博士学位論文

側鎖による三環性骨格制御を基盤とする新規 PPARγ 作動薬の創製研究

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略語表

本報では以下の略語を用いた。

ADFP: adipocyte differentiation-related protein

ANGPTL4: angiopoietin-like 4

aP2: adipocyte Protein 2

DPPA: diphenylphosphoryl azide

DTBAD: di-*tert*-butyl azodicarboxylate

EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

FBS: fetal bovine serum

HOBt: 1-hydroxybenzotriazole

KRT20: keratin 20

PPAR: peroxisome proliferator-activated receptor

PPRE: peroxisome proliferator response element

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

LBD: ligand binding domain

Ms: methansulfonyl

qPCR: quantitative polymerase chain reaction

SCID: severe combined immunodeficiency

TFA: trifluoroacetic acid

TFAA: trifluoroacetic anhydride

TMS: trimethylsilyl

TZD: thiazolidinedione

1 緒言

PPAR (peroxisome proliferator-activated receptor) γ は核内受容体スーパーファミリーの一つであり、リガンド依存的に標的遺伝子の転写活性を調節することにより、末梢のインスリン感受性、脂肪前駆細胞から脂肪細胞への分化誘導などを制御している。[1,2,3] PPARγ は、同じく核内受容体であるレチノイド X 受容体 α とヘテロ 2 量体を形成し、下流制御遺伝子のプロモーター領域に存在する DNA 応答配列に結合、転写因子として働くことが知られている。[4]

これまでに様々な PPARγ 作動薬が開発されている。チアゾリジンジオン (TZD) 誘導体であるピオグリタゾンやロシグリタゾンを含む代表的な PPARγ 作動薬の構造を Figure 1 に示す。[5,6,7,8,9,10,11,12,13] これら PPARγ 作動薬は 2 型糖尿病の治療薬として有用であることが示されている。PPARγ 作動薬はアディポサイトプロテイン 2 (*aP2*)、 アディポフィリン (*adfp*)、アディポネクチンなどの脂肪関連分子群の遺伝子の転写を活性化し、脂肪前駆細胞から脂肪細胞への分化を誘導する。[14,15,16] 例えば、ロシグリタゾンなどによるマウス線維芽細胞様細胞株 3T3-L1 の脂肪細胞への分化誘導がよく知られている。[17,18,19]

上記のような PPARγ 活性化に基づく脂肪前駆細胞の分化誘導作用と同様に、PPARγ 作動薬は低分化がん細胞の分化誘導を促進すると考えられている。例えば、PPARγ 完全作動薬である TZD 誘導体エファツタゾン (CS-7017, Figure 1) が、未分化甲状腺がん[20]、非小細胞肺がん[21]、すい臓がん [8] など種々のがん細胞に対して分化誘導作用を示す可能性が報告されている。未分化甲状腺がん細胞株 DRO にエファツタゾン約 1 nM を添加すると、DRO 細胞株のコロニー形成阻害作用が認められた(63 %阻害)。[9] この時、DRO 細胞でのエファツタゾンの PPARγ 活性化能は、PPRE3-tk-Luc 遺伝子 (レポーター遺伝子：ルシフェラーゼ) を導入したレポータージーンアッセイにおいて確認されている。すなわち、PPARγ 作動薬は、新規メカニズムの抗がん剤になることが期待される。

一方、エファツタゾンの投与により末梢の浮腫に代表される有害事象が第 1 相臨床試験で確認されている。[20] また、他の PPARγ 完全作動薬の非臨床および臨床研究において同様の有害事象が報告されている。[22,23] これまでの研究で、PPARγ 作動薬の骨格の違いによって下流遺伝子発現のプロファイルが異なることが知られている。[24] そこで、新規骨格を有する PPARγ 作動薬を取

得することができれば、浮腫に繋がる体液貯留作用を回避した新規の抗腫瘍剤になりうると考え、PPARγ リガンドの探索研究を開始した。

以下に、シード化合物の配座解析を鍵とするリード化合物ジベンゾオキセピン誘導体の創出、代謝安定化のための構造最適化、特徴的な PPARγ タンパク質に対する結合様式および *in vitro* および *in vivo* 抗腫瘍活性について順に述べる。

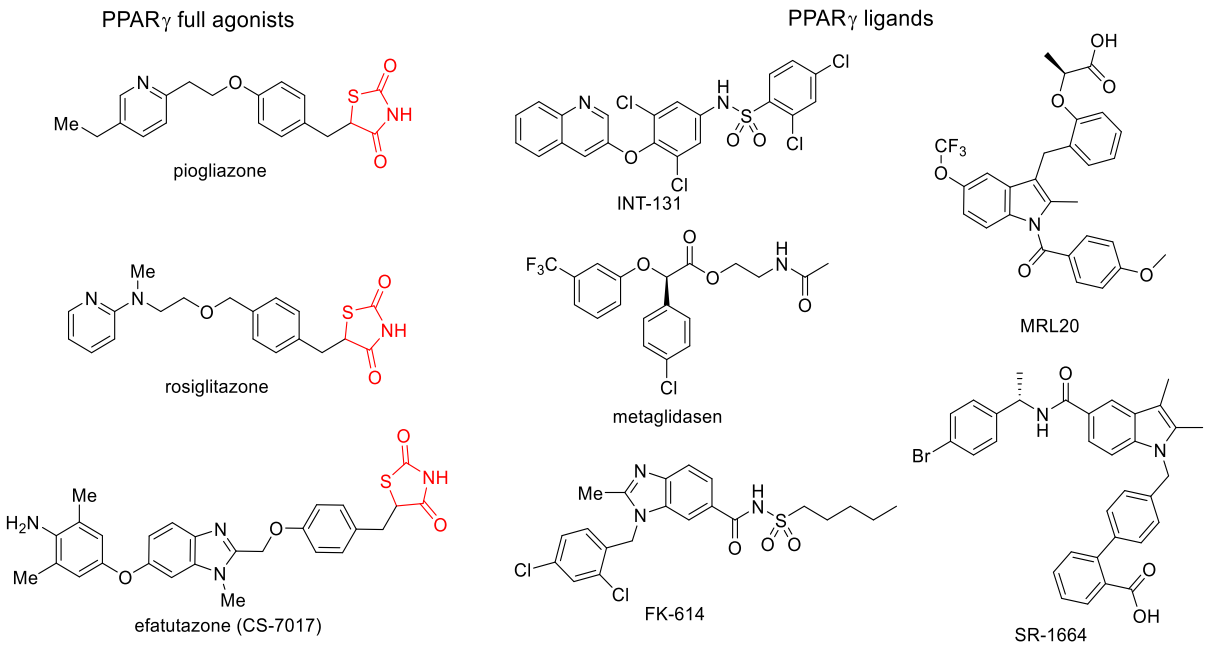

Figure 1. Chemical structures of PPARγ full agonists and ligands. The structures of TZD are showed in red.

2 リード化合物の取得とそのプロファイル

2.1　ヒット化合物の取得とメチル基の導入

社内で別の創薬標的を狙ったテーマの中で PPARγ 作動活性を有するジベンゾアゼピン誘導体 **1-DM** を偶然見出した (Figure 2)。*in vitro* 活性評価は、PPARγ レポータージーンアッセイ[a] で実施した。筆者は、PPARγ 作動活性が不十分なものの (EC_{50} = 4,564 nM) TZD 構造を持たず既存の PPARγ 作動薬とは異なる母核を有することから **1-DM** に着目し、本化合物をシード化合物として誘導体展開することとした。

これまでの構造活性相関研究から、ロシグリタゾンによる PPARγ 活性発現には、酸性構造である TZD 部と PPARγ helix 12 の Tyr473 との水素結合形成が必須であることがわかっている。[25] すなわち、**1-DM** の酸性構造であるテトラゾール部分の空間配置を適切に制御すれば、PPARγ 作動活性が向上し得ると考えた。一般にマジカルメチルと呼ばれるメチル基導入による低分子化合物の配座制御による結合活性の向上について多くの知見がある。[26] また、筆者の所属研究室では、シクロプロパン環のような高いひずみ構造を有する環の隣接位にメチル基を導入すると、メチル基の立体障害によって、立体障害が少ない水素原子がシクロプロパン環を向くように側鎖の配座が制御される知見を得ている (Figure 3)。[27] そこで、ジベンゾアゼピン窒素原子に隣接する炭素原子上にメチル基を導入してテトラゾールの配向を制御することで、PPARγ 作動活性の向上を狙った。

メチル基導入体 **1** の光学純度 86%ee のユートマー (Figure 2) の PPARγ レポーター活性は、期待通り、**1-DM** に比べ 20 倍以上活性向上した (EC_{50} = 197 nM)。この結果は、三環構造の隣接位に導入したメチル基によって化合物 **1** の配座が活性配座に制御されたことを示唆する。ただし、残念ながら、化合物 **1** はジベンゾアゼピン窒素原子に隣接する位置に不斉炭素を有するため、誘導体合成の過程で不斉点を構築する必要があり、効率的な最適化研究が困難であると想定された。そこで、不斉炭素を回避した代替母核への変換を検討することとした。

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a 酵母の転写因子である GAL4 の DNA 結合ドメインと PPARγ リガンド結合領域とのキメラ核内レセプターを用いたトランスアクティベーションアッセイ法。GAL4 応答性ルシフェラーゼを組み込んだ HEK293 細胞において、ルシフェラーゼタンパク質による化学発光を定量することにより、PPARγ 作動による転写活性を評価。

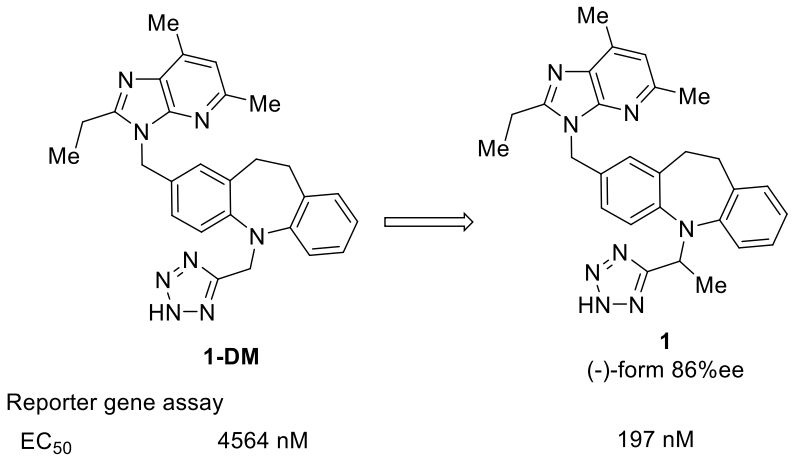


Figure 2. Chemical structure and reporter activity of compound **1**.

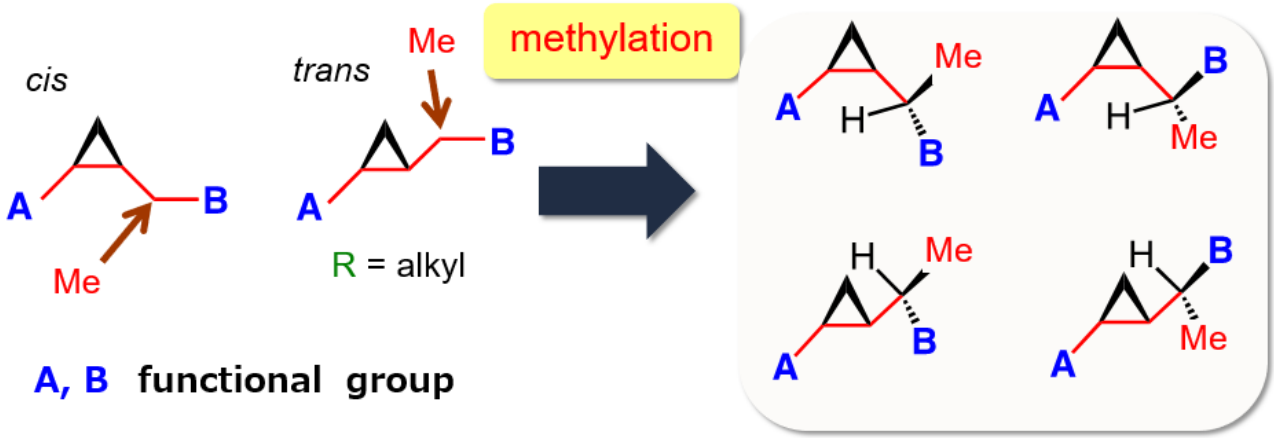


Figure 3. Conformational restriction by methyl group in cyclopropane scaffolds.

2. 2　三環系母核の配座解析に基づく代替母核のデザイン

母核を変換するにあたり、まず、化合物 **1** の立体配座について詳細に解析した。先に述べたように、化合物 **1** のテトラゾール部と PPARγ の Tyr473 との相互作用が PPARγ 作動活性に重要と予想される。 すなわち、窒素原子の隣接位に導入したメチル基による三環系骨格ジベンゾアゼピンと酸性構造テトラゾールとの相対的な空間配置（＝配向）の制御が、化合物 **1** の活性発現に大きく寄与していると考えられる。同時に、メチル基が三環系骨格ジベンゾアゼピンの配座にも影響していると考えられた。

化合物 **1** の 6-7-6 三環系ジベンゾアゼピン骨格は、代表的な三環系抗うつ薬イミプラミンと同一であり、その配座研究は既に報告されている。[28] 7 員環の両側のベンゼン環が上下にフリップし、それに伴って橋頭位の置換基もベンゼン環との衝突を避けるようにコンベックス面側に配置する (Figure 4)。すなわち、導入したメチル基によって三環系骨格の配座変化にも影響が出てい

る可能性がある。これらの配座を考慮して新たな骨格をデザインすべく、ヒット化合物の **1-DM** および化合物 **1** の配座を解析し、安定配座を比較することとした。

化合物 **1-DM** および **1** の正確な安定配座探索を実施する上で、着目したいテトラゾールと三環構造以外の部分構造、すなわちイミダゾピリジン側鎖を簡略化したモデル化合物を設定した (Figure 5)。また、化合物 **1** の絶対立体配置は未決定のため、両エナンチオマーの配座探索が必要であった。**1-DM** のモデル化合物を **1-DM'**、**1** の *R* 体のモデル化合物を(*R*)-**1'**、同様に *S* 体を(*S*)-**1'**とした。

分子モデリングソフト MacroModel (version 10.9) を用いて、まず、**1-DM'**の最安定配座を探索した。その結果を、Figure 6 に示す。また、最安定配座のポテンシャルエネルギーから 0.13 kcal/mol 以内に、二つの主な準安定配座が算出された。前述したように三環構造がフリップした配座に加え、テトラゾールの配向が 2 位メチル基と *anti, syn* の相対配置の配座の四つである。ここで、母核 2 位メチル基が紙面で下向きの配座を *down,* 上向きのものを *up* とし、**1-DM'**の 4 つの配座を *down-syn, up-anti, up-syn, down-anti* と定義した。次に、同様に計算して求めた(*R*)-**1'**および(*S*)-**1'**の最安定配座を Figure 6(B),(C)に示す。ただし、最安定配座から 0.1 kcal/mol 以内に確認された準安定配座は、それぞれ一つずつであった。通常であれば、**1-DM'**の結果と同様に、母核のフリップとテトラゾール配向の組み合わせにより、それぞれのエナンチオマーで **1-DM'**のような 4 種類の安定配座が確認されると考えられる。しかし、最安定配座から 0.1 kcal/mol 以内において、(*R*)-**1'**は、*down-syn* と *up-anti,* (*S*)-**1'**は *up-syn* と *down-anti* の二種類ずつの安定配座が確認されるのみであり、(*R*)-**1'**の *down-syn* と(*S*)-**1'**は *up-syn* との関係、及び(*R*)-**1'**の *up-anti* と(*S*)-**1'**の *down-anti* の関係は、互いに鏡像異性体に相当する。それぞれのエナンチオマーで、テトラゾールの配向に関しては *syn, anti* の両方の配向がみられたが、それに伴うフリップ構造はそれぞれ一種類であった。この結果は、三環構造と橋頭位に隣接する炭素原子上のメチル基とテトラゾールの立体障害により、テトラゾール部の配向にともなってより安定な母核の配座に制御されている可能性が考えられる。2.1 章で述べた、メチル基の立体効果に基づく化合物デザインの妥当性を支持するものである。

次に、不斉炭素を回避し、かつテトラゾール部の *syn* 及び *anti* 配向を不飽和結合の *E/Z* 異性体によって模倣できることを期待して、ジベンゾシクロヘプタンメチリデン構造を考案した。即ち、

母核 5 位とテトラゾールが結合する炭素間を炭素－炭素二重結合にして自由回転を固定した、テトラゾール部が 2 位メチル基に対して *syn* 配置である化合物 **2** およびそのモデル化合物 **2'**、および、*anti* 配置である **3** およびそのモデル化合物 **3'**を設計した (Figure 5)。MacroModel を用いて **2'**, **3'**の最安定配座を計算した結果、Figure 6(D), (E)に示す配座が得られた。いずれも母核がフリップし、 *down, up* の両配座が確認され、テトラゾールとメチル基との立体反発を避けるよう三環構造の配座が制御されていた。得られた **2'**および **3'**の計四つの安定配座は、**1'**の両エナンチオマーの四つの安定配座、*down-syn, up-anti, up-syn, down-anti* に相当する。即ち、*Z* 体である **2'**の二つの安定配座 *down-Z* および *up-Z* は(*R*)-**1'**の *down-syn* と(*S*)-**1'**の *up-syn* を、一方、*E* 体である **3'**の二つの安定配座 *down-E* および *up-E* は(*R*)-**1'**の *up-anti* と(*S*)-**1'**の *down-anti* をそれぞれ効果的に模倣する。実際に、**1** の各エナンチオマーの安定配座と **2'**, **3'**の対応する安定配座をそれぞれ重ね合わせると、いずれも三環骨格およびテトラゾール部の良好な重なりが確認された (Figure 7)。

以上のことから、ジベンゾシクロヘプタンメチリデン体 **2**, **3** は、**1** のユートマーの活性配座を模倣できる可能性が高い。化合物 **2**, **3** は、強い PPARγ 作動活性を示し、不斉炭素を回避したリード化合物になりえると期待した。そこで、**2**, **3**、および、テトラゾール環の代替構造として報告のある[29]オキサジアゾロンに変換した **4**, **5** を合成した。(誘導体合成に関しては、4.1 章で述べる。)

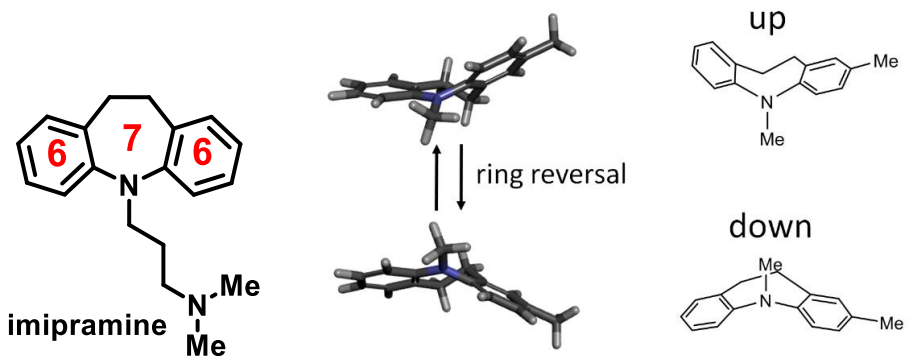


Figure 4. Ring reversal motion of the 6-7-6-tricyclic ring systems.

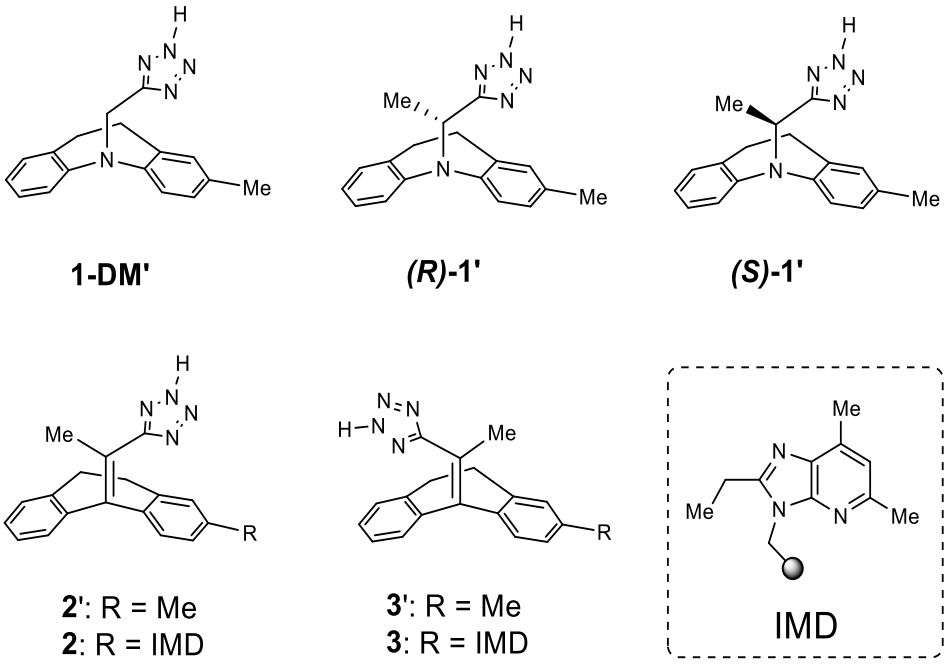
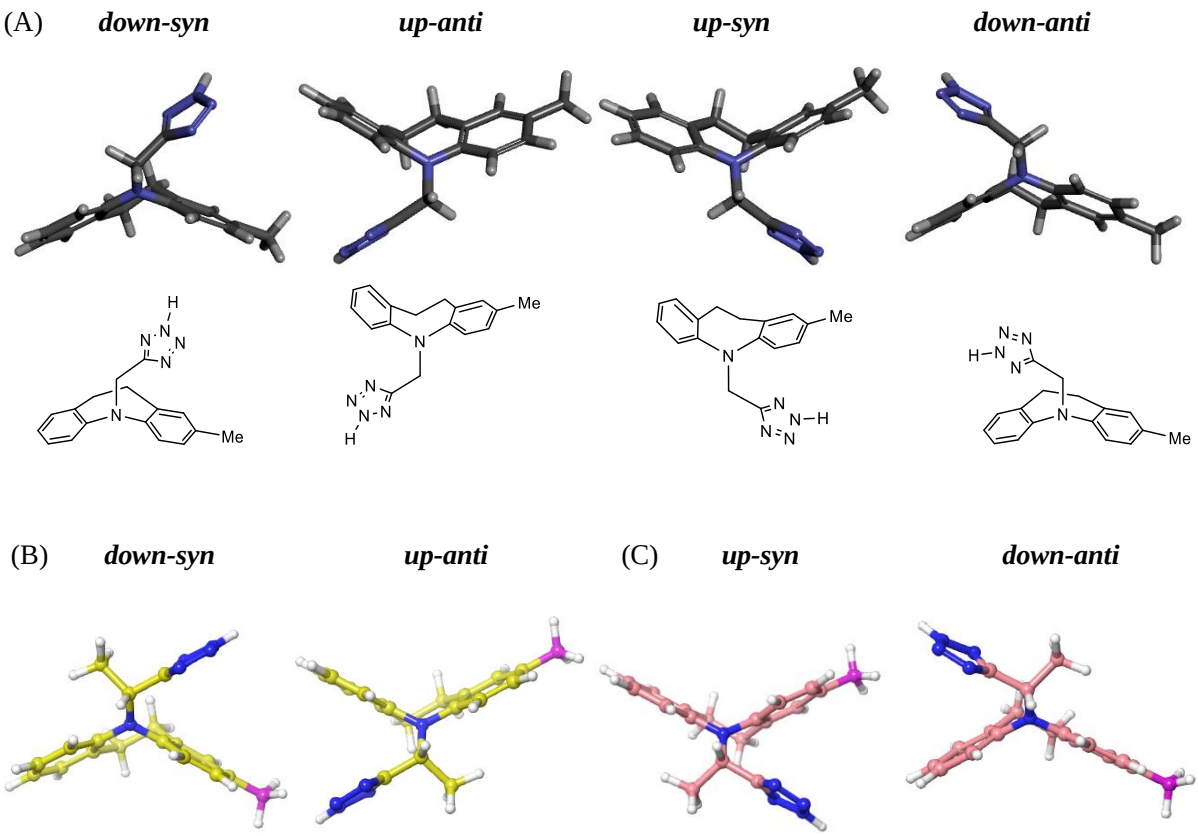


Figure 5. Chemical structures of model substrate for conformation studies and compound **2**,**3**.



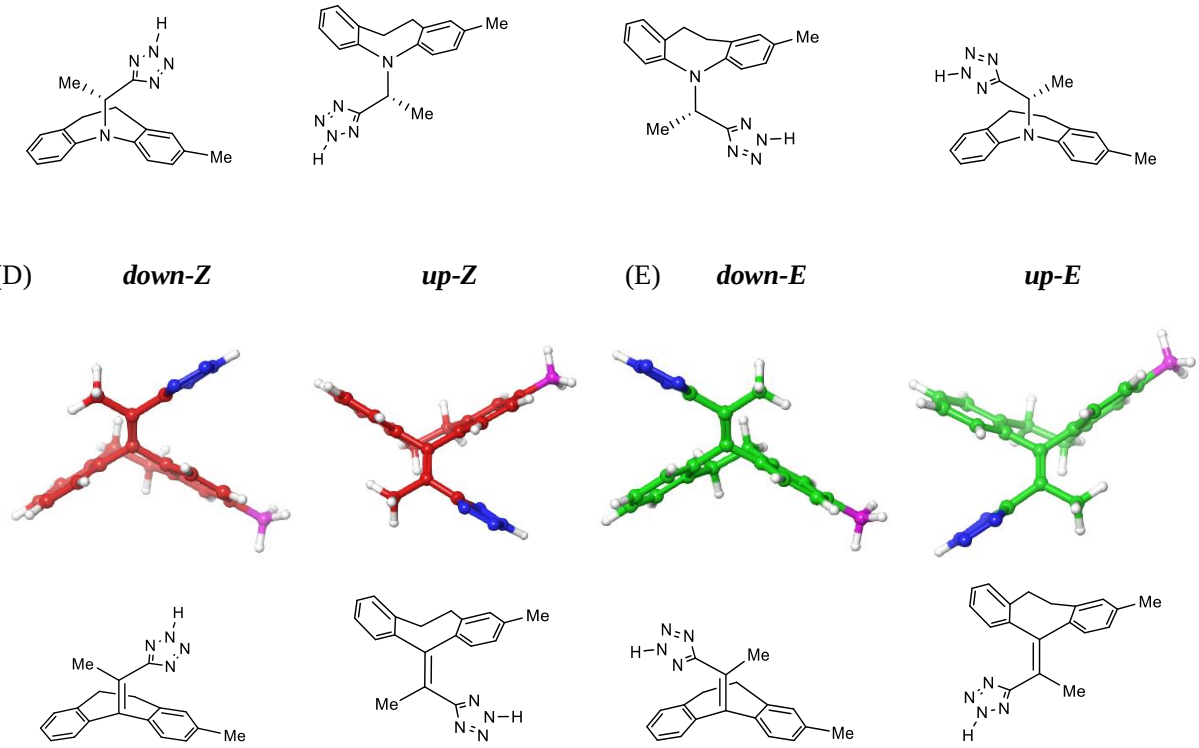
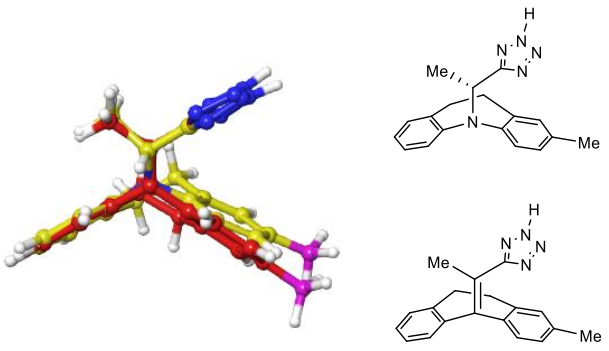


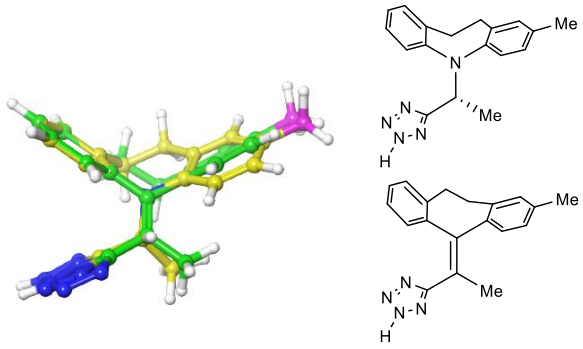
Figure 6. Stable conformations of (A) **1-DM'** (gray), (B) (*R*)-**1'** (yellow), (C) (*S*)-**1'** (pink), (D) **2'** (red), (E) **3'** (green) as calculated by the MacroModel. The hydrogens, nitrogens, and 2-methyl group are colored white, blue and magenta respectively.

1-DM' (*up-anti, down-anti*): ΔE = 0.0 kcal/mol (global minimum), **1-DM'** (*down-syn, up-syn*): ΔE = 0.13 kcal/mol, (*R*)-**1'** *up-anti*: ΔE = 0.0 kcal/mol (global minimum), (*R*)-**1'** *down-syn*: ΔE = 0.1 kcal/mol, (*S*)-**1'** *down-anti*: ΔE = 0.0 kcal/mol (global minimum), (*S*)-**1'** *up-syn*: ΔE = 0.1 kcal/mol, **2'** *down-Z,up-Z*: ΔE = 0.0 kcal/mol (global minimum), **3'** *down-E,up-E*: ΔE = 0.0 kcal/mol (global minimum),

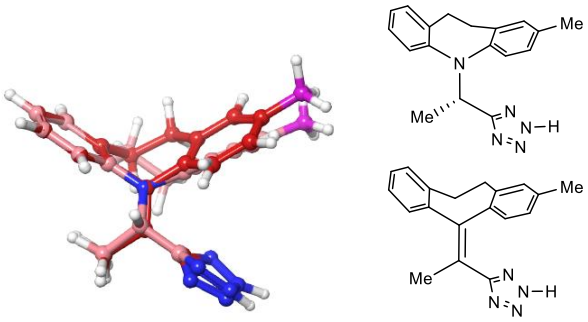
(A) ***down-syn*** ((*R*)-**1'**) /***down-Z*** (**2'**)



(B) ***up-anti*** ((*R*)-**1'**) /***up-E*** (**3'**)



(C) ***up-syn*** ((*S*)-**1'**) /***up-Z*** (**2'**)



(D) ***down-anti*** ((*S*)-**1'**) /***down-E*** (**3'**)

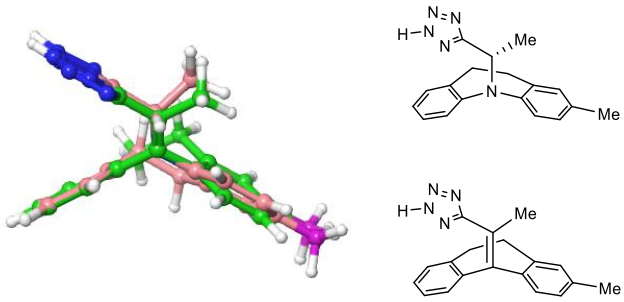


Figure 7. Superimposition of the most stable conformations between compound (*R*)-**1'** (yellow), (*S*)-**1'** (pink), **2'** (red) and **3'** (green) as calculated by the MacroModel. The hydrogens, nitrogens and 2-methyl group are colored white, blue and magenta respectively.

