg, 1.88 mmol) and **131** (600 mg, 1.79 mmol). ^{1}H NMR ($CDCl_3$) δ: –0.17 (3H, s), –0.14 (3H, s), 0.74 (9H, s), 1.22–1.63 (5H, m), 1.73–1.84 (3H, m), 2.17–2.31 (3H, m), 2.80–2.84 (1H, m), 3.12–3.16 (1H, m), 3.77–3.82 (4H, m), 4.12 (1H, q, *J* = 7.3 Hz), 4.60 (1H, t, *J* = 8.2 Hz), 5.05–5.26 (2H, m), 6.80–6.83 (2H, m), 7.17–7.19 (2H, m), 7.29–7.38 (5H, m).

***tert*-Butyl 5-{[(4*R*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-1-{[1-(4-methoxyphenyl)cyclohexyl]carbonyl}-D-prolyl]amino}-1*H*-pyrazolo[4,3-*b*]pyridine-1-carboxylate (134).**



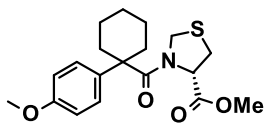
To a solution of **132** (840 mg, 1.52 mmol) in ethanol (15 mL) was added 10% Pd/C (300 mg), after which the mixture was stirred at rt under a H_2 atmosphere for 3 h. The reaction solution was filtered through celite using DCM and methanol, and the solvent was distilled off under reduced pressure to obtain intermediate **133** (646 mg, 1.40 mmol, 92% yield) as a white solid. This intermediate was dissolved in pyridine (9 mL), and $POCl_3$ (0.186 mL, 2.02 mmol) and **123** (729 mg, 3.11 mmol) were added at 0 °C, after which the mixture was stirred at rt overnight. The resultant mixture was diluted with diethyl ether, washed with 10% aqueous citric acid solution three times and with saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure and then the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **134** (214 mg, 20% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: –0.17 (3H, s), –0.13 (3H, s), 0.64 (9H, s), 1.28–1.31 (1H, m), 1.57–1.77 (17H, m), 2.08–2.40 (4H, m), 3.11–3.18 (1H, m), 3.81 (3H, s), 4.10–4.15 (1H, m), 4.67–4.69 (1H, m), 6.93 (2H, d, *J* = 7.9 Hz), 7.27–7.29 (2H, m), 8.18 (1H, s), 8.38 (1H, d, *J* = 9.1 Hz), 8.52 (1H, d, *J* = 9.1 Hz), 8.80 (1H, s).

(4*R*)-4-Hydroxy-1-{[1-(4-methoxyphenyl)cyclohexyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (105).



A solution of **134** (223 mg, 0.328 mmol) in acetonitrile (4.8 mL) was cooled to 0 °C. Then, TFA (2.4 mL) and water (2.4 mL) were added and the mixture was stirred at rt for 1 h. After the reaction solution was concentrated under reduced pressure, a saturated aqueous $NaHCO_3$ solution was added to the obtained residue. This mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was subjected to silica gel column chromatography (hexane/ethyl acetate) to obtain compound **105** (140 mg, 0.302 mmol, 92% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.22–1.39 (1H, m), 1.60–1.82 (7H, m), 2.08–2.15 (2H, m), 2.30–2.42 (2H, m), 2.89–2.97 (1H, m), 3.31–3.38 (1H, m), 3.81 (3H, s), 4.15–4.22 (1H, m), 4.58–4.74 (2H, m), 6.90 (2H, d, *J* = 8.5 Hz), 7.22–7.25 (2H, m), 7.75 (1H, d, *J* = 9.1 Hz), 8.08 (1H, s), 8.18 (1H, d, *J* = 9.1 Hz), 8.99 (1H, s), 11.04 (1H, s). MS (ESI/APCI) *m/z*: 464.3 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{30}N_5O_4$ $(M + H)^+$, 464.2292; found, 464.2309.

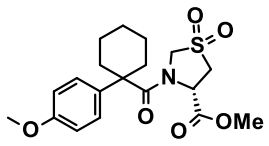
Methyl (4*S*)-3-{[1-(4-methoxyphenyl)cyclohexyl]carbonyl}-1,3-thiazolidine-4-carboxylate (136).



To a solution of **56** (406 mg, 1.73 mmol) in DCE (8 mL) were added oxalyl chloride (0.297 mL, 3.47 mmol) and DMF (2 drops), followed by stirring of the mixture at rt for 25 min. To the reaction solution were further added

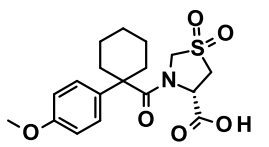
oxalyl chloride (0.297 mL, 3.47 mmol) and DMF (1 drop), followed by stirring at rt for 50 min and then at 75 °C for 45 min. A residue obtained by distilling off the solvent under reduced pressure was dissolved in DCE (4 mL), followed by the addition of a solution of methyl (4*S*)-thiazoridine-4-carboxylate (**135**, 232 mg, 1.58 mmol) in DCE (6 mL), pyridine (0.635 mL, 7.88 mmol), and DMAP (23 mg, 0.189 mmol), with the resultant mixture then being stirred at 75 °C for 3.3 h. The resultant mixture was allowed to cool to rt and supplemented with a saturated aqueous NH_4Cl solution. The resultant mixture was extracted with DCM, washed with a 10% aqueous citric acid solution and saturated brine, and dried over anhydrous sodium sulfate. A residue obtained by distilling off the solvent under reduced pressure was subjected to amino silica gel column chromatography (hexane/ethyl acetate) and further to silica gel column chromatography (hexane/ethyl acetate) to obtain compound **136** (328 mg, 0.902 mmol, 57% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.26–1.29 (1H, m), 1.58–1.70 (7H, m), 2.32–2.35 (2H, m), 2.97 (1H, dd, *J* = 11.8, 5.8 Hz), 3.13–3.15 (1H, m), 3.76 (3H, s), 3.80 (3H, s), 4.01–4.04 (1H, m), 4.12–4.14 (1H, m), 5.03–5.06 (1H, m), 6.88–6.89 (2H, m), 7.21 (2H, d, *J* = 8.5 Hz).

Methyl (4*S*)-3-{[1-(4-methoxyphenyl)cyclohexyl]carbonyl}-1,3-thiazolidine-4-carboxylate 1,1-dioxide (137).



To a solution of **136** (118 mg, 0.325 mmol) in DCM (5 mL) was added *m*-CPBA (purity ≤77%, 140 mg, 0.812 mmol), followed by stirring of the mixture at rt for 6.4 h. The mixture was then diluted with ethyl acetate and supplemented with water. The resultant mixture was extracted with ethyl acetate, washed with saturated brine, and dried over anhydrous sodium sulfate. A residue obtained by distilling off the solvent under reduced pressure was subjected to silica gel column chromatography (hexane/ethyl acetate) to obtain compound **137** (68 mg, 0.172 mmol, 53% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.24–1.81 (8H, m), 2.17–2.27 (2H, m), 3.22 (1H, dd, *J* = 13.3, 5.4 Hz), 3.37–3.40 (1H, m), 3.77–3.80 (7H, m), 4.24–4.27 (1H, m), 5.38–5.40 (1H, m), 6.91 (2H, dd, *J* = 8.7, 2.0 Hz), 7.19 (2H, dd, *J* = 8.7, 2.0 Hz).

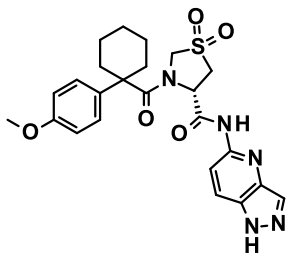
(4*S*)-3-{[1-(4-Methoxyphenyl)cyclohexyl]carbonyl}-1,3-thiazolidine-4-carboxylic acid 1,1-dioxide (138).



A solution of **137** (28 mg, 0.066 mmol) in THF (2 mL) was allowed to cool to 0 °C, supplemented with water (1 mL) and lithium hydroxide monohydrate (9 mg, 0.21 mmol), and then stirred at rt for 24.5 h. The organic solvent was distilled off under reduced pressure and 1 mol/L aqueous HCl solution was added to obtain a pH of ~2. The resultant mixture was extracted with ethyl acetate, washed with saturated brine, and dried over anhydrous sodium sulfate. A residue obtained by

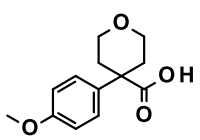
distilling off the solvent under reduced pressure was formed into a slurry with diisopropyl ether, and the resultant sample was filtered off to obtain compound **138** (25 mg, 0.0066 mmol, 93% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.24–1.26 (1H, m), 1.44–1.79 (7H, m), 2.18–2.22 (2H, m), 3.39 (1H, dd, *J* = 13.6, 6.3 Hz), 3.73–3.75 (4H, m), 4.04–4.05 (1H, m), 4.11–4.13 (1H, m), 5.07–5.10 (1H, m), 6.92 (2H, d, *J* = 9.1 Hz), 7.19 (2H, d, *J* = 9.1 Hz), 13.27 (1H, s).

(4*S*)-3-{[1-(4-Methoxyphenyl)cyclohexyl]carbonyl}-*N*-(1*H*-pyrazolo[4,3-*b*]pyridin-5-yl)-1,3-thiazolidine-4-carboxamide 1,1-dioxide (106).



A suspension of **138** (400 mg, 1.05 mmol) and **123** (491 mg, 2.10 mmol) in pyridine (6 mL) was cooled to 0 °C, supplemented with $POCl_3$ (0.288 mL, 3.15 mmol), and then stirred at a temperature from 0 °C to rt for 19.2 h. The resultant mixture was diluted with ethyl acetate, washed with 10% aqueous citric acid solution three times, with a saturated aqueous $NaHCO_3$ solution, and with saturated brine, and then dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) and then by amino silica gel column chromatography (hexane/ethyl acetate) to obtain intermediate **139** as a pale-yellow solid. This intermediate was dissolved in DCM (6 mL), followed by the addition of TFA (6 mL), after which the mixture was stirred at rt for 2.3 h. The resultant mixture was concentrated under reduced pressure, ethyl acetate and a saturated aqueous $NaHCO_3$ solution were added to the obtained residue, and the mixture was then extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **106** (168 mg, 0.338 mmol, 32% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.23–1.38 (2H, m), 1.61–1.85 (6H, m), 2.25–2.37 (2H, m), 3.24–3.36 (1H, m), 3.67–3.83 (2H, m), 3.77 (3H, s), 4.42–4.54 (1H, m), 5.64–5.71 (1H, m), 6.85 (2H, d, *J* = 8.5 Hz), 7.19 (2H, d, *J* = 8.5 Hz), 7.88 (1H, d, *J* = 9.1 Hz), 8.20–8.23 (2H, m), 9.17 (1H, s), 10.43 (1H, s). MS (ESI/APCI) *m/z*: 498.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{24}H_{28}N_5O_5S$ $(M + H)^+$, 498.1806; found, 498.1825.

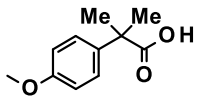
4-(4-Methoxyphenyl)tetrahydro-2*H*-pyran-4-carboxylic acid (142a).



