Development of a novel class of peroxisome proliferator-activated receptor (PPAR) gamma ligands as an anticancer agent with a unique binding mode based on a non-thiazolidinedione scaffold

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ABSTRACT

We previously identified dibenzooxepine derivative **1** as a potent PPARγ ligand with a unique binding mode owing to its non-thiazolidinedione scaffold. However, while **1** showed remarkably potent MKN-45 gastric cancer cell aggregation activity, an indicator of cancer differentiation-inducing activity induced by PPARγ activation, we recognized that **1** was metabolically unstable. In the present study, we identified a metabolically soft spot, and successfully discovered 3-fluoro dibenzooxepine derivative **9** with better metabolic stability. Further optimization provided imidazo[1,2-*a*]pyridine derivative **17**, which showed potent MKN-45 gastric cancer cell aggregation activity and excellent PK profiles compared with **9**. Compound **17** exerted a growth inhibitory effect on AsPC-1/AG1 pancreatic tumor in mice. Furthermore, the decrease in the hematocrit (an indicator of localized edema, a serious adverse effect of PPARγ ligands) was tolerable even with oral administration at 200 mg/kg in healthy mice.

1. Introduction

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear receptor superfamily and regulates the expression of genes involved in peripheral insulin sensitivity, adipogenesis and glucose homeostasis.[1,2,3] PPARγ heterodimerizes with retinoid X receptor α (RXRα), a nuclear receptor, and binds to the DNA response element (PPAR response element [PPRE]) of each target gene to induce their transactivation activities.[4]

A number of selective PPARγ agonists have been developed, and the structures of well-known PPARγ agonists, containing the thiazolidinedione (TZD) structure, e.g., pioglitazone and rosiglitazone, are shown in Figure 1. [5,6,7,8,9,10,11,12,13] PPARγ agonists have been reported to be beneficial for treating type 2 diabetes mellitus. PPARγ plays a key role in adipogenesis,[14] particularly the differentiation from pre-adipocytes to adipocytes, by regulating the expression of adipose-related genes, such as adipocyte fatty acid-binding protein 2 (*aP2*),[15] adipose differentiation-related protein (*Adfp*) and *Adiponectin*.[16] For instance, PPARγ agonists strongly promote the differentiation of fibroblast-like cells such as 3T3-L1 cells to adipocytes.[17,18,19]

Considering the differentiation of pre-adipocytes based on PPARγ activation, compounds that activate PPARγ might lead to the differentiation induction of cancer cells. Indeed, the TZD derivative efatutazone (CS-7017), a potent PPARγ full agonist, has been reported as a cancer differentiation-inducing agent,[8,20] inducing the differentiation of several cancer, including anaplastic thyroid carcinoma,[20] non-small cell lung cancer,[21] and pancreatic cancer.[8] However, in the clinical studies of efatutazone, some adverse effects, such as localized edema, were observed, and these adverse effects are likely due to the structural properties of the TZD moiety.[20,22] Similar adverse effects, largely characterized by fluid retention, have also been reported in both preclinical and clinical studies of other PPARγ full agonists. Thus, avoiding of fluid retention should be attempted in the development of PPARγ ligands as antitumor drugs.

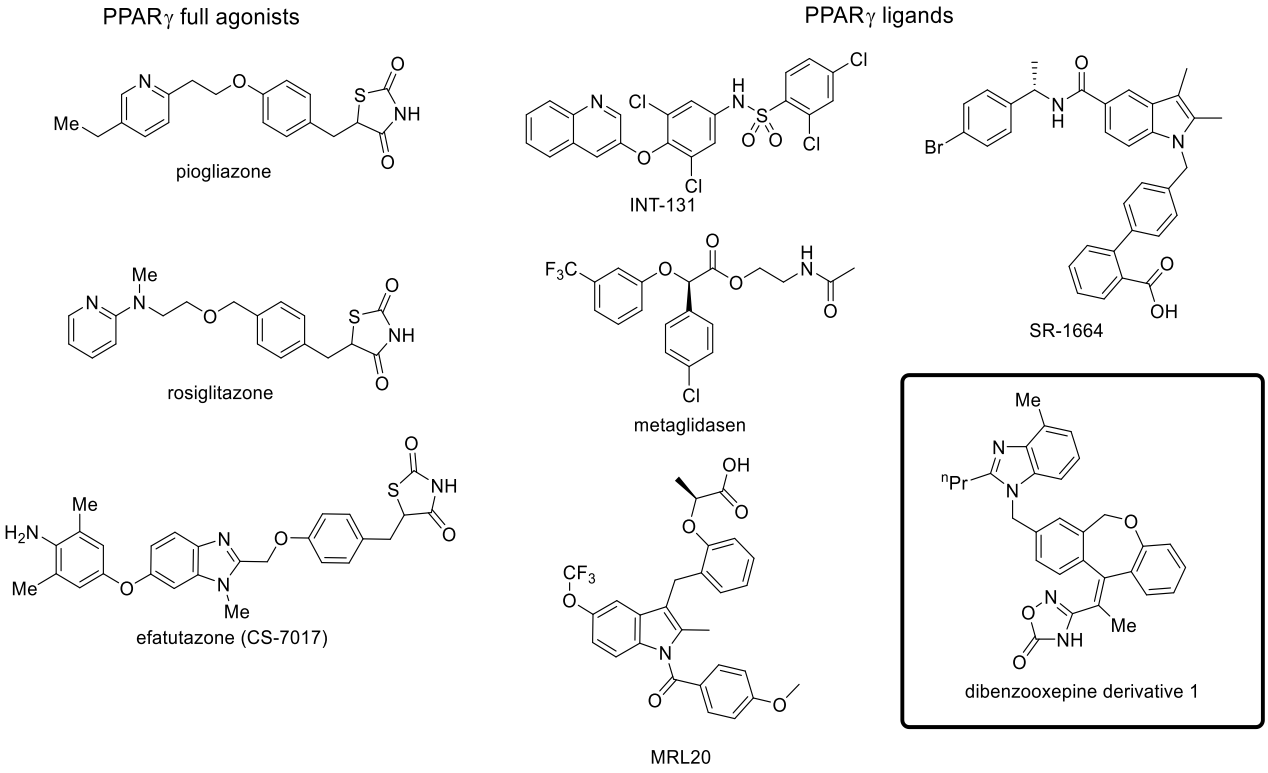