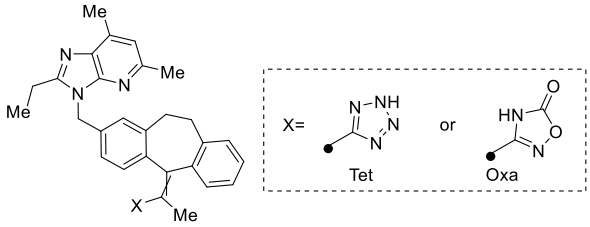
2.3　配座解析結果を反映した新規母核を有する誘導体の PPARγ 作動活性

合成したジベンゾシクロヘプタンメチリデン誘導体の *E/Z* 異性体 **2**–**5** の PPARγ 作動活性を HEK293 レポーター遺伝子評価系で評価した (Table 1)。レポーター遺伝子発現の最大値は、代表的な PPARγ 作動薬であるピオグリタゾンを 1 μM 処置した場合の効果に対する比として算出している。ジベンゾシクロヘプタンメチリデンの *E* 体 **2** および **4** は、ほぼ PPARγ の作動活性を示さなかった。一方で、*Z* 体 **3** および **5** は 1 μM においてピオグリタゾン対比で 20%以上のレポーター遺伝子発現の最大値を示し、シード化合物 **1** と同等以上の活性を保持した。これらの結果から、ジベンゾシクロヘプタンメチリデン *Z* 体の安定配座が、化合物 **1** の活性配座を模倣している可能性が高いと考えらえる。

筆者が取得した三環系誘導体は、ピオグリタゾンのような完全作動薬と比較すると何れも PPARγ 作動活性の最大値が低い。これまでの PPARγ 作動薬の研究から、糖尿病改善作用を示すためには、必ずしもレポーター遺伝子評価系で高い最大値を示す必要がないことが知られている。例えば、PPARγ の部分作動薬として知られている INT-131 やメタグリダセンは、筆者の化合物と同様に HEK293 レポーター評価系の遺伝子発現の最大値が 20%以下と低いが、非臨床もしくは臨床研究において高い糖尿病改善作用を示すことが報告されている。[30,31,32,33] また、*db/db* 糖尿病マウスにおいて、INT-131 は溶媒投与群に対して約 60%の血糖降下作用を示し、その効果はピオグリタゾンと同等である。がん疾患に関してはレポーターアッセイにおける PPARγ 作動活性と *in vivo* 薬効の相関に関する前例がないが、糖尿病改善作用と同様の傾向を示す可能性が考えられる。

そこで、筆者はジベンゾシクロヘプタンメチリデン誘導体 **5** を新たなリード化合物に設定し、さらなる最適化研究を実施することとした。化合物 **3** よりも化合物 **5** を選択して誘導体展開を進めることにしたのは、**5** のほうが分子の脂溶性が低く、後の誘導体展開に有利であることが想定されたからである (ClogP of **3** and **5**: 6.03 vs. 5.76, calculated by ChemBioDraw Ultra 14.0)。

Table 1. PPARγ reporter activities of *E*/*Z* isomers of dibenzocycloheptanemethylidene[a]



Compd.	Geometric isomer	X	efficacy % at 1 μM	EC_{50} (nM)
1	-	Tet	24	197
2	*E*	Tet	0.4	-
4	*E*	Oxa	1.5	-
3	*Z*	Tet	59	251
5	*Z*	Oxa	23	177
pioglitazone			100	2053
INT-131			8.3	59
metaglidasen			11	7365

[a]The efficacies and EC_{50} values of compounds **1**–**5** in human PPARγ /GAL4 transfected HEK293EBNA cells at 24 h after drug treatment. The efficacy of each compound was calculated as the percentage of the maximum activation obtained with pioglitazone at 1000 nM. EC_{50} values were determined using the XLFit.

2.4　リード化合物の構造活性相関と高活性化合物 **9** の取得

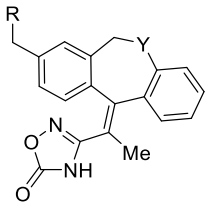
不斉炭素を持たず、活性配座をとっていると考えられるリード化合物**5**の活性向上を目指し、母核およびイミダゾピリジンの変換を実施した (Table 2)。作動活性は、化合物最適化の指標として上記と同様のルシフェラーゼをレポーター遺伝子とするHEK293レポーター遺伝子評価系で評価した。リード化合物**5**は比較的高い脂溶性を有しており(CLogP 5.76)、また、母核のジベンゾシクロヘプタンメチリデン骨格の合成には多工程を要した。本課題を解決すべく、母核のジベンゾシクロヘプタンメチリデンのシクロヘプテン環に酸素原子を導入した化合物**6** (CLogP 5.00) を設計・合成した。ジベンゾシクロヘプタンメチリデンの合成に必要な工程は8工程であるのに対し、化合物**6**の母核ジベンゾオキセピン環の合成に必要な工程は5工程である。(Scheme 1,2を参照)

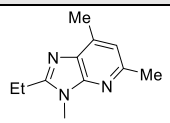
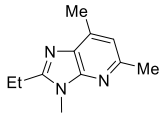
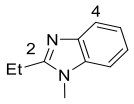
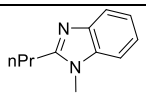
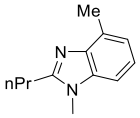
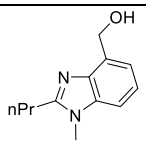
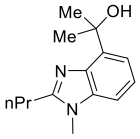
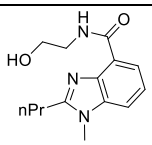
化合物**6**は、リード化合物**5**よりも2倍程度活性が向上した (EC_{50} = 84 nM)。さらに、イミダゾピリジン環の窒素原子およびメチル基を除去したベンゾイミダゾールとした化合物**7**を合成したところ、さらにレポーター活性が向上した (EC_{50} = 17 nM)。

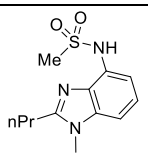
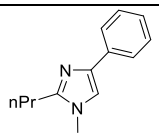
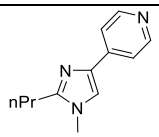
次に、ベンゾイミダゾール部2位の置換基をエチル基からノルマルプロピル基に変換したところ、さらに活性が向上した (化合物**8**, EC_{50} = 2.4 nM)。また、化合物**8**のベンゾイミダゾール4位にメチル基を導入した**9**も同等の活性を示したことから、同部位の置換基許容性が伺えた。そこで、脂溶性の低下を目的に、ベンゾイミダゾール4位に水酸基やスルホンアミド基などの高極性の置換基を導入した化合物**10**–**13**を合成し、評価した。残念ながら、これらの誘導体の活性は減弱したことから、同部位への極性の高い置換基の導入は活性面で許容されないことが明らかとなった。また、ベンゾイミダゾールを、4位に芳香環を有するイミダゾールで置換した化合物**14**および**15**も合成したが、これらの活性も低下した。

上記のようにして得られた化合物**9**は、起点化合物**5**と比較して非常に低濃度からレポーター活性を示すが、PPARγ作動活性の最大値に関しては低い化合物であった。3.3項で述べたように糖尿病治療薬研究においては、レポーター評価におけるPPARγ作動活性の最大値の程度によって、*in vivo*における薬理作用の強さには影響しないという知見が得られている。そこで、筆者は、化合物**9**の強力なEC_{50}値に着目し、本化合物のPPARγタンパク質への結合様式の解析やがん細胞を用いた評価に進めることとした。

Table 2. in vitro activities of dibenzocycloheptanemethylidene and dibenzo[*b,e*]oxepine derivatives in PPARγ reporter gene assay[a]



Compd.	R	Y	Reporter gene assay	
			EC_{50} (nM)	Efficacy (%)
5		CH_2	177	24
6		O	84	19
7		O	17	9.7
8		O	2.7	14
9		O	2.4	9.5
10		O	33	9.1
11		O	13	4.8
12		O	57	11

13		O	166	7.0
14		O	151	13
15		O	593	7.2
pioglitazone			2053	100
INT-131			59	8.3
metaglidasen			7365	11
FK-614			163	11

[a]The efficacies and EC_{50} values of compounds **5**–**15**, other PPARγ agonists in human PPARγ/GAL4 transfected HEK293EBNA cells at 24h after drug treatment. The efficacy of each compound was calculated as the percentage of the maximum activation obtained with pioglitazone at 1000 nM. EC_{50} values were determined using the XLFit.

2.5　化合物 **9** の低分化がん細胞株 MKN-45 に対する分化誘導作用評価

非常に低濃度からレポーター活性を示す化合物 **9** のがん細胞に対する分化誘導作用を評価した (Figure 8)。低分化がん細胞株として胃がん細胞株 MKN-45 を用いた。[34] MKN-45 は通常の培養条件で細胞同士が接着せず、浮遊細胞のような形態で増殖する。PPARγ 作動薬を MKN-45 に処置すると細胞の形質が変化し、細胞が凝集したような塊が観察される。この細胞の凝集塊を IN Cell Analyzer で定量化することにより、がん細胞の分化誘導活性の指標とした。[35] 代表的な PPARγ 作動薬であるピオグリタゾン、INT-131、FK-614 およびメタグリダセンを評価したところ、メタグリダセン以外の薬剤は高濃度域でのみ細胞凝集活性が認められ、メタグリダセンは同活性を示さなかった。一方で、化合物 **9** は非常に低濃度から細胞凝集活性を示した (94% at 30 nM)。この結果から化合物 **9** の細胞凝集活性、すなわちがん細胞の分化誘導作用は、他の PPARγ 作動薬よりも著しく強力であることが明らかとなった。

また、化合物 **9** 処置による MKN-45 細胞の各種遺伝子変動を定量的ポリメラーゼ連鎖反応 (qPCR) にて確認した (Figure 9)。 その結果、PPARγ で制御されるアンジオポエチン様タンパク質 4 (*angptl4*) 遺伝子[36]の発現量が、がん細胞の凝集作用を示す濃度 (10 nM) で有意に上昇した。さらに、細胞の分化マーカーの一つとして知られるアディポフィリン (*adfp*)[37]の発現量は、細胞凝集が見られる 100 nM で上昇し、また、間葉系のマーカーであるビメンチン[38]は同濃度で低下していたことから、化合物 **9** による PPARγ の活性化と MKN-45 細胞株の細胞凝集活性、すなわち分化誘導が関連している可能性が示唆された。

次に、化合物 **9** が PPARγ の転写活性化を介して、がん細胞の分化誘導を示しているかを確認するため、レポーター評価の EC_{50} 値と MKN-45 凝集活性の EC_{50} 値を比較した。Figure 10 は、化合物 **8**–**15** の両アッセイにおける EC_{50} 値の散布図であり、レポーターアッセイと細胞凝集アッセイの EC_{50} 値はよく相関した (r^2 = 0.924)。 この結果より、筆者の化合物は、PPARγ 作動活性に基づいてがん細胞の分化誘導を示している可能性があるが、このメカニズムを解明するためには、今後より詳細な検討を実施する必要がある。例えば、PPARγ 機能阻害による分化誘導作用のキャンセルや、他の PPARγ 作動薬との下流制御遺伝子変動プロファイルの差異の確認などである。

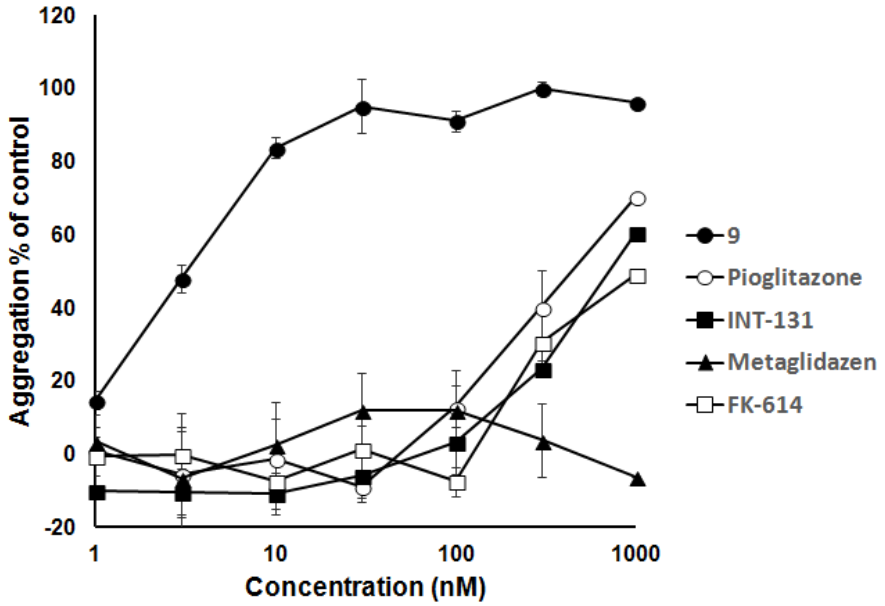


Figure 8. Aggregation activities of PPARγ ligands in MKN-45 cells. The aggregation of MKN-45 cells was evaluated using an IN Cell Analyzer 1000 (GE Healthcare) after treatment of compounds for 5 days. The efficacy of **9** was calculated using its ratio of cell-aggregated clusters as the control value. The

aggregation % values of other PPARγ ligands (pioglitazone, INT-131, metaglidasen, FK-614) are shown as the values relative to the maximum efficacy of **9**.

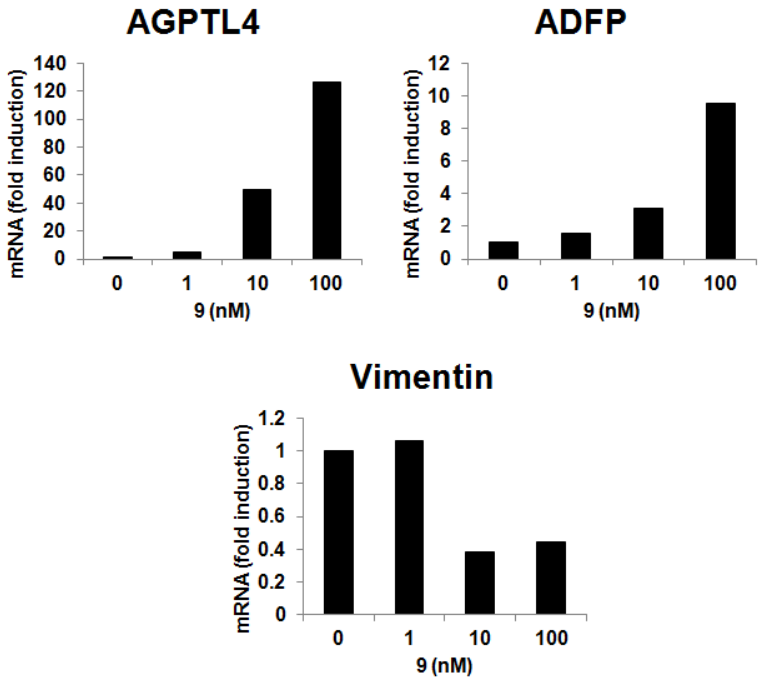


Figure 9. The gene expression in MKN-45 cells after the treatment of **9** at 1–100 nM. The gene expression was determined by quantitative PCR using an ABI PCR system. The fold-inductions are shown as the values relative to baseline.

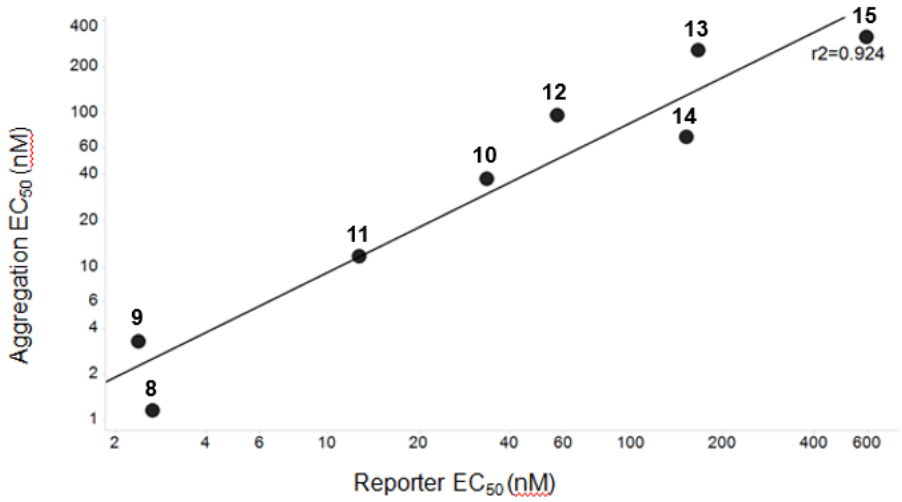


Figure 10. The correlation of the EC_{50} values of tricyclic compounds **8**–**15** on the aggregation of MKN-45

cells with those obtained in a reporter gene assay for HEK293 cells. There was a strong correlation between the two assays.

2.6　化合物 **9** の PPARγ タンパク質との複合体構造

PPARγ リガンドバインディングドメイン (LBD) と PPARγ リガンドとの複合体構造は、複数報告されている。[24,39,40] 化合物 **9** は、PPARγ に作用しがん細胞を低濃度から強力に分化誘導させるという特徴的な生理活性を示すことから、PPARγ LBD との結合様式は興味深い。Figure 11A に示すように、筆者は化合物 **9** と PPARγ LBD 複合体の X 線結晶構造解析を取得することに成功した。化合物 **9** のオキサジアゾロンの酸性プロトンは、PPARγ 作動活性発現に重要と言われている Try473 (helix 12) の酸素原子[41] と水素結合を形成していた。また、ベンゾイミダゾール部位は、helix 3 および helix 5 で形成されるカノニカルサイト[b]方向に伸長していた。また、三環性母核のベンゼン環が、helix 11 近傍にできた小さな疎水性のポケットと相互作用していた。この疎水性ポケットは、本化合物の結合に伴って helix 3 の Phe282 の配置が変化したことで形成されたと思われる。このように、化合物 **9** は、PPARγ 作動活性発現に重要な AF-2 領域を構成する helix 3 および helix 11 の構造に影響を与えており、特定のコファクタータンパク質の AF-2 領域へのリクルートにも影響を与えている可能性がある。本化合物を用いたコファクタープロファイルの解析に興味がもたれる。

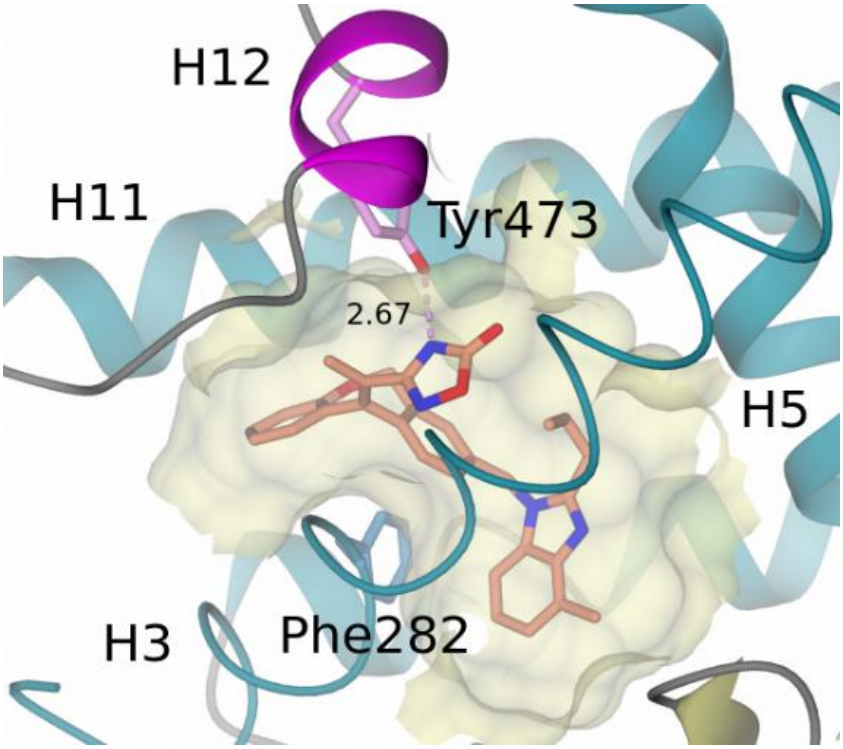
次に、化合物 **9** の上記結合様式を、他の PPARγ 作動薬と比較した。Figure 11(B)はロシグリタゾンの、Figure 11(C)は化合物 **9** と他の PPARγ 作動薬であるロシグリタゾン、INT-131、メタグリダセン、MRL20[36] の X 線結晶構造解析を重ね合わせた図である。その結果、化合物 **9** のオキサジアゾロンは、ロシグリタゾンおよび MRL20 の酸性構造とおおよそ重なり、Tyr473 (helix 12) との水素結合形成は共通していた。また、ベンゾイミダゾール部のカノニカルサイトとの相互作用も他のリガンドと同様であることが確認された。その一方、ジベンゾオキセピン母核が形成する helix 3 および helix 11 との疎水性相互作用は、他のリガンドには見られない独自の相互作用であ

b PPARγ タンパク質のリガンド結合領域に存在し、helix 3, 5 および 11 で囲まれた領域。PPARγ の内因性リガンドと言われている 15-デオキシプロスタグランジン J2 が結合する疎水性の領域である。helix 3,5 および helix 12 は activation function-2 (AF-2) 領域を構成する。Figure 11 のように helix 12 が作動活性型に安定化された場合、AF-2 領域に PPARγ 作動活性発現に必要なコファクタータンパク質がリクルートされ、転写活性を発現する。

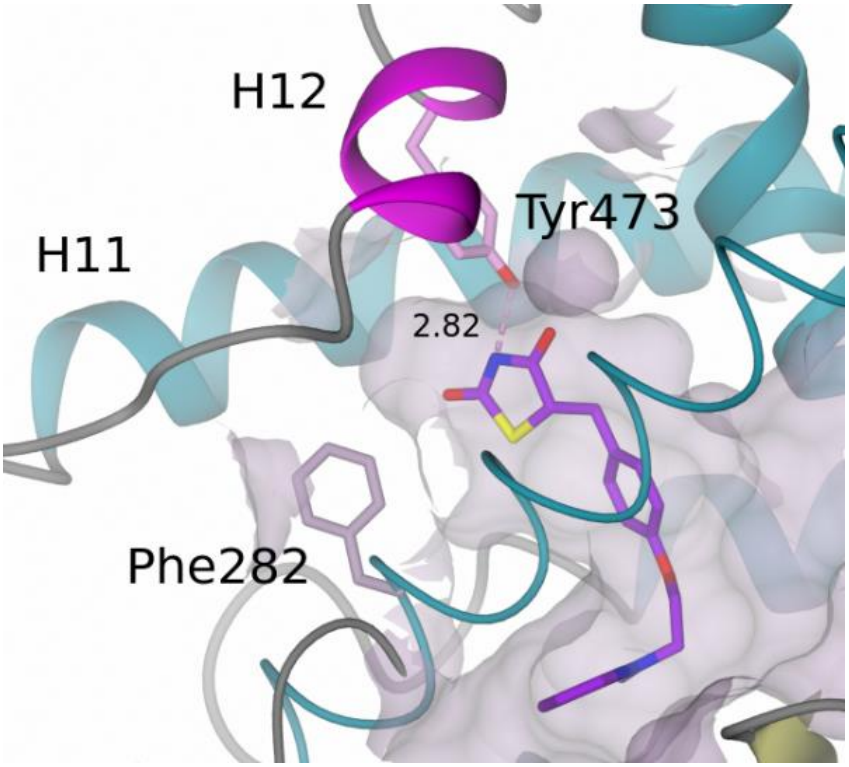
ることがわかった。この化合物 **9** 独自の結合様式が、低濃度でのがん細胞の分化誘導活性という既存の PPARγ 作動薬にはない特徴的な活性に繋がっている可能性がある。

X 線結晶構造解析の結果から、化合物 **1** の活性配座を考察した。化合物 **1** の *R/S* 体からは 4 種類の安定配座が算出されているが、(*R*)-**1** の *down-syn* 配座および化合物 **3** の *down-Z* の配座は、化合物 **9** のX線結晶構造解析中の配座と良好な重なりを示した (Figure 12)。酸性構造および母核もよく重なっていることから、モデル基質 **1'**、**2'**および **3'**を用いた配座解析の比較による化合物デザインは妥当であったと考察した。化合物 **1** および **9** の安定配座の重なりから、(*R*)-**1** がユートマーであり、(*R*)-**1** の *down-syn* 配座 (aquamarine)、化合物 **3** の *down-Z* (bluepurple) が生理活性を示す配座であると推察した。

(A)



(B)



(C)

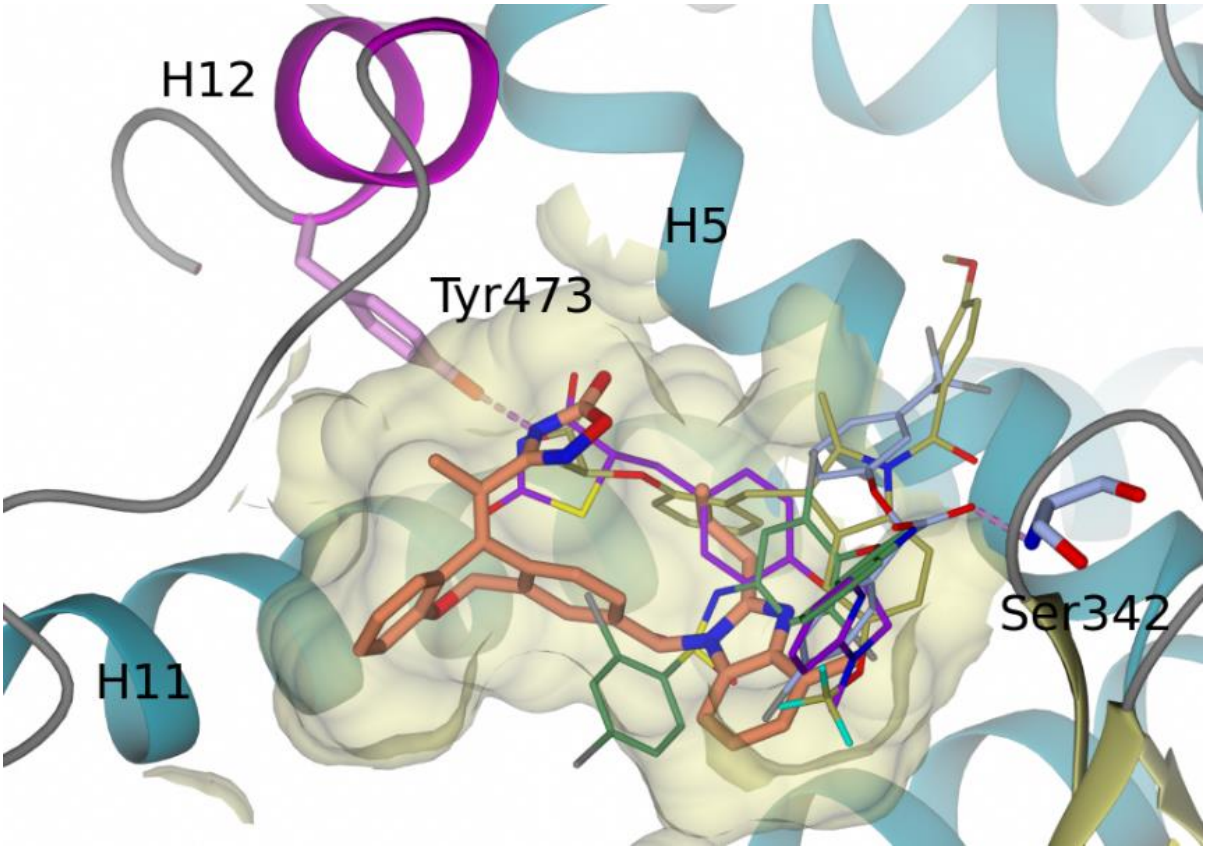


Figure 11. The crystal structures of PPARγ agonists in the PPARγ LBD. (A) The binding mode of **9** (orange) to the PPARγ LBD. The proton of the oxadiazolone ring of **9** interacted with the oxygen atom of Tyr473. (B) The binding mode of rosiglitazone to the PPARγ LBD. Rosiglitazone bound to the canonical site. (C) The overlay of the complex structure of **9**, rosiglitazone (purple, PDB:1FM6), INT-131 (deepgreen, PDB:3FUR), metaglidasen (lightblue, PDB:4PVU), and MRL20 (gold, PDB:2Q59). The acidic group of the ligands interacted with Tyr473.

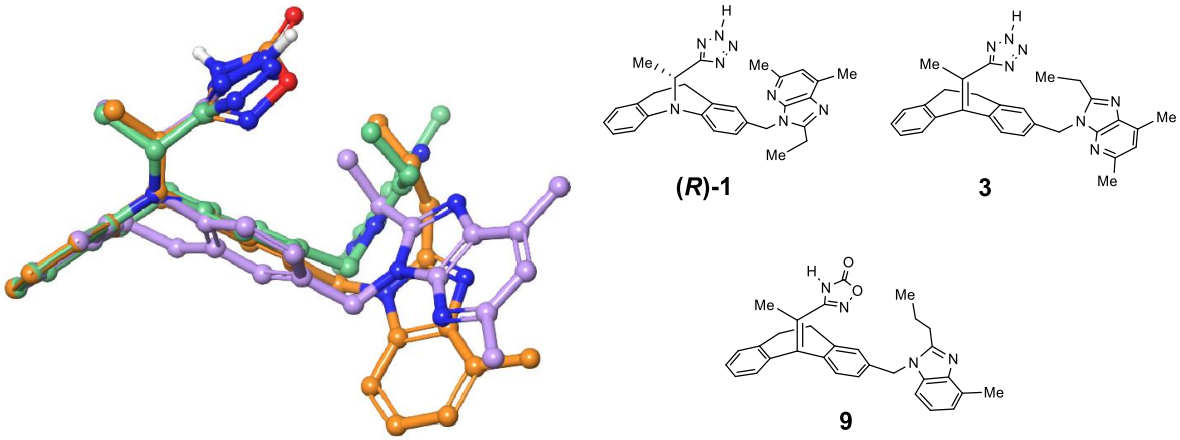


Figure 12. The overlay of the stable conformations *down-syn* of (*R*)-**1** (aquamarine), and *down-Z* of **3** (bluepurple) as calculated by the MacroModel and the structure of **9** (orange) from the crystal structure with the PPARγ LBD.