Under a N_2 atmosphere, a suspension of NaH (7.51 g, 172 mmol) in DMF (150 mL) was ice-cooled, supplemented in a dropwise manner with a solution of 4-methoxyphenylacetonitrile (**140**, 10.5 mL, 78.1 mmol) and 1-bromo-2-(2-bromoethoxy)ethane (20.2 g, 86.0 mmol) in DMF (50 mL) over a period of 30 min, and stirred under ice-cooling for 1 h and at rt for 4 h. After another session of ice-cooling, water

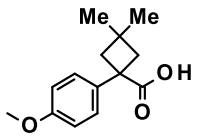
was added to the reaction solution, the mixture was extracted with diethyl ether three times, and the organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The organic layer was filtered and concentrated under reduced pressure to obtain crude compound **141a** (22.4 g) as an orange oil. To this crude product (22.4 g) were added ethylene glycol (100 mL) and KOH (13.01 g, 234 mmol), followed by stirring for 8 h under heated reflux. After allowing the mixture to cool to rt, water was added to the reaction solution and the mixture was washed with diethyl ether twice. The aqueous layer was acidified by adding 1 mol/L aqueous HCl solution and stirred at rt overnight. The precipitated solid was collected by filtration and purified by silica gel column chromatography (chloroform/methanol) to obtain compound **142a** (13.6 g, 57.6 mmol, 74% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.93–2.00 (2H, m), 2.47–2.53 (2H, m), 3.58–3.64 (2H, m), 3.80 (3H, s), 3.89–3.94 (2H, m), 6.89 (2H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.5 Hz).

2-(4-Methoxyphenyl)-2-methylpropanoic acid (142b).



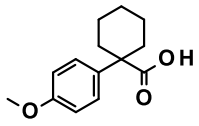
In a manner similar to that employed for the synthesis of **142a**, the title compound **142b** (15.62 g, 80.4 mmol, 49% yield) was obtained using **140** (24.08 g, 163.6 mmol) and iodomethane (22.4 mL, 360 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.44 (6H, s), 3.73 (3H, s), 6.88 (2H, d, J = 8.5 Hz), 7.26 (2H, d, J = 8.5 Hz), 12.22 (1H, brs).

1-(4-Methoxyphenyl)-3,3-dimethylcyclobutanecarboxylic acid (142c).



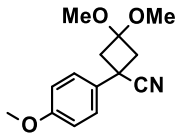
To a suspension of NaH (1.50 g, 34.0 mmol) in DMSO (40 mL) was added a solution of **140** (2.30 g, 16.0 mmol) and 1,3-dibromo-2,2-dimethylpropane (4.00 g, 17.0 mmol) in diethyl ether (10 mL) in a dropwise manner, followed by stirring at rt for 20 h. Isopropyl alcohol was added to the reaction mixture, and then the mixture was diluted with water and extracted with hexane. The organic layer was concentrated, diluted with diethyl ether, and washed with water. The organic layer was dried over sodium sulfate, filtered, and concentrated to obtain crude compound **141c** (3.55 g) as a pale-yellow oil. To this crude product (3.55 g) were added ethylene glycol (60 mL) and KOH (9.20 g, 140 mmol), followed by stirring for 30 min under heated reflux. After allowing the reaction solution to cool to rt, water was added to it, and the mixture was then washed with diethyl ether twice. The aqueous layer was acidified by adding 1 mol/L aqueous HCl solution, and the precipitated solid was collected by filtration and dried to obtain compound **142c** (2.77 g, 11.8 mmol, 74% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 0.99 (3H, s), 1.15 (3H, s), 2.33 (2H, d, J = 12.1 Hz), 2.73 (2H, d, J = 12.1 Hz), 3.79 (3H, s), 6.86 (2H, d, J = 9.1 Hz), 7.24 (2H, d, J = 9.1 Hz).

1-(3-Fluoro-4-methoxyphenyl)cyclohexanecarboxylic acid (142d).



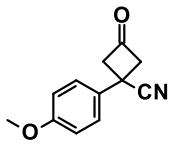
In a manner similar to that employed for the synthesis of **142a**, the title compound **142d** (616 mg, 2.44 mmol, 23% yield) was obtained using **140** (2.20 g, 13.3 mmol) and 1.5-dibromopentane (2.00 mL, 14.7 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.26–1.73 (8H, m), 2.39–2.42 (2H, m), 3.87 (3H, s), 6.89–6.94 (1H, m), 7.13–7.21 (2H, m).

3,3-Dimethoxy-1-(4-methoxyphenyl)cyclobutanecarbonitrile (143).



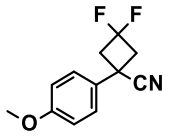
To a suspension of NaH (1.42 g, 32.6 mmol) in DMSO (40 mL) was added a solution of **140** (2.18 g, 14.8 mmol) and 1,3-dibromo-2,2-dimethoxypropane (4.27 g, 16.3 mmol) in diethyl ether (10 mL) in a dropwise manner, followed by stirring at rt for 16 h. Isopropyl alcohol was added to the reaction mixture, and then the mixture was diluted with water and extracted with hexane. The organic layer was concentrated, diluted with diethyl ether, and then washed with water. The organic layer was dried over sodium sulfate, filtered, and concentrated to obtain compound **143** (3.64 g, 14.7 mmol, 99% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.65–2.72 (2H, m), 3.05–3.12 (2H, m), 3.17 (3H, s), 3.28 (3H, s), 3.82 (3H, s), 6.91 (2H, d, J = 8.5 Hz), 7.38 (2H, d, J = 8.5 Hz).

1-(4-Methoxyphenyl)-3-oxocyclobutanecarbonitrile (144).



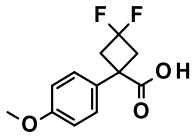
To a solution of **143** (3.64 g, 14.7 mmol) in acetone (40 mL) was added 6 mol/L aqueous HCl solution (10 mL), followed by heating to 60 °C and stirring for 1 h. The organic solvent was distilled off, neutralized with 5 mol/L aqueous NaOH and a saturated aqueous $NaHCO_3$ solution, and then extracted with diethyl ether. The organic layer was washed with saturated brine and dried over sodium sulfate. The residue obtained by filtration and concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **144** (2.23 g, 11.1 mmol, 75% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 3.64–3.72 (2H, m), 3.84 (3H, s), 3.99–4.07 (2H, m), 6.93–6.99 (2H, m), 7.37–7.43 (2H, m).

3,3-Difluoro-1-(4-methoxyphenyl)cyclobutanecarbonitrile (145).



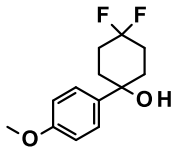
A solution of **144** (2.00 g, 9.94 mmol) in DCM (20 mL) was cooled to 0 °C and supplemented in a dropwise manner with a solution of DAST (3.30 mL, 25.2 mmol) in DCM (10 mL), followed by stirring at rt for 20 h. The reaction mixture was diluted with DCM and then sequentially washed with a saturated aqueous $NaHCO_3$ solution and with saturated brine. The organic layer was dried over sodium sulfate, filtered, and concentrated, and the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **145** (1.85 g, 8.29 mmol, 83% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 3.10–3.25 (2H, m), 3.43–3.55 (2H, m), 3.83 (3H, s), 6.92–6.99 (2H, m), 7.33–7.41 (2H, m).

3,3-Difluoro-1-(4-methoxyphenyl)cyclobutanecarboxylic acid (146).



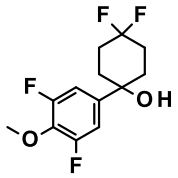
A mixture of KOH (955 mg, 17.0 mmol) and ethylene glycol (7.6 mL) was heated and dissolved. The solution was cooled to rt, and then **145** (380 mg, 1.70 mmol) was added and stirred at 150 °C for 5 min under a N_2 atmosphere. The reaction mixture was cooled to rt, diluted with water, and then washed with diethyl ether. The aqueous layer was acidified with concentrated HCl and then extracted with diethyl ether. The organic layer was dried over sodium sulfate, filtered, and concentrated, and the obtained residue was purified by silica gel column chromatography (chloroform/methanol) to obtain compound **146** (257 mg, 1.06 mmol, 62% yield) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 2.96–3.11 (2H, m), 3.38–3.51 (2H, m), 3.80 (3H, s), 6.86–6.93 (2H, m), 7.19–7.28 (2H, m).

4,4-Difluoro-1-(4-methoxyphenyl)cyclohexanol (148).



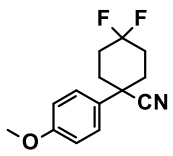
A mixture of (4-methoxyphenyl)magnesium bromide (0.5 mol/L, THF solution, 44 mL, 20.0 mmol) and THF (40 mL) was cooled to 0 °C, supplemented with a solution of 4,4-difluorocyclohexanone (**147**, 2.68 g, 20.0 mmol) in THF (10 mL) in a dropwise manner, and then stirred for 1 h while increasing the temperature to rt. Next, the reaction mixture was cooled to 0 °C, slowly supplemented with 0.5 mol/L aqueous HCl solution, and then extracted with diethyl ether. The residue obtained by washing the organic layer with saturated brine, drying over anhydrous sodium sulfate, filtering, and concentrating was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **148** (3.62 g, 14.9 mmol, 75% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.43 (1H, s), 1.83–1.94 (2H, m), 1.97–2.18 (4H, m), 2.19–2.39 (2H, m), 3.81 (3H, s), 6.86–6.93 (2H, m), 7.39–7.45 (2H, m).

1-(3,5-Difluoro-4-methoxyphenyl)-4,4-difluorocyclohexanol (149).



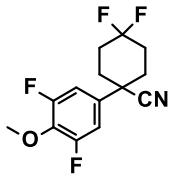
Under ice-cooling, to a solution of 4-bromo-2,6-difluoroanisole (5.00 g, 22.4 mmol) in THF (50 mL) was added isopropyl magnesium chloride-lithium chloride (1.3 mol/L, THF solution, 19 mL, 24.7 mmol), followed by stirring for 1 h. **147** (3.61 g, 26.9 mmol) was added and stirred at the same temperature for 1 h, then 1 mol/L aqueous HCl solution was added, and the mixture was extracted with ethyl acetate, washed with saturated brine, and dried over anhydrous magnesium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **149** (3.88 g, 13.9 mmol, 62% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.81–1.85 (2H, m), 2.02–2.11 (4H, m), 2.17–2.35 (2H, m), 3.99 (3H, s), 7.01–7.05 (2H, m).

4,4-Difluoro-1-(4-methoxyphenyl)cyclohexanecarbonitrile (151).



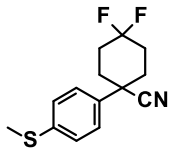
Under a N_2 atmosphere, to a solution of indium(III) bromide (266 mg, 0.751 mmol) and trimethylsilyl cyanide (1.90 mL, 15.0 mmol) in DCM (15 mL) was added a solution of **148** (1.82 g, 7.51 mmol) in DCM (15 mL) in a dropwise manner, followed by stirring for 30 min. The residue obtained by concentrating the reaction mixture was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **151** (1.82 g, 7.24 mmol, 96% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 2.06–2.40 (8H, m), 3.82 (3H, s), 6.90–6.96 (2H, m), 7.37–7.44 (2H, m).

1-(3,5-Difluoro-4-methoxyphenyl)-4,4-difluorocyclohexanecarbonitrile (152).



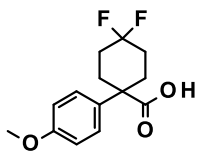
In a manner similar to that employed for the synthesis of **151**, the title compound **152** (3.128 g, 10.89 mmol, 78% yield) was obtained using **149** (3.88 g, 13.9 mmol). ^{1}H NMR ($CDCl_3$) δ: 2.03–2.11 (2H, m), 2.18–2.36 (6H, m), 4.02 (3H, s), 7.02–7.09 (2H, m).