3　化合物 **9** の最適化研究

3.1　化合物 **9** の課題と誘導体展開の方向性

低分化がん細胞に対して強力な分化誘導作用を示し、かつ独自の PPARγ タンパク質に対する結合様式を示す化合物 **9** を取得できたものの、**9** を新規の抗がん剤として開発する上での課題が明らかとなった。Figure 13 に化合物 **9** のマウス経口投与時の血中濃度を示すが、化合物 **9** の血中半減期は非常に短く、*in vivo* において薬効を発揮することは期待できない結果であった。薬物動態が不良である原因としては、化合物 **9** の肝固有クリアランス (CL_{int})[c] が高いことがあげられる (Figure 14, >24.9 L/h/kg)。筆者は、この分子の高い脂溶性がシトクローム P450 による酸化的代謝に対する安定性の低さの原因であると推察した。そこで、社内別プロジェクトにおいて、ジベンゾオキセピン母核のベンゼン環に窒素原子を導入し、母核の脂溶性が低下した化合物 **6a**，**7a** が得られていたことに着目し、これらの化合物の活性および代謝安定性について評価した。

化合物 **6a**、**7a** をレポーターアッセイで評価した結果、左側のベンゼン環に窒素原子を導入した化合物 **6a** は EC_{50} が 86 nM であり、化合物 **9** よりは活性が減弱した。一方で、右側ベンゼン環上に窒素原子を導入した化合物 **7a** は、大幅に活性が減弱した (EC_{50} = 2194 nM)。しかし、化合物 **7a** のヒト肝固有クリアランスは化合物 **6a** や化合物 **9** よりも著しく低下し (**7a**: 2.06 L/h/kg vs. **6a**,**9**: >24.9 L/h/kg)、優れた代謝安定性を示すことが明らかとなった。この結果から、母核右側ベンゼン環が主な酸化代謝部位であることが推察された。

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c 肝固有クリアランス(intrinsic clearance; CL_{int})：肝臓における薬物を代謝する酵素の固有の能力を表す。(創薬化学 下巻 改訂第 2 版から引用)

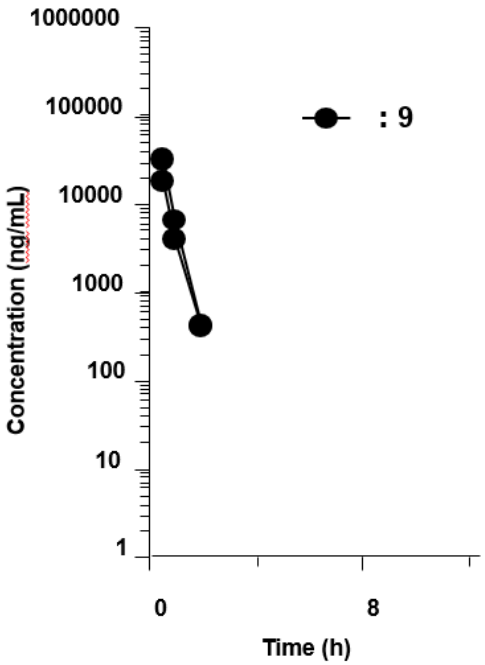


Figure 13. Plasma concentrations of **9** after its oral administration in BALB/c mice (30 mg/kg, *n* = 2).

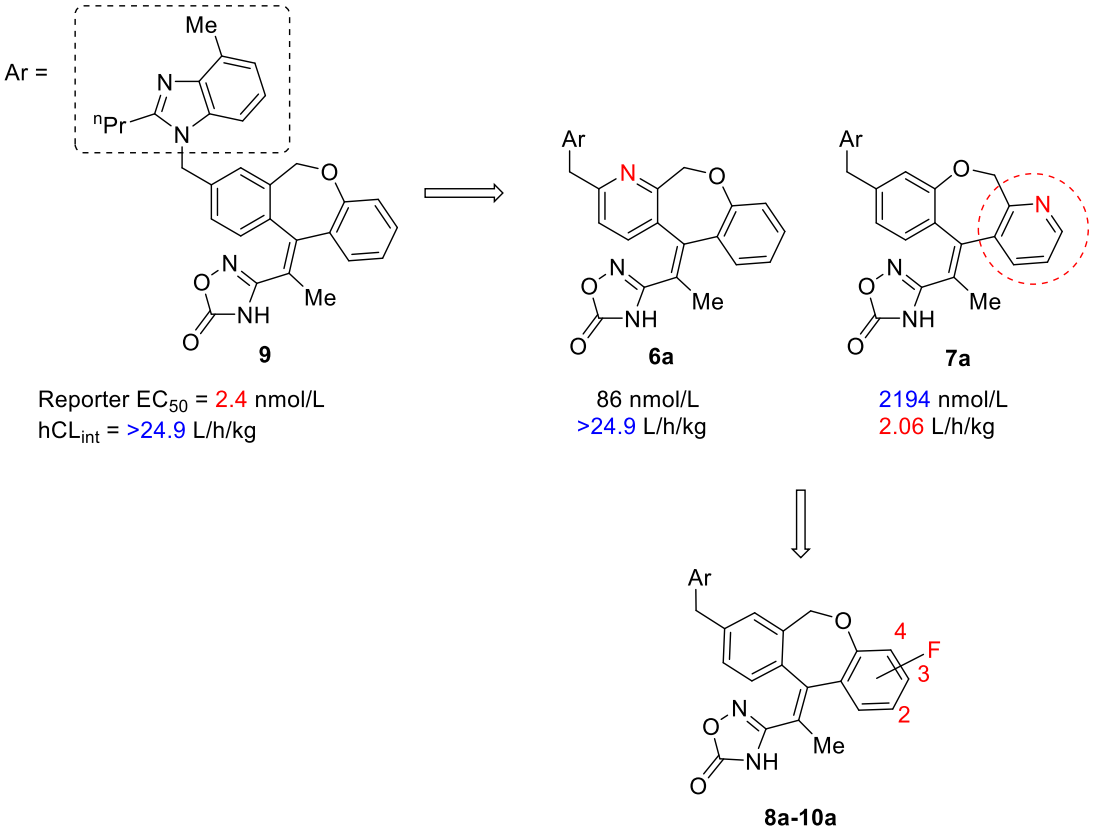


Figure 14. Improvement of the human metabolic stability of lead compound **9** by modification of the tricyclic core-structure.

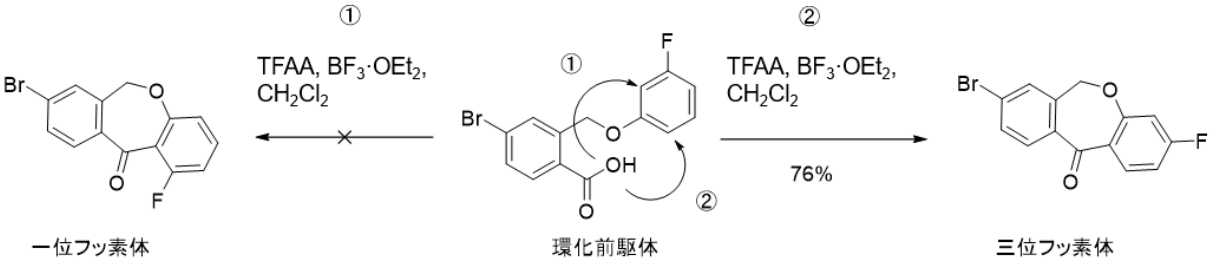
3.2　代謝安定な起点化合物の取得

上記のように母核構造変換体の合成により主酸化代謝部位が右側のベンゼン環であることが推定されたので、CYP による酸化代謝をブロックする方策として、右側ベンゼン環にフッ素原子を導入することを考えた。これまでのメディシナルケミストリー研究において、代謝部位へのフッ素原子導入により CYP の酸化代謝をブロックできることが報告されている。[42]

ジベンゾオキセピンの 1–4 位にフッ素原子の導入を試みたところ、1 位フルオロ体以外は所望の誘導体 **8a**–**10a** を合成することができた。1 位フルオロ体に関しては、閉環反応時の位置選択性の問題で合成することができなかった。[d] フッ素導入体はいずれも非常に強力なレポーター活性および MKN-45 の凝集活性を示した (Table 3)。[43] さらに、ヒト肝ミクロソームを用いた代謝実験の結果、3 位にフッ素原子を導入した化合物 **9a** においてのみ大幅な代謝安定性の向上 (3.2 L/h/kg) が確認され、2 位および 4 位フルオロ体では同様の代謝安定化は確認できなかった (>24.9 and 22.6 L/h/kg)。このことから、ジベンゾオキセピンの 3 位が主酸化代謝部位であることが示唆された。

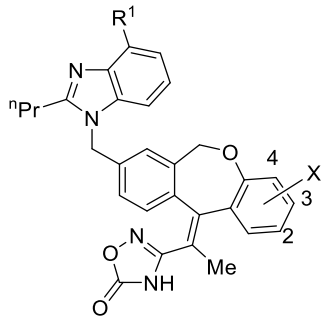
in vitro での代謝安定性が向上した化合物 **9a** のマウスにおける薬物動態を評価した。3 位フルオロ体 **9a** は、マウス経口投与において化合物 **9** よりも血中半減期が延長し、それに伴って AUC が改善した (Figure 15)。このように筆者は、比較的良好なマウス PK を有するジベンゾオキセピン 3 位フルオロ体 **9a** を見出した。しかし、そのマウス PK および薬効は十分とは言えなかった。そこで、フルオロ体 **9a** をもとに、さらなる構造最適化を計画した。X 線結晶構造解析 (Figure 11A) よりオキサジアゾロン部位は活性発現に必須であり、また、三環性骨格は活性配座をとる

d ジベンゾオキセピン環の構築工程において、環化前駆体の Friedel-Crafts 反応での位置選択性により、一位フッ素体と三位フッ素体が生成する可能性があった。筆者は、本反応において一挙に両位置異性体を取得するつもりであったが、下記の条件では、三位フッ素体のみが得られた。



ために必要な構造であるため構造変換は不適であった。そこで、筆者はベンゾイミダゾール部の最適化研究に着手した。

Table 3. Effects on PPARγ reporter, MKN-45 aggregation activities and human hepatic intrinsic clearance of fluoro dibenzooxepine derivatives



Compd.	R^1	X	Reporter gene assay		MKN-45 Aggregation assay		hCL_{int}
			EC_{50}(nM)	Efficacy(%)	EC_{50}(nM)	Efficacy(%)	(L/h/kg)
9	Me	H	2.4	9.5±0.6	3.3	100	>24.9
8a	H	2-F	12	6.9±1.5	7.0	102	>24.9
9a	H	3-F	7.4	11.7±3.6	1.0	104	3.2
10a	H	4-F	4.4	8.7±1.6	3.8	101	22.6

The efficacies and EC_{50} values of compounds **9** and **8a**–**10a** in human PPARγ/GAL4 transfected HEK293EBNA cells at 24 h after drug treatment. The efficacy of each compound was calculated as the percentage of the maximum activation obtained with pioglitazone at 1000 nM. EC_{50} values were determined using the XLFit.

The aggregation of MKN-45 cells was evaluated using an IN Cell Analyzer 1000 (GE Healthcare) after treatment of compounds **9** and **8a**–**10a** for 5 days. The aggregation efficacies were shown as the values relative to the maximum efficacy of **9**. EC_{50} values were determined using the XLFit. The hCLint was calculated from the elimination rate constant of each compound in human liver microsomes (n = 2).

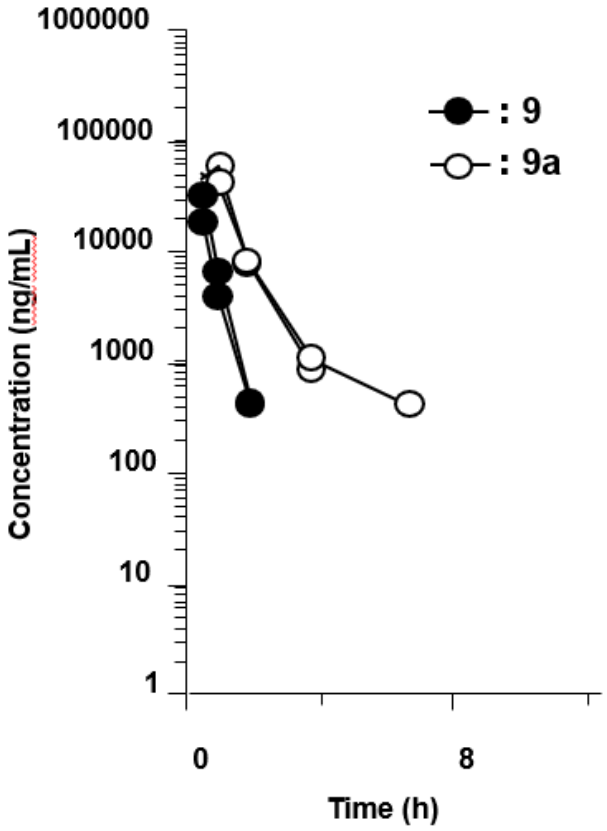


Figure 15. Plasma concentrations of **9** and 3-fluoro-dibenzooxepine derivative **9a** in BALB/c mice orally administered (30 mg/kg, *n* = 2).

3. 3　ベンゾイミダゾール部の最適化

化合物 **9** と PPARγ LBD との複合体のX線結晶構造解析 (Figure 11A)[44]から、ベンゾイミダゾール部位はカノニカルサイトの疎水性アミノ酸残基に囲まれ、その結合ポケットも狭いことが確認された。したがって、ベンゾイミダゾール部への嵩高い置換基の導入や高極性を付与するような置換基の導入は許容されない可能性が高いと考えられた。実際に、2.4 節に記載の通り、化合物 **6** から **7** への変換においてベンゾイミダゾールから窒素原子を除去することによって活性が向上している。3 位フルオロ体でも同様の傾向か検証すべく、ベンゾイミダゾールのベンゼン環上に窒素原子を導入した化合物 **11a** および **12a** を合成した。その結果、X線結晶構造解析から想定されるように PPARγ レポーター活性が減弱する結果であった (それぞれ、EC_{50} = 32nM および 24 nM, Table 4)。そこで、脂溶性を保持した他の誘導体へ展開することとした。チエノ[3,4-*d*]イミダゾール **13a** およびイミダゾ[1,2-*a*]ピリジン誘導体 **14a** は、化合物 **9** と同等の非常に強力な細胞凝集活性を示した (それぞれ、EC_{50} = 3.0 nM、 5.3 nM)。化合物 **13a** のマウス PK は著しく不良であった

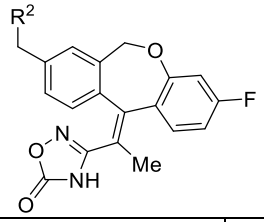
一方で、化合物 **14a** のマウス AUC は化合物 **9** の 2 倍程度であった。そこで、化合物 **14a** の周辺誘導体 **15a**–**21a** の合成・評価を実施した (Table 4)。

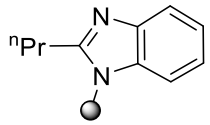
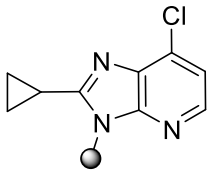
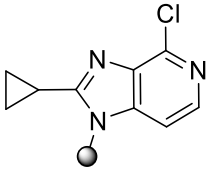
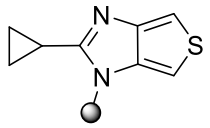
クロロ基を導入した化合物 **15a**、**16a** および **17a** は、MKN-45 凝集活性の EC_{50} 値がそれぞれ 2.0、1.4、2.5 nM と非常に強力であった。7 位クロロ体 **16a** のマウス PK (AUC = 10200 ng·h/mL) は化合物 **14a** (AUC = 3750 ng·h/mL) よりも良好であったがヒト肝固有クリアランスが化合物 **17a** よりも高い値を示した。7 位フルオロ体 **18a** は、強力な凝集活性 (EC_{50} = 1.7 nM) および良好なマウス AUC (5510 ng·h/mL) を示したが、ヒト肝固有クリアランス (hCL_{int} = 3.9 L/h/kg) に課題が残った。また、イミダゾ[1,2-*a*]ピリジン 2 位に酸素原子を導入した誘導体は、強い細胞凝集活性を示したが (**19a**: 1.0 nM, **20a**: 4.8 nM)、マウス PK が不十分であった (**19a**: AUC = 2450, **20a**: 1086 ng·h/mL)。対照的に、8,9-ジヒドロフロ[3,2-*c*]イミダゾ[1,2-*a*]ピリジン誘導体 **21a** は、優れたマウス AUC (8760 ng·h/mL) を示したが、細胞凝集活性 (EC_{50} = 19 nM) が化合物 **14a** (EC_{50} = 5.3 nM) に及ばなかった。

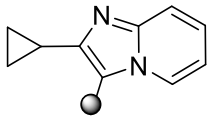
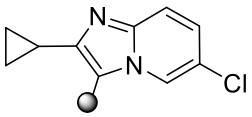
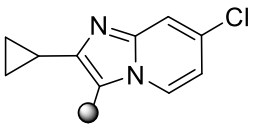
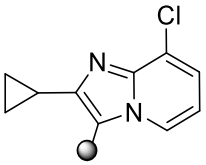
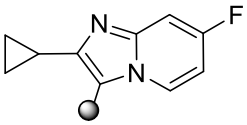
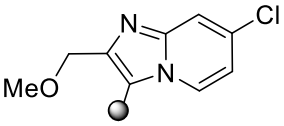
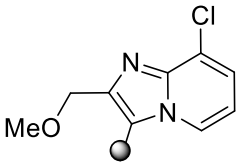
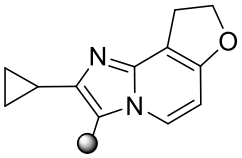
イミダゾ[1,2-*a*]ピリジン部位の最適化研究により、化合物 **17a** が活性および薬物動態の両観点で最有望である結果が得られた。そこで、化合物 **17a** の PPARγ LBD に対する結合様式を確認することとした。Figure 16(A)は、化合物 **17a** と PPARγ の複合体のX線結晶構造解析データである。化合物 **17a** は、オキサジアゾロンの酸性プロトンは Tyr473 の酸素原子と、イミダゾ[1,2-*a*]ピリジン部位はカノニカルサイトと相互作用していた。さらに、ジベンゾオキセピンのベンゼン環は、helix 3 および helix 11 で形成される疎水性ポケットを占有していた。化合物 **17a** と化合物 **9** のX線構造の重ね合わせを Figure 16(B)に示す。ベンゾイミダゾール上の窒素原子の位置を変換しても、化合物 **17a** の配座は化合物 **9** とよく重なる結果であった。

以上の結果より、化合物 **17a** は、化合物 **9** の示すがん細胞の分化誘導作用および特徴的な結合様式を踏襲し、薬物動態が改善した誘導体であることが明らかとなった。そこで、化合物 **17a** を用いて、*in vivo* における薬効および毒性評価を実施することとした。

Table 4. Effects on PPARγ reporter, MKN-45 aggregation activities and pharmacokinetic parameters of dibenzooxepine derivatives modified at the 8-substituted heterocycles



Compd.	R^2	Reporter gene assay		MKN-45 Aggregation assay		AUC (ng·h/mL)	hCL_{int} (L/h/kg)
		EC_{50} (nM)	Efficacy (%)	EC_{50} (nM)	Efficacy (%)		
9a		7.4	11.7±3.6	1.0	104	1480	3.2
11a		38	10.3±1.3	32	101	N.T.	2.3
12a		59	13.7±2.4	22	105	N.T.	10
13a		21	13.7±2.6	3.0	105	196	2.5

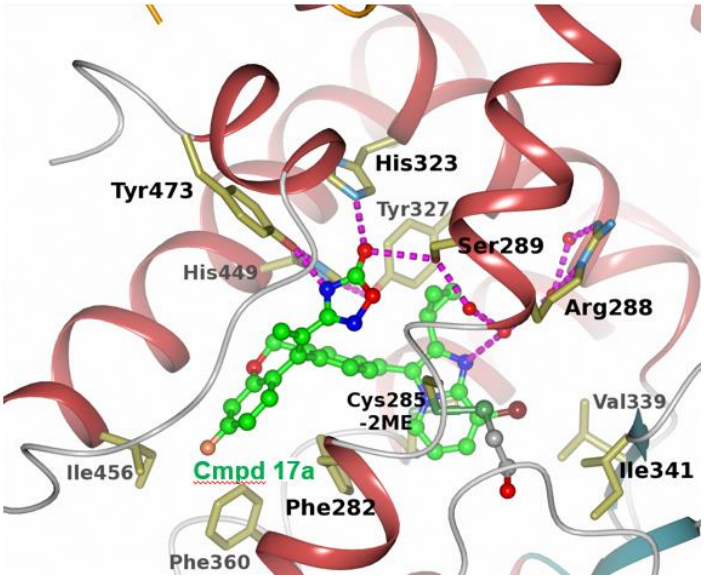
14a		19	9.8±3.1	5.3	106	3750	2.4
15a		11	15.1±1.5	2.0	102	1710	4.0
16a		3.6	12.6±1.7	1.4	101	10200	6.1
17a		4.2	10.6±0.4	2.5	103	6890	2.2
18a		13	13.5±2.2	1.7	112	5510	3.9
19a		5.3	9.0±1.2	1.0	106	2450	2.4
20a		7.3	7.4±0.3	4.8	96	1086	1.3
21a		23	9.7±0.7	19	110	8760	2.0

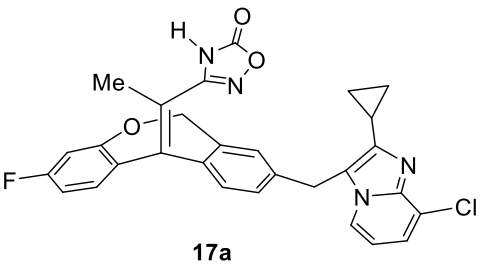
The efficacies and EC_{50} values of compounds **9a** and **11a**–**21a** in human PPARγ/GAL4 transfected HEK293EBNA cells at 24 h after drug treatment. The efficacy of each compound was calculated as the percentage of the maximum activation obtained with pioglitazone at 1000 nM. EC_{50} values were determined using the XLFit.

The aggregation of MKN-45 cells was evaluated using an IN Cell Analyzer 1000 (GE Healthcare) after treatment of compounds **9a** and **11a**–**21a** for 5 days. The aggregation efficacies were shown as the values relative to the maximum efficacy of **9**. EC_{50} values were determined using the XLFit.

The AUC was calculated from plasma drug concentrations in BALB/c mice treated orally at 10 mg/kg (*n* = 2). The hCLint was calculated from the elimination rate constant of each compound in human liver microsomes (*n* = 2). N.T.; not tested.

(A)





17a

(B)

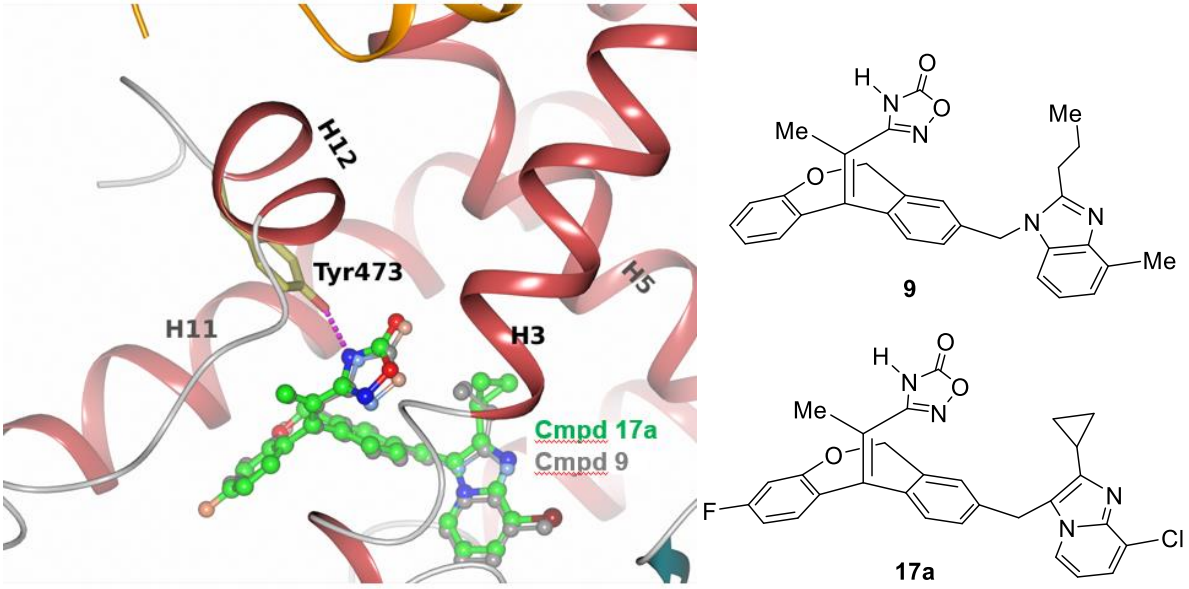


Figure 16. The X-ray crystal structures of ligands complexed with PPARγ(PDB: 6K0T). (A) The binding mode of **17a** (ball and stick; green) to the PPARγ LBD. The proton of the oxadiazolone ring of **17a** interacted with the phenolic oxygen of Tyr473. 2ME: 2-mercaptoethanol, which was used in the protein purification buffer and found in the ligand binding pocket as a covalent adduct to Cys285. (B) The superimposition of the complex structures of **17a** (green) and **9** (gray, PDB: 6AD9).

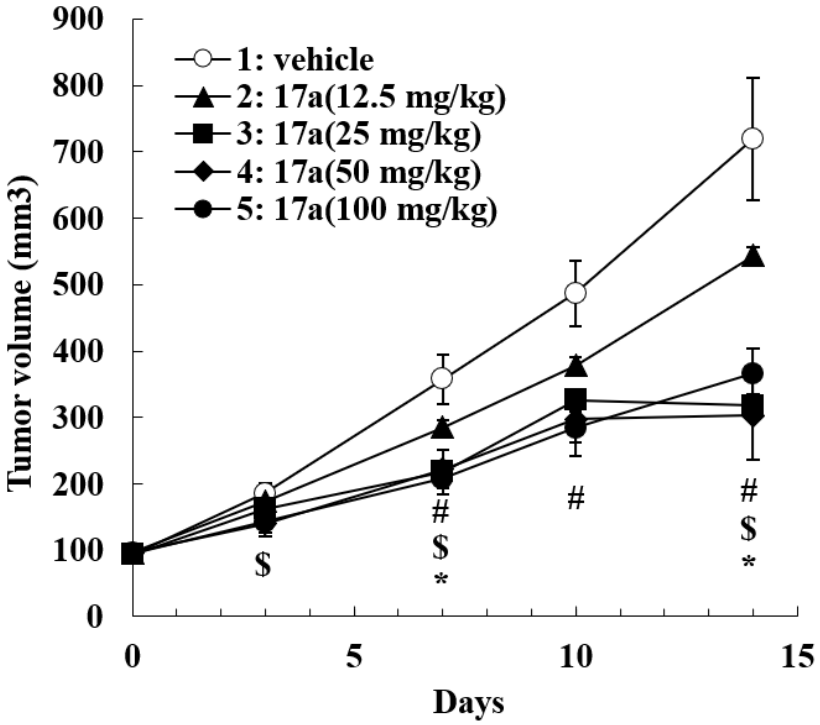
3. 4　化合物 **17a** の薬効評価

すい癌細胞株AsPC-1マウス皮下移植マウスを用いて化合物**17a**の抗腫瘍作用を評価した。*in vitro*評価で用いた胃がん細胞株MKN-45は低分化型のがん細胞株であり、SCIDマウスに皮下移植したところがん細胞の生着が認められなかった。筆者の化合物と同様にがん細胞の分化誘導作用を示すエファツタゾンでは、AsPC-1細胞においても分化誘導作用が確認されていたことから[8]、同細胞株の皮下移植マウスを用いて薬効を評価した。コントロール群と比較し、化合物**17a**を12.5, 25, 50, 100 mg/kgの各用量で1日2回、14日間経口投与したところ、投与開始から10日後には25 mg/kgの用量でコントロール群と比較し、有意に腫瘍増殖を抑制した。25 mg/kg投与群では薬効が飽和していたため、本評価系における薬効用量は12.5–25 mg/kgと考えられる(Figure 17(A))。

次に、化合物**17a** 100 mg/kg投与群において、薬物投与14日目最終投与の4時間後の腫瘍を採取し、AsPC-1腫瘍組織中のアンジオポエチン様タンパク質 4 (*angptl4*, PPARγの下流制御遺伝子) お

よび ケラチン20 (細胞分化マーカー、*krt20*[45]) のmRNA発現量をqPCRにて測定した。Figure 17(B)に示すように、化合物**17a**の投与により、コントロールに比べPPARγ の薬力学的マーカーである*angptl4*と同様に、細胞の分化度の指標である*krt20*のmRNA発現量が顕著に上昇していた。この結果から、化合物**17a**は*in vivo*においてもPPARγ作動活性に基づいて、がん細胞を分化誘導している可能性があると考えられる。

(A)



(B)

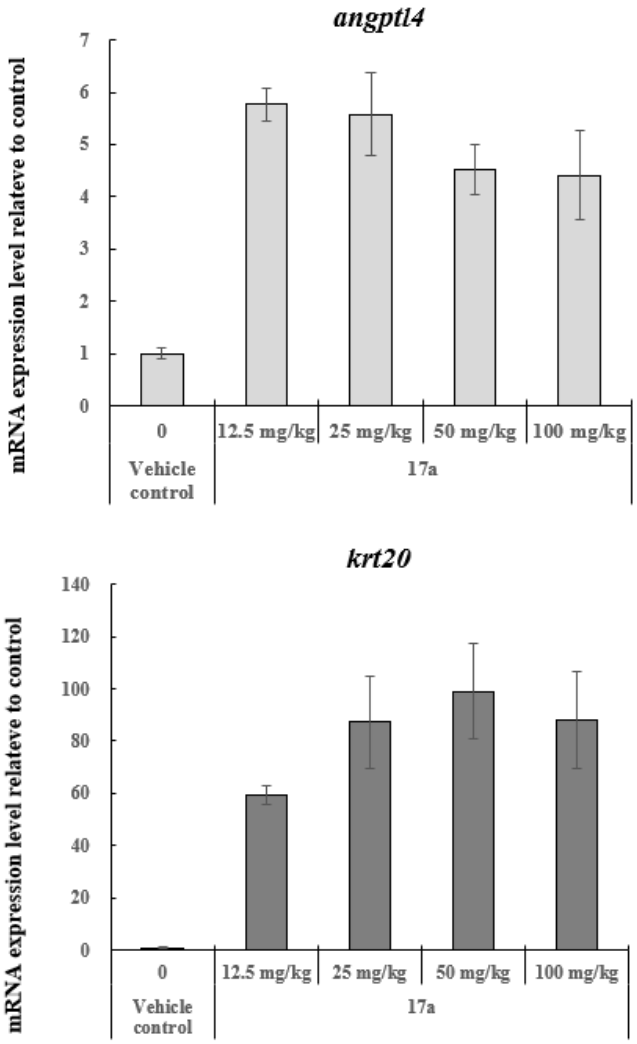


Figure 17. Antitumor effect of **17a** on AsPC-1/AG1 xenotransplanted mice. (A) Tumor growth inhibition after the administration of **17a** in the xenotransplanted mice. Each plot represents the mean ± SE of the relative tumor volume (n = 5). #: $p<0.05$ between the vehicle-treated group and the **17a** 25 mg/kg-treated group. $: $p<0.05$ between the vehicle-treated group and the **17a** 50 mg/kg-treated group. *: $p<0.05$ between the vehicle-treated group and the **17a** 100 mg/kg-treated group. All of the p values were calculated by the Steel test.

(B) The mRNA expression of *angptl4* and *krt20* in the xenotransplanted tumor. Compound **17a** was orally administered at 12.5, 25, 50 and 100 mg/kg twice a daily for 14 days. The gene expression was determined by quantitative PCR using an ABI PCR system. The fold-inductions are shown as the values relative to baseline.

3.5　化合物 **17a** の体液貯留の評価

PPARγ 作動薬の非臨床および臨床試験において、体液貯留に伴う浮腫が有害事象として報告されている。[46,47] この副作用はチアゾリジンジオン誘導体のファーマコフォアに基づく可能性が高いと考えられている。[48,49] すなわち、非チアゾリジンジオン構造を母核とする化合物 **17a** は、この体液貯留作用を示さない可能性が期待できる。そこで、**17a** の体液貯留作用を実際に評価した。体液貯留の評価は、健常の CD-1 マウスに化合物 **17a** を投与し、ヘマトクリット値を測定することで行った。化合物 **17a** を 1 日 2 回、4 日間経口投与したところ、AsPC-1 皮下移植マウスの薬効用量の 2 倍に相当する 50 mg/kg 投与群で、軽微なヘマトクリット値の低下に留まった (Figure 18, **17a**, 42.9%; vehicle, 44.1%)。さらに、薬効用量の約 8 倍以上である 200 mg/kg 投与群においても、ヘマトクリット値の低下は vehicle 群と比較して有意差が付かない程度であった (42.0%)。臨床でも体液貯留が報告されているピオグリタゾンと比較しても、化合物 **17a** の過剰量投与時のヘマトクリット値の低下は軽微であった。[41]

このように、非チアゾリジンジオン構造を有する化合物からのリード最適化によって、重篤な体液貯留を回避することに成功した。

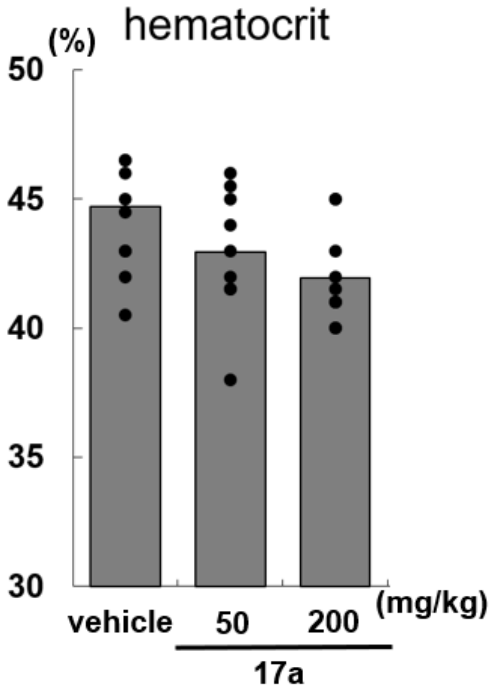


Figure 18. Hematocrit values in CD-1 mice treated orally with **17a** twice a daily at 50 and 200 mg/kg. p = 0.671 between the vehicle-treated group and the **17a** 50 mg/kg-treated group. p = 0.176 between the vehicle-treated group and the **17a** 200 mg/kg-treated group.

4　三環性化合物の合成

4. 1　化合物 **4**–**15** の合成

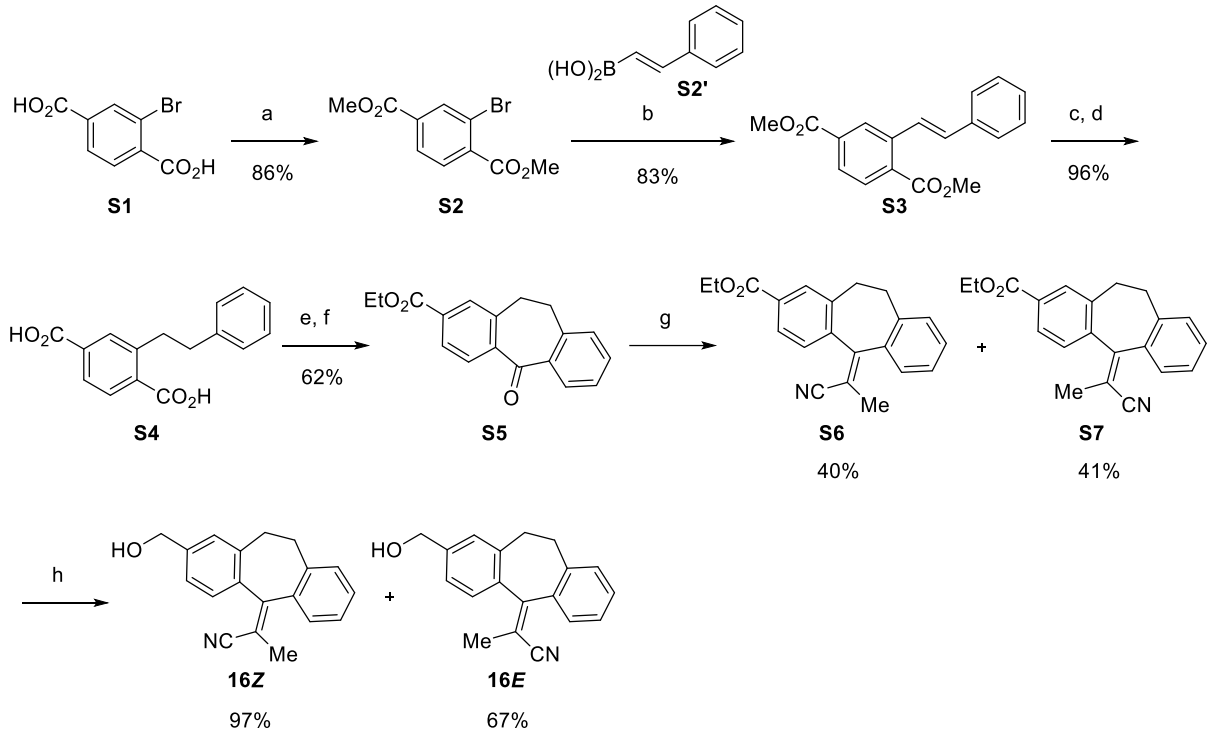
2-(2-(ヒドロキシメチル)-10,11-ジヒドロ-5*H*-ジベンゾシクロヘプテン-5-イリデン)プロパンニトリル **16*Z*** および **16*E*** は 2-ブロモテレフタル酸 **S1** より 8 工程にて合成した (Scheme 1)。また、(*E*)-2-(8-(ヒドロキシメチル)ジベンゾ[*b*,*e*]オキセピン-11(6*H*)-イリデン)プロパンニトリル **17*E*** は 5-ブロモイソベンゾフラン-1-オン **S8** から 5 工程にて調製した (Schme 2)。化合物 **2**–**11**,**14** および **15** は、**16*Z***、**16*E*** および **17*E*** より調製した (Scheme 3)。

イミダ[1,2-*a*]ピリジンを光延反応により **16*Z***、**16*E*** のベンジル位に導入し、化合物 **20*E***、**20*Z*** および **21*E*** を得た。**17*E*** のベンジル位の水酸基をブロモ化もしくはクロロ化することにより、化合物 **18** および **19** へとし、続いてアゾール環を導入することにより化合物 **22*E***–**29*E*** を取得した。

20*E*、**20*Z*** のシアノ基をトリメチルシリルアジド用いた[3+2]付加環化反応によりテトラゾール環へと変換し、化合物 **2** および **3** を合成した。さらに、**22*E***–**24*E***、**26*E***–**29*E*** のシアノ基に対するヒドロキシアミンの付加反応、続くエチルクロロホルメートを用いたアシル化およびカリウム *tert*-ブトキシドによる閉環反応により、オキサジアゾロン環を構築し目的物 **4**–**11**, **14** および **15** を合成した。

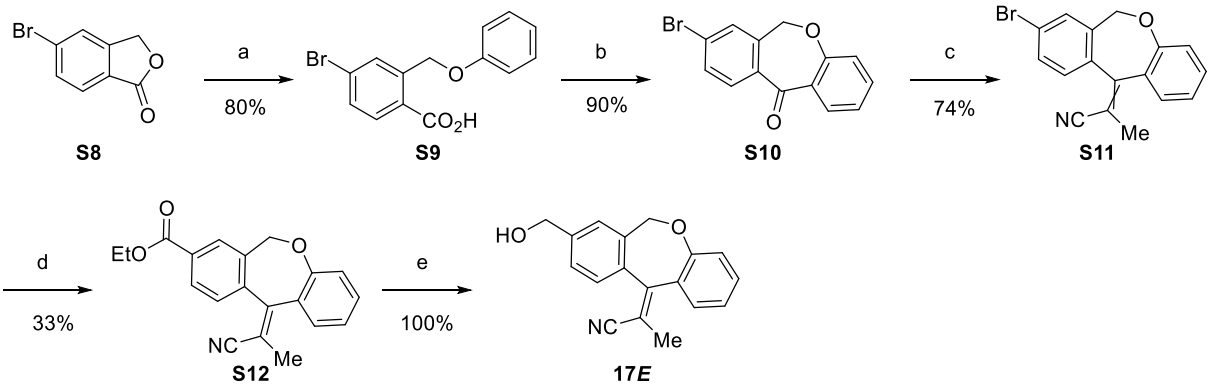
化合物**25*E***のベンゾイミダゾール4位置換基変換は、Scheme 4に従って進めた。化合物**25*E***のエステル基を加水分解した後、2-アミノエタノールと縮合しアミド体**31**を得た。また、ベンゾイミダゾール4位カルボン酸をCurtius転移によりBoc基に変換し、Boc基を脱保護したのちスルホニルクロライドを反応させることでスルホニルアミド体**32**を合成した。上記と同様の方法により、**31**および**32**のシアノ基をオキサジアゾロン環に変換し、化合物**12**および**13**をそれぞれ調製した。

Scheme 1. Synthesis of dibenzocycloheptane compounds [a]



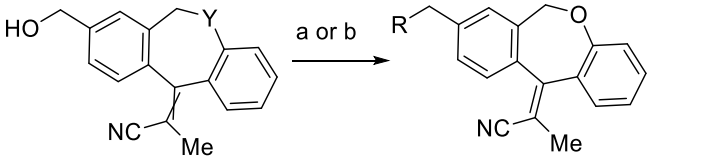
[a]Reagents: a) $HC(OMe)_3$, *conc*. H_2SO_4, MeOH; b) **S2'**, *cat*. $PdCl_2[P(o\text{-tolyl})_3]_2$, Na_2CO_3, DME/H_2O(5:1); c) 4 M NaOH, MeOH; d) *cat*. 10 wt% Pd on activated carbon, H_2 gas, DMF; e) polyphosphoric acid, sulfolane; f) $HC(OEt)_3$, *conc*. H_2SO_4, EtOH; g) diethyl chlorophosphate, propionitrile, LDA, THF/DMF; h) $LiBH_4$, THF.

Scheme 2. Synthesis of dibenzooxepine compounds [a]



[a]Reagents: a) Phenol, NaOMe/MeOH, NMP; b) TFAA, $BF_3 \cdot OEt_2$, ^{n}heptane; c) diethyl phosphorochloridate, propiononitrile, LDA, THF/DMF; d) $Pd(OAc)_2$, dppp, EtOH, Cs_2CO_3, CO gas, DMF; e) $LiBH_4$, THF.

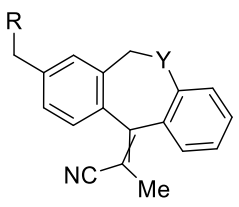
Scheme 3. Synthesis of dibenzocycloheptane and dibenzooxepine compounds [a]



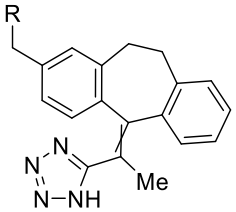
16*E*:Y = CH_2
16*Z*:Y = CH_2
17*E*:Y = O

18:R = Cl(condition a) 96%
19:R = Br(condition b) 38%

c d



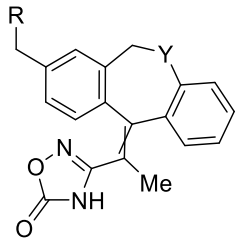
e



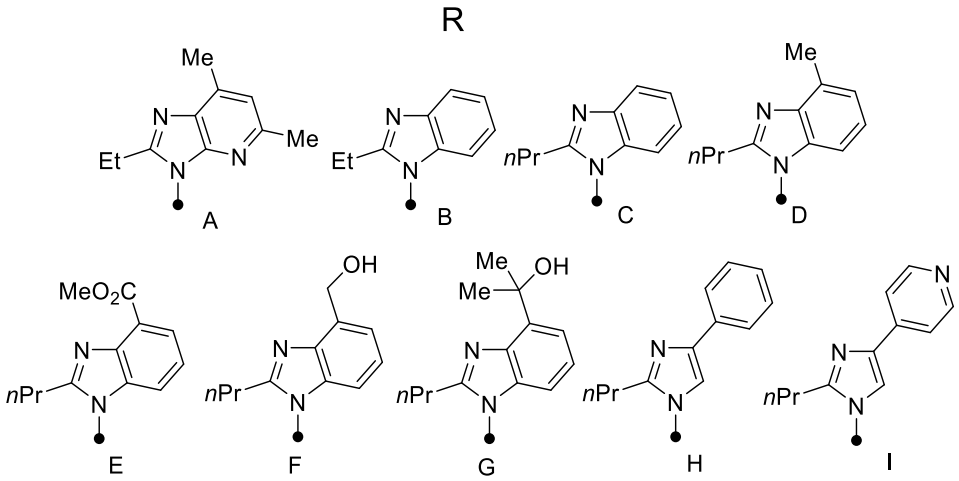
2:R = A,*E*-form 90%
3:R = A,*Z*-form 74%

20*E*:R = A,Y = CH_2	69%
20*Z*:R = A,Y = CH_2	68%
21*E*:R = A,Y = O	63%
22*E*:R = B,Y = O	77%
23*E*:R = C,Y = O	100%
24*E*:R = D,Y = O	57%
25*E*:R = E,Y = O	74%
26*E*:R = F,Y = O	89%
27*E*:R = G,Y = O	100%
28*E*:R = H,Y = O	91%
29*E*:R = I,Y = O	40%

f,g,h

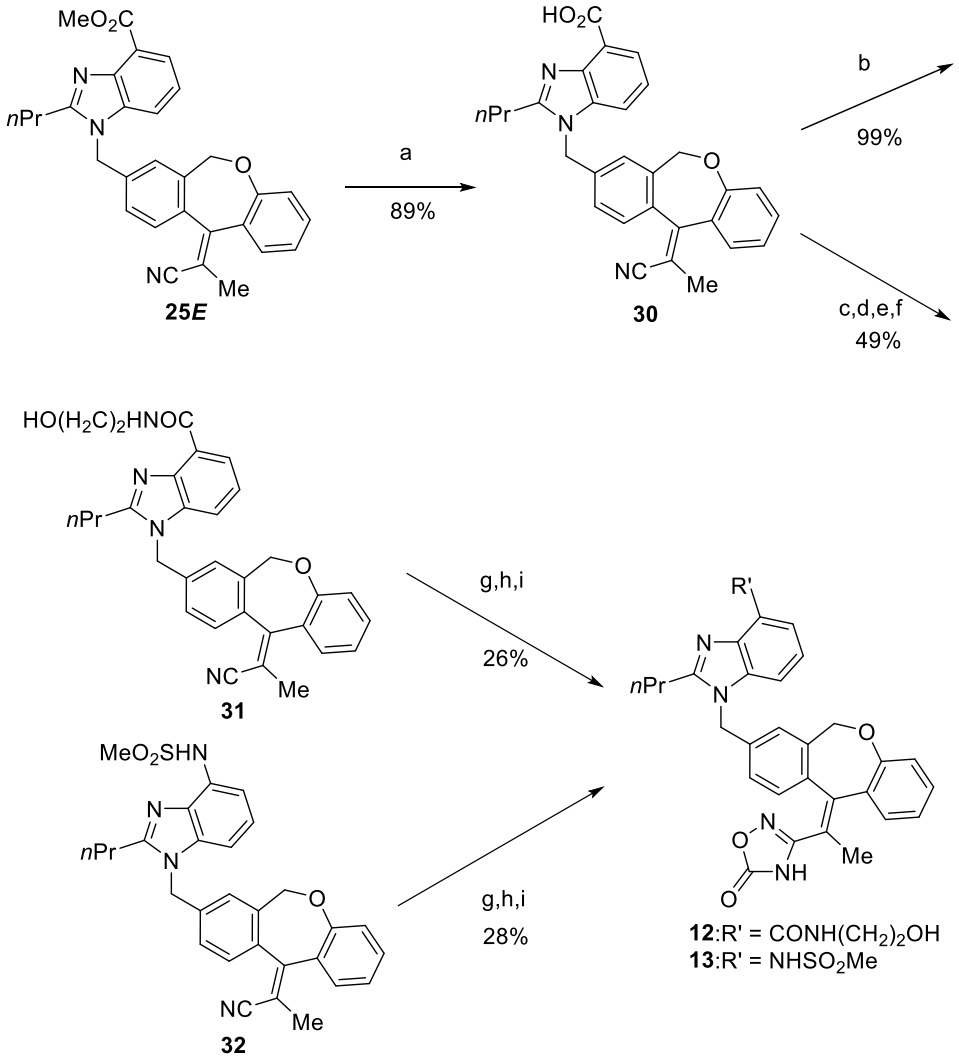


4:R = A,Y = CH_2	76%	**9**:R = D,Y = O	22%
5:R = A,Y = CH_2	56%	**10**:R = F,Y = O	10%
6:R = A,Y = O	36%	**11**:R = G,Y = O	37%
7:R = B,Y = O	4.8%	**14**:R = H,Y = O	36%
8:R = C,Y = O	12%	**15**:R = I,Y = O	8.3%



[a]Reagents: a) MsCl, LiCl, Et_3N, THF; b) Ms_2O, LiBr, 2,6-lutidine, THF; c) RH, DTBAD, ps-PPh_2, THF; d) RH, K_2CO_3, DMF; e) $TMSN_3$, $^nBu_2SnO$, toluene, 90 ^oC; f) 50% H_2NOH aq., EtOH, reflux; g) $ClCO_2Et$, pyridine or Et_3N, CH_2Cl_2; h) *tert*-BuOK, toluene, THF.

Scheme 4. Modification of the substituents in 4-position on benzimidazole ring [a]



[a]Reagents: a) 4 M NaOH, EtOH, 60 °C; b) $NH_2(CH_2)_2OH$, EDCI･HCl, HOBt, DMF; c) DPPA, Et_3N, $CHCl_3$; d) *tert*-BuOH, reflux; e) TFA, CH_2Cl_2; f) $MeSO_2Cl$, DMAP, pyridine; g) 50% H_2NOH aq., EtOH, reflux; h) $ClCO_2Et$, pyridine or Et_3N, CH_2Cl_2; i) *tert*-BuOK, toluene, THF.

4.2　化合物 **8a**–**21a** の合成

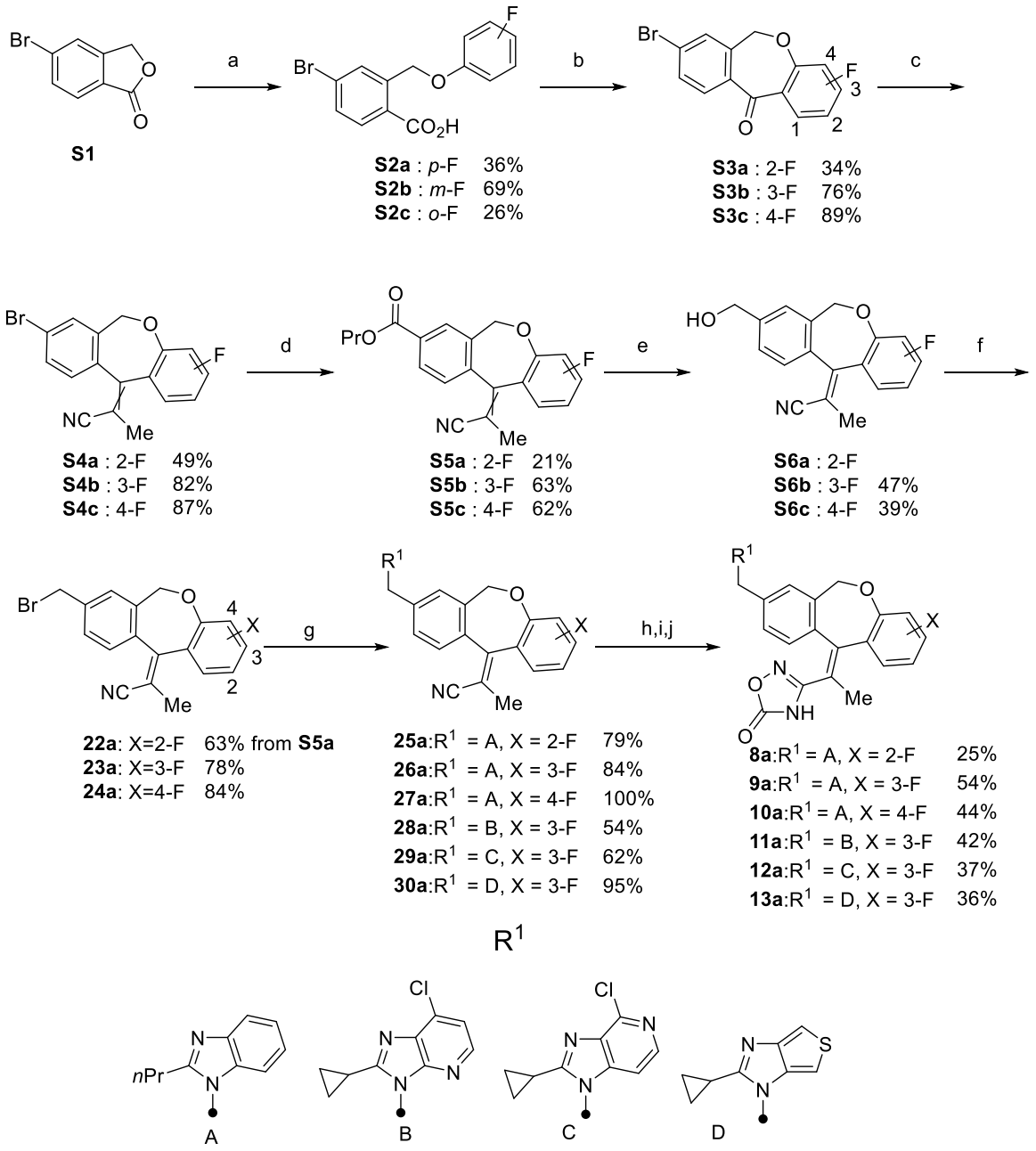
化合物 **8a**–**13a** は 8-(ブロモメチル)-ジベンゾ[*b*,*e*]オキセピン誘導体 **22a**–**24a** より合成した (Scheme 5)。**22a**–**24a** は、5 工程にて 5-ブロモイソベンゾフラン-1-オンから得た。各種イミダゾールを炭酸カリウム存在下、**22a**–**24a** と室温で反応させ、カップリング体 **25a**–**30a** を取得した。続いて、前述のようなシアノ基の閉環反応に付すことにより化合物 **8a**–**13a** を取得した。

イミダゾ[1,2-*a*]ピリジン **14a**–**18a** は銅触媒存在下による 3 成分縮合反応により合成した (Scheme 6)。[50] 銅(II)トリフルオロメタンスルホネートの存在下、2-アミノピリジン、アルデヒドおよび別途 **S4b** より薗頭反応により調製した 2-(8-エチル-3-フルオロジベンゾ[*b*,*e*]オキセピン-

11(6*H*)-イリデン)プロパンニトリル **31a** を加熱することにより、**32a**–**36a** を合成した。化合物 **31a** は幾何異性体の混合物であり、3 成分縮合反応の生成物も幾何異性体の混合物として得られた。これらの異性体は、シリカゲルカラムクロマトグラフィーで所望の *E* 体を分離して **32a**–**36a** を取得している。最後に、定法通りオキサジアゾロン環を構築することにより、**14a**–**18a** を合成した。

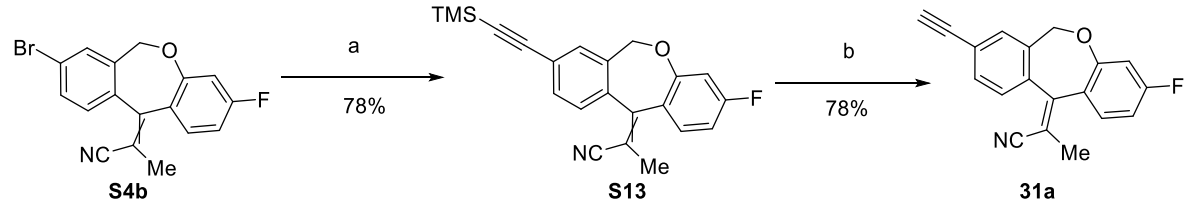
(*E*)-2-(3-フルオロ-8-ホルミルジベンゾ[*b*,*e*]オキセピン-11(6*H*)-イリデン)プロパンニトリル **37a** を用いたイミダゾ[1,2-*a*]ピリジン誘導体の合成法を Scheme 7 に示す。中間体 **37a** は、**S6b** のベンジルアルコールを Dess-Martin 試薬により酸化することで調製した。Grignard 試薬存在下、3-ヨードイミダゾ[1,2-*a*]ピリジンをアルデヒドに反応させた後、生じたベンジル位の水酸基を還元することにより、カップリング体 **38a**–**40a** を合成した。最後に、上記と同様にシアノ基からオキサジアゾロン環を構築し、化合物 **19a**–**21a** を合成した。

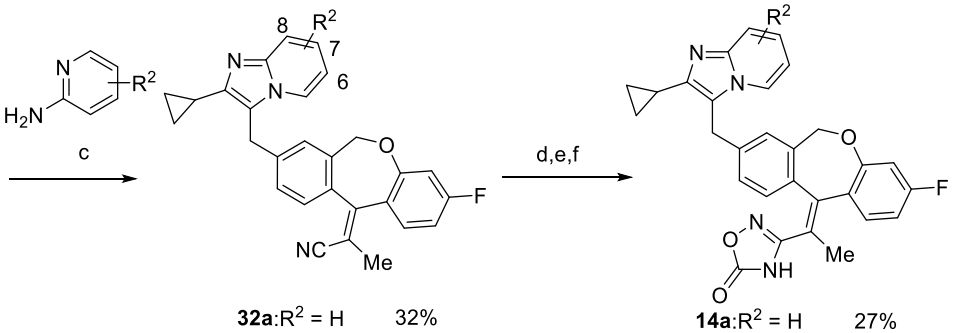
Scheme 5. Synthesis of benzimidazole analogues [a]



[a]Reagents:a) ArOH, 28%NaOMe in MeOH, DMF; b) TFAA, $BF_3 \cdot OEt_2$, CH_2Cl_2; c) diethyl chlorophosphate, propionitrile, LDA, THF; d) $Pd(OAc)_2$, dppp, PrOH, Cs_2CO_3, CO gas, DMF; e) $LiBH_4$, THF; f) Ms_2O, LiBr, 2,6-lutidine, THF; g) R^1H, K_2CO_3, DMF; h) 50% H_2NOH aq., EtOH, reflux; i) $ClCO_2Et$, Et_3N, CH_2Cl_2; j) *tert*-BuOK, toluene, THF.