4,4-Difluoro-1-[4-(methylsulfanyl)phenyl]cyclohexanecarbonitrile (153).



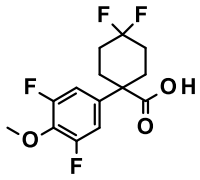
To a suspension of Mg (906 mg, 37.3 mmol) in THF (100 mL) was added 1-bromo-4-(methylsulfamoyl)benzene (3.81 mL, 28.0 mmol), followed by stirring at 65 °C for 2 h. **147** (2.50 g, 18.6 mmol) in THF (20 mL) was added, the mixture was stirred at rt for 3 h, and then 2 mol/L aqueous HCl solution was added, after which the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain crude compound **150** (2.94 g, 11.4 mmol, 61% yield) as a pale-yellow oil. Under a N_2 atmosphere, to a solution of indium(III) bromide (403 mg, 1.14 mmol) and trimethylsilyl cyanide (2.26 g, 22.8 mmol) in DCM (30 mL) was added a solution of crude compound **150** (2.94 g) in DCM (10 mL) in a dropwise manner, followed by stirring for 3 h. The residue obtained by concentrating the reaction mixture was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **153** (1.12 g, 4.17 mmol, 37% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 2.10–2.35 (8H, m), 2.49 (3H, s), 7.28 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 8.5 Hz).

4,4-Difluoro-1-(4-methoxyphenyl)cyclohexane carboxylic acid (154).



In a manner similar to that employed for the synthesis of **146**, the title compound **154** (477 mg, 1.76 mmol, 88% yield) was obtained using **151** (503 mg, 2.00 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.88–2.15 (6H, m), 2.47–2.62 (2H, m), 3.80 (3H, s), 6.86–6.93 (2H, m), 7.32–7.38 (2H, m).

1-(3,5-Difluoro-4-methoxyphenyl)-4,4-difluorocyclohexanecarboxylic acid (155).

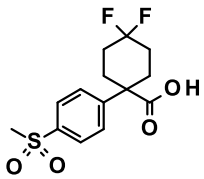


To a solution of **152** (1.00 g, 3.48 mmol) in toluene (10 mL) was added diisobutylaluminum hydride (0.97 mol/L, toluene solution, 5.2 mL, 5.22 mmol). After stirring for 20 min, a saturated aqueous L-(+)-potassium sodium tartrate solution was added and stirred at rt for 5 min. The mixture was extracted with ethyl acetate, and the organic layer was washed with 1 mol/L aqueous HCl solution and with saturated brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated to obtain crude aldehyde (0.982 g, 3.38 mmol) as a colorless oil. Under ice-cooling, to a mixture of this crude compound (982 mg), *tert*-butyl alcohol (15 mL), and water (3 ml) were sequentially added 2-methyl-2-butene (1.8 mL, 16.9 mmol), sodium dihydrogenphosphate (812 mg, 6.77 mmol), and sodium chlorite (612 mg, 6.77 mmol) and stirred at rt for 3 h. Then, 1 mol/L aqueous HCl solution was added, the mixture was extracted with ethyl acetate, and the organic layer was washed with saturated brine and dried over anhydrous magnesium sulfate. The residue obtained by concentration was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **155** (603 mg, 1.97 mmol, 57% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.90–2.18 (6H, m), 2.51–2.53 (2H, m), 4.00 (3H, s), 6.96–7.01 (2H, m).

4,4-Difluoro-1-[4-(methylsulfanyl)phenyl]cyclohexanecarboxylic acid (156).

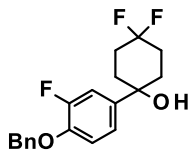
In a manner similar to that employed for the synthesis of **146**, the title compound **156** (944 mg, 3.30 mmol, 79% yield) was obtained using **153** (1.12 g, 4.17 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.82–2.09 (6H, m), 2.37–2.44 (2H, m), 2.46 (3H, s), 7.25 (2H, d, J = 8.5 Hz), 7.36 (2H, d, J = 8.5 Hz), 12.80 (1H, brs).

4,4-Difluoro-1-[4-(methylsulfonyl)phenyl]cyclohexanecarboxylic acid (157).



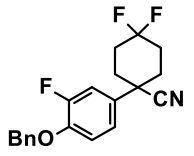
To a solution of **156** (400 mg, 1.40 mmol) in acetic acid (4 mL) was added hydrogen peroxide water (concentration 35%, 0.575 mL) at 0 °C. After stirring at 50 °C for 7 h, the mixed reaction solution was diluted with water, and the resulting solid was collected by filtration to obtain compound **157** (208 mg, 0.654 mmol, 47% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.87–2.10 (6H, m), 2.40–2.49 (2H, m), 3.22 (3H, s), 7.71 (2H, d, J = 8.5 Hz), 7.93 (2H, d, J = 8.5 Hz), 13.07 (1H, brs).

1-[4-(Benzyloxy)-3-fluorophenyl]-4,4-difluorocyclohexanol (158).



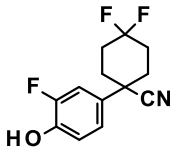
In a manner similar to that employed for the synthesis of **149**, the title compound **158** (6.21 g, 18.5 mmol, 66% yield) was obtained using 1-benzyloxy-4-bromo-2-fluoro-benzene (9.45 g, 33.6 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.47 (1H, s), 1.78–2.76 (8H, m), 5.14 (2H, s), 6.95–7.00 (1H, m), 7.10–7.14 (1H, m), 7.23–7.28 (1H, m), 7.31–7.45 (5H, m).

1-[4-(Benzyloxy)-3-fluorophenyl]-4,4-difluorocyclohexanecarbonitrile (159).



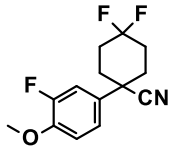
In a manner similar to that employed for the synthesis of **151**, the title compound **159** (2.50 g, 7.24 mmol, 55% yield) was obtained using **158** (4.40 g, 13.1 mmol). ^{1}H NMR ($CDCl_3$) δ: 2.01–2.38 (8H, m), 5.16 (2H, s), 6.98–7.04 (1H, m), 7.15–7.24 (2H, m), 7.31–7.46 (5H, m).

4,4-Difluoro-1-(3-fluoro-4-hydroxyphenyl)cyclohexanecarbonitrile (160).



In a manner similar to that employed for the synthesis of **126**, the title compound **160** (1.46 g, 5.72 mmol, 99% yield) was obtained using **159** (2.00 g, 5.79 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.99–2.35 (8H, m), 6.95–7.03 (1H, m), 7.15–7.21 (1H, m), 7.31–7.38 (1H, m), 10.20 (1H, brs).

4,4-Difluoro-1-(3-fluoro-4-methoxyphenyl)cyclohexanecarbonitrile (161).

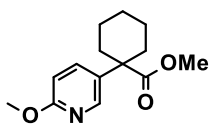


To a solution of **160** (500 mg, 1.96 mmol) in acetone (10 mL) were added K_2CO_3 (541 mg, 3.92 mmol) and iodomethane (556 mg, 3.92 mmol), followed by stirring for 4 h. The reaction solution was filtered through celite using DCM and the solvent was distilled off under reduced pressure. Crude product **161** was given as a pale-brown oil and could be used in the next step without further purification.

4,4-Difluoro-1-(3-fluoro-4-methoxyphenyl)cyclohexanecarboxylic acid (162).

In a manner similar to that employed for the synthesis of **155**, the title compound **162** (348 mg, 1.21 mmol, 59% yield) was obtained using **161** (550 mg, 2.04 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.78–2.15 (6H, m), 2.37–2.46 (2H, m), 3.83 (3H, s), 6.99–7.26 (3H, m), 12.83 (1H, brs).

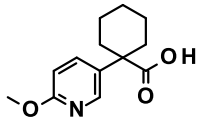
Methyl 1-(6-methoxypyridin-3-yl)cyclohexanecarboxylate (164).



Under a N_2 atmosphere, a solution of dicyclohexylamine (3.50 mL, 17.6 mmol) in toluene (150 mL) was ice-cooled, supplemented with *n*BuLi (1.6 mol/L, hexane solution, 11 mL, 17.6 mmol), and stirred at rt for 30 min. Methyl cyclohexanecarboxylate (3.0 mL, 20.7 mmol) was added at rt, stirred for 15 min,

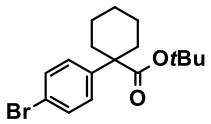
and then 5-bromo-2-methoxypyridine (**163**, 1.9 mL, 16.0 mmol), $Pd_2(dba)_3$ (735 mg, 0.798 mmol), and TTBP·HBF_4 (461 mg, 1.60 mmol) were added and stirred at rt overnight. Next, 1 mol/L aqueous HCl solution was added to the reaction solution and insoluble matter was filtered off. The filtrate was extracted with ethyl acetate three times, and the organic layer was washed with saturated brine and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) and then purified again by amino silica gel column chromatography (hexane/ethyl acetate) to obtain compound **164** (1.61 g, 6.46 mmol, 40% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.24–1.33 (1H, m), 1.41–1.52 (2H, m), 1.56–1.75 (5H, m), 2.42–2.47 (2H, m), 3.64 (3H, s), 3.92 (3H, s), 6.70 (1H, d, *J* = 8.8 Hz), 7.60 (1H, dd, *J* = 8.8, 2.7 Hz), 8.18 (1H, d, *J* = 2.7 Hz).

1-(6-Methoxypyridin-3-yl)cyclohexanecarboxylic acid (165).



To a solution of **164** (1.60 g, 6.42 mmol) in THF (40 mL) were added methanol (20 mL) and a 1 mol/L aqueous NaOH solution (19 mL), followed by stirring at 50 °C for 10 h. The reaction solution was acidified by adding 1 mol/L aqueous HCl solution and then extracted with ethyl acetate three times, and the organic layer was washed with saturated brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography (chloroform/methanol) to obtain compound **165** (1.17 g, 4.97 mmol, 78% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.25–1.34 (1H, m), 1.48–1.78 (7H, m), 2.42–2.45 (2H, m), 3.92 (3H, s), 6.72 (1H, d, *J* = 9.1 Hz), 7.66 (1H, d, *J* = 9.1 Hz), 8.23 (1H, s).

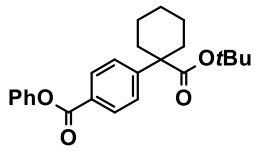
***tert*-Butyl 1-(4-bromophenyl)cyclohexanecarboxylate (167).**



To a suspension of 1-(4-bromophenyl)cyclohexanecarboxylic acid (**166**, 11.5 g, 41.0 mmol) in DCM (200 mL) were added thionyl chloride (5.6 mL, 81.9 mmol) and DMF (0.100 mL), followed by stirring at 40 °C for 4 h and then concentration under reduced pressure. The procedure of adding toluene to the residue and concentrating under reduced pressure was carried out twice to obtain a crude acid chloride. To a solution of the crude acid chloride in THF (200 mL) was added potassium *tert*-butoxide (1.0 mol/L, THF solution, 50 mL) in a dropwise manner at 0 °C, followed by stirring at rt overnight. After concentrating the reaction solution under reduced pressure, a 10% aqueous citric acid solution was added to the residue and the mixture was extracted with ethyl acetate three times. The organic layer was sequentially washed with a saturated aqueous $NaHCO_3$ solution and with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **167** (10.9 g, 32.1 mmol, 78% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.16–1.24

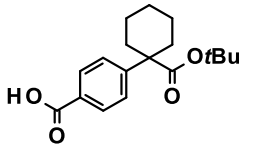
(1H, m), 1.36 (9H, s), 1.45–1.68 (7H, m), 2.37–2.40 (2H, m), 7.27 (2H, d, *J* = 8.5 Hz), 7.43 (2H, d, *J* = 8.5 Hz).