Scheme 6. Introduction of the substituents to benzo[1,2-*a*]imidazole ring [a]



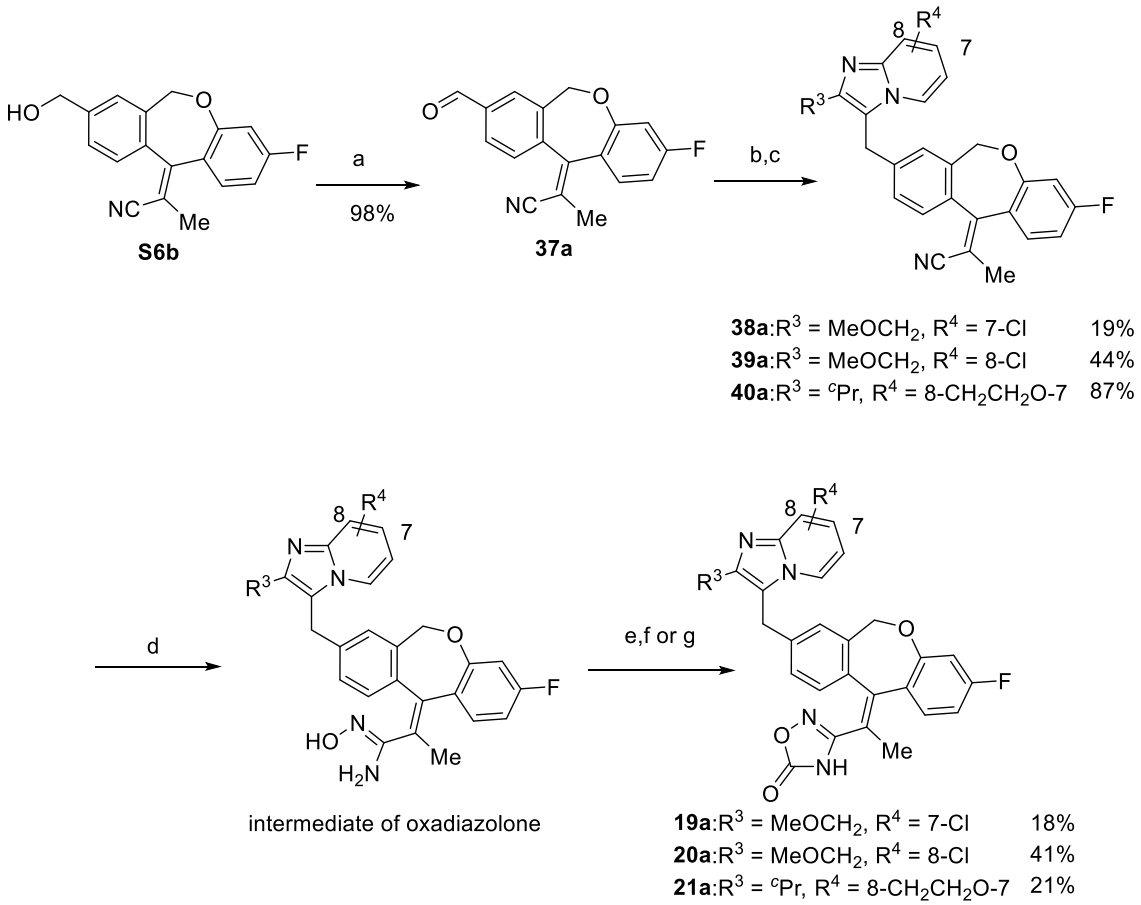


32a:R^2 = H 32%
33a:R^2 = 6-Cl 14%
34a:R^2 = 7-Cl 13%
35a:R^2 = 8-Cl 15%
36a:R^2 = 7-F 4.3%

14a:R^2 = H 27%
15a:R^2 = 6-Cl 36%
16a:R^2 = 7-Cl 46%
17a:R^2 = 8-Cl 27%
18a:R^2 = 7-F 37%

[a]Reagents: a) TMSacetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, DMF; b) K_2CO_3, MeOH; c) [c]PrCHO, CuCl, $Cu(OTf)_2$, toluene, 120 °C; d) 50% H_2NOH aq., EtOH, reflux; e) $ClCO_2Et$, Et_3N, CH_2Cl_2; f) *tert*-BuOK, toluene, THF.

Scheme 7. Synthesis of the side chain structure from 8-formyldibenzooxepine [a]



[a]Reagents: a) Dess-Martin periodinane, CH_2Cl_2; b) 3-iodoimidazo[1,2-*a*]pyridine, ^{i}PrMgCl, THF, -40 ^{o}C; c) NaI, Me_2SiCl_2, CH_2Cl_2, acetone, 0 ^{o}C or NaI, Me_3SiCl, hexane, acetonitrile, rt or TFA, Et_3SiH, 60 ^{o}C; d) 50% H_2NOH aq., EtOH or DMSO, reflux; e) $ClCO_2Et$, Et_3N, CH_2Cl_2; f) *tert*-BuOK, toluene, THF; g) 1,1'-carbonyldiimidazole, DBU, 1,4-dioxane, 110 ^{o}C.

5　結論

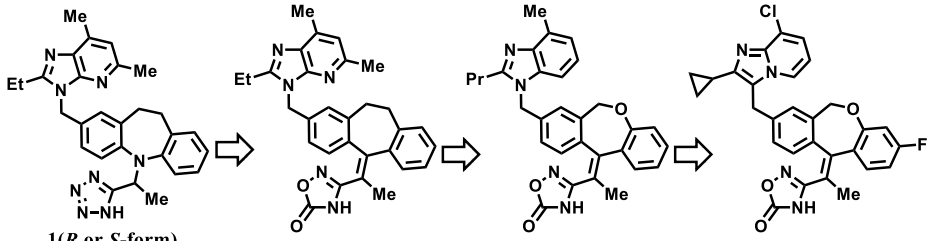
シード化合物 **1-DM** に対して、テトラゾールおよびジベンゾアゼピン母核の相対的空間立体配置を規定すべくメチル基を導入した化合物 **1** は、大幅にレポーター活性が向上した (EC_{50} = 197 nM)。さらに、配座解析の結果より、化合物 **1** のテトラゾールの *syn/anti* 配向性をジベンゾシクロヘプタンメチリデン *exo*-オレフィンの *E/Z* 異性体で模倣することができると考え、**1** の不斉炭素を回避すべく対応する *E/Z* 異性体を設計、合成した。その結果、*Z* 体 **5** はヒット化合物 **1** と同等の PPARγ 作動活性を示し (EC_{50} = 177 nM)、さらなる母核とイミダゾピリジン部位の最適化研究により、化合物 **1** の 100 倍程度強力なレポーター活性 (EC_{50} = 2.4 nM) を示すリード化合物 **9** を見出した (Table 5)。化合物 **9** の PPARγ LBD との複合体の X 線結晶構造解析は、PPARγ の構造変化を伴う、既存の PPARγ 作動薬と異なる特徴的な結合様式であることが明らかとなった。興味深いことに、(*R*)-**1** (*down-syn*) の安定配座は、化合物 **9** のX線結晶構造中の配座と良好な重なりを示したことから、(*R*)-**1** の生理活性配座は *down-syn* 配座であることが示唆された。さらに、化合物 **9** は、低分化胃がん細胞株 MKN-45 の強力な分化誘導作用を示した。しかし、化合物 **9** は、酸化的代謝に不安定な誘導体であったため、薬物動態プロファイルの改善が必要であった。

化合物 **9** のジベンゾオキセピンの右側ベンゼン環に窒素原子を導入し脂溶性を低下させた化合物 **7a** では、レポーター活性が大幅に低下したものの大幅な代謝安定化が確認された。この右側ベンゼン環が酸化代謝に感受性が高い部位であると考え、代謝ブロックを目的に右側ベンゼン環にフッ素原子を導入したところ、3 位フルオロ体 **9a** においてのみ大幅な代謝安定化が確認され、マウス PK が改善した。さらなる有望化合物を取得すべく、ベンゾイミダゾール部位の最適化研究を進めた結果、イミダゾ[1,2-*a*]ピリジン誘導体 **17a** において、強力な MKN-45 の凝集活性を示し、マウス PK も良好な化合物を取得するに至った。また、X線結晶構造解析の結果から、化合物 **17a** は化合物 **9** の特徴的な PPARγ LBD に対する結合様式を保持していた。

このように得られた有望化合物 **17a** の AsPC-1 皮下腫瘍マウスにおける薬効を評価したところ、経口投与において有意な腫瘍の増殖抑制作用が認められた。さらに、化合物 **17a** は、非チアゾリジンジオン構造の新規骨格を有していることから、PPARγ 作動薬の課題である体液貯留作用を回避していることが期待された。本化合物のマウス過剰量投与時 (AsPC-1 薬効用量の 8 倍、200 mg/kg、1 日 2 回、4 日間投与) における体液貯留は、vehicle 群に対し有意差が付かない程度であり重篤な体液貯留を示さないことを確認している。

以上のように、筆者は有望な薬効および安全性プロファイルを有する、新たな抗腫瘍剤の候補化合物 **17a** を創製することに成功した。

Table 5.起点化合物からの有望化合物**17a**創製までの流れ



1(*R* or *S*-form)
(-)-form 86%ee

5

9

17a

HEK293 reporter EC_{50} (nM)	197	177	2.4	4.2
MKN-45 aggregation EC_{50} (nM)	N.T.	N.T.	3.3	2.5
hCL_{int} (L/h/kg)	4.3	>24.9	>24.9	2.2

6 試験方法

Chimeric GAL4-PPARγ transactivation reporter assay.

Test compounds were screened for agonist activity on PPARγ-GAL4 chimeric receptors in transiently transfected HEK293EBNA cells. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific) containing 10% fetal bovine serum (Thermo Fisher Scientific), 100 U/mL penicillin, and 100 μg/ml streptomycin (Thermo Fisher Scientific) and incubated at 37 ºC in 5% CO_2. To transfect the reporter constract into HEK293EBNA cells, cells were seeded at 1x 10^5 cells/ml in tissue culture dish (Iwaki, Chiba, Japan). After 24h incubation, transfections were performed with Superfect transfection reagent (QIAGEN) according to the instructions of the manufacturer. In brief, pM-human PPARγ/GAL4 expression vector and pZAC19-Luc vector were premixed and transfected into the cells followed by 2.5 h incubation. After a further 24 h incubation with growth medium, the transfected cells were seeded into 96-well assay plates, and test compounds were added (1-3000 nM, N = 3 per concentration). The test compounds were initially dissolved in DMSO, and then diluted in DMEM without any supplement. Steady-Glo luciferase assay reagent (Promega) was used as a substrate, and the luciferase activity was measured using the Microplate Scintillation and luminescence counter TopCount NXT (Packard, Groningen, Netherlands). The luciferase activity was normalized to that of pioglitazone at 1000 nM. The maximum activation (efficacy) of pioglitazone was taken as 100%. The efficacy of each compound was calculated as the percentage of the maximum activation obtained with pioglitazone. EC_{50} values were determined by the concentration that was 50% of its maximum activity using the XLFit.

MKN45 cell aggregation assay.

MKN45 cells were aggregated when cells were incubated with PPARγ agonists. The cells were maintained in RPMI1640 medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (Thermo Fisher Scientific), 100 U/mL penicillin, and 100 μg/ml streptomycin(Thermo Fisher Scientific) and incubated at 37 ºC in 5% CO_2. Cells were seeded at 2500 cells/well in 96-well assay plates and incubated with test compounds, (1-1000 nM, N = 3 per concentration), for 5 days. Cell images were captured using an IN Cell Analyzer 1000 (GE healthcare). Nuclei were stained with the Hoechst 33342 (SIGMA) dyes, and the nuclei area was calculated. An area exceeding 1,015.58 μm^2 was defined as an aggregated cell cluster. The area

ratio of aggregated cell clusters to the total cell area was calculated and taken as the formation rate of aggregation. The cell aggregation-inducing activity was normalized to that of **9**, with the maximum activity of **9** taken as 100%. The maximum activity of each compound was then calculated as the percentage of the maximum activity of **9**. EC_{50} values was determined as the concentration achieving 50% of its maximum activity using the XLFit.

MKN-45 cell gene expression analyses.

MKN45 cells were seeded in assay plate and incubated with test compounds, (N = 3 per concentration), for 72 h. Total RNA was isolated from MKN45 cells using a RNeasy Mini Kit (QIAGEN). The cells were washed with cold PBS and lysed with buffer according to the instructions of the manufacturer. The RNA was reverse-transcribed using a SuperScript VILO cDNA Synthesis kit (Thermo Fisher Scientific) and synthesized to cDNA. Quantitative PCR was performed with Taqman fluorescent dye using an ABI PCR system. For PCR primers and probes, we used the Taqman® Gene Expression Assays system (Thermo Fisher Scientific) for ANGPTL4 (Hs_01101127_m1), VIM (Hs00958116_m1), and ADFP (Hs_00765634_m1). The PCR primer/probe sequences for GAPDH were as follows. Forward: ACAGTCAGCCGCATCTTCTTT, Reverse: CCCAATACGACCAAATCCGT, Probe: 6FAM-CGAGCCACATCGCTCAGACACCAT-Tamra (Operon). The gene expression value was corrected based on the value of GAPDH. The fold-induction ratio to the control was calculated.

Molecular modelling methods.

The three-dimensional molecule structures of **1-DM'**, **1'-3'** were built using Schrödinger MacroModel10.9. The conformational search of each compound was performed using mixed torsional/low-mode sampling as implemented in MacroModel 10.9 with OPLS_2005 force field. The conformational analysis was carried out without solvent. The default values were used for all other settings. The obtained global minimum energy conformations of the ligands were superimposed.

Animals.

Male BALB/c mice were purchased from Charles River Ltd. (Tokyo, Japan). They were maintained on a constant 12-h light/dark cycle in a temperature- and humidity-controlled room and were given food and water. Male severe combined immunodeficient (SCID) mice were purchased from CLEA Japan, Inc., and

used at eight weeks of age. All animal experiments were performed in compliance with protocols approved by the Institutional Animal Care and Use Committee of Kyowa Kirin.

Pharmacokinetics Studies.

Each compound was orally administered to mice. Blood samples in mice were collected from the tail vein at appropriate time-points and centrifuged to obtain plasma. Diluted plasma samples were extracted using protein precipitation with acetonitrile containing an internal standard. The collected supernatants were subjected to LC-MS/MS analysis using an atmospheric pressure chemical ionization interface in positive ion mode. The compound concentrations in the plasma samples were calculated using calibration standards curve in control plasma. The AUC was obtained from the plasma concentration-time profiles using noncompartmental analysis.

Metabolic stability assay in human liver microsomes.

An incubation mixture was prepared consisting of human liver microsomes, each compound and a potassium phosphate buffer containing $MgCl_2$ (pH 7.4). The reactions were initiated with the addition of NADPH and held for 30 min at 37 °C, and then terminated by adding ice-cold acetonitrile containing internal standards. The supernatant was subjected to LC-MS/MS analysis. The *in vitro* disappearance rate constant for each compound was determined by the slope of the linear regression from log percentage remaining versus incubation time relationships, and the intrinsic clearance was calculated from the elimination rate constant, the microsomes concentration and liver microsomes amount per body weight.

Xenograft Study.

AsPC-1/AG1 cells (3×10^7) suspended in 0.1mL PBS were inoculated subcutaneously into SCID mice. **17a** or control (0.5 % MC400) administration into tumor-bearing SCID mice was started 7 days after the tumor inoculation. Twenty-five mice were divided into 5 groups of 5 mice per group for **17a** (12.5, 25, 50 or 100 mg/kg) or control, such that the average tumor volumes were equivalent among the groups. The tumor volume was monitored twice weekly and calculated by the following formula: tumor volume (mm^3) = 0.5 × (major diameter) × (minor diameter)^2. Mice were treated with oral administration of **17a** (12.5, 25, 50 or 100 mg/kg) or control twice a day for 14 days. The relative tumor volume in each mouse was represented as V/V_0, where V_0 was the volume on Day0. The difference in the V/V_0 between the control group and each

treatment group was assessed by the Steel test. Statistical analyses were performed using the SAS software program (Release 9.1.3, SAS institute Inc.). In our study, $P<0.05$ was considered significant.

RT-qPCR.

Tumor tissues were collected from AsPC-1/AG1 tumor-bearing mice 4 h after the last dose of bid ×14 oral administration. Total RNA was extracted using RNeasy mini Kit (Catalog no. 74104; Qiagen). RNA was converted to cDNA using SuperScript VILO cDNA synthesis kit (Cat No.11754250; Invitrogen) according to the manufacturer's protocol. qPCR was performed using Taqman Gene Expression Master Mix (Catalog no. 4352042; Applied Biosysems) with cDNA and Taqman probes (#KRT20 Hs000300643_m1 and ANGPTL4 Hs01191127_m1; Applied Biosystems) on 7500 Fast Real-Time PCR system (Applied Biosystems).

Hematocrit test.

Male Crlj:CD1(ICR) mice (n = 10 per group) 7 to 8 weeks old at the start of dosing were orally administered derivative **17a** twice a day (10 mL/kg/time) at doses of 0 (vehicle: 0.5%[w/v] methyl cellulose 400), 50 and 200 mg/kg for 4 days. On the day after the dosing period, blood samples were collected in hematocrit tubes and the hematocrit values were determined by the micro-hematocrit method.

7 実験項

General Methods.

All reagents and solvents were procured from commercial sources and used as received. Thin layer chromatography (TLC) was carried out using Merck GmbH Precoated silica gel 60 F254. Chromatography on silica gel was carried out using prepacked silica gel cartridges (Yamazen Hi-Flash Column Silicagel or Wako Presep® Silicagel). Chemical shifts in ^{1}H NMR spectra were reported in δ values (ppm) relative to trimethylsilane. HPLC analyses were performed following conditions: Waters Xbrige® C18 column (3.5 μm, 4.6 mm × 50 mm), 30 °C column temperature, 1.0 mL/min flow rate, photodiode array detection (254 nm), and linear mobile phase gradient of 20%−90% B over 5 min, holding for 3.5 min at 20% B (mobile phase A, 0.05% trifluoroacetic acid in water; mobile phase B, acetonitrile), by which the purities of final compounds were confirmed as >95%. Mass spectra were recorded on a Waters 2695 (ESI-MS).

(*E*)-3-((5-(1-(1*H*-Tetrazol-5-yl)ethylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-yl)methyl)-2-ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridine (2). To a solution of **20*E*** (193 mg, 0.447 mmol) and dibutyltin oxide (44 mg, 0.18 mmol) in toluene (5.5 mL) was added trimethylsilylazide (0.474 mL, 3.57 mmol) and the solution was stirred at 80 °C for 48 h. To the reaction mixture was added methanol and the solution was concentrated to dryness. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 96:4 $CHCl_3$/methanol) to afford **2** (190 mg, 90%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.5 Hz, 3H), 2.31 (s, 3H), 2.53 (s, 3H), 2.58 (s, 3H), 2.66–2.78 (m, 4H), 3.09–3.25 (m, 2H), 5.41 (s, 2H), 6.88–6.91 (m, 2H), 6.99–7.02 (m, 2H), 7.13–7.32 (m, 4H). The proton of tetrazole was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 476. HPLC: purity 99%, R_T 3.25 min.

(*Z*)-3-((5-(1-(1*H*-Tetrazol-5-yl)ethylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-yl)methyl)-2-ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridine (3). A solution of **20*Z*** (212 mg, 0.49 mmol) in toluene (6.0 mL) at room temperature was treated with dibutyltin oxide (48 mg, 0.19 mmol) and trimethylsilylazide (0.516 mL, 3.88 mmol), and then the mixture was stirred at 80 °C for 48 h. To the reaction mixture was added methanol and the solution was concentrated to dryness. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 96:4 $CHCl_3$/methanol) to afford **3** (171 mg, 74%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.6 Hz, 3H), 2.33 (s, 3H), 2.54 (s, 3H), 2.61 (s,

3H), 2.68–2.82 (m, 2H), 2.75 (q, J = 7.6 Hz, 2H), 3.15–3.34 (m, 2H), 5.38 (s, 2H), 6.83–6.94 (m, 4H), 7.14–7.24 (m, 4H). The proton of tetrazole was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 476. HPLC: purity 99%, R_T 3.57 min.

(*E*)-3-(1-(2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (4). A solution of **20*E*** (192 mg, 0.44 mmol) in ethanol (4.4 mL) was treated with NH_2OH solution 50wt.% in water (0.54 mL, 8.9 mmol), and the resulting mixture was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in DMF (4.4 mL). To a stirred solution was added pyridine (44 μL, 0.53 mmol) and ethyl chloroformate (52 μL, 0.53 mmol) and the solution was stirred for 1h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (4.4 mL) was added *tert*-BuOK (100 mg, 0.888 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 98:2 $CHCl_3$/methanol) to give **4** (166 mg, 76%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.5 Hz, 3H), 2.09 (s, 3H), 2.57 (s, 3H), 2.62 (s, 3H), 2.72–2.87 (m, 4H), 3.21–3.37 (m, 2H), 5.40 (s, 2H), 6.91–6.94 (m, 3H), 7.06–7.11 (m, 2H), 7.19–7.35 (m, 3H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 492. HPLC: purity 97%, R_T 3.63 min.

(*Z*)-3-(1-(2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (5). To a stirred solution of **20*Z*** (197 mg, 0.456 mmol) in ethanol (4.5 mL) was added NH_2OH solution 50wt.% in water (0.56 mL, 9.24 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic

layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in DMF (4.5 mL). To a stirred solution was added pyridine (0.046 mL, 0.55 mmol) and ethyl chloroformate (0.054 mL, 0.55 mmol) and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (4.5 mL) was added *tert*-BuOK (104 mg, 0.924 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 98:2 $CHCl_3$/methanol) to give **5** (126 mg, 56%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, J = 7.5 Hz, 3H), 2.11 (s, 3H), 2.55 (s, 3H), 2.63 (s, 3H), 2.75–2.83 (m, 2H), 2.79 (q, J = 7.5 Hz, 2H), 3.24–3.34 (m, 2H), 5.42 (s, 2H), 6.89–6.94 (m, 3H), 7.05–7.24 (m, 5H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 492. HPLC: purity 95%, R_T 3.85 min.

(*E*)-3-(1-(8-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (6). To a stirred solution of **21*E*** (145 mg, 0.334 mmol) in ethanol (1.5 mL) was added NH_2OH solution 50wt.% in water (0.41 mL, 6.7 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in DMF (1.0 mL). To a stirred solution was added pyridine (0.053 mL, 0.66 mmol) and ethyl chloroformate (0.090 mL, 0.7 mmol) and the solution was stirred for 1h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (3.0 mL) was added *tert*-BuOK (70 mg, 0.66 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate.

The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 98:2 $CHCl_3$/methanol) to give **6** (60 mg, 36%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 1.23 (t, J = 7.5 Hz, 3H), 2.06 (s, 3H), 2.54 (s, 3H), 2.58 (s, 3H), 2.68 (q, J = 7.5 Hz, 2H), 4.51 (d, J = 12.6 Hz, 1H), 5.28 (d, J = 12.6 Hz, 1H), 5.44 (s, 2H), 6.65 (dd, J = 8.3, 1.1 Hz, 1H), 6.80 (td, J = 7.5, 1.1 Hz, 1H), 6.90 (s, 1H), 7.01 (dd, J = 7.8, 1.7 Hz, 1H), 7.06 (s, 1H), 7.11–7.15 (m, 1H), 7.17 (br s, 2H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 494. HPLC: purity 99%, R_T 3.6 min.

(*E*)-3-(1-(8-((2-Ethyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (7). To a stirred solution of **22*E*** (157 mg, 0.387 mmol) in ethanol (3.0 mL) was added NH_2OH solution 50wt.% in water (0.712 mL, 11.6 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in THF (2.2 mL). To a stirred solution was added Et_3N (81 μL, 0.58 mmol) and ethyl chloroformate (56 μL, 0.58 mmol) and the solution was stirred for 1h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (3.9 mL) was added *tert*-BuOK (65 mg, 0.58 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (99:1 to 95:5 $CHCl_3$/methanol) to to give **7** (8.7 mg, 4.8%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 1.30 (t, J = 7.4 Hz, 3H), 2.31 (s, 3H), 2.90 (q, J = 7.4 Hz, 2H), 4.67 (d, J = 12.6 Hz, 1H), 5.29–5.44 (m, 2H), 5.53 (d, J = 12.6 Hz, 1H), 6.77–6.86 (m, 1H), 6.87–7.07 (m, 3H), 7.11–7.37 (m, 6H), 7.70–7.84 (m, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 465. HPLC: purity 98%, R_T 3.45 min.

(*E*)-3-(1-(8-((2-Propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (8). To a stirred solution of **23*E*** (344 mg, 0.821 mmol) in ethanol (8.2 mL) was added NH_2OH solution 50wt.% in water (0.754 mL, 34.6 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in DMF (4.1 mL). To a stirred solution was added pyridine (80 μL, 0.98 mmol) and ethyl chloroformate (94 μL, 0.98 mmol) and the solution was stirred for 1h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (8.2 mL) was added *tert*-BuOK (138 mg, 1.23 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 99:1 $CHCl_3$/methanol) to give **8** (46 mg, 12%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.94–1.06 (m, 3H), 1.80–1.96 (m, 2H), 2.28 (s, 3H), 2.74–2.84 (m, 2H), 4.71 (d, J = 12.6 Hz, 1H), 5.35 (s, 2H), 5.48 (d, J = 12.6 Hz, 1H), 6.74–6.86 (m, 1H), 6.86–6.97 (m, 1H), 6.98–7.04 (m, 1H), 7.04–7.32 (m, 7H), 7.64–7.78 (m, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 479. HPLC: purity 99%, R_T 3.6 min.

(*E*)-3-(1-(8-((4-Methyl-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (9). To a stirred solution of **24*E*** (449 mg, 1.04 mmol) in ethanol (10 mL) was added NH_2OH solution 50wt.% in water (1.9 mL, 31 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in DMF (5.2 mL). To a stirred solution was added pyridine (0.10 mL, 1.2 mmol) and ethyl chloroformate (0.12 mL, 1.2 mmol) the solution was stirred for 1h at room temperature. The reaction mixture was poured

into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (10 mL) was added *tert*-BuOK (190 mg, 1.55 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 99:1 $CHCl_3$/methanol) to give **9** (109 mg, 22%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 0.80–0.93 (m, 3H), 1.59–1.72 (m, 2H), 2.14 (s, 3H), 2.44–2.54 (m, 3H), 2.70–2.83 (m, 2H), 4.74–4.94 (m, 1H), 5.35–5.52 (m, 3H), 6.74–6.80 (m, 1H), 6.85–7.06 (m, 5H), 7.10–7.32 (m, 4H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 493. HPLC: purity 99%, R_T 3.7 min.

(*E*)-3-(1-(8-((4-(Hydroxymethyl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (10). To a stirred solution of **26*E*** (90 mg, 0.20 mmol) in ethanol (2 mL) was added NH_2OH solution 50wt.% in water (0.368 mL, 6.00 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in THF (1 mL). To a stirred solution was added Et_3N (0.042 mL, 0.30 mmol) and ethyl chloroformate (0.029 mL, 0.30 mmol) the solution was stirred for 1h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (2 mL) was added *tert*-BuOK (34 mg, 0.30 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 $CHCl_3$/methanol) to give **10** (10 mg, 10%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 0.85-1.00 (m, 3H), 1.67–1.84 (m, 2H), 2.27 (s, 3H), 2.93–3.04 (m, 2H),

4.76 (d, J = 12.6 Hz, 1H), 5.06 (s, 2H), 5.41 (s, 2H), 5.56 (d, J = 12.6 Hz, 1H), 6.78–6.87 (m, 1H), 6.86–7.01 (m, 2H), 7.04–7.24 (m, 7H). The protons of oxadiazolone and OH were not observed. LC/MS (ESI, $[M + H]^+$, m/z) 509. HPLC: purity 98%, R_T 3.4 min.

(*E*)-3-(1-(8-((4-(2-Hydroxypropan-2-yl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (11). To a stirred solution of **27*E*** (147 mg, 0.308 mmol) in ethanol (2 mL) was added NH_2OH solution 50wt.% in water (0.566 mL, 9.24 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in THF (2 mL). To a stirred solution was added Et_3N (0.064 mL, 0.46 mmol) and ethyl chloroformate (0.044 mL, 0.46 mmol) the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (2 mL) was added *tert*-BuOK (52 mg, 0.46 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 $CHCl_3$/methanol) to give **11** (61 mg, 37%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 0.92–1.06 (m, 3H), 1.71 (s, 6H), 1.78–1.98 (m, 2H), 2.31 (s, 3H), 2.70–2.82 (m, 2H), 4.77 (d, J = 12.6 Hz, 1H), 5.33 (s, 2H), 5.51 (d, J = 12.6 Hz, 1H), 6.70–6.87 (m, 1H), 6.87–6.98 (m, 1H), 6.98–7.24 (m, 8H). The protons of oxadiazolone and OH were not observed. LC/MS (ESI, $[M + H]^+$, m/z) 537. HPLC: purity 96%, R_T 3.7 min.

(*E*)-*N*-(2-Hydroxyethyl)-1-((11-(1-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)ethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepin-8-yl)methyl)-2-propyl-1*H*-benzo[*d*]imidazole-4-carboxamide (12). To a stirred solution of **31** (240 mg, 0.474 mmol) in ethanol (2.4 mL) was added NH_2OH solution 50wt.% in water (0.73 mL, 24 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined

organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2.4 mL). To a stirred solution was added Et_3N (99 μL, 0.71 mmol) and ethyl chloroformate (68 μL, 0.71 mmol) the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1.2 mL) was added *tert*-BuOK (106 mg, 0.948 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 97:3 $CHCl_3$/methanol) to give **12** (70 mg, 26%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 0.85–1.01 (m, 3H), 1.72–1.90 (m, 2H), 2.18 (s, 3H), 2.77–3.01 (m, 2H), 3.43–3.65 (m, 4H), 4.72–5.05 (m, 2H), 5.38–5.52 (m, 1H), 5.59 (s, 2H), 6.73–6.86 (m, 1H), 6.86–6.99 (m, 1H), 7.02–7.13 (m, 2H), 7.13–7.36 (m, 4H), 7.61–7.75 (m, 1H), 7.75–7.93 (m, 1H), 9.93–10.13 (m, 1H), 12.19 (br s, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 566. HPLC: purity 99%, R_T 3.3 min.

(*E*)-*N*-(1-((11-(1-(5-Oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)ethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepin-8-yl)methyl)-2-propyl-1*H*-benzo[*d*]imidazol-4-yl)methanesulfonamide (13). To a stirred solution of **32** (45 mg, 0.088 mmol) in ethanol (1.0 mL) was added NH_2OH solution 50wt.% in water (0.27 mL, 4.4 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (1.0 mL). To a stirred solution was added Et_3N (18 μL, 0.13 mmol) and ethyl chloroformate (13 μL, 0.13 mmol) the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1.0 mL) was added *tert*-BuOK (20 mg, 0.18 mmol) and the solution was stirred for 30 minutes at

room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 97:3 $CHCl_3$/methanol) to give **13** (14 mg, 28%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.89–1.07 (m, 3H), 1.74–1.90 (m, 2H), 2.32 (s, 3H), 2.78–2.92 (m, 2H), 3.11 (s, 3H), 4.79 (d, J = 12.6 Hz, 1H), 5.38 (s, 2H), 5.55 (d, J = 12.6 Hz, 1H), 6.80–6.88 (m, 1H), 6.91–7.01 (m, 2H), 7.01–7.09 (m, 2H), 7.09–7.26 (m, 4H), 7.41–7.51 (m, 1H). The protons of oxadiazolone and sulfonamide were not observed. LC/MS (ESI, $[M + H]^+$, m/z) 572. HPLC: purity 99%, R_T 3.52 min.