***tert*-Butyl 1-(4-acetylphenyl)cyclohexanecarboxylate (168).**



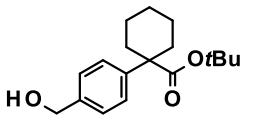
Under a N_2 atmosphere, to a mixture of compound **167** (10.9 g, 32.1 mmol), palladium(II) acetate (361 mg, 1.61 mmol), and TTBP·HBF_4 (1.86 g, 6.43 mmol) were added acetonitrile (50 mL), triethylamine (13.5 mL, 96.4 mmol), and phenyl formate (10.5 mL, 96.4 mmol), followed by stirring at 80 °C overnight. After allowing the reaction solution to cool to rt, water was added to it, the mixture was extracted with ethyl acetate three times, and the organic layer was washed with saturated brine and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **168** (8.62 g, 22.7 mmol, 71% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.24–1.33 (1H, m), 1.38 (9H, s), 1.49–1.58 (2H, m), 1.64–1.73 (5H, m), 2.44–2.47 (2H, m), 7.19–7.29 (3H, m), 7.40–7.45 (2H, m), 7.55 (2H, d, *J* = 8.5 Hz), 8.15 (2H, d, *J* = 8.5 Hz).

4-[1-(*tert*-Butoxycarbonyl)cyclohexyl]benzoic acid (169).



In a manner similar to that employed for the synthesis of **165**, the title compound **169** (6.82 g, 22.4 mmol, 99% yield) was obtained using **168** (8.62 g, 22.7 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.21–1.31 (1H, m), 1.37 (9H, s), 1.46–1.58 (2H, m), 1.62–1.72 (5H, m), 2.40–2.47 (2H, m), 7.51 (2H, d, *J* = 8.5 Hz), 8.06 (2H, d, *J* = 8.5 Hz).

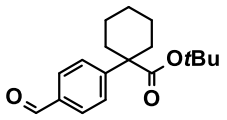
***tert*-Butyl 1-[4-(hydroxymethyl)phenyl]cyclohexanecarboxylate (170).**



To a solution of **169** (1.00 g, 3.29 mmol) in THF (15 mL) was added triethylamine (0.687 mL, 4.93 mmol), followed by ice-cooling, supplementation with isobutyl chloroformate (0.518 mL, 3.94 mmol), and then stirring at the same temperature for 30 min. After separating insoluble matter by filtration, the filtrate was ice-cooled, supplemented with sodium borohydride (620 mg, 16.4 mmol) and water (3 mL), and then stirred at the same temperature for 15 min and at rt for 1 h. A saturated aqueous NH_4Cl solution was added to the reaction solution, the mixture was extracted with ethyl acetate three times, and the organic layer was washed with saturated brine and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **170** (861 mg, 2.96 mmol, 90% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.21–1.29 (1H, m), 1.37 (9H, s), 1.41–1.54 (2H, m), 1.60–1.71 (6H, m), 2.39–2.45 (2H, m), 4.67 (2H, d, *J* = 5.4 Hz), 7.31 (2H, d, *J* = 8.5 Hz), 7.39 (2H, d,

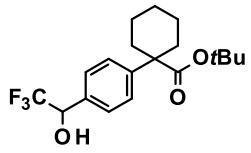
J = 8.5 Hz).

***tert*-Butyl 1-(4-formylphenyl)cyclohexanecarboxylate (171).**



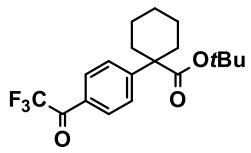
To a suspension of Dess-Martin periodinane (4.56 g, 10.7 mmol) in DCM (24 mL) was added a solution of **170** (2.08 g, 7.16 mmol) in DCM (35 mL), followed by stirring at rt for 3.5 h. To the reaction solution were added diethyl ether, a saturated aqueous $NaHCO_3$ solution, and sodium thiosulfate pentahydrate (18.9 g, 75.2 mmol), followed by stirring at rt for 1 h, after which the liquid was separated. The aqueous layer was extracted with diethyl ether twice, and the organic layers were combined, washed with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **171** (1.39 g, 4.82 mmol, 67% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.24–1.37 (10H, m), 1.48–1.57 (2H, m), 1.62–1.72 (5H, m), 2.44 (2H, d, J = 11.5 Hz), 7.57 (2H, d, J = 8.5 Hz), 7.84 (2H, d, J = 8.5 Hz), 10.00 (1H, s).

***tert*-Butyl 1-[4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl]cyclohexanecarboxylate (172).**



Under a N_2 atmosphere, to a solution of **171**(1.39 g, 4.82 mmol) in DMF (25 mL) were added (trifluoromethyl)trimethylsilane (0.855 mL, 5.78 mmol) and K_2CO_3 (12.0 mg, 0.0964 mmol), followed by stirring at rt for 4 h, after which K_2CO_3 (53.0 mg, 0.386 mmol) was added and stirring was performed at rt for 2.5 h. (Trifluoromethyl)trimethylsilane (1.07 mL, 7.23 mmol) and K_2CO_3 (65.0 mg, 0.482 mmol) were further added, followed by stirring at rt for 1.5 h. After ice-cooling the reaction solution, TBAF (1 mol/L, THF solution, 19.5 mL) was added and stirring was performed at rt for 30 min. Water and saturated brine were added to the reaction solution, the mixture was extracted with diethyl ether three times, and the organic layer was washed with saturated brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **172** (1.65 g, 4.60 mmol, 96% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.21–1.29 (1H, m), 1.37 (9H, s), 1.46–1.70 (7H, m), 2.43 (2H, d, J = 10.9 Hz), 2.53–2.58 (1H, m), 4.97–5.03 (1H, m), 7.40–7.45 (4H, m).

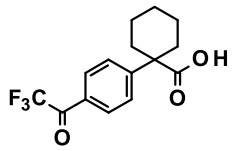
***tert*-Butyl 1-[4-(trifluoroacetyl)phenyl]cyclohexanecarboxylate (173).**



To a solution of **172** (1.65 g, 4.60 mmol) in DCM (25 mL) was added manganese dioxide (2.35 g, 23.0 mmol), followed by stirring at rt overnight. Manganese dioxide (2.35 g, 23.0 mmol) was further added and stirred at rt overnight. After stirring at 40 °C for 8 h, the reaction solution was filtered through celite, the filtrate was concentrated under reduced pressure, and then the obtained residue was

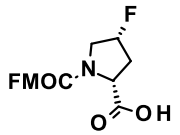
purified by silica gel column chromatography (chloroform) to obtain compound **173** (1.50 g, 4.21 mmol, 91% yield) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.21–1.34 (1H, m), 1.38 (9H, s), 1.48–1.58 (2H, m), 1.61–1.73 (5H, m), 2.42–2.45 (2H, m), 7.58 (2H, d, *J* = 8.5 Hz), 8.03 (2H, d, *J* = 8.5 Hz).

1-[4-(Trifluoroacetyl)phenyl]cyclohexanecarboxylic acid (174).



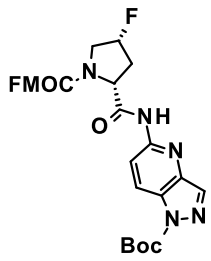
To a solution of **173** (1.50 g, 4.21 mmol) in DCM (20 mL) was added TFA (20 mL) under ice-cooling and stirred at rt for 2 h. After concentrating the reaction solution under reduced pressure, DCM was added to the residue, the mixture was washed with water, and then the organic layer was dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained solid was suspended in hexane, collected by filtration, and dried to obtain compound **174** (1.11 g, 3.70 mmol, 88% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.26–1.35 (1H, m), 1.52–1.72 (5H, m), 1.77–1.84 (2H, m), 2.46–2.49 (2H, m), 7.63 (2H, d, *J* = 8.5 Hz), 8.05 (2H, d, *J* = 8.5 Hz).

(4*R*)-1-[(9H-Fluoren-9-ylmethoxy)carbonyl]-4-fluoro-D-proline (175).



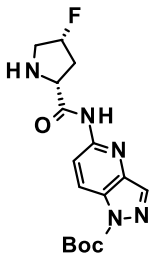
To (4*R*)-1-(*tert*-butoxycarbonyl)-4-fluoro-D-proline (**80**, 3.00 g, 12.9 mmol) was added 4 mol/L HCl (1,4-dioxane solution, 30 mL), followed by stirring at rt for 4 h, after which the mixture was concentrated under reduced pressure to obtain a solid. This solid was dissolved in water (60 mL), ice-cooled, supplemented with $NaHCO_3$ (5.40 g, 64.3 mmol), 1,4-dioxane (60 mL), and 9-fluorenylmethyl chloroformate (4.00 g, 15.4 mmol), and stirred at rt overnight. Water was added to the reaction solution, the mixture was washed with diethyl ether twice, and the aqueous layer was acidified by adding 1 mol/L aqueous HCl solution. The layer was extracted with chloroform three times, and the organic layer was dried over anhydrous sodium sulfate, and then filtered and concentrated under reduced pressure. Water was added to the obtained residue, and the mixture was solidified by ultrasonication and left to stand in a refrigerator overnight. The precipitated solid was collected by filtration and dried to obtain compound **175** (4.00 g, 11.3 mmol, 88% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 2.23–2.68 (2H, m), 3.54–3.75 (2H, m), 4.18–4.51 (4H, m), 5.32 (1H, dd, *J* = 52.9, 3.9 Hz), 7.31–7.35 (2H, m), 7.42–7.44 (2H, m), 7.65–7.68 (2H, m), 7.90 (2H, t, *J* = 7.0 Hz), 12.73 (1H, brs).

***tert*-Butyl 5-({(4*R*)-1-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-4-fluoro-D-prolyl}amino)-1*H*-pyrazolo[4,3-*b*]pyridine-1-carboxylate (177).**



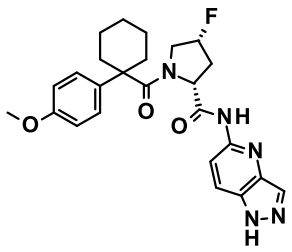
To a solution of **175** (300 mg, 0.844 mmol) in DCM (5 mL) were added thionyl chloride (0.612 mL, 8.44 mmol) and DMF (0.010 mL), followed by stirring at rt for 3 h and then at 40 °C for 30 min. After concentrating the reaction solution under reduced pressure, the residue was dissolved in DCM (1 mL), supplemented with hexane (10 mL), and the precipitated solid was collected by filtration and dried to obtain the acid chloride **176** (261 mg) as a pale-yellow solid. To a mixture of **123** (162 mg, 0.696 mmol), DIPEA (0.157 mL, 0.904 mmol), and DCM (3 mL) was added a solution of this intermediate **176** in DCM (3 mL) in a dropwise manner under ice-cooling, followed by stirring at rt for 1.5 h. Then, 1 mol/L aqueous HCl solution was added to the reaction solution, the mixture was extracted with DCM three times, and the organic layer was dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **177** (320 mg, 0.560 mmol, 66% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.66 (9H, s), 2.32–2.41 (1H, m), 2.52–2.70 (1H, m), 3.66–3.83 (2H, m), 4.23–4.32 (3H, m), 4.65 (1H, d, J = 9.7 Hz), 5.29 (1H, d, J = 53.8 Hz), 7.15–7.38 (4H, m), 7.63 (2H, s), 7.81 (2H, s), 8.26–8.29 (1H, m), 8.39–8.42 (2H, m), 10.44 (1H, s).

***tert*-Butyl 5-{[(4*R*)-4-fluoro-D-prolyl]amino}-1*H*-pyrazolo[4,3-*b*]pyridine-1-carboxylate (178).**



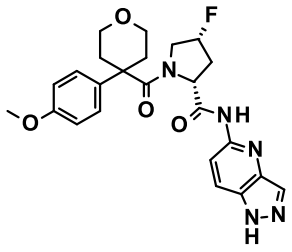
To a solution of **177** (310 mg, 0.542 mmol) in DMF (10 mL) was added piperidine (0.500 mL, 5.05 mmol) under ice-cooling, followed by stirring under ice-cooling for 15 min and at rt for 15 min. Water was added to the reaction solution, the mixture was extracted with ethyl acetate three times, and the organic layer was washed with saturated brine and then dried over anhydrous sodium sulfate. Next, the aqueous layers were combined, extracted with DCM twice, and the organic layer was dried over anhydrous sodium sulfate. The obtained organic layers were combined, filtered, concentrated under reduced pressure, and then the residue was purified by silica gel column chromatography (ethyl acetate/methanol) to obtain compound **178** (174 mg, 0.498 mmol, 92% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.66 (9H, s), 2.21–2.41 (2H, m), 3.07–3.26 (2H, m), 3.54 (1H, s), 3.92 (1H, d, J = 7.3 Hz), 5.26 (1H, d, J = 54.4 Hz), 8.41–8.48 (3H, m), 10.57 (1H, s).

(4*R*)-4-Fluoro-1-{[1-(4-methoxyphenyl)cyclohexyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (104).



To a solution of **56** (2.58 g, 11.0 mmol) in DCM (78 mL) were added thionyl chloride (1.60 mL, 22.1 mmol) and DMF (0.085 mL), and the mixture was stirred at 40 °C for 30 min. The reaction mixture was concentrated under reduced pressure to obtain the crude acid chloride. To a solution of **178** (2.40 g, 6.87 mmol) in DCM (40 mL) was added the crude acid chloride (2.60 g, 10.3 mmol) in DCM (14 mL) at 0 °C, and the mixture was stirred at 0 °C for 3 h. To the resultant mixture was added a saturated aqueous NH_4Cl solution, followed by extraction with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was subjected to silica gel column chromatography (hexane/ethyl acetate) to obtain the intermediate (3.88 g) as a colorless solid. To a solution of this intermediate in DCM (40 mL) was added TFA (40 mL) under ice-cooling, followed by stirring at rt for 2 h. After concentrating the reaction solution under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain **104** (1.62 g, 3.48 mmol, 51% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.25–2.23 (10H, m), 2.31–2.47 (2H, m), 2.54 (1H, t, *J* = 16.9 Hz), 3.42–3.64 (1H, br m), 3.82 (3H, s), 4.77–4.92 (1H, m), 5.02 (1H, d, *J* = 53.2 Hz), 6.93 (2H, d, *J* = 8.5 Hz), 7.23–7.31 (2H, m), 7.84 (1H, d, *J* = 9.1 Hz), 8.14 (1H, s), 8.38 (1H, d, *J* = 9.1 Hz), 8.57 (1H, brs), 10.22 (1H, brs). MS (ESI/APCI) *m/z*: 466.3 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{29}FN_5O_3$ $(M + H)^+$, 466.2249; found, 466.2266.

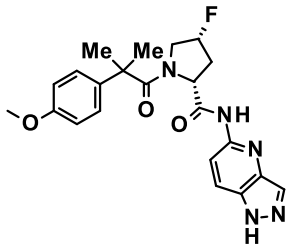
(4*R*)-4-Fluoro-1-{[4-(4-methoxyphenyl)tetrahydro-2*H*-pyran-4-yl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (107).



To a suspension of **142a** (180 mg, 0.751 mmol) in DCM (5 mL) were added oxalyl chloride (0.318 mL, 3.76 mmol) and DMF (0.0100 mL), and the mixture was stirred at rt for 1 h. Then, the mixture was concentrated under reduced pressure to obtain the crude acid chloride as a pale-yellow oil. To a solution of **178** (175 mg, 0.501 mmol) in DCM (5 mL) was added DIPEA (0.262 mL, 1.50 mmol). Then, the mixture was cooled with ice, supplemented with a solution of the acid chloride obtained above in DCM (5 mL), and stirred at rt for 30 min. To the reaction solution was added a saturated aqueous NH_4Cl solution and the mixture was extracted with DCM three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and then dried over anhydrous sodium sulfate. The resultant mixture was filtered, concentrated under reduced pressure, and then the obtained residue was purified by silica gel column chromatography (ethyl acetate/methanol) to obtain the crude compound (305 mg) as a colorless solid. To a solution of this crude compound in THF (2 mL) were added methanol (5 mL) and K_2CO_3 (140 mg, 1.00 mmol), and the mixture was stirred at rt for 2 h. To the reaction solution was

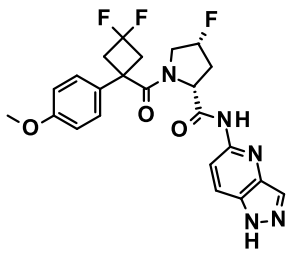
added 1 mol/L aqueous HCl solution, after which the mixture was extracted with ethyl acetate three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (chloroform/methanol) to obtain compound **107** (103 mg, 0.220 mmol, 44% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6, 100 °C) δ: 1.83–2.38 (6H, m), 3.22–3.36 (2H, m), 3.67–3.76 (7H, m), 4.77 (1H, d, J = 9.1 Hz), 5.05 (1H, d, J = 53.2 Hz), 6.95 (2H, d, J = 8.5 Hz), 7.26 (2H, d, J = 8.5 Hz), 7.98–8.08 (3H, m), 9.91 (1H, s), 13.03 (1H, s). MS (ESI/APCI) *m/z*: 468.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{24}H_{27}FN_5O_4$ $(M + H)^+$, 468.2042; found, 468.2044.

(4*R*)-4-Fluoro-1-[2-(4-methoxyphenyl)-2-methylpropanoyl]-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (108).



In a manner similar to that employed for the synthesis of **107**, the title compound **108** (145 mg, 0.341 mmol, 68% yield) was obtained using **142b** (150 mg, 0.751 mmol) and **178** (175 mg, 0.501 mmol). ^{1}H NMR (DMSO-d_6, 100 °C) δ: 1.45 (6H, s), 2.05–2.16 (1H, m), 2.27–2.43 (1H, m), 3.02–3.39 (2H, m), 3.75 (3H, s), 4.74 (1H, d, J = 8.5 Hz), 5.07 (1H, d, J = 54.4 Hz), 6.93 (2H, d, J = 8.5 Hz), 7.26 (2H, d, J = 8.5 Hz), 7.97–8.10 (3H, m), 9.86 (1H, s), 13.03 (1H, s). MS (ESI/APCI) *m/z*: 426.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{22}H_{25}FN_5O_3$ $(M + H)^+$, 426.1936; found, 426.1945.

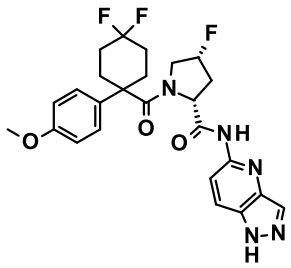
(4*R*)-1-{[3,3-Difluoro-1-(4-methoxyphenyl)cyclobutyl]carbonyl}-4-fluoro-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (109).



To a solution of **146** (173 mg, 0.716 mmol) in DMF (7 mL) were added COMU (368 mg, 0.859 mmol) and DIPEA (0.25 mL, 1.43 mmol), and the mixture was stirred at rt for 15 min. **178** (250 mg, 0.716 mmol) was then added and the mixture was stirred at rt overnight. A saturated aqueous NH_4Cl solution was added to the reaction solution and the mixture was extracted with ethyl acetate three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the intermediate (323 mg) as a white solid. To a solution of this intermediate in DCM (6 mL) was added TFA (3 mL) under ice-cooling, and the mixture was stirred at rt for 2 h. After the reaction solution was concentrated under reduced pressure, a saturated aqueous $NaHCO_3$ solution was added to the obtained residue. The mixture was extracted with DCM three times and the organic layer was dried over

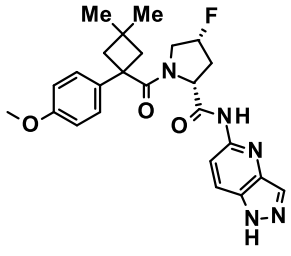
anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **109** (157 mg, 0.332 mmol, 46% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 100 °C) δ: 2.14–2.28 (1H, m), 2.30–2.52 (1H, m), 2.93–3.10 (2H, m), 3.19–3.57 (4H, m), 3.75 (3H, s), 4.66–4.87 (1H, m), 5.07–5.27 (1H, m), 6.94 (2H, d, J = 7.9 Hz), 7.36 (2H, d, J = 7.9 Hz), 7.92–8.08 (3H, m), 9.85 (1H, brs), 12.95 (1H, brs). MS (ESI/APCI) *m/z*: 474.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{23}H_{23}F_3N_5O_3$ $(M + H)^+$, 474.1748; found, 474.1749.

(4*R*)-1-{[4,4-Difluoro-1-(4-methoxyphenyl)cyclohexyl]carbonyl}-4-fluoro-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (110, DS-9300).



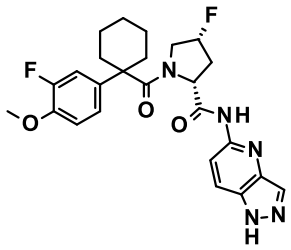
In a manner similar to that employed for the synthesis of **109**, the title compound **110** (247 mg, 0.493 mmol, 69% yield) was obtained using **154** (193 mg, 0.716 mmol) and **178** (250 mg, 0.716 mmol). ^{1}H NMR (DMSO-d_6, 100 °C) δ: 1.74–1.86 (1H, m), 1.88–2.53 (9H, m), 3.19–3.42 (2H, m), 3.72–3.80 (3H, m), 4.74–4.84 (1H, m), 4.9–5.15 (1H, m), 6.90–6.98 (2H, m), 7.22–7.31 (2H, m), 7.93–8.09 (3H, m), 9.91 (1H, brs), 12.95 (1H, brs). MS (ESI/APCI) *m/z*: 502.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{27}F_3N_5O_3$ $(M + H)^+$, 502.2061; found, 502.2060. Anal. calculated for $C_{25}H_{26}F_3N_5O_3$: C, 59.87; H, 5.23; N, 13.97; F, 11.37. Found: C, 59.76; H, 5.28; N, 13.81; F, 11.46.

(4*R*)-4-Fluoro-1-{[1-(4-methoxyphenyl)-3,3-dimethylcyclobutyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (111).