(*E*)-3-(1-(8-((4-Phenyl-2-propyl-1*H*-imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (14). To a stirred solution of **28*E*** (182 mg, 0.409 mmol) in ethanol (2.0 mL) was added NH_2OH solution 50wt.% in water (1.35 mL, 20.5 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2.0 mL). To a stirred solution was added Et_3N (86 μL, 0.62 mmol) and ethyl chloroformate (59 μL, 0.62 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (2.0 mL) was added *tert*-BuOK (91 mg, 0.82 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 90:10 $CHCl_3$/methanol) to give **14** (75 mg, 36%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.92–1.02 (m, 3H), 1.67–1.87 (m, 2H), 2.26 (s, 3H), 2.58–2.69 (m, 2H), 4.78 (d, J = 12.6 Hz, 1H), 5.11 (s, 2H), 5.52 (d, J = 12.6 Hz, 1H), 6.81–6.87 (m, 1H), 6.87–6.95 (m, 1H), 6.98–7.11 (m, 4H), 7.11–

7.27 (m, 3H), 7.27–7.39 (m, 2H), 7.68–7.79 (m, 2H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 505. HPLC: purity 95%, R_T 3.9 min.

(*E*)-3-(1-(8-((2-Propyl-4-(pyridin-4-yl)-1*H*-imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (15). To a stirred solution of **29*E*** (43 mg, 0.097 mmol) in ethanol (1.0 mL) was added NH_2OH solution 50wt.% in water (0.32 mL, 4.8 mmol), and the solution was refluxed for 3 days. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (1.0 mL). To a stirred solution was added Et_3N (20 μL, 0.15 mmol) and ethyl chloroformate (14 μL, 0.15 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1.0 mL) was added *tert*-BuOK (22 mg, 0.19 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 90:10 $CHCl_3$/methanol) to give **15** (3.6 mg, 8.3%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.93–1.04 (m, 3H), 1.67–1.85 (m, 2H), 2.32 (s, 3H), 2.55–2.71 (m, 2H), 4.67 (d, J = 12.6 Hz, 1H), 5.12 (s, 2H), 5.45 (d, J = 12.6 Hz, 1H), 6.79–6.89 (m, 2H), 6.89–7.02 (m, 1H), 7.07–7.32 (m, 5H), 7.55–7.68 (m, 2H), 8.32–8.45 (m, 2H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 506. HPLC: purity 96%, R_T 3.2 min.

(*E*)-2-(2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)propanenitrile (20*E*). To a stirred solution of **16*E*** (367 mg, 1.33 mmol), 2-ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridine (367 mg, 2.09 mmol) and PPh_3 (1.4 g, 2.8 mmol, polymer-bound, ~approximately 3 mmol/g triphenylphosphine loading, Sigma-Aldrich) in THF (13 mL) was added di-*tert*-butyl azodicarboxylate (642 mg, 2.79 mmol), and the solution was stirred for 2 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated. The obtained residue was

then purified by flash column chromatography on silica gel (80:20 to 65:35 hexane/ethyl acetate) to give **20*E*** (399 mg, 69%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.6 Hz, 3H), 1.99 (s, 3H), 2.58 (s, 3H), 2.63 (s, 3H), 2.73–2.88 (m, 2H), 2.76 (q, J = 7.6 Hz, 2H), 3.18–3.34 (m, 2H), 5.41 (s, 2H), 6.90–7.01 (m, 4H), 7.13 (dd, J = 7.1, 1.6 Hz, 1H), 7.18–7.28 (m, 2H), 7.39 (dd, J = 7.1, 1.6 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 433.

(*Z*)-2-(2-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)propanenitrile (20*Z*). To a stirred solution of **16*Z*** (399 mg, 1.45 mmol), 2-ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridine (367 mg, 2.09 mmol) and PPh_3 (1.4 g, 2.8 mmol, polymer-bound, ~3 mmol/g triphenylphosphine loading, Sigma-Aldrich) in THF (13 mL) was added di-*tert*-butyl azodicarboxylate (642 mg, 2.79 mmol), and the solution was stirred for 2 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated. The obtained residue was then purified by flash column chromatography on silica gel (80:20 to 65:35 hexane/ethyl acetate) to give **20*Z*** (376 mg, 68%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.4 Hz, 3H), 2.00 (s, 3H), 2.56 (s, 3H), 2.63 (s, 3H), 2.74–2.84 (m, 2H), 2.75 (q, J = 7.6 Hz, 2H), 3.20–3.30 (m, 2H), 5.40 (s, 2H), 6.83 (s, 1H), 6.88 (s, 1H), 6.97 (dd, J = 8.0, 1.6 Hz, 1H), 7.03–7.06 (m, 1H), 7.15–7.26 (m, 3H), 7.36 (d, J = 7.9 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 433.

(*E*)-2-(8-((2-Ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (21*E*). 2-ethyl-5,7-dimethyl-3*H*-imidazo[4,5-*b*]pyridine (218 mg, 1.25 mmol) was added to a solution of **17*E*** (231 mg, 0.834 mmol), PPh_3 (1.4 g, 2.8 mmol, polymer-bound, ~approximately 3 mmol/g triphenylphosphine loading) and di-*tert*-butyl azodicarboxylate (382 mg, 1.66 mmol) in THF (4 mL), and the solution was stirred for 2 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated. The obtained residue was then purified by flash column chromatography on silica gel (70:30 to 20:80 hexane/ethyl acetate) to give **21*E*** (228 mg, 63%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.6 Hz, 3H), 2.24 (s, 3H), 2.58 (s, 3H), 2.65 (s, 3H), 2.77 (q, J = 7.6 Hz, 2H), 4.74 (d, J = 12.6 Hz, 1H), 5.40 (d, J = 12.6 Hz, 1H), 5.44–5.51 (m, 2H), 6.79–6.86 (m, 1H), 6.86–6.95 (m, 2H), 7.00–7.10 (m, 2H), 7.12–7.25 (m, 2H), 7.37–7.46 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 435.

(*E*)-2-(8-((2-Ethyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (22*E*). 18 (300 mg, 1.01 mmol) was added to a solution of 2-ethyl-1*H*-

benzo[*d*]imidazole (163 mg, 1.12 mmol) and K_2CO_3 (701 mg, 5.07 mmol) in DMF (5 mL), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (90:10 to 50:50 hexane/ethyl acetate) to give **22*E*** (318 mg, 77%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, J = 7.6 Hz, 3H), 2.24 (s, 3H), 2.83 (q, J = 7.6 Hz, 2H), 4.72 (d, J = 12.6 Hz, 1H), 5.35 (s, 2H), 5.41 (d, J = 12.6 Hz, 1H), 6.80–6.99 (m, 3H), 7.02–7.15 (m, 2H), 7.16–7.33 (m, 4H), 7.43–7.50 (m, 1H), 7.72–7.83 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 406.

(*E*)-2-(8-((2-Propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (23*E*). 18 (300 mg, 1.01 mmol) was added to a solution of 2-propyl-1*H*-benzo[*d*]imidazole (179 mg, 1.12 mmol) and K_2CO_3 (701 mg, 5.07 mmol) in DMF (5 mL), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (90:10 to 70:30 hexane/ethyl acetate) to give **23*E*** (448 mg, 100%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95–1.12 (m, 3H), 1.82–1.98 (m, 2H), 2.26 (s, 3H), 2.72–2.89 (m, 2H), 4.73 (d, J = 12.6 Hz, 1H), 5.35 (s, 2H), 5.41 (d, J = 12.6 Hz, 1H), 6.81–6.99 (m, 3H), 7.01–7.15 (m, 2H), 7.15–7.30 (m, 4H), 7.40–7.48 (m, 1H), 7.74–7.84 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 420.

(*E*)-2-(8-((4-Methyl-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (24*E*). 19 (1.15 g, 3.61 mmol) was added to a solution of 4-methyl-2-propyl-1*H*-benzo[*d*]imidazole (691 mg, 3.97 mmol) and K_2CO_3 (2.49 g, 18.0 mmol) in DMF (20 mL), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (90:10 to 70:30 hexane/ethyl acetate) to give **24*E*** (891 mg, 57%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95–1.05 (m, 3H), 1.74–1.91 (m, 2H), 2.25 (s,

3H), 2.69 (s, 3H), 2.78–2.88 (m, 2H), 4.73 (d, *J* = 12.6 Hz, 1H), 5.34 (s, 2H), 5.41 (d, *J* = 12.6 Hz, 1H), 6.80–6.98 (m, 3H), 6.98–7.18 (m, 5H), 7.17–7.28 (m, 1H), 7.38–7.48 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 434.

Methyl (*E*)-1-((11-(1-Cyanoethylidene)-6,11-dihydrodibenzo[*b,e*]oxepin-8-yl)methyl)-2-propyl-1*H*-benzo[*d*]imidazole-4-carboxylate (25*E*). 19 (853 mg, 2.89 mmol) was added to a solution of methyl 2-propyl-1*H*-benzo[*d*]imidazole-4-carboxylate (600 mg, 2.75 mmol) and K_2CO_3 (1.9 g, 14 mmol) in DMF (16 mL), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (80:20 to 20:80 hexane/ethyl acetate) to give **25*E*** (970 mg, 74%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97–1.10 (m, 3H), 1.83–1.97 (m, 2H), 2.24 (s, 3H), 2.84–2.99 (m, 2H), 4.04 (s, 3H), 4.64–4.75 (m, 1H), 5.28–5.49 (m, 3H), 6.82–6.96 (m, 3H), 7.02–7.14 (m, 2H), 7.18–7.30 (m, 2H), 7.31–7.39 (m, 1H), 7.39–7.46 (m, 1H), 7.89–8.00 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 478.

(*E*)-2-(8-((4-(Hydroxymethyl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (26*E*). 19 (137 mg, 0.465 mmol) was added to a solution of (2-propyl-1*H*-benzo[*d*]imidazole-4-yl)methanol (97 mg, 0.51 mmol) and K_2CO_3 (321 mg, 2.33 mmol) in DMF (2.3 mL), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (50:50 to 20:80 hexane/ethyl acetate) to give **26*E*** (185 mg, 89%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91–1.06 (m, 3H), 1.77–1.92 (m, 2H), 2.25 (s, 3H), 2.72–2.84 (m, 2H), 4.22–4.33 (m, 1H), 4.73 (d, *J* = 12.6 Hz, 1H), 5.09–5.19 (m, 2H), 5.35 (s, 2H), 5.41 (d, *J* = 12.6 Hz, 1H), 6.83–6.99 (m, 3H), 7.02–7.17 (m, 5H), 7.17–7.29 (m, 1H), 7.40–7.50 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 450.

(*E*)-2-(8-((4-(2-Hydroxypropan-2-yl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (27*E*). A solution of K_2CO_3 (404 mg, 2.92 mmol) and 2-(2-propyl-1*H*-benzo[*d*]imidazol-4-yl)propan-2-ol (190 mg, 0.642 mmol) in DMF (3 mL) was treated with **19** (173 mg,

0.584 mmol), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (90:10 to 60:40 hexane/ethyl acetate) to give **27*E*** (295 mg, 100%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89–1.07 (m, 3H), 1.73 (s, 6H), 1.78–1.98 (m, 2H), 2.25 (s, 3H), 2.70–2.85 (m, 2H), 4.75 (d, J = 12.6 Hz, 1H), 5.32 (s, 2H), 5.43 (d, J = 12.6 Hz, 1H), 6.76–7.01 (m, 4H), 7.01–7.34 (m, 5H), 7.39–7.51 (m, 1H). The proton of OH was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 478.

(*E*)-2-(8-((4-Phenyl-2-propyl-1*H*-imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (28*E*). To a stirred solution of **19** (131 mg, 0.445 mmol) and 4-phenyl-2-propyl-1*H*-imidazole (91 mg, 0.49 mmol) in DMF (2 mL) was added K_2CO_3 (307 mg, 2.22 mmol), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 70:30 hexane/ethyl acetate) to give **28*E*** (182 mg, 91%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92–1.04 (m, 3H), 1.65–1.84 (m, 2H), 2.26 (s, 3H), 2.57–2.70 (m, 2H), 4.79 (d, J = 12.6 Hz, 1H), 5.11 (s, 2H), 5.43 (d, J = 12.6 Hz, 1H), 6.80–6.97 (m, 2H), 7.00–7.12 (m, 3H), 7.13–7.30 (m, 3H), 7.30–7.40 (m, 2H), 7.43–7.52 (m, 1H), 7.72–7.83 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 446.

(*E*)-2-(8-((2-Propyl-4-(pyridin-4-yl)-1*H*-imidazol-1-yl)methyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (29*E*). To a stirred solution of **19** (69 mg, 0.24 mmol) and 2-propyl-4-(pyridine-4-yl)-1*H*-imidazole (44 mg, 0.235 mmol) in DMF (1.5 mL) was added K_2CO_3 (160 mg, 1.18 mmol), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 90:10 $CHCl_3$/methanol) to give **29*E*** (43 mg, 40%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93–1.06 (m, 3H), 1.68–1.85 (m, 2H), 2.25 (s, 3H), 2.61–2.73 (m, 2H), 4.81 (d, J = 12.6 Hz, 1H), 5.13 (s, 2H), 5.43 (d, J = 12.6 Hz, 1H), 6.79–6.97 (m,

2H), 7.03–7.32 (m, 4H), 7.35–7.55 (m, 2H), 7.57–7.67 (m, 2H), 8.47–8.60 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 447.

(*E*)-1-((11-(1-Cyanoethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepin-8-yl)methyl)-2-propyl-1*H*-benzo[*d*]imidazole-4-carboxylic acid (30). To a stirred solution of **25*E*** (100 mg, 0.209 mmol) in ethanol (1 mL) was added 4M NaOH aqueous solution (1.0 mL, 4.0 mmol) and the solution was stirred for 2 h at 70 °C. The reaction mixture was acidified with 4 M HCl and the resultant solid was filtered, washed with water, and dried under reduced pressure to give **30** (86 mg, 89%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87–1.01 (m, 3H), 1.60–1.82 (m, 2H), 2.18 (s, 3H), 2.96–3.12 (m, 2H), 4.93 (d, J = 12.6 Hz, 1H), 5.44 (d, J = 12.6 Hz, 1H), 5.76 (s, 2H), 6.80–6.87 (m, 1H), 6.90– 7.00 (m, 1H), 7.14–7.35 (m, 3H), 7.35–7.41 (m, 1H), 7.41–7.51 (m, 2H), 7.85–8.01 (m, 2H). The proton of CO_2H was not observed. LC/MS (ESI, $[M - H]^-$, m/z) 462.

(*E*)-*N*-(1-((11-(1-Cyanoethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepin-8-yl)methyl)-2-propyl-1*H*-benzo[*d*]imidazol-4-yl)-3-hydroxypropanamide (31). To a stirred solution of **30** (220 mg, 0.475 mmol), EDCI·HCl (109 mg, 0.571 mmol) and HOBt·H_2O (87 mg, 0.57 mmol) in DMF (4 mL) was added 2-aminoethanol (57 μL, 0.95 mmol) and the solution was stirred overnight at room temparature. The reaction mixture was poured into saturated sodium hydrogen carbonae solution and the resultant solid was filtered, washed with water, and dried under reduced pressure. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 hexane/$CHCl_3$) to give **31** (241 mg, 99%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88–1.13 (m, 3H), 1.79–1.97 (m, 2H), 2.26 (s, 3H), 2.72–2.88 (m, 2H), 3.38–3.59 (m, 1H), 3.71–3.86 (m, 2H), 3.86–3.97 (m, 2H), 4.68–4.78 (m, 1H), 5.33–5.49 (m, 3H), 6.81–6.96 (m, 3H), 7.03–7.15 (m, 2H), 7.20–7.37 (m, 3H), 7.41–7.50 (m, 1H), 8.03–8.17 (m, 1H), 10.22–10.35 (m, 1H). The protons of OH and amide were not observed. LC/MS (ESI, $[M + H]^+$, m/z) 507.

(*E*)-*N*-(1-((11-(1-Cyanoethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepin-8-yl)methyl)-2-propyl-1*H*-benzo[*d*]imidazol-4-yl)methanesulfonamide (32). To a stirred solution of **30** (810 mg, 1.86 mmol) and Et_3N (1.3 ml, 9.3 mmol) in $CHCl_3$ (9 mL) was added DPPA (2.1 mL, 9.3 mmol) and the solution was stirred for 5 h at room temparature. *tert*-BuOH (9 ml) was added and the reaction mixture was stirred overnight at 100 °C. The reaction mixture was poured into saturated sodium hydrogen carbonate solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium

sulfate, and filtered. The organic layer was concentrated to give the residue. To a stirred solution of the obtained residue in CH_2Cl_2 (1 mL) was added TFA (0.31 mL) and the solution was stirred for 2 h at room temparature. The reaction mixture was poured into saturated sodium hydrogen carbonate solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was purified by flash column chromatography on silica gel (70:30 to 20:80 hexane/ethyl acetate). The resulting residue was dissolved in CH_2Cl_2 (2 mL) and DMAP (2 mg, 0.02 mmol) , $MeSO_2Cl$ (7.8 μL, 0.10 mmol) were added. The reaction mixture was stirred 5 h at room temparature. After the consumption of starting material, the mixture was poured into 2 mol/L HCl and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (80:20 to 20:80 hexane/ethyl acetate) to afford **32** (47 mg, 49%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93–1.03 (m, 3H), 1.73–1.88 (m, 2H), 2.25 (s, 3H), 2.72–2.84 (m, 2H), 3.09 (s, 3H), 4.76 (d, J = 12.6 Hz, 1H), 5.33 (s, 2H), 5.43 (d, J = 12.6 Hz, 1H), 6.79–6.88 (m, 1H), 6.88–7.01 (m, 3H), 7.03–7.13 (m, 2H), 7.13–7.20 (m, 1H), 7.20–7.25 (m, 1H), 7.36–7.42 (m, 1H), 7.42–7.47 (m, 1H), 7.67–8.26 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 513.

(*E*)-3-(1-(2-Fluoro-8-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (8a). To a stirred solution of **25a** (63 mg, 0.13 mmol) in ethanol (2 mL) was added NH_2OH solution 50wt.% in water (0.42 mL, 6.4 mmol), and the solution was refluxed overnight. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2 mL). To a stirred solution was added triethylamine (26 μL, 0.19 mmol) and ethyl chloroformate (18 μL, 0.19 mmol) the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (2 mL) was added *tert*-BuOK (28 mg, 0.25 mmol) and the solution was stirred for 30 minutes at room temperature. The

reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 $CHCl_3$/methanol) to give **8a** (16 mg, 25%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, J = 7.4 Hz, 3H), 1.73-1.91 (m, 2H), 2.31 (s, 3H), 2.72-2.82 (m, 2H), 4.59 (d, J = 12.8 Hz, 1H), 5.28-5.42 (m, 3H), 6.71-6.97 (m, 4H), 6.97-7.06 (m, 1H), 7.08-7.25 (m, 4H), 7.58-7.73 (m, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 497. HPLC: purity 96%, R_T 3.82 min.

(*E*)-3-(1-(3-Fluoro-8-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (9a). To a stirred solution of **26a** (110 mg, 0.251 mmol) in ethanol (1.3 mL) was added NH_2OH solution 50wt.% in water (0.769 mL, 12.3 mmol), and the solution was refluxed overnight. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (1.3 mL). To a stirred solution was added triethylamine (70 μL, 0.50 mmol) and ethyl chloroformate (48 μL, 0.50 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL)/THF (1 mL) was added *tert*-BuOK (85 mg, 0.75 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 $CHCl_3$/methanol) to give **9a** (68 mg, 54%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (t, J = 7.4 Hz, 3H), 1.74–1.94 (m, 2H), 2.28 (s, 3H), 2.79 (t, J = 7.4 Hz, 2H), 4.65 (d, J = 12.0 Hz, 1H), 5.34 (s, 2H), 5.45 (d, J = 12.0 Hz, 1H), 6.48–6.56 (m, 1H), 6.60–6.70 (m, 1H), 6.95–7.30 (m, 7H), 7.67–7.74 (m, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 497. HPLC: purity 99%, R_T 3.90 min.

(*E*)-3-(1-(4-Fluoro-8-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (10a). To a stirred solution of **27a** (160 mg, 0.366 mmol) in ethanol (1.8 mL) was added NH_2OH solution 50wt.% in water (1.12 mL, 18.3 mmol), and the solution was refluxed overnight. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (1.8 mL). To a stirred solution was added triethylamine (102 μL, 0.732 mmol) and ethyl chloroformate (70 μL, 0.73 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL)/THF (1 mL) was added *tert*-BuOK (123 mg, 1.09 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 92:8 $CHCl_3$/methanol) to give **10a** (80 mg, 44%) as an amorphous. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, J = 7.4 Hz, 3H), 1.61-1.83 (m, 2H), 2.18 (s, 3H), 2.77 (t, J = 7.6 Hz, 2H), 5.06 (d, J = 12.4 Hz, 1H), 5.44-5.61 (m, 3H), 6.86-6.98 (m, 1H), 6.98-7.25 (m, 6H), 7.24-7.32 (m, 1H), 7.42-7.52 (m, 1H), 7.53-7.66 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 497. The proton of oxadiazolone was not observed. HPLC: purity 99%, R_T 3.78 min.

(*E*)-3-(1-(8-((7-Chloro-2-cyclopropyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (11a). To a stirred solution of **28a** (175 mg, 0.372 mmol) in ethanol (1.9 mL) was added NH_2OH solution 50wt.% in water (1.14 mL, 18.6 mmol), and the solution was refluxed overnight. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (1.9 mL). To a stirred solution was added triethylamine (104 μL, 0.744 mmol) and ethyl chloroformate (71 μL, 0.74 mmol), and the solution was stirred for 1 h at

room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL) /THF (1 mL) was added *tert*-BuOK (125 mg, 1.12 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 90:10 $CHCl_3$/methanol) to give **11a** (82 mg, 42%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05–1.17 (m, 2H), 1.24–1.33 (m, 2H), 1.85–1.97 (m, 1H), 2.28 (s, 3H), 4.78 (d, J = 12.8 Hz, 1H), 5.45–5.68 (m, 3H), 6.47–6.58 (m, 1H), 6.58–6.71 (m, 1H), 6.98–7.29 (m, 5H), 8.14 (d, J = 5.4 Hz, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 530. HPLC: purity 98%, R_T 5.13 min.

(*E*)-3-(1-(8-((4-Chloro-2-cyclopropyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)methyl)-3-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (12a). To a stirred solution of **29a** (91 mg, 0.19 mmol) in ethanol (2.0 mL) was added NH_2OH solution 50wt.% in water (0.64 mL, 9.7 mmol), and the solution was refluxed overnight. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2.0 mL). To a stirred solution was added triethylamine (54 μL, 0.39 mmol) and ethyl chloroformate (37 μL, 0.39 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL)/THF (1 mL) was added *tert*-BuOK (43 mg, 0.39 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by recrystallized from isopropyl alcohol to give **12a** (37 mg, 37%) as an amorphous. ^{1}H NMR (300

MHz, $CDCl_3$): δ 1.02-1.17 (m, 2H), 1.24-1.39 (m, 2H), 1.83-1.97 (m, 1H), 2.29 (s, 3H), 4.70 (d, J = 12.6 Hz, 1H), 5.46-5.59 (m, 3H), 6.46-6.60 (m, 1H), 6.60-6.73 (m, 1H), 6.96-7.25 (m, 5H), 7.99-8.15 (m, 1H), 8.57 (br s, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 530. HPLC: purity 99%, R_T 4.57 min.

(*E*)-3-(1-(8-((2-Cyclopropyl-1*H*-thieno[3,4-*d*]imidazol-1-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (13a). To a stirred solution of **30a** (160 mg, 0.360 mmol) in ethanol (1.8 mL) was added NH_2OH solution 50wt.% in water (1.11 mL, 18.1 mmol), and the solution was refluxed overnight. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (1.8 mL). To a stirred solution was added triethylamine (101 μL, 0.725 mmol) and ethyl chloroformate (69 μL, 0.73 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL)/THF (1 mL) was added *tert*-BuOK (81 mg, 0.73 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by recrystallized from isopropyl alcohol to give **13a** (65 mg, 36%) as an amorphous. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.90–1.19 (m, 4H), 2.04–2.20 (m, 1H), 2.17 (s, 3H), 4.96 (d, J = 12.5 Hz, 1H), 5.34 (s, 2H), 5.52 (d, J = 12.5 Hz, 1H), 6.58–6.72 (m, 2H), 6.74–6.86 (m, 1H), 6.95 (d, J = 2.6 Hz, 1H), 7.09 (d, J = 7.9 Hz, 1H), 7.19–7.29 (m, 2H), 7.39–7.44 (m, 1H), 12.16 (br s, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 501. HPLC: purity 98%, R_T 3.77 min.

(*E*)-3-(1-(8-((2-Cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (14a). To a stirred solution of **32a** (201 mg, 0.462 mmol) in ethanol (3 mL) was added NH_2OH solution 50wt.% in water (1.5 mL, 23 mmol), and the solution was refluxed overnight. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over

sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2 mL). To a stirred solution was added triethylamine (129 μL, 0.923 mmol) and ethyl chloroformate (89 μL, 0.92 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (2 mL) was added *tert*-BuOK (104 mg, 0.923 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 $CHCl_3$/methanol) to give **14a** (62 mg, 27%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.93–1.07 (m, 2H), 1.07–1.21 (m, 2H), 1.95–2.09 (m, 1H), 2.29 (s, 3H), 4.29–4.47 (m, 2H), 4.74 (d, J = 12.8 Hz, 1H), 5.50 (d, J = 12.8 Hz, 1H), 6.47–6.58 (m, 1H), 6.59–6.75 (m, 2H), 7.01–7.25 (m, 5H), 7.44–7.54 (m, 1H), 7.54–7.63 (m, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 495. HPLC: purity 95%, R_T 3.78 min.

(*E*)-3-(1-(8-((6-Chloro-2-cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (15a). To a stirred solution of **33a** (88 mg, 0.19 mmol) in ethanol (2 mL) was added NH_2OH solution 50wt.% in water (33 μL, 5.6 mmol), and the solution was refluxed for 1 day. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2 mL). To a stirred solution was added triethylamine (52 μL, 0.38 mmol) and ethyl chloroformate (36 μL, 0.38 mmol), and the solution was stirred for 1h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (2 mL) was added *tert*-BuOK (42 mg, 0.38 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer

was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 $CHCl_3$/methanol) to give **15a** (37 mg, 36%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 0.95–1.20 (m, 4H), 1.94–2.07 (m, 1H), 2.28 (s, 3H), 4.34 (s, 2H), 4.80 (d, *J* = 12.7 Hz, 1H), 5.54 (d, *J* = 12.7 Hz, 1H), 6.50–6.73 (m, 2H), 7.00–7.30 (m, 5H), 7.45 (d, *J* = 9.8 Hz, 1H), 7.67 (s, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 529. HPLC: purity 96%, R_T 3.97 min.

(*E*)-3-(1-(8-((7-Chloro-2-cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (16a). To a stirred solution of **34a** (83 mg, 0.18 mmol) in ethanol (2 mL) was added NH_2OH solution 50wt.% in water (35 μL, 5.3 mmol), and the solution was refluxed for 1 day. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2 mL). To a stirred solution was added triethylamine (49 μL, 0.35 mmol) and ethyl chloroformate (34 μL, 0.35 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL) /THF (1 mL) was added *tert*-BuOK (40 mg, 0.35 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 95:5 $CHCl_3$/methanol) to give **16a** (42 mg, 46%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 0.82–0.99 (m, 4H), 2.09–2.20 (m, 1H), 2.15 (s, 3H), 4.38 (d, *J* = 17.1 Hz, 1H), 4.44 (d, *J* = 17.1 Hz, 1H), 4.90 (d, *J* = 12.7 Hz, 1H), 5.49 (d, *J* = 12.7 Hz, 1H), 6.65 (dd, *J* = 10.8, 2.0 Hz, 1H), 6.74–6.84 (m, 1H), 6.84 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.02 (d, *J* = 7.8 Hz, 1H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.19–7.20 (m, 1H), 7.26 (s, 1H), 7.57 (d, *J* = 2.0 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H).

The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 529. HPLC: purity 95%, R_T 3.88 min.

(*E*)-3-(1-(8-((8-Chloro-2-cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (17a). To a stirred solution of **35a** (100 mg, 0.213 mmol) in ethanol (2 mL) was added NH_2OH solution 50wt.% in water (0.42 mL, 6.4 mmol), and the solution was refluxed for 1 day. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2 mL). To a stirred solution was added triethylamine (59 μL, 0.43 mmol) and ethyl chloroformate (41 μL, 0.43 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL) /THF (1 mL) was added *tert*-BuOK (48 mg, 0.43 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 90:10 $CHCl_3$/methanol) to give **17a** (30 mg, 27%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.86–1.16 (m, 4H), 1.92–2.10 (m, 1H), 2.27 (s, 3H), 4.27–4.42 (m, 2H), 4.68 (d, J = 12.6 Hz, 1H), 5.48 (d, J = 12.6 Hz, 1H), 6.43–6.57 (m, 1H), 6.57–6.68 (m, 2H), 7.02–7.20 (m, 5H), 7.50–7.58 (m, 1H).The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 529. HPLC: purity 97%, R_T 3.93 min.

(*E*)-3-(1-(8-((2-Cyclopropyl-7-fluoroimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (18a). To a stirred solution of **36a** (3.9 g, 8.9 mmol) in DMSO (43 mL) was added NH_2OH solution 50wt.% in water (26.0 mL, 430 mmol), and the solution was refluxed for 1 day. After the consumption of the starting material, the reaction mixture was poured into water and the resulting suspension was filtered. The obtained residue was dissolved in CH_2Cl_2 (43 mL). To a stirred solution was added triethylamine (2.3 mL, 17 mmol) and ethyl chloroformate (1.6 mL, 17 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give

the residue. To a solution of the residue in toluene (15 mL) /THF (15 mL) was added *tert*-BuOK (1.9 g, 17 mmol) and the solution was stirred for 30 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and the suspension was filtered. The obtained residue was recrystallized in ethanol (20 mL) to give **18a** (1.7 g, 37%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.91–1.18 (m, 4H), 1.89–2.08 (m, 1H), 2.29 (s, 3H), 4.26–4.50 (m, 2H), 4.78 (d, J = 12.8 Hz, 1H), 5.53 (d, J = 12.8 Hz, 1H), 6.39–6.77 (m, 3H), 7.00–7.30 (m, 5H), 7.44–7.59 (m, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 513. HPLC: purity 98%, R_T 3.85 min.

(*E*)-3-(1-(8-((7-Chloro-2-(methoxymethyl)imidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (19a). To a stirred solution of **38a** (72 mg, 0.15 mmol) in ethanol (10 mL) was added NH_2OH solution 50%wt in water (0.467 mL, 7.63 mmol), and the solution was refluxed for 20 h. After the consumption of starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (2 mL). To a stirred solution was added Et_3N (43 μL, 0.31 mmol) and ethyl chloroformate (29 μL, 0.31 mmol) , and the solution was stirred for 1 h at room temperature. The reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL)/THF (1 mL) was added *tert*-BuOK (34 mg, 0.31 mmol) and the solution was stirred for 5 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by reverse phase chromatography (80:20 to 10:90 0.05% trifluoroacetic acid in water /acetonitrile) to give **19a** (8.2 mg, 18%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 2.15 (s, 3H), 3.27 (s, 3H), 4.39 (s, 2H), 4.56 (s, 2H), 4.87 (d, J = 12.5 Hz, 1H), 5.48 (d, J = 12.5 Hz, 1H), 6.65 (dd, J = 2.6, 10.8 Hz, 1H), 6.79 (d t, J = 2.7, 8.3 Hz, 1H), 6.81–6.96 (m, 1H), 7.18 (d, J = 7.9 Hz, 1H), 7.22 (d, J = 6.9 Hz, 1H), 7.25 (d, J = 6.9 Hz, 1H), 7.27 (s, 1H), 7.70 (s, 1H), 8.16 (d, J = 7.6 Hz, 1H), 12.08 (br s, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 533. HPLC: purity 98%, R_T 3.77 min.

(*E*)-3-(1-(8-((8-Chloro-2-(methoxymethyl)imidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (20a). To a stirred solution of **39a** (100 mg, 0.211 mmol) in ethanol (1.0 mL) was added NH_2OH solution 50%wt in water (0.388 mL, 6.33 mmol), and the solution was refluxed overnight. After the consumption of starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in 1,4-dioxane (1.0 mL). To a stirred solution was added DBU (48 μL, 0.32 mmol) and 1,1'-carbonyldiimidazole (38 mg, 0.23 mmol) and the solution was stirred for 1 h at 110 °C. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1 mL)/THF (1 mL) was added *tert*-BuOK (34 mg, 0.31 mmol) and the solution was stirred for 5 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by reverse phase chromatography (80:20 to 20:80 0.05% trifluoroacetic acid in water /acetonitrile) to give **20a** (47 mg, 41%) as an amorphous. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 2.52 (s, 3H), 3.28 (s, 3H), 4.38-4.45 (m, 2H), 4.62 (s, 2H), 4.88 (d, J = 12.4 Hz, 1H), 5.48 (d, J = 12.4 Hz, 1H), 6.59-6.68 (m, 1H), 6.75-6.91 (m, 2H), 6.97-7.07 (m, 1H), 7.16-7.27 (m, 2H), 7.27-7.33 (m, 1H), 7.40-7.48 (m, 1H), 8.08-8.17 (m, 1H), 12.11 (br s, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 533. HPLC: purity 98%, R_T 3.77 min.

(*E*)-3-(1-(8-((2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]imidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)ethyl)-1,2,4-oxadiazol-5(4*H*)-one (21a). To a stirred solution of **40a** (377 mg, 0.789 mmol) in DMSO (4 mL) was added NH_2OH solution 50%wt in water (1.45 mL, 23.7 mmol), and the solution was refluxed for 6 h. After the consumption of starting material, the reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to dryness and the obtained residue was dissolved in CH_2Cl_2 (4 mL). To a stirred solution was added Et_3N (218 μL, 1.56 mmol) and ethyl chloroformate (150 μL, 1.56 mmol), and the solution was stirred for 1 h at room

temperature. The reaction mixture was poured into water and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. To a solution of the residue in toluene (1.7 mL) / THF (1.7 mL) was added *tert*-BuOK (154 mg, 137 mmol) and the solution was stirred for 40 minutes at room temperature. The reaction mixture was poured into 5% aqueous citric acid solution and extracted twice with $CHCl_3$. The combined layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by reverse phase chromatography (80:20 to 10:90 0.05% trifluoroacetic acid in water /acetonitrile) to give **21a** (90 mg, 21%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.93–1.02 (m, 2H), 1.03–1.10 (m, 2H), 1.93–2.07 (m, 1H), 2.27 (s, 3H), 3.39 (t, J = 9.2 Hz, 2H), 4.25–4.39 (m, 2H), 4.60–4.72 (m, 3H), 5.46 (d, J = 12.5 Hz, 1H), 6.46 (d, J = 7.3 Hz, 1H), 6.52 (dd, J = 10.3, 2.6 Hz, 1H), 6.59–6.68 (m, 1H), 7.04–7.21 (m, 4H), 7.39 (d, J = 7.3 Hz, 1H). The proton of oxadiazolone was not observed. LC/MS (ESI, $[M + H]^+$, m/z) 537. HPLC: purity 99%, R_T 4.13 min.

(*E*)-2-(2-Fluoro-8-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (25a). To a stirred solution of **22a** (65 mg, 0.18 mmol) and 2-propylbenzo[*d*]imidazole (32 mg, 0.20 mmol) in DMF (1.5 mL) was added potassium carbonate (125 mg, 0.908 mmol), and the solution was stirred for 4 h at 60 °C. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 50:50 hexane/ethyl acetate) to give **25a** (63 mg, 79%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (t, J = 7.4 Hz, 3H), 1.71-1.93 (m, 2H), 2.23 (s, 3H), 2.74-2.91 (m, 2H), 4.73 (d, J = 12.4 Hz, 1H), 5.23-5.38 (m, 3H), 6.72-6.85 (m, 2H), 6.90-7.18 (m, 7H), 7.39-7.47 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 438.

(*E*)-2-(3-Fluoro-8-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (26a). To a stirred solution of **23a** (117 mg, 0.330 mmol) and 2-propylbenzo[*d*]imidazole (50 mg, 0.31 mmol) in DMF (1.8 mL) was added potassium carbonate (216 mg, 1.56 mmol), and the solution was stirred over night at room temparature. After the consumption of the

starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 50:50 hexane/ethyl acetate) to give **26a** (115 mg, 84%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02 (t, J = 7.4 Hz, 3H), 1.77–1.97 (m, 2H), 2.23 (s, 3H), 2.80 (t, J = 7.4 Hz, 2H), 4.73 (d, J = 12.6 Hz, 1H), 5.32–5.48 (m, 3H), 6.51–6.58 (m, 1H), 6.59–6.69 (m, 1H), 6.94–7.07 (m, 3H), 7.11–7.29 (m, 3H), 7.43 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 7.5 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 438.

(*E*)-2-(4-Fluoro-8-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (27a). To a stirred solution of **24a** (141 mg, 0.393 mmol) and 2-propylbenzo[*d*]imidazole (60 mg, 0.37 mmol) in DMF (2.2 mL) was added potassium carbonate (258 mg, 1.87 mmol), and the solution was stirred for 4 h at 60 °C. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 50:50 hexane/ethyl acetate) to give **27a** (164 mg, 100%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (t, J = 7.4 Hz, 3H), 1.82-1.95 (m, 2H), 2.24 (s, 3H), 2.80 (t, J = 7.4 Hz, 2H), 4.91 (d, J = 12.4 Hz, 1H), 5.30-5.40 (m, 2H), 5.46 (d, J = 12.4 Hz, 1H), 6.79-6.89 (m, 2H), 6.98-7.31 (m, 6H), 7.40-7.50 (m, 1H), 7.72-7.82 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 438.

(*E*)-2-(8-((7-Chloro-2-cyclopropyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (28a). To a stirred solution of **23a** (250 mg, 0.698 mmol) and 7-chloro-2-cyclopropylimidazo[4,5-*b*]pyridine (135 mg, 0.698 mmol) in DMF (3.5 mL) was added potassium carbonate (480 mg, 3.49 mmol), and the solution was stirred overnight at room temparature. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 50:50 hexane/ethyl acetate) to give **28a** (177 mg, 54%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02–1.16 (m, 2H), 1.25–1.38

(m, 2H), 1.83–1.97 (m, 1H), 2.26 (s, 3H), 4.76 (d, *J* = 12.8 Hz, 1H), 5.41 (d, *J* = 12.8 Hz, 1H), 5.59–5.63 (m, 2H), 6.51–6.59 (m, 1H), 6.59–6.70 (m, 1H), 6.97–7.08 (m, 1H), 7.13–7.35 (m, 3H), 7.38–7.48 (m, 1H), 8.11–8.24 (m, 1H).LC/MS (ESI, $[M + H]^+$, m/z) 471.

(*E*)-2-(8-((4-Chloro-2-cyclopropyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (29a). To a stirred solution of **23a** (120 mg, 0.335 mmol) and 4-chloro-2-cyclopropylimidazo[4,5-*c*]pyridine (66 mg, 0.34 mmol) in DMF (2.0 mL) was added potassium carbonate (231 mg, 1.68 mmol), and the solution was stirred overnight at room temparature. After the consumption of the starting material, the reaction mixture was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 44:66 hexane/ethyl acetate) to give **29a** (98 mg, 62%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04-1.17 (m, 2H), 1.27-1.38 (m, 2H), 1.83-1.96 (m, 1H), 2.25 (s, 3H), 4.71-4.81 (m, 1H), 5.32-5.53 (m, 3H), 6.53-6.73 (m, 2H), 6.95-7.11 (m, 3H), 7.15-7.22 (m, 1H), 7.42-7.53 (m, 1H), 8.09-8.15 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 471.

(*E*)-2-(8-((2-Cyclopropyl-1*H*-thieno[3,4-*d*]imidazol-1-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (30a). To a stirred solution of 2-cyclopropyl-1*H*-thieno[3,4-*d*]imidazole (65 mg, 0.40 mmol) in DMF (2.3 mL) was added potassium carbonate (274 mg, 1.98 mmol) and (*E*)-2-(8-(bromomethyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-yliden)propanenitrile **23a** (145 mg, 0.404 mmol), and the solution was stirred overnight at room temperature. The reaction mixture was poured into water and the resulting suspension was filtered and washed with water twice. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 50:50 hexane/ethyl acetate) to obtain **30a** (166 mg, 95%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00–1.09 (m, 2H), 1.18–1.26 (m, 2H), 1.73–1.84 (m, 1H), 2.25 (s, 3H), 4.80 (d, *J* = 12.8 Hz, 1H), 5.19–5.35 (m, 2H), 5.43 (d, *J* = 12.8 Hz, 1H), 6.29 (d, *J* = 2.6 Hz, 1H), 6.57 (dd, *J* = 9.9, 2.6 Hz, 1H), 6.61–6.69 (m, 1H), 6.94 (d, *J* = 2.6 Hz, 1H), 7.04 (d, *J* = 8.8, 6.6 Hz, 1H), 7.16–7.19 (m, 1H), 7.27–7.32 (m, 1H), 7.47 (d, *J* = 8.1 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 442.

(*E*)-2-(8-((2-Cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (32a). To a stirred solution of 2-aminopyridine (136 mg, 1.44 mmol) in toluene (5

mL) was added copper (I) chloride (10 mg, 0.10 mmol), copper (II) trifluoromethanesulfonate (36 mg, 0.10 mmol), cyclopropanecarbaldehyde (0.32 ml, 4.4 mmol) and 2-(8-ethynyl-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-yliden)propanenitrile **31a** (500 mg, 1.73 mmol), and the solution was stirred for 5 h at 120 °C. The reaction mixture was filtered with celite pad. The filtrate was poured into water and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 34:66 hexane/ethyl acetate) to obtain **32a** (205 mg, 32%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): 0.92–1.08 (m, 2H), 1.08–1.18 (m, 2H), 1.91–2.09 (m, 1H), 2.23 (s, 3H), 4.24–4.51 (m, 2H), 4.76 (d, *J* = 12.8 Hz, 1H), 5.41 (d, *J* = 12.8 Hz, 1H), 6.50–6.70 (m, 3H), 6.94–7.15 (m, 3H), 7.21–7.30 (m, 1H), 7.35–7.46 (m, 1H), 7.49–7.58 (m, 1H), 7.58–7.66 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 436.

(*E*)-2-(8-((6-Chloro-2-cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (33a). To a stirred solution of 5-chloropyridin-2-amine (178 mg, 1.38 mmol) in toluene (7 mL) was added copper (I) chloride (27 mg, 0.28 mmol), copper (II) trifluoromethanesulfonate (100 mg, 0.277 mmol), cyclopropanecarbaldehyde (0.156 ml, 2.07 mmol) and 2-(8-ethynyl-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-yliden)propanenitrile **31a** (400 mg, 1.38 mmol), and the solution was irradiated for 4 h with the reaction temperature controlled around 120 °C. The reaction mixture was filtered with a celite pad. The filtrate was poured into saturated ammonium chloride solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (90:10 to 40:60 hexane/ethyl acetate) to obtain **33a** (88 mg, 14%) as an amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99–1.04 (m, 2H), 1.09–1.15 (m, 2H), 1.95–2.02 (m, 1H), 2.24 (s, 3H), 4.30–4.35 (m, 2H), 4.78 (d, *J* = 12.7 Hz, 1H), 5.43 (d, *J* = 12.7 Hz, 1H), 6.54–6.58 (m, 1H), 6.62–6.67 (m, 1H), 7.01–7.07 (m, 2H), 7.11 (br s, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 9.8 Hz, 1H), 7.67 (d, *J* = 2.0 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 470.

(*E*)-2-(8-((7-Chloro-2-cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (34a). To a stirred solution of 4-chloropyridin-2-amine (156 mg, 1.21

mmol) in toluene (6 mL) was added copper (I) chloride (24 mg, 0.24 mmol), copper (II) trifluoromethanesulfonate (88 mg, 0.24 mmol), cyclopropanecarbaldehyde (0.136 ml, 1.82 mmol) and 2-(8-ethynyl-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-yliden)propanenitrile **31a** (350 mg, 1.21 mmol), and the solution was stirred overnight at 120 °C. The reaction mixture was filtered with celite pad. The filtrate was poured into saturated ammonium chloride solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (90:10 to 40:60 hexane/ethyl acetate) to obtain **34a** (73 mg, 13%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.98–1.05 (m, 2H), 1.08–1.15 (m, 2H), 1.94–2.03 (m, 1H), 2.23 (s, 3H), 4.30 (d, *J* = 17.2 Hz, 1H), 4.38 (d, *J* = 17.2 Hz, 1H), 4.76 (d, *J* = 12.8 Hz, 1H), 5.41 (d, *J* = 12.8 Hz, 1H), 6.53–6.59 (m, 1H), 6.61–6.68 (m, 2H),6.99–7.06 (m, 1H), 7.08–7.10 (m, 1H), 7.20–7.24 (m, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.50–7.54 (m, 2H).

LC/MS (ESI, $[M + H]^+$, m/z) 470.

(*E*)-2-(8-((8-Chloro-2-cyclopropylimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (35a). To a stirred solution of 3-chloropyridin-2-amine (101 mg, 0.786 mmol) in toluene (4 mL) was added copper (I) chloride (5.4 mg, 0.055 mmol), copper (II) trifluoromethanesulfonate (20 mg, 0.055 mmol), cyclopropanecarbaldehyde (88 μL, 1.2 mmol) amd 2-(8-ethynyl-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-yliden)propanenitrile **31a** (250 mg, 0.864 mmol), and the solution was stirred for 30 h at 120 °C. The reaction mixture was filtered with celite pad. The filtrate was poured into saturated ammonium chloride solution and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 40:60 hexane/ethyl acetate) to obtain **35a** (61 mg, 15%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.92–1.09 (m, 2H), 1.09–1.28 (m, 2H), 1.93–2.10 (m, 1H), 2.23 (s, 3H), 4.24–4.52 (m, 2H), 4.75 (d, *J* = 12.6 Hz, 1H), 5.40 (d, *J* = 12.6 Hz, 1H), 6.45–6.73 (m, 3H), 6.95–7.31 (m, 4H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 470.

(*E*)-2-(8-((2-Cyclopropyl-7-fluoroimidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (36a). To a stirred solution of 4-fluoropyridin-2-amine (141 mg, 1.23

mmol) in toluene (5 mL) was added copper (I) chloride (11 mg, 0.11 mmol), copper (II) trifluoromethanesulfonate (41 mg, 1.1 mmol), cyclopropanecarbaldehyde (13 μL, 1.7 mmol) and 2-(8-ethynyl-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-yliden)propanenitrile **31a** (330 mg, 0.864 mmol), and the solution was stirred for 8 h at 120 °C. The reaction mixture was filtered with celite pad. The filtrate was poured into water and extracted twice with ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 50:50 hexane/ethyl acetate) to obtain **36a** (25 mg, 4.3%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 0.90–1.18 (m, 4H), 1.92–2.07 (m, 1H), 2.23 (s, 3H), 4.21–4.44 (m, 2H), 4.75 (d, J = 12.8 Hz, 1H), 5.43 (d, J = 12.8 Hz, 1H), 6.43–6.74 (m, 3H), 6.99–7.31 (m, 4H), 7.33–7.62 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 454.

(*E*)-2-(8-((7-Chloro-2-(methoxymethyl)imidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (38a). To a stirred solution of 7-chloro-3-iodo-2-(methoxymethyl)imidazo[1,2-*a*]pyridine (700 mg, 2.17 mmol) in THF (4.5 mL) was added isopropylmagnesium chloride (2 mol/L solution in THF, 1.1 ml, 2.0 mmol) and the solution was stirred for 1 h at -50 °C. To the reaction mixture was added **37a** (318 mg, 1.09 mmol) and stirred for 1 h at -20 °C. After the consumption of the starting material, the reaction mixture was poured into saturated aqueous ammonium chloride solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 70:30 $CHCl_3$/methanol) to give the residue (400 mg). To a stirred solution of chlorotrimethylsilane (1.04 mL, 8.16 mmol) in hexane (0.8 mL) / acetonitrile (0.4 mL) was added sodium iodide (1.2 g, 8.2 mmol) and the mixture was stirred overnight at room temperature. To the reaction mixture was added the obtained residue (400 mg) and the mixture was stirred overnight at room temperature. The reaction mixture was poured into saturated aqueous ammonium chloride solution / saturated aqueous sodium thiosulfate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 70:30 $CHCl_3$/methanol) to give **38a** (72 mg, 19%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 2.23 (s, 3H), 3.47 (s, 3H), 4.35–4.37 (m, 1H),

4.63–4.76 (m, 4H), 5.38–5.42 (m, 1H), 6.53–6.71 (m, 3H), 7.00–7.10 (m, 2H), 7.20–7.24 (m, 2H), 7.39–7.42 (m, 1H), 7.58–7.60 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 474.

(*E*)-2-(8-((8-Chloro-2-(methoxymethyl)imidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (39a). To a stirred solution of 8-chloro-3-iodo-2-(methoxymethyl)imidazo[1,2-*a*]pyridine (500 mg, 1.55 mmol) in THF (2.0 mL) was added isopropylmagnesium chloride (2 mol/L solution in THF, 0.775 ml, 1.55 mmol) and the solution was stirred for 1 h at -50 °C. To the reaction mixture was added **37a** (227 mg, 0.775 mmol) and stirred for 1 h at -20 °C. After the consumption of the starting material, the reaction mixture was poured into saturated aqueous ammonium chloride solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 70:30 $CHCl_3$/methanol) to give the residue (180 mg). To a stirred solution of chlorotrimethylsilane (0.471 mL, 3.71 mmol) in hexane (0.4 mL) / acetonitrile (0.4 mL) was added sodium iodide (556 mg, 3.71 mmol) and the mixture was stirred overnight at room temperature. To the reaction mixture was added the residue (180 mg) and the mixture was stirred overnight at room temperature. The reaction mixture was poured into saturated aqueous ammonium chloride solution / saturated aqueous sodium thiosulfate solution and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 70:30 $CHCl_3$/methanol) to give **39a** (77 mg, 44%) as an amorphous. ^{1}H-NMR (300 MHz, $CDCl_3$):δ 2.23 (s, 3H), 3.47 (s, 3H), 4.33–4.46 (m, 2H), 4.76 (s, 3H), 5.39 (d, J = 12.7 Hz, 1H), 6.53–6.57 (m, 1H), 6.62–6.68 (m, 2H), 7.00–7.04 (m, 1H), 7.10–7.11 (m, 1H), 7.23–7.30 (m, 2H), 7.39–7.41 (m, 1H), 7.62–7.64 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 474.

(*E*)-2-(8-((2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]imidazo[1,2-*a*]pyridin-3-yl)methyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (40a). To a stirred solution of 2-cyclopropyl-3-iodo-8,9-dihydrofuro[3,2-*c*]imidazo[1,2-*a*]pyridine (435 mg, 1.33 mmol) in THF (4.5 mL) was added isopropylmagnesium chloride lithium chloride complex (1.3 mol/L solution in THF, 1.15 ml, 1.50 mmol) and the solution was stirred for 1 h at -40 °C. To the reaction mixture was added **37a** (244 mg, 0.843 mmol) and stirred for 1 h at -20 °C. After the consumption of the starting material, the reaction mixture was

poured into saturated aqueous ammonium chloride solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then recrystallized from isopropyl alcohol (20 mL) to afford a white solid (362 mg). To a stirred solution of the resulting residue (362 mg, 0.729 mmol) in TFA (3.6 mL) was added triethylsilane (0.583 mL, 3.65 mmol) and the mixture was stirred for 1 h at 60 °C. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 90:10 $CHCl_3$/methanol) to give **40a** (386 mg, 87%) as an amorphous.^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.91–1.01 (m, 2H), 1.03–1.11 (m, 2H), 1.95–2.03 (m, 1H), 2.23 (s, 3H), 3.48 (t, J = 8.8 Hz, 2H), 4.25–4.38 (m, 2H), 4.69 (t, J = 8.8 Hz, 2H), 4.75 (d, J = 12.7 Hz, 1H), 5.40 (d, J = 12.7 Hz, 1H), 6.41 (d, J = 6.8 Hz, 1H), 6.55 (dd, J = 10.2, 2.4 Hz, 1H), 6.60–6.67 (m, 1H), 6.99–7.05 (m, 1H), 7.07–7.11 (m, 1H), 7.21–7.25 (m, 1H), 7.37–7.43 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 537.

Dimethyl 2-bromoterephthalate (S2). To a stirred solution of commercially available **1** (7.52 g, 30.7 mmol, Aldrich, 115274, CAS :586-35-6) in MeOH (50 mL) was added trimethyl orthoformate (8.7 mL, 80.0 mmol) and *conc*. H_2SO_4 (0.65 mL, 12.0 mmol), and the solution was refluxed for 12h. The reaction mixture was cooled at room temperature, and concentrated ubder reduced pressure. The obtain residue was pored into saturated aqueous sodium hydrogen carbonate solution at 0°C. The resultant soid was filtered, washed with water, and dried under reduced pressure to give dimetyl 2-bromoterephthalate (**S2**) (7.19 g, 86%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.95 (d, J = 0.7 Hz, 3H), 3.96 (d, J = 0.7 Hz, 3H), 7.81 (d, J = 8.3 Hz, 1H), 7.99–8.03 (m, 1H), 8.32 (s, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 273.

Dimethyl (*E*)-2-styrylterephthalate (S3). To a stirred solution of dimetyl 2-bromoterephthalate (**S2**) (7.0 g, 25.6 mmol) in 1,2-dimethoxyethane (240 mL) was added (*E*)-styrylboronic acid (4.9 g, 33.3 mmol), dichlorobis(tri-*o*-tolylphosphine)palladium(II) (1.0 g, 1.28 mmol), sodium carbonate (8.1 g, 76.8 mmol) and water (60 mL), and the solution was refluxed for 2h. The reaction mixture was cooled at room temperature, and filtered on celite pad. The filtrate was pored into saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (300 mL). The combined organic layers were washed with brine,

dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by recrystallization from 10% ethyl acetate in *n*-hexane to afford the residue. The resultant solid washed with diisopropyl ether, and dried under reduced pressure to give dimethyl (*E*)-2-styrylterephthalate (**S3**) (6.28 g, 83%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.96 (d, J = 0.7 Hz, 3H), 3.98 (d, J = 0.7 Hz, 3H), 7.13 (d, J = 16.2 Hz, 1H), 7.28–7.40 (m, 3H), 7.55–7.59 (m, 2H), 7.88–7.91 (m, 3H), 8.39 (s, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 297.

2-Phenethylterephthalic acid (S4). To a stirred solution of dimethyl (*E*)-2-styrylterephthalate (**S3**) (6.2 g, 20.9 mmol) in EtOH (30 mL) was added 4 mol/L NaOH aqueous solution (55 mL, 209 mmol), and the solution was refluxed for 3h. The reaction mixture was cooled at room temperature, and concentrated ubder reduced pressure. The obtain residue was pored into 2 mol/L HCl aqueous solution at 0°C. The resultant soid was filtered, washed with water, and dried under reduced pressure. The obtained residue was solved in DMF (20 mL). To a stirred solution was added Palladium 10% on carbon (0.1 g), and the solution was stirred for 3h at room temperature under a hydrogen atmosphere. The reaction mixture was filtered on celite pad, and the filtrate was concentrated ubder reduced pressure. The obtained residue was then purified by recrystallization from 50% EtOH aqueous solution to afford the residue. The resultant solid washed with water, and dried under reduced pressure to give 2-phenetylterephthalic acid (**S4**) (5.57 g, 96%) as a white solid. ^{1}H NMR (270 MHz, DMSO-d_6): δ 2.81–2.87 (m, 2H), 3.18–3.24 (m, 2H), 7.16–7.32 (m, 5H), 7.82–7.90 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 271.

Ethyl 5-*oxo*-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-2-carboxylate (S5). To a stirred solution of 2-phenetylterephthalic acid (**S4**) (4.5 g, 16.6 mmol) in sulfolane (30 mL) was added polyphosphoric acid (30 mL), and the solution was stirred for 12h at 140°C. The reaction mixture was cooled at room temperature, and pored into water. The solution was stirred for 1h at room temperature. The resultant soid was filtered, washed with water, and dried under reduced pressure. The obtained residue was solved in EtOH (20 mL). To a stirred solution was added triethyl orthoformate (3.6 mL, 21.6 mmol) and *conc*. H_2SO_4 (0.35 mL, 6.6 mmol), and the solution was refluxed for 12h. The reaction mixture was cooled at room temperature, and concentrated ubder reduced pressure. The obtained residue was pored into saturated aqueous sodium hydrogen carbonate solution at 0 °C and extracted with ethyl acetate (50 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to

give ethyl 5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-carboxylate (**S5**) (2.9 g, 62%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.42 (t, J = 7.1 Hz, 3H), 3.25 (s, 4H), 4.40 (q, J = 7.2 Hz, 2H), 7.21–7.28 (m, 1H), 7.34 (td, J = 1.3, 7.6 Hz, 1H), 7.46 (td, J = 1.3, 7.6 Hz, 1H), 7.92–8.04 (m, 4H). LC/MS (ESI, $[M + H]^+$, m/z) 281.

Ethyl (*Z*)-5-(1-cyanoethylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-carboxylate (S6).

Ethyl (*E*)-5-(1-cyanoethylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-carboxylate (S7).

To a stirred solution of LDA (2.0 mol/L, 268 mL, 0.54 mol) in THF (90 mL) was added to a solution of propanenitrile (15 g, 0.27 mol) in THF (90 mL) at 0°C, and the solution was stirred for 0.5h at same temperature. The reaction mixture was added to a solution of diethyl chlorophosphate (47 g, 0.27 mol) in THF (90 mL) at 0°C, and the solution was stirred for 1h at room temperature. The reaction mixture was added to a solution of ethyl 5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-carboxylate (**S5**) (30 g, 0.11 mol) in DMF (270 mL) at room temperature, and the solution was stirred for 3h at 80°C. The reaction mixture was concentrated under reduced pressure. The obtained residue was pored into water (500 mL), and neutralized by 6 mol/L HCl aqueous solution (pH = 7.1). The solution was extracted with ethyl acetate (800 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtain residue was then purified by recrystallization from IPA (150 mL) at 0°C to afford the residue. The resultant solid washed with IPA, and dried under reduced pressure to give ethyl (*Z*)-5-(1-cyanoethylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-carboxylate (**S6**) (12.98 g, 40%, *E*:*Z* = 1:99) as a white solid. The obtained mother liquid was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (95:5 to 90:10 *n*-hexane/ethyl acetate) to give ethyl (*E*)-5-(1-cyanoethylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-carboxylate (**S7**) (13.4 g, 41%, *E*:*Z* = 85:15) as a oil.

(**S6**) : ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (t, J = 7.2 Hz, 3H), 2.06 (s, 3H), 2.84–3.01 (m, 2H), 3.28–3.45 (m, 2H), 4.36 (q, J = 7.2 Hz, 2H), 7.06–7.12 (m, 1H), 7.17–7.31 (m, 3H), 7.48 (d, J = 8.1 Hz, 1H), 7.84–7.93 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 318.

(**S7**) : ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, J = 7.2 Hz, 3H), 2.01 (s, 3H), 2.88–3.00 (m, 2H), 3.28–3.44 (m, 2H), 4.37 (q, J = 7.2 Hz, 2H), 7.12–7.18 (m, 2H), 7.20–7.31 (m, 2H), 7.41–7.47 (m, 1H), 7.84–7.94 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 318.

(*Z*)-2-{2-(Hydroxymethyl)-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene}propanenitrile (16*Z*). To a stirred solution of ethyl (*Z*)-5-(1-cyanoethylidene)-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-2-carboxylate (**S6**) (53 g, 0.17 mol) in THF (530 mL) was added lithium borohydride (7.6 g, 0.34 mol), and the solution was refluxed for 6h. The reaction mixture was cooled at room temperature, and pored into ice water (1000 mL). The resultant solution was pored into 6 mol/L HCl aqueous solution to pH 5 and extracted with ethyl acetate (500 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (chloroform) to give (*Z*)-2-{2-(hydroxymethyl)-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene}propanenitrile (**16*Z***) (46.8 g, 97%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H), 2.82–2.96 (m, 2H), 3.27–3.40 (m, 2H), 4.65 (d, J = 5.9 Hz, 2H), 7.06–7.08 (m, 1H), 7.18–7.23 (m, 5H), 7.43 (d, J = 7.7 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 276.

(*E*)-2-{2-(Hydroxymethyl)-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene}propanenitrile (16*E*). To a stirred solution of ethyl (*E*)-5-(1-cyanoethylidene)-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-2-carboxylate (**S7**) (13 g, 0.042 mol, *E*:*Z* = 85:15) in THF (134 mL) was added lithium borohydride (1.9 g, 0.088 mol), and the solution was refluxed for 6h. The reaction mixture was cooled at room temperature, and pored into ice water (1000 mL). The resultant solution was pored into 6 mol/L HCl aqueous solution to pH 5 and extracted with ethyl acetate (150 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (chloroform) to give (*E*)-2-{2-(hydroxymethyl)-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene}propanenitrile (**16*E***) (7.7 g, 67%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 3H), 2.83–2.95 (m, 2H), 3.27–3.40 (m, 2H), 4.66 (d, J = 5.7 Hz, 2H), 7.08 (d, J = 5.9 Hz, 1H), 7.13–7.24 (m, 5H), 7.40–7.43 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 276.