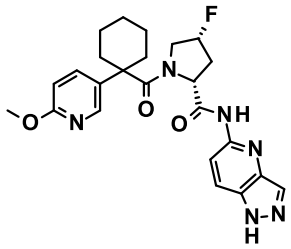
In a manner similar to that employed for the synthesis of **109**, the title compound **111** (198 mg, 0.425 mmol, 59% yield) was obtained using **142c** (168 mg, 0.716 mmol) and **178** (250 mg, 0.716 mmol). ^{1}H NMR (DMSO-d_6, 100 °C) δ: 1.07 (3H, s), 1.09 (3H, s), 2.11–2.45 (4H, m), 2.63–2.77 (2H, m), 3.26–3.48 (2H, m), 3.72 (3H, s), 4.72 (1H, d, J = 8.5 Hz), 5.14 (1H, d, J = 53.5 Hz), 6.89 (2H, d, J = 8.5 Hz), 7.29 (2H, d, J = 8.5 Hz), 7.91–8.10 (3H, m), 9.64 (1H, brs), 12.95 (1H, brs). MS (ESI/APCI) *m/z*: 466.3 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{29}FN_5O_3$ $(M + H)^+$, 466.2249; found, 466.2247.

(4*R*)-4-Fluoro-1-{[1-(3-fluoro-4-methoxyphenyl)cyclohexyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (112).



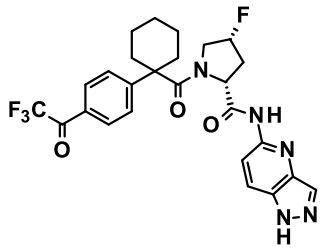
In a manner similar to that employed for the synthesis of **104**, the title compound **112** (186 mg, 0.385 mmol, 81% yield) was obtained using **142d** (200 mg, 0.793 mmol) and **178** (166 mg, 0.475 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.23–1.37 (1H, m), 1.60–1.82 (7H, m), 2.07–2.59 (4H, m), 3.01–3.40 (1H, m), 3.50–3.65 (1H, m), 3.91 (3H, s), 4.77–4.93 (1H, m), 5.06 (1H, d, *J* = 52.6 Hz), 6.99–7.13 (3H, m), 7.82 (1H, d, *J* = 9.1 Hz), 8.12 (1H, s), 8.33 (1H, d, *J* = 9.1 Hz), 8.52 (1H, s), 10.50 (1H, s). MS (ESI/APCI) *m/z*: 484.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{28}F_2N_5O_3$ $(M + H)^+$, 484.2155; found, 484.2162.

(4*R*)-4-Fluoro-1-{[1-(6-methoxypyridin-3-yl)cyclohexyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (113).



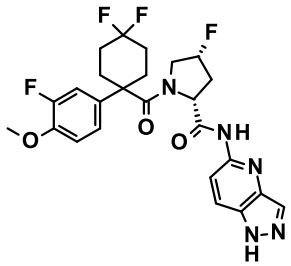
To a suspension of **165** (270 mg, 1.15 mmol) in DCM (10 mL) were added oxalyl chloride (0.147 mL, 1.71 mmol) and DMF (1 drop), and the mixture was stirred at rt for 30 min. Then, the mixture was concentrated under reduced pressure to obtain the crude acid chloride as a pale-yellow oil. To a solution of **178** (200 mg, 0.572 mmol) in DCM (10 mL) was added DIPEA (0.350 mL, 2.00 mmol). Then, the mixture was cooled with ice, a solution of the acid chloride obtained above in DCM (5 mL) was added, and the mixture was stirred at rt for 2 h. To the reaction solution was added a saturated aqueous NH_4Cl solution and the mixture was extracted with DCM three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and then dried over anhydrous sodium sulfate. The resultant sample was filtered, concentrated under reduced pressure, and then the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain the intermediate (170 mg) as a pale-yellow solid. To a solution of this intermediate in DCM (3 mL) was added TFA (3 mL) under ice-cooling, followed by stirring at rt for 1 h. After concentrating the reaction solution under reduced pressure, the obtained residue was purified by silica gel column chromatography (ethyl acetate/methanol) to obtain **113** (95.9 mg, 0.206 mmol, 36% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6, 100 °C) δ: 1.24–2.40 (12H, m), 3.04–3.53 (2H, m), 3.85 (3H, s), 4.74–4.83 (1H, m), 5.09 (1H, d, *J* = 53.8 Hz), 6.75–6.79 (1H, m), 7.59–7.64 (1H, m), 7.96–8.17 (4H, m), 10.00 (1H, s), 13.06 (1H, s). MS (ESI/APCI) *m/z*: 467.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{24}H_{28}FN_6O_3$ $(M + H)^+$, 467.2201; found, 467.2217.

(4*R*)-4-Fluoro-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-1-({1-[4-(trifluoroacetyl)phenyl]cyclohexyl}carbonyl)-D-prolinamide (114).



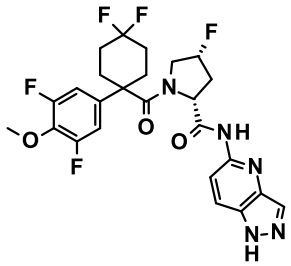
In a manner similar to that employed for the synthesis of **113**, the title compound **114** (149 mg, 0.280 mmol, 56% yield) was obtained using **174** (180 mg, 0.601 mmol) and **178** (175 mg, 0.501 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.27–1.43 (1H, m), 1.66–1.96 (7H, m), 2.11–2.59 (4H, m), 3.09–3.59 (2H, m), 4.75–4.92 (1H, m), 5.06 (1H, d, *J* = 52.0 Hz), 7.60 (2H, d, *J* = 8.5 Hz), 7.84 (1H, d, *J* = 9.1 Hz), 8.14–8.19 (3H, m), 8.34 (1H, d, *J* = 9.1 Hz), 8.44 (1H, s), 10.60 (1H, s). MS (ESI/APCI) *m/z*: 530.2 $(M - H)^-$. HRMS (ESI) *m/z*: calculated for $C_{26}H_{24}F_4N_5O_3$ $(M - H)^-$, 530.1821; found, 530.1796.

(4*R*)-1-{[4,4-Difluoro-1-(3-fluoro-4-methoxyphenyl)cyclohexyl]carbonyl}-4-fluoro-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (116).



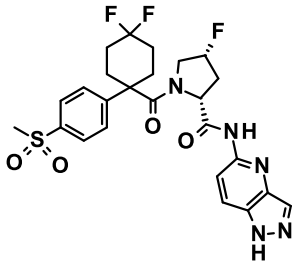
To a solution of **162** (330 mg, 1.15 mmol) in DCM (6 mL) were added thionyl chloride (0.087 mL, 1.20 mmol) and DMF (0.012 mL), and the mixture was stirred at 40 °C for 2 h. The reaction mixture was concentrated under reduced pressure to obtain the crude acid chloride. To a solution of **178** (210 mg, 0.601 mmol) in DCM (6 mL) was added the crude acid chloride obtained above in DCM (6 mL) at 0 °C, and the mixture was stirred at rt for 30 min. To the resultant mixture was added 1 mol/L aqueous HCl solution, followed by extraction of the mixture with DCM. The organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure to obtain the crude compound (328 mg) as a white solid. To a solution of this crude compound in methanol (5 mL) was added K_2CO_3 (146 mg, 1.06 mmol), and the mixture was stirred at rt for 2 h. To the reaction solution was added 1 mol/L aqueous HCl solution, followed by extraction of the mixture with ethyl acetate three times. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution and with saturated brine, and then dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate) to obtain compound **116** (193 mg, 0.372 mmol, 62% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.66–1.78 (1H, m), 1.91–2.49 (9H, m), 3.02–3.16 (1H, m), 3.27–3.36 (1H, m), 3.85 (3H, s), 4.79–4.85 (1H, m), 5.09 (1H, d, *J* = 54.1 Hz), 7.11–7.28 (3H, m), 8.06 (1H, d, *J* = 9.1 Hz), 8.12–8.18 (2H, m), 10.59 (1H, s), 13.29 (1H, s). MS (ESI/APCI) *m/z*: 520.3 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{26}F_4N_5O_3$ $(M + H)^+$, 520.1966; found, 520.1966.

(4*R*)-1-{[1-(3,5-Difluoro-4-methoxyphenyl)-4,4-difluorocyclohexyl]carbonyl}-4-fluoro-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (117).



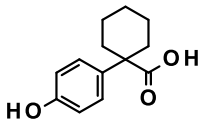
In a manner similar to that employed for the synthesis of **113**, the title compound **117** (158 mg, 0.294 mmol, 38% yield) was obtained using **155** (200 mg, 0.653 mmol) and **178** (288 mg, 0.784 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.80–2.20 (6H, m), 2.23–2.47 (4H, m), 3.35–3.49 (2H, m), 3.92 (3H, s), 4.82 (1H, d, J = 7.9 Hz), 5.12 (1H, d, J = 54.1 Hz), 7.08 (2H, d, J = 9.7 Hz), 7.95–8.06 (3H, m), 10.04 (1H, s), 12.89 (1H, brs). MS (ESI/APCI) *m/z*: 538.3 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{25}F_5N_5O_3$ $(M + H)^+$, 538.1872; found, 538.1871.

(4*R*)-1-({4,4-Difluoro-1-[4-(methylsulfonyl)phenyl]cyclohexyl}carbonyl)-4-fluoro-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (118).



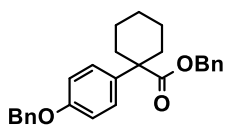
In a manner similar to that employed for the synthesis of **109**, the title compound **118** (19.5 mg, 0.0355 mmol, 5.5% yield) was obtained using **157** (206 mg, 0.647 mmol) and **178** (250 mg, 0.716 mmol). ^{1}H NMR (DMSO-d_6, 100 °C) δ: 1.82–2.45 (10H, m), 3.16 (3H, s), 3.21–3.37 (2H, m), 4.78–4.85 (1H, m), 5.07 (1H, d, J = 53.7 Hz), 7.65 (2H, d, J = 7.9 Hz), 7.91–8.07 (5H, m), 10.06 (1H, s), 13.00 (1H, s). MS (ESI/APCI) *m/z*: 550.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{25}H_{27}F_3N_5O_4S$ $(M + H)^+$, 550.1730; found, 550.1745.

1-(4-Hydroxyphenyl)cyclohexanecarboxylic acid (179).



Under a N_2 atmosphere, **56** (5.00 g, 21.3 mmol) was suspended in DCM (50.0 mL), cooled to −78 °C, supplemented with boron tribromide (1 mol/L, DCM solution, 28.2 mL) in a dropwise manner, and stirred at the same temperature for 1 h and at rt for 2 h. Iced water was poured into the reaction solution and the mixture was stirred at rt for 40 min. After concentration under reduced pressure, the obtained solid was collected by filtration and dried to obtain compound **179** (4.60 g, 20.9 mmol, 98% yield) as a pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.17–1.63 (8H, m), 2.25–2.34 (2H, m), 6.70 (2H, d, J = 8.5 Hz), 7.17 (2H, d, J = 8.5 Hz), 9.26 (1H, s).

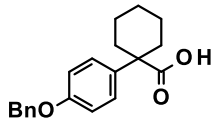
Benzyl 1-[4-(benzyloxy)phenyl]cyclohexanecarboxylate (180).



To a mixture of **179** (1.00 g, 4.54 mmol) and K_2CO_3 (1.26 g, 9.08 mmol) were added DMF (20.0 mL) and benzyl bromide (1.27 mL, 10.4 mmol), followed by stirring at rt overnight. Water was added to the reaction solution, the mixture was extracted with ethyl acetate, and then the organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. After filtration and concentration under

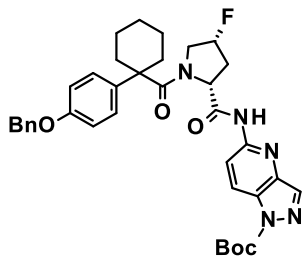
reduced pressure, the obtained residue was purified by silica gel column chromatography (hexane/chloroform) to obtain compound **180** (1.24 g, 3.10 mmol, 68% yield) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.18–1.70 (8H, m), 2.33–2.39 (2H, m), 5.05–5.09 (4H, m), 6.96 (2H, d, J = 9.2 Hz), 7.17–7.45 (12H, m).

1-[4-(Benzyloxy)phenyl]cyclohexanecarboxylic acid (181).



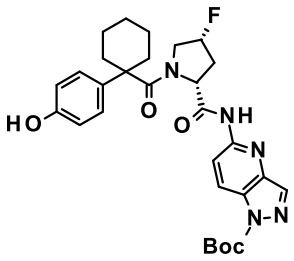
In a manner similar to that employed for the synthesis of **154**, the title compound **181** (898 mg, 2.89 mmol, 97% yield) was obtained using **180** (1.20 g, 3.00 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.15–1.31 (1H, m), 1.33–1.48 (2H, m), 1.50–1.66 (5H, m), 2.24–2.36 (2H, m), 5.08 (2H, s), 6.96 (2H, d, J = 8.5 Hz), 7.25–7.48 (7H, m), 12.20 (1H, brs).

***tert*-Butyl 5-{[(4*R*)-1-({1-[4-(benzyloxy)phenyl]cyclohexyl}carbonyl)-4-fluoro-D-prolyl]amino}-1*H*-pyrazolo[4,3-*b*]pyridine-1-carboxylate (182).**



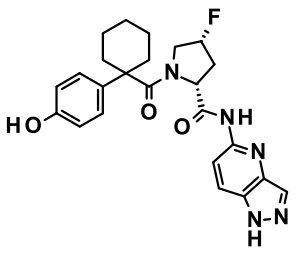
In a manner similar to that employed for the synthesis of **177**, the title compound **182** (1.18 g, 1.84 mmol, 95% yield) was obtained using **181** (600 mg, 1.93 mmol) and **178** (675 mg, 1.93 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.25–1.39 (1H, m), 1.50–1.89 (17H, m), 2.29–2.47 (2H, m), 2.50–2.64 (1H, m), 2.97–3.31 (1H, m), 3.40–3.61 (1H, m), 4.71–5.12 (4H, m), 7.00 (2H, d, J = 8.5 Hz), 7.26 (2H, d, J = 8.5 Hz), 7.30–7.46 (5H, m), 8.21 (1H, s), 8.41 (1H, d, J = 9.2 Hz), 8.48 (1H, d, J = 9.2 Hz), 8.69 (1H, s).

(4*R*)-4-Fluoro-1-{[1-(4-hydroxyphenyl)cyclohexyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (183).



In a manner similar to that employed for the synthesis of **126**, the title compound **183** (960 mg, 1.74 mmol, 95% yield) was obtained using **182** (1.18 g, 1.84 mmol). 1H NMR (DMSO-d_6) δ: 1.20–1.32 (1H, m), 1.44–1.83 (16H, m), 2.01–2.46 (4H, m), 3.05–3.34 (2H, m), 4.69–4.81 (1H, m), 5.05 (1H, d, J = 53.1 Hz), 6.76 (2H, d, J = 7.9 Hz), 7.13 (2H, d, J = 7.9 Hz), 8.33–8.53 (3H, m), 9.34 (1H, s), 10.51 (1H, s).

(4*R*)-4-Fluoro-1-{[1-(4-hydroxyphenyl)cyclohexyl]carbonyl}-*N*-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-D-prolinamide (115).



To a solution of **183** (150 mg, 0.272 mmol) in DCM (3 mL) was added TFA (1.5 mL), and the mixture was stirred at rt for 6 h. The resultant sample was concentrated under reduced pressure, a saturated aqueous $NaHCO_3$ solution was added to the obtained residue, then the mixture was extracted with ethyl acetate. The resultant sample was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure, after which the obtained residue was purified by silica gel column chromatography (chloroform/methanol) to obtain compound **115** (120 mg, 0.266 mmol, 98% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.15–1.35 (1H, m), 1.42–1.83 (7H, m), 1.93–2.45 (4H, m), 3.04–3.36 (2H, m), 4.63–4.82 (1H, m), 5.04 (1H, d, J = 53.7 Hz), 6.76 (2H, d, J = 7.9 Hz), 7.13 (2H, d, J = 7.9 Hz), 7.99–8.22 (3H, m), 9.34 (1H, s), 10.18 (1H, s), 13.26 (1H, s). MS (ESI/APCI) *m/z*: 452.2 $(M + H)^+$. HRMS (ESI) *m/z*: calculated for $C_{24}H_{27}FN_5O_3$ $(M + H)^+$, 452.2092; found, 452.2096.

2. X-ray crystallography

Procedure of protein production, purification, crystallization and structure determination

Human EP300 (1159-1666, Y1467F(Δ1520-1580), hereafter hEP300(1159-1666)) with an *N*-terminal His tag was overexpressed by *E. coli* BL21 (DE3) and purified to a single band on SDS-PAGE by Ni-affinity chromatography, 3c-protease treatment followed by reverse affinity chromatography, and size exclusion chromatography. The fractions containing hEP300(1159-1666) were collected and concentrated up to 30 mg/mL in buffer [20mM Tris(pH 7.5), 150mM NaCl, 5% (v/v) Glycerol(w/v), 0.5 mM TCEP].

Purified hEP300(1159-1666) was mixed with final 2 mM of compound **(+)-35**, **38**, **43**, **104** and co-crystallized by the vapor diffusion method with a 1:1 mixture of protein solution and reservoir solution (11-20% PEG3350 and 0.1 M HEPES (pH 7.0)). Three-dimensional crystals were obtained within a few days by the seeding method and flash-frozen with 20% Glycerol in the reservoir solution as a cryoprotectant.

Diffraction data were collected with in-house CuKα X-ray source (compound **(+)-35**, **43**) or at the Photon Factory BL1A beamline (Tsukuba, Japan; compound **38**, **104**) at cryogenic temperature and processed by XDS[49]. Phases were determined by molecular replacement using Phaser[50] with PDB 4BHW, 4PZS, and 7VHY as search models. Refinement and model building were performed using the REFMAC5[51], Phenix[52] and Coot[53] programs. The coordinates and statistics for the co-crystal structures with compound **(+)-35**, **38**, **43**, and **104** are shown in Table E1 and available from the PDB using accession code 7VHY, 7VHZ, 7VI0, 8GZC respectively.

Table E1. Data collection and refinement statistics.

	(+)-35	38	43	104
Wavelength	1.5418	1.100	1.5418	1.100
Resolution range	19.87–2.3 (2.382–2.3)	19.78–2.001 (2.073–2.001)	19.24–2.102 (2.177–2.102)	19.91–2.0 (2.071–2.0)
Space group	P1	P1	P1	P1
Unit cell	44.49 88.781 89.949 116.577 99.444 85.295	43.676 89.219 91.132 114.986 95.327 92.022	43.555 88.361 90.655 116.519 94.38 91.358	43.5 89.35 91.6 116.598 95.182 91.782
Total reflections	111546 (9432)	144538 (13125)	262338 (24190)	423002 (24591)
Unique reflections	50892 (4621)	78659 (7716)	66781 (6410)	81804 (7946)
Multiplicity	2.2 (2.0)	1.8 (1.7)	3.9 (3.8)	5.2 (3.1)
Completeness (%)	94.28 (85.83)	94.28 (92.14)	95.16 (91.43)	98.86 (95.87)
Mean I/sigma(I)	7.54 (0.80)	10.69 (1.08)	8.75 (0.97)	8.15 (0.48)
Wilson B-factor	39.84	37.98	35.06	46.71
R-merge	0.1123 (1.037)	0.04389 (0.6288)	0.1279 (1.323)	0.1066 (2.068)
R-meas	0.1517 (1.402)	0.06206 (0.8891)	0.1481 (1.537)	0.1179 (2.498)
R-pim	0.1014 (0.9374)	0.04387 (0.6285)	0.07458 (0.7803)	0.04968 (1.386)
CC1/2	0.991 (0.338)	0.998 (0.521)	0.996 (0.466)	0.998 (0.2)
CC*	0.998 (0.711)	1 (0.828)	0.999 (0.797)	0.999 (0.577)
Reflections used in refinement	50874 (4621)	78649 (7716)	66634 (6410)	81784 (7944)
Reflections used for R-free	2590 (239)	3904 (392)	3320 (325)	4086 (404)
R-work	0.2139 (0.3309)	0.1940 (0.3303)	0.1992 (0.3159)	0.1979 (0.3998)
R-free	0.2498 (0.3437)	0.2212 (0.3306)	0.2343 (0.3585)	0.2299 (0.4034)
CC(work)	0.951 (0.565)	0.962 (0.673)	0.961 (0.686)	0.968 (0.465)
CC(free)	0.926 (0.534)	0.939 (0.695)	0.939 (0.683)	0.937 (0.376)
Number of non-hydrogen atoms	7299	7579	7551	7280
macromolecules	6951	6970	6970	6925
ligands	38	70	74	74
solvent	310	539	507	281

Protein residues	876	878	874	880
RMS (bonds)	0.003	0.005	0.004	0.003
RMS (angles)	0.59	0.74	0.76	0.64
Ramachandran favored (%)	97.81	97.70	97.57	96.56
Ramachandran allowed (%)	2.19	2.30	2.43	3.44
Ramachandran outliers (%)	0.00	0.00	0.00	0.00
Rotamer outliers (%)	0.94	1.20	1.33	2.31
Clashscore	3.67	4.47	5.26	3.62
Average B-factor	54.75	48.93	46.93	65.39
macromolecules	55.16	49.09	47.13	65.87
ligands	42.41	39.93	42.63	52.13
solvent	46.94	48.08	44.81	57.25
Number of TLS groups	1	1	1	1

Statistics for the highest-resolution shell are shown in parentheses.

3. Biological assay procedures

HAT selectivity assay procedure

IC_{50} values of compounds inhibiting EP300 were determined by time-resolved fluorescence resonance energy transfer technology (LANCE; PerkinElmer) by detecting H4 peptide acetylation. Test compounds were incubated with 200 ng/mL recombinant human full-length EP300 produced by Daiichi Sankyo RD NOVARE, 400 nM biotinylated H4 peptide (Anaspec, #65242-1), and 8 μM Acetyl-CoA (Sigma-Aldrich, #A2056) in 50 mM Tris-HCl buffer (pH 8.0) containing 0.1 mM EDTA, 0.01% Tween 20, 1 mM dithiothreitol, and 0.01% bovine serum albumin (BSA), and 330 nM trichostatin A with a total volume of 10 μL in each well of a 384-well plate (PerkinElmer, #6008289) for 60 min at 28 °C. The enzymatic reaction was stopped by the addition of LANCE detection buffer (PerkinElmer, #CR97-100) containing 30 μM Lys-CoA (Daiichi Sankyo Co., Ltd.) with a total volume of 5 μL. To detect H4 acetylation, LANCE detection buffer containing 2 nM Eu-anti-acetyl-Lysine Antibody (PerkinElmer, TRF0412) and 50 nM ULight-labeled Streptavidin (PerkinElmer, TRF-0102) was added to each well and then allowed to react overnight at rt. After incubation, the fluorescence (Ex 615 nm, Em 665 nm) of each well in the microplate was measured with an EnVision Xcite Multimode Plate Reader (PerkinElmer). Data (n = 4) were analyzed by the program GraphPad Prism