4-Bromo-2-(phenoxymethyl)benzoic acid (S9). To a stirred solution of 5-bromophthalide (**S8**) (350 g, 1.64 mol) and phenol (232 g, 2.46 mol) in NMP (350 mL) was added sodium methoxide 28% solution in methanol (495 mL, 2.50 mol), and the solution was stirred for 3h at 120°C. The reaction mixture was cooled at 90°C, and pored into water (350 mL) and MeOH (700 mL). The resultant solution was pored into 4 mol/L HCl aqueous solution (570 mL) at 50°C, and the solution was stirred for 30 min at 5°C. The resultant soid was filtered, washed with water/MeOH = 3/2 (350 mL × 2), and dried under reduced pressure to give 4-bromo-2-(phenoxymethyl)benzoic acid (**S9**) (476 g, 80%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.43 (s, 2H), 6.93–7.87 (m, 8H). LC/MS (ESI, $[M + H]^+$, m/z) 307.

8-Bromodibenzo[*b,e*]oxepin-11(6*H*)-one (S10). To a stirred suspension of 4-bromo-2-(phenoxymethyl)benzoic acid (**S9**) (470 g, 1.29 mol) in *n*-heptane (4.70 L) was added TFAA (324 mL, 2.30 mol) and $BF_3{\cdot}OEt_2$ (19.4 mL, 0.153 mol), and the solution was stirred for 30 ·min at room temperature. The reaction mixture was added TFAA (108 mL, 0.765 mol) and $BF_3{\cdot}OEt_2$ (19.4 mL, 0.153 mol), and the solution was stirred for 50 min at room temperature. The reaction mixture was added TFAA (108 mL, 0.765 mol) and $BF_3{\cdot}OEt_2$ (19.4 mL, 0.153 mol), and the solution was stirred for 1h at room temperature. The reaction mixture was cooled at 0°C, and pored into water (2.35 L), and extracted with ethyl acetate (7.99 L). The combined organic layer was pored into saturated aqueous sodium bicarbonate solution (3.29L) and 4 mol/L NaOH aqueous solution (1.2 L), and stirred. The resultant emulsion was filtered on celite pad, and washed with ethyl acetate (470 mL). The filtrate was separated, and the organic layers were washed with brine. The resultant organic layers were stirred for 30 min at room temperature with activated carbon (23.5 g), and filtered. The filtrate was concentrated to give the residue. The obtained residue was then purified by recrystallization from EtOH (1.88 L) to afford the residue. The resultant solid washed with EtOH, and dried under reduced pressure to give 8-bromodibenzo[*b,e*]oxepin-11(6*H*)-one (**S10**) (342 g, 90%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.15 (s, 2H), 7.05–7.17 (m, 2H), 7.47–7.63 (m, 3H), 7.80 (d, J = 8.1 Hz, 1H), 8.24 (dd, J = 8.1, 1.8 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 289.

2-(8-Bromodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S11). To a stirred solution of LDA (2.0 mol/L, 12.1 mL, 24.2 mmol) in THF (3 mL) was added to a solution of propanenitrile (0.67 g, 12 mmol) in THF (3 mL) at 0°C, and the solution was stirred for 0.5h at same temperature. The reaction mixture was added to a solution of diethyl chlorophosphate (2.1 g, 12 mmol) in THF (3 mL) at 0°C, and the solution

was stirred for 3h at room temperature. The reaction mixture was added to a solution of 8-bromodibenzo[*b*,*e*]oxepin-11(6*H*)-one (**S10**) (1.0 g, 3.5 mmol) in DMF (9 mL) at room temperature, and the solution was stirred for 3h at 80°C. The reaction mixture was pored into saturated aqueous ammonium chloride solution and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (97:3 to 93:7 hexane/ethyl acetate) to give 2-(8-bromodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S11**) (*E*/*Z* = 6/4, 840 mg, 74%) as a amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.00–2.28 (m, 3H), 4.75–4.87 (m, 1H), 5.43 (d, *J* = 12.6 Hz, 1H), 6.82–7.08 (m, 3H), 7.21–7.37 (m, 2H), 7.48–7.58 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 326.

Ethyl (*E*)-11-(1-cyanoethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepine-8-carboxylate (S12). To a stirred solution of 2-(8-bromodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S11**) (*E*/*Z* = 6/4, 840 mg, 2.58 mmol) in DMF (4.3 mL) was added palladium (II) acetate (88 mg, 0.39 mmol), 1,3-bis(diphenylphosphino)propane (dppp; 161 mg, 0.390 mmol), cesium carbonate (1.26 g, 3.86 mmol) and EtOH (4.3 mL), and the solution was stirred for 5h at 70°C under a carbon monoxide atmosphere. The reaction mixture was cooled at room temperature, and concentrated ubder reduced pressure. The obtain residue was pored into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (95:5 to 90:10 hexane/ethyl acetate) to give ethyl (*E*)-11-(1-cyanoethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepine-8-carboxylate (**S12**) (274 mg, 33%) as a amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, *J* = 7.1 Hz, 3H), 2.28 (s, 3H), 4.35–4.43 (m, 2H), 4.95 (d, *J* = 12.6 Hz, 1H), 5.50 (d, *J* = 12.6 Hz, 1H), 6.87–6.96 (m, 2H), 7.08 (dd, *J* = 1.7, 7.8 Hz, 1H), 7.23–7.28 (m, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 8.07–8.12 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 320.

(*E*)-2-{8-(Hydroxymethyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene}propanenitrile (17*E*). To a stirred solution of ethyl (*E*)-11-(1-cyanoethylidene)-6,11-dihydrodibenzo[*b*,*e*]oxepine-8-carboxylate (**S12**) (909 mg, 2.85 mmol) in THF (14.5 mL) was added lithium borohydride (310 mg, 14.3 mmol), and the solution was stirred for 4h at 50°C. The reaction mixture was cooled at room temperature, and pored into ice water (1000 mL). The resultant solution was pored into 2 mol/L HCl aqueous solution to pH 5 and extracted with

ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (80:20 to 60:40 hexane/ethyl acetate) to give (*E*)-2-{8-(hydroxymethyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene}propanenitrile (**17*E***) (815 mg, 100%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H), 4.72 (d, J = 5.9 Hz, 2H), 4.87 (d, J = 12.5 Hz, 1H), 5.49 (d, J = 12.5 Hz, 1H), 6.86–6.94 (m, 2H), 7.07 (dd, J = 1.6, 7.9 Hz, 1H), 7.21–7.26 (m, 1H), 7.38–7.49 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 278.

4-Bromo-2-((4-fluorophenoxy)methyl)benzoic acid (S2a). To a stirred solution of 5-bromoisobenzofuran-1(3*H*)-one (6.0 g, 28 mmol) in DMF (10 mL) was added 4-fluorophenol (6.1 mL, 67 mmol) and sodium methoxide (28% methanol solution, 5.4 g, 28 mmol), and the solution was stirred for 23 h at 120 °C. The reaction mixture was cooled at room temperature, and was pored into 4 M NaOH. The mixture was washed with toluene and diethyl ether. The water layer was acidified with 4 M HCl, and diluted by ethanol. The mixture was stirred overnight and the resultant soid was filtered, washed with water/EtOH = 1/1, and dried under reduced pressure to give 4-bromo-2-((4-fluorophenoxy)methyl)benzoic acid (**S2a**) (3.4 g, 36%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.41 (s, 2H), 6.67–6.78 (m, 1H), 6.89–7.07 (m, 2H), 7.07–7.20 (m, 1H), 7.67 (dd, J = 8.6, 2.0 Hz, 1H), 7.83 (d, J = 2.0 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H). The proton of carboxylic acid was not observed. LC/MS (ESI, $[M - H]^-$, m/z) 323.

4-Bromo-2-((3-fluorophenoxy)methyl)benzoic acid (S2b). To a stirred solution of 5-bromoisobenzofuran-1(3*H*)-one (9.5 g, 45 mmol) in DMF (10 mL) was added 3-fluorophenol (6.1 mL, 67 mmol) and sodium methoxide (28% methanol solution, 13 g, 67 mmol), and the solution was stirred for 23 h at 120 °C. The reaction mixture was cooled at room temperature, and was pored into 4 M NaOH. The mixture was washed with toluene and diethyl ether. The water layer was acidified with 4 M HCl, and diluted by ethanol. The mixture was stirred overnight and the resultant soid was filtered, washed with water/EtOH = 1/1, and dried under reduced pressure to give 4-bromo-2-((3-fluorophenoxy)methyl)benzoic acid (**S2b**) (10 g, 69%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.48 (s, 2H), 6.66–6.86 (m, 3H), 7.21–7.32 (m, 1H), 7.58 (dd, J = 8.5, 2.0 Hz, 1H), 7.99 (d, J = 2.0 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H). The proton of carboxylic acid was not observed. LC/MS (ESI, $[M - H]^-$, m/z) 323.

4-Bromo-2-((2-fluorophenoxy)methyl)benzoic acid (S2c). To a stirred solution of 5-

bromoisobenzofuran-1(3*H*)-one (7.0 g, 26 mmol) in DMF (9 mL) was added 2-fluorophenol (5.3 mL, 60 mmol) and sodium methoxide (28% methanol solution, 12 g, 60 mmol), and the solution was stirred for 23 h at 120 °C. The reaction mixture was cooled at room temperature, and was pored into 4 M NaOH. The mixture was washed with toluene and diethyl ether. The water layer was acidified with 4 M HCl, and diluted by ethanol. The mixture was stirred overnight and the resultant soid was filtered, washed with water/EtOH = 1/1, and dried under reduced pressure to 4-bromo-2-((2-fluorophenoxy)methyl)benzoic acid (**S2c**) (3.7 g, 26%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.55 (s, 2H), 6.90–7.19 (m, 4H), 7.58 (dd, J = 8.2, 1.6 Hz, 1H), 8.02 (d,J = 8.2 Hz, 1H), 8.08 (d, J = 1.6 Hz, 1H). The proton of carboxylic acid was not observed. LC/MS (ESI, $[M - H]^-$, m/z) 323.

8-Bromo-2-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (S3a). To a stirred solution of 4-bromo-2-((4-fluorophenoxy)methyl)benzoic acid (**S2a**) (3.4 g, 10 mmol) in CH_2Cl_2 (35 mL) was added trifluoroacetic anhydride (2.2 mL, 16 mmol) and borane trifluoride diethyl ether complex (0.091 mL, 0.73 mmol), and the mixture was stirred for 7 h at room tempareture. The reaction mixture was pored into 2M NaOH and extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was purified by recrystallization from chloroform to afford 8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (**S3a**) (1.1 g, 34%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.13 (s, 2H), 6.91–7.00 (m, 1H), 7.09–7.17 (m, 1H), 7.53 (d, J = 2.0 Hz, 1H), 7.57–7.65 (m, 1H), 7.83 (d, J = 8.6 Hz, 1H), 7.90 (dd, J = 9.5, 3.3 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 307.

8-Bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (S3b). To a stirred solution of 4-bromo-2-((2-fluorophenoxy)methyl)benzoic acid (**S2b**) (6.0 g, 18 mmol) in CH_2Cl_2 (62 mL) was added trifluoroacetic anhydride (5.2 mL, 37 mmol) and borane trifluoride diethyl ether complex (2.3 mL, 18 mmol), and the mixture was stirred for 7 h at room tempareture. The reaction mixture was pored into 2M NaOH and extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was purified by recrystallization from chloroform to afford 8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (**S3b**) (4.3 g, 76%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.16 (s, 2H), 6.76 (dd, J = 9.9, 2.6 Hz, 1H), 6.83–6.91 (m, 1H), 7.54 (d,J = 2.0 Hz, 1H), 7.63 (dd, J = 8.2, 2.0 Hz, 1H), 7.80 (d, J = 8.2

Hz, 1H), 8.28 (dd, *J* = 9.0, 6.8 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 307.

8-Bromo-4-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (S3c). To a stirred solution of 4-bromo-2-((2-fluorophenoxy)methyl)benzoic acid (**S2c**) (3.4 g, 11 mmol) in CH_2Cl_2 (35 mL) was added trifluoroacetic anhydride (6.1 mL, 67 mmol) and borane trifluoride diethyl ether complex (1.3 mL, 11 mmol), and the mixture was stirred for 7 h at room tempareture. The reaction mixture was pored into 2M NaOH and extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was purified by recrystallization from chloroform to afford 8-bromo-4-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (**S3c**) (2.9 g, 89%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.24 (s, 2H), 7.02–7.12 (m, 1H), 7.28–7.36 (m, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.63 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.96–8.02 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 307.

2-(8-Bromo-2-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S4a). To a stirred solution of propionitrile (0.19 mL, 2.7 mmol) in THF (2 mL) was added LDA (2 M heptane/THF/ethylbenzene solution, 2.7 mL, 5.4 mmol) and the mixture was stirred for 1 h at room temparature. A solution of 8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (**S3a**) (1.1 g, 3.6 mmol) in THF (1 mL)/DMF (1 mL) was added to the mixture, and the mixture was stirred for 2 h at 80 °C. The reaction mixture was pored into saturated NH_4Cl solution and extracted with ethylacetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 85:15 hexane/ethyl acetate) to afford 2-(8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S4a**) (182 mg, 49%, *E/Z* mixture) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.03 (s, 1.5H), 2.26 (s, 1.5H), 4.71–4.87 (m, 1H), 5.38 (d, *J* = 13.2 Hz, 1H), 6.74–6.89 (m, 1.5H), 6.92–7.08 (m, 1.5H), 7.18–7.25 (m, 0.5H), 7.35 (d, *J* = 7.9 Hz, 0.5H), 7.47–7.59 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 344.

2-(8-Bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S4b). To a stirred solution of propionitrile (36 mL, 2.58 mmol) in THF (30 mL) was added LDA (2 M heptane/THF/ethylbenzene solution, 36 mL, 72 mmol) and the mixture was stirred for 1 h at room temparature. A solution of 8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (**S3b**) (4.5 g, 14 mmol) in THF (14 mL)/DMF (14 mL) was added to the mixture, and the mixture was stirred for 2 h at 80 °C. The reaction mixture was pored into

saturated NH_4Cl solution and extracted with ethylacetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 85:15 hexane/ethyl acetate) to afford 2-(8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S4b**) (4.1 g, 82%, *E/Z* mixture) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.01 (s, 1.5H), 2.24 (s, 1.5H), 4.74–4.88 (m, 1H), 5.42 (d, J = 12.8 Hz, 1H), 6.51–6.62 (m, 1H), 6.62–6.74 (m, 1H), 6.99–7.08 (m, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.48 (dd, J = 8.8, 6.6 Hz, 1H), 7.51–7.60 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 344.

2-(8-Bromo-4-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S4c). To a stirred solution of propionitrile (3.3 mL, 47 mmol) in THF (40 mL) was added LDA (2 M heptane/THF/ethylbenzene solution, 47 mL, 93 mmol) and the mixture was stirred for 1 h at room temparature. A solution of 8-bromo-4-fluorodibenzo[*b,e*]oxepin-11(6*H*)-one (**S3c**) (5.7 g, 19 mmol) in THF (18 mL)/DMF (19 mL) was added to the mixture, and the mixture was stirred for 2 h at 80 °C. The reaction mixture was pored into saturated NH_4Cl solution and extracted with ethylacetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 85:15 hexane/ethyl acetate) to afford 2-(8-bromo-4-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S4c**) (5.5 g, 87%, *E/Z* mixture) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.03 (s, 1.5H), 2.25 (s, 1.5H), 4.90–5.01 (m, 1H), 5.48 (d, J = 12.8 Hz, 1H), 6.79–6.96 (m, 1.5H), 7.00–7.14 (m, 1.5H), 7.23–7.38 (m, 1H), 7.50–7.62 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 344.

Propyl (*E*)-11-(1-cyanoethylidene)-2-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (S5a). To a stirred solution of 2-(8-bromo-2-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S4a**) (980 mg, 2.85 mmol, *E/Z* mixture) in DMF (8 mL) and propanol (4 mL) was added cesium carbonate (1.1 g, 3.4 mmol), palladium acetate (II) (190 mg, 0.855 mmol) and 1,3-bis(diphenylphosphino)propane (350 mg, 0.855 mmol), and the mixture was stirred for 6 h at 70 °C under a carbon monoxide atmosphere. The reaction mixture was filtered through a celite pad. The filtrate was pored into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash

column chromatography on silica gel (100:0 to 40:60 hexane/ethyl acetate) to afford propyl (*E*)-11-(1-cyanoethylidene)-2-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (**S5a**) (210 mg, 21%) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.03 (t, J = 7.4 Hz, 3H), 1.71–1.87 (m, 2H), 2.29 (s, 3H), 4.25–4.34 (m, 2H), 4.94 (d, J = 12.9 Hz, 1H), 5.46 (d, J = 12.9 Hz, 1H), 6.77–6.89 (m, 2H), 6.94–7.03 (m, 1H), 7.56 (d,J = 7.9 Hz, 1H), 8.05 (d, J = 1.7 Hz, 1H), 8.11 (dd, J = 7.9, 1.7 Hz, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 352.

Propyl 11-(1-cyanoethylidene)-3-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (S5b). To a stirred solution of 2-(8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S4b**) (14 g, 41 mmol, *E/Z* mixture) in DMF (105 mL) and propanol (52 mL) was added cesium carbonate (15.9 g, 48.8 mmol), palladium acetate (II) (2.74 g, 12.2 mmol) and 1,3-bis(diphenylphosphino)propane (5.04 g, 12.2 mmol), and the mixture was stirred for 6 h at 70°C under a carbon monoxide atmosphere. The reaction mixture was filtered through a celite pad. The filtrate was pored into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 75:25 hexane/ethyl acetate) to afford propyl 11-(1-cyanoethylidene)-3-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (**S5b**) (15.5 g, 63%, *E/Z* mixture) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.98–1.08 (m, 3H), 1.73–1.87 (m, 2H), 2.02 (s, 1.5H), 2.27 (s, 1.5H), 4.25–4.35 (m, 2H), 4.89–4.99 (m, 1H), 5.49 (d, J = 12.8 Hz, 1H), 6.50–6.63 (m, 1H), 6.63–6.75 (m, 1H), 7.05 (dd, J = 8.6, 6.4 Hz, 0.5H), 7.23–7.29 (m, 0.5H), 7.50 (dd, J = 8.8, 5.9 Hz, 0.5H), 7.55 (d, J = 7.8 Hz, 0.5H), 8.00–8.16 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 352.

Propyl 11-(1-cyanoethylidene)-4-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (S5c). To a stirred solution of 2-(8-bromo-4-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S4c**) (5.5 g, 16 mmol, *E/Z* mixture) in DMF (41 mL) and propanol (21 mL) was added cesium carbonate (6.3 g, 19 mmol), palladium acetate (II) (1.1 g, 4.8 mmol) and 1,3-bis(diphenylphosphino)propane (2.0g, 4.8 mmol), and the mixture was stirred for 6 h at 70 °C under a carbon monoxide atmosphere. The reaction mixture was filtered through a celite pad. The filtrate was pored into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column

chromatography on silica gel (100:0 to 70:30 hexane/ethyl acetate) to afford propyl 11-(1-cyanoethylidene)-4-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (**S5c**) (3.5 g, 62%, *E/Z* mixture) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.99–1.08 (m, 3H), 1.71–1.88 (m, 2H), 2.04 (s, 1.5H), 2.28 (s, 1.5H), 4.25–4.36 (m, 2H), 5.03–5.15 (m, 1H), 5.55 (d, *J* = 12.9 Hz, 1H), 6.83–6.96 (m, 1.5H), 7.02–7.15 (m, 1H), 7.25–7.33 (m, 1H), 7.56 (d, *J* = 7.9 Hz, 0.5H), 8.07–8.17 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 352.

(*E*)-2-(2-Fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S6a). To a stirred solution of propyl (*E*)-11-(1-cyanoethylidene)-2-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (**S5a**) (210 mg, 0.600 mmol) in THF (3 mL) was added lithium borohydride (65 mg, 3.0 mmol) and the mixture was stirred for 3 h at 60 °C. The reaction mixture was cooled in ice bath and pored into into 4 M HCl and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 40:60 hexane/ethyl acetate) to afford (*E*)-2-(3-fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S6a**) (230 mg, 130%) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.20–1.31 (m, 1H), 2.27 (s, 3H), 4.72–5.01 (m, 3H), 5.35–5.50 (m, 1H), 6.74–6.89 (m, 2H), 6.90–7.01 (m, 1H), 7.25–7.51 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 296.

(*E*)-2-(3-Fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S6b). To a stirred solution of propyl 11-(1-cyanoethylidene)-3-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (**S5b**) (16 g, 44 mmol, *E/Z* mixture) in THF (220 mL) was added lithium borohydride (4.80 g, 220 mmol) and the mixture was stirred for 3 h at 60°C. The reaction mixture was cooled in ice bath and pored into into 4 M HCl and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 40:60 hexane/ethyl acetate) to afford (*E*)-2-(3-fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S6b**) (6.1 g, 47%) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.70 (t, *J* = 5.9 Hz, 1H), 2.25 (s, 3H), 4.73 (d, *J* = 5.9 Hz, 2H), 4.87 (d, *J* = 12.5 Hz, 1H), 5.48 (d, *J* = 12.5 Hz, 1H), 6.58 (dd, *J* = 10.4, 2.5 Hz, 1H), 6.61–6.69 (m, 1H), 7.04 (dd,*J* = 8.6, 6.6 Hz, 1H), 7.38–7.50 (m, 3H). LC/MS (ESI, $[M + H]^+$,

m/z) 296.

(*E*)-2-(4-Fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S6c). To a stirred solution of propyl 11-(1-cyanoethylidene)-4-fluoro-6,11-dihydrodibenzo[*b,e*]oxepine-8-carboxylate (**S5c**) (3.5 g, 10 mmol, *E/Z* mixture) in THF (50 mL) was added lithium borohydride (2.2 g, 99 mmol) and the mixture was stirred for 3 h at 60°C. The reaction mixture was cooled in ice bath and pored into into 4 M HCl and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 40:60 hexane/ethyl acetate) to afford (*E*)-2-(4-fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S6c**) (1.1 g, 39%) as a white amorphous. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.80 (br s, 1H), 2.26 (s, 3H), 4.67–4.76 (m, 2H), 5.02 (d, J = 12.8 Hz, 1H), 5.54 (d, J = 12.8 Hz, 1H), 6.81–6.88 (m, 2H), 7.00–7.10 (m, 1H), 7.39–7.50 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 296.

(*E*)-2-(8-(Bromomethyl)-2-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (22a). To a stirred solution of (*E*)-2-{3-fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (**S6a**) (230 mg, 0.600 mmol) in THF (3 mL) was added 2,6-lutidine (0.42 mL, 3.6 mmol), Ms_2O (0.26 g, 1.5 mmol) and lithium bromide (0.31 g, 3.6 mmol), and the solution was stirred for 15 h at room temperature. The reaction mixture was pored into water, and extracted with ethyl acetate. The combined organic layers were washed with 1 M HCl aqueous solution, saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 70:30 hexane/ethyl acetate) to afford (*E*)-2-{8-(bromomethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (**22a**) (135 mg, 63%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.27 (s, 3H), 4.48 (s, 2H), 4.86 (d, J = 13.2 Hz, 1H), 5.42 (d, J = 13.2 Hz, 1H), 6.76–6.88 (m, 2H), 6.93–7.01 (m, 1H), 7.37–7.41 (m, 1H), 7.43–7.47 (m, 2H). LC/MS (ESI, $[M + H]^+$, m/z) 358.

(*E*)-2-(8-(Bromomethyl)-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (23a). To a stirred solution of (*E*)-2-{3-fluoro-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (**S6b**) (2.1 g, 7.3 mmol) in THF (73 mL) was added 2,6-lutidine (5.1 mL, 44 mmol), Ms_2O (3.2 g, 18 mmol) and lithium bromide (3.8 g, 44 mmol), and the solution was stirred for 15 h at room temperature. The reaction

mixture was pored into water, and extracted with ethyl acetate. The combined organic layers were washed with 1 M HCl aqueous solution, saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 50:50 hexane/ethyl acetate) to afford (*E*)-2-{8-(bromomethyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene}propanenitrile (**23a**) (2.0 g, 78%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.25 (s, 3H), 4.48 (s, 2H), 4.86 (d, J = 12.8 Hz, 1H), 5.45 (d, J = 12.8 Hz, 1H), 6.58 (dd, J = 10.2, 2.6 Hz, 1H), 6.61–6.70 (m, 1H), 7.04 (dd, J = 8.9, 6.6 Hz, 1H), 7.39–7.49 (m,3H). LC/MS (ESI, $[M + H]^+$, m/z) 358.

(*E*)-2-(8-(Bromomethyl)-4-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (24a). To a stirred solution of (*E*)-2-{4-fluoro-8-(hydroxymethyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene}propanenitrile (**S6c**) (1.1 g, 3.8 mmol) in THF (38 mL) was added 2,6-lutidine (2.7 mL, 23 mmol), Ms_2O (1.7 g, 9.6 mmol) and lithium bromide (2.0 g, 23 mmol), and the solution was stirred for 15 h at room temperature. The reaction mixture was pored into water, and extracted with ethyl acetate. The combined organic layers were washed with 1 M HCl aqueous solution, saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 97:3 $CHCl_3$/MeOH) to afford (*E*)-2-{8-(bromomethyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene}propanenitrile (**24a**) (1.2 g, 84%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.26 (s, 3H), 4.48 (s, 2H), 5.01 (d, J = 12.5 Hz, 1H), 5.52 (d, J = 12.5 Hz, 1H), 6.80–6.91 (m, 2H), 7.01–7.11 (m, 1H), 7.40–7.48 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 358.

(*E*)-2-(3-fluoro-8-formyldibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (37a). To a stirred solution of (*E*)-2-{3-fluoro-8-(hydroxymethyl)dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene}propanenitrile (**S6b**) (800 mg, 2.71 mmol) in CH_2Cl_2 (9 mL) was added Dess-Martin periodinane (1.38 g, 3.25 mmol), and the solution was stirred for 15 h at room temperature. The reaction mixture was pored into saturated aqueous sodium bicarbonate solution, and extracted with $CHCl_3$. The combined organic layers were washed with saturated aqueous sodium thiosulfate solution, and brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to afford (*E*)-2-(3-fluoro-8-formyldibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile (**37a**) (780 mg, 98%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): 2.04 (s, 3H),

4.96 (d, *J* = 12.4 Hz, 1H), 5.50 (d, *J* = 12.4 Hz, 1H), 6.43–6.62 (m, 1H), 6.66–6.80 (m, 1H), 7.33–7.42 (m, 1H), 7.41–7.59 (m, 1H), 7.89–8.01 (m, 2H), 9.98–10.17 (m, 1H). LC/MS (ESI, $[M + H]^+$, m/z) 294.

2-(3-Fluoro-8-((trimethylsilyl)ethynyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (S13). To a stirred solution of 2-(8-bromo-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S4b**) (5.0 g, 15 mmol) in DMF (42 mL) was added bis(triphenylphosphine)palladium(II) chloride (1.0 g, 15 mmol), copper(I) idodide (0.28 g, 1.5 mmol) , triethylamine (8.1 mL, 58 mmol) and trimethylsilylacetylene (4.1 mL, 29 mmol), and the solution was stirred for 15 h at room temperature. The reaction mixture was pored into water, and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 80:20 hexane/ethyl acetate) to afford 2-(3-fluoro-8-((trimethylsilyl)ethynyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (**S13**) (4.1 g, 78%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): 0.33–0.20 (m, 9H), 2.01 (s, 1.2H), 2.24 (s, 1.8H), 4.89–4.73 (m, 1H), 5.48–5.37 (m, 1H), 6.76–6.49 (m, 2H), 7.17–6.98 (m, 1H), 7.56–7.37 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 362.

2-(8-Ethynyl-3-fluorodibenzo[*b,e*]oxepin-11(6*H*)-ylidene)propanenitrile (31a). To a stirred solution of 2-(3-fluoro-8-((trimethylsilyl)ethynyl)dibenzo[b,e]oxepin-11(6H)-ylidene)propanenitrile (**S13**) (4.1 g, 11 mmol) in MeOH (45 mL) was added potassium carbonate (1.56 g, 11.3 mmol), and the solution was stirred for 5 h at room temperature. The reaction mixture was pored into water, and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by flash column chromatography on silica gel (100:0 to 80:20 hexane/ethyl acetate) to afford 2-(8-ethynyl-3-fluorodibenzo[b,e]oxepin-11(6H)-ylidene)propanenitrile (**31a**) (4.1 g, 78%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): 2.01 (s, 1.2H), 2.27 (s, 1.8H), 3.06–3.19 (m, 1H), 4.74–4.92 (m, 1H), 5.33–5.52 (m, 1H), 6.47–6.77 (m, 2H), 6.98–7.18 (m, 1H), 7.36–7.60 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 290.

(*E*)-2-{8-(Chloromethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (18). To a stirred solution of (*E*)-2-{8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (**17*E***) (10 g, 36.1 mmol) in

THF (100 mL) was added Et_3N (7.5 mL, 54 mmol), MsCl (4.2 mL, 54 mmol) and lithium chloride (2.3 g, 54 mmol) at 0°C, and the solution was stirred for 3.5h at 50°C. The reaction mixture was cooled at room temperature, and pored into ice water (1000 mL). The resultant solution was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give the residue. The obtained residue was then purified by recrystallization from diisopropyl ether (50 mL) to afford the residue. The resultant solid washed with diisopropyl ether, and dried under reduced pressure to give (*E*)-2-{8-(chloromethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (**18**) (10.2 g, 96%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.27 (s, 3H), 4.59 (s, 2H), 4.88 (d, J = 12.4 Hz, 1H), 5.48 (d, J = 12.4 Hz, 1H), 6.84–6.95 (m, 2H), 7.03–7.11 (m, 1H), 7.20–7.28 (m, 1H), 7.39–7.52 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 296.

(*E*)-2-{8-(Bromomethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (19). To a stirred solution of (*E*)-2-{8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (**17*E***) (10 g, 36 mmol) in THF (361 mL) was added 2,6-lutidine (25.0 mL, 216 mmol), Ms_2O (15.7 g, 90.2 mmol) and lithium bromide (18.8 g, 216.4 mmol), and the solution was stirred for 15 h at room temperature. The reaction mixture was pored into water, and extracted with ethyl acetate. The combined organic layers were washed with 1 mol/L HCl aqueous solution, saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, and filtered. The organic layer was concentrated to give (*E*)-2-{8-(bromomethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}propanenitrile (**19**) (4.7 g, 38%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.26 (s, 3H), 4.48 (s, 2H), 4.86 (d, J = 12.7 Hz, 1H), 5.46 (d, J = 12.7 Hz, 1H), 6.84–6.95 (m, 2H), 7.07 (dd, J = 1.6, 7.8 Hz, 1H), 7.20–7.28 (m, 1H), 7.38–7.49 (m, 3H). LC/MS (ESI, $[M + H]^+$, m/z) 340.

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