6 (GraphPad Software Inc.). CBP, MYST2, and MYST4 were produced and purified in our laboratory. TIP60, PCAF, and GCN5 were purchased from SignalChem Biotech. Reaction buffer was composed of 25 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% Tween-20, 0.01% bovine serum albumin, 330 nM trichostatin A. 2.5 μL of reaction buffer containing Acetyl-CoA (Sigma-Aldrich, #A2056) at the indicated concentration shown in Table E2 and 50 nM Histone H4 (1-25)-GSGSK(Biotin) (Anaspec, #65242-1) or Histone H3 (1-21)-GGK(Biotin)-NH2 (Anaspec, #AS-61702) as shown in Table E2 was added to respective wells of a 384-well plate which contained 25 nL of dimethyl sulfoxide containing 50-0.0031 μM compounds at enzyme reaction. A total of 2.5 μL of each enzyme was added to start the reaction and the plates were incubated at rt for 1 h. Thereafter, 2.5 μL of AlphaLISA 1× Epigenetics Buffer 1 (Perkin Elmer Co., Ltd., #AL008F) containing 30 μM Lys-CoA or 600 μM Anacardic Acid (CALBIOCHEM, #172050) for terminating the enzyme reactions, 30 μM Acetylated-Lysine Antibody (Cell Signaling, #9441L), 15 μg/ml Protein A AlphaLISA Acceptor Beads (Perkin Elmer Co., Ltd., #AL101M), and 15 μg/ml AlphaScreen Streptavidin Donor Beads (Perkin Elmer Co., Ltd., #6760002) were added to each well, and the resultant sample was cultured at rt for 1 h. EnVision Xcite (PerkinElmer Co., Ltd.) was used to measure the AlphaLISA signal. This measured signal was used to determine the enzyme inhibition rates at the respective concentrations of the compounds, and the data (n = 4) thus obtained were analyzed using the medical statistical analysis software GraphPad Prism (GraphPad Software, Inc.) to calculate IC_{50} values.

Table E2. Components of enzymatic reaction. Each concentration at the enzyme reaction is shown.

	EP300	CBP	TIP60	MYST2	MYST4	PCAF	GCN5
Acetyl-CoA (μM)	0.4	1	4	11	2	3	3
Histone peptide	H4	H4	H4	H4	H4	H3	H3
Enzyme (nM)	0.6	0.3	30	50	2	1	1
Stop compound	Lys-CoA	Lys-CoA	Anacardic Acid	Anacardic Acid	Anacardic Acid	Anacardic Acid	Anacardic Acid

Cell line culture

LK2 cells were purchased from Human Science Research Resources Bank. VCaP, 22Rv1, LNCaP, and PC3 were purchased from American Type Culture Collection (ATCC). VCaP cell line was cultured in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific Inc.) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone). LK2, 22Rv1, LNCaP, and PC3 were cultured in ATCC-

modified RPMI-1640 medium (Thermo Fisher Scientific Inc., #11095) containing 10% FBS. All cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2.

Evaluation of Intracellular H3K27ac Inhibitory Activity

LK2 cells were seeded in a 96-well plate at 20000 cells/100 μL/well and were cultured overnight at 37 °C in 5% CO_2. Thereafter, 11 μL of a solution of the compound (in which the final concentration of DMSO was 0.1%) was added to each well, and the resultant sample was cultured at 37 °C in 5% CO_2 for 3 h. The supernatant was discarded, 4% paraformaldehyde was added at 100 μL/well, and the resultant mixture was left to stand at rt for 15 min. Then, the 4% paraformaldehyde was discarded and the resultant sample was washed with PBS-T. A quenching buffer (PBS-T containing 1% H_2O_2) was added at 100 μL/well and the resultant sample was left to stand at rt for 10 min. The sample was then washed with PBS-T, supplemented with a blocking buffer [StartingBlocK T20 (TBS) Blocking Buffer (Thermo Fisher Scientific, #37543)] at 200 μL/well, and left to stand at rt for 1 h. Next, the supernatant was discarded, Acetyl-Histone H3 (Lys27) (D5E4) XP™ Rabbit mAb (Cell Signaling, #8173) diluted with a blocking buffer was added at 50 μL/well, and the resultant mixture was left to stand at 4 °C overnight. The resultant sample was then washed with PBS-T, supplemented with Anti-Rabbit IgG-HRP (Cell Signaling, #7074S) diluted with a blocking buffer at 50 μL/well, and the resultant mixture was cultured at rt for 1 h. The resultant sample was washed with PBS-T, supplemented with SuperSignal™ ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific, #37069) at 50 μL/well, and the signal was measured using EnVision. Based on the measured signal, the enzyme inhibition rates at the respective concentrations of the compounds were measured, and the data thus obtained were analyzed using the medical statistical analysis software GraphPad Prism (GraphPad Software, Inc.) to calculate IC_{50} values.

Evaluation of *in vitro* SOX2 mRNA expression (LK2 cells)

LK2 cells were seeded in a 12 well plate at 10000 cells/1000 μL/well, and were cultured overnight at 37 °C in 5% CO_2. Thereafter, 10 μL of a solution of **DS17701585** (in which the final concentration of dimethyl sulfoxide was 0.25%) was added thereto, and the resultant was cultured at 37 °C in 5% CO_2 for 6 h. SOX2 mRNA abundance in LK2 cells were quantified by qRT-PCR.

Mouse Xenograft Model (*in vivo* SOX2 mRNA expression assay)

LK2 cells were trypsinized, counted and resuspended in a 1:1 mixture of PBS and Matrigel (Corning, #354234) on ice. Cell suspension was then injected (5.0×10^6 cells/mouse) subcutaneously into the right axilla of 5-week-old female BALB/c-nude mice (Charles River Laboratories). At 12 days post-inoculation, mice with 200–400 mm^3 of tumor were selected and randomized to each PK/PD study groups (n=3).

PK/PD experiment (*in vivo* SOX2 mRNA expression assay)

Mice bearing LK2 tumors were administered vehicle (0.5w/v% Methyl Cellulose 400) or **DS17701585 (**50 and 200 mg/kg, p.o., 0.5w/v% Methyl Cellulose 400 suspension), followed by blood and tumor tissue collections at 6 h post-treatment. Compound concentration in plasma and tumors were determined using LC/MS/MS methods. SOX2 mRNA abundance in tumors was quantified by qRT-PCR.

Determination of tumor SOX2 mRNA level (qRT-PCR)

RNAs from tumor samples were isolated with RNeasy Mini Kit (QIAGEN, Venlo, the Netherlands) following a manufactured standard protocol. 10 ng of RNA was directly subjected to qRT-PCR using TaqMan Fast Virus 1-step Master Mix (Thermo Fisher Scientific, Waltham, MA). qRT-PCR was performed in triplicate. The $2^{-\Delta\Delta CT}$ method was used to analyze the relative changes in gene expression with GAPDH as a reference gene. TaqMan probes were purchased from Thermo Fisher Scientific (SOX2; Hs01053049_s1, GAPDH; Hs02758991_g1)

Cell Proliferation Assay. VCaP (8000 cells/well), 22Rv1 (2000 cells/well), and PC3 (2000 cells/well) were plated in 96-well plates. These cells were treated for 7 days with various concentrations of DS-9300. After treatment with DS-9300, CellTiter-Glo (Promega Corporation, G9241) solution was added to each well and cell proliferation was assessed by measuring the luminescence using a 96-well plate reader (PerkinElmer, EnVision 2104 Multi-label Reader).

Western Blotting Assay. VCaP and LNCaP cells were seeded in six-well plates at 2×10^5 cells/well and treated with DS-9300 at the indicated concentrations for 3 days. After the treatment, cells were lysed in RIPA buffer (Thermo Fisher Scientific, #89900) supplemented with PhosSTOP (Sigma-Aldrich, #4906837001) and cOmplete Mini (Sigma-Aldrich, #04693116001). Protein concentration was determined using the DC-protein Assay Kit (Bio-Rad Laboratories, Inc., #500-0116). Protein samples were subjected to 10%–20% SDS-polyacrylamide gradient gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. These membranes were blocked in PVDF blocking reagent (TOYOBO Co., Ltd.) for 1 h and incubated with primary antibody for H3 (Cell Signaling Technology, Inc., #4499), H3K18ac (Cell Signaling Technology, Inc., #13998), H3K27ac (Cell Signaling Technology, Inc., #8173), PSA (Abcam, #ab76113), or actin (Cell Signaling Technology, Inc., #4970) diluted with Can Get Signal Solution 1 (TOYOBO Co., Ltd.) at 4 °C. The next day, the membranes were washed three times with TBS-T and incubated with anti-rabbit IgG antibody (GE Healthcare, #NA934V) diluted with Can Get Signal Solution 2 (TOYOBO Co., Ltd.) for 1 h. After washing three times, the membranes were visualized with a chemiluminescence reagent (Millipore,

#WBLUF0500) and the labeled proteins were detected with ImageQuant LAS 4000 (GE Healthcare).

Measurement of LogD. Equal amounts of PBS and 1-octanol were shaken and left overnight. The upper layer (1-octanol) and lower layer (PBS) were collected separately. Each test compound was dissolved in 1-octanol or PBS. The same amount of either PBS or 1-octanol was added and the mixture was shaken vigorously for 30 min at rt followed by centrifugation at 2100 *g* for 5 min at rt. Subsequently, both phases were separated and assayed by HPLC and LC-MS. $LogD_{7.4}$ was calculated using the following equation:
$LogD_{7.4}$ = log(peak area of compound in 1-octanol / peak area of compound in PBS).

Pharmacokinetic Experiments. BALB/cAnNCrlCrlj mice (Charles River Laboratories Japan, Inc.) were used for the experiments. The tested compounds were administered intravenously via the tail vein at 1 mg/5 mL/kg or orally by gavage to mice at 10 mg/10 mL/kg under fed conditions. Blood was drawn from the jugular vein and plasma was generated by centrifugation at 10,000 *g* for 5 min. Plasma samples were stored at −20 °C until analysis. The plasma concentration–time data were analyzed by noncompartmental analysis. AUC was calculated using the trapezoidal rule. Total plasma clearance (CL) was estimated as dose/AUC. Bioavailability (BA) was calculated using AUC data obtained upon oral and intravenous administration.

***In Vivo* Study (for DS-9300).** Four-week-old NSG male mice (Charles River Laboratories Japan, Inc.) were inoculated subcutaneously with 1.0×10^7 VCaP suspended tumor cells in the right flank (Day 0). Surgical removal of both testes was conducted on each mouse (Day 11). On Day 21, the mice were divided by stratified randomization into treatment groups (N = 5) and treated orally once daily for 33 days with the test compound DS-9300 at different doses. The length and width of each tumor were measured using a digital caliper on Days 21, 24, 28, 31, 35, 38, 42, 46, 50, and 53. Tumor volume was calculated using the following formula: TV = L × W × W/2. The mice were weighed daily with a digital balance for animals from Day 21 to Day 53. Plasma samples and tumor samples (Day 53) were collected from all mice in each group. The PSA concentration of each plasma sample was determined using Human PSA Total (KLK3) ELISA kit (Thermo Fisher Scientific Inc.), in accordance with the manufacturer's instructions.

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主論文目録

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1) Kanada, R., Suzuki, T., Murata, T., Miyazaki, M., Shimada, T., Kuroha, M., Minami, M., Higuchi, S., Tominaga, Y., Naito, H. **4-Pyridone-3-carboxylic acid as a benzoic acid bioisostere: Design, synthesis, and evaluation of EP300/CBP histone acetyltransferase inhibitors.** *Bioorg. Med. Chem. Lett.* **2021**, *51*, 128358.

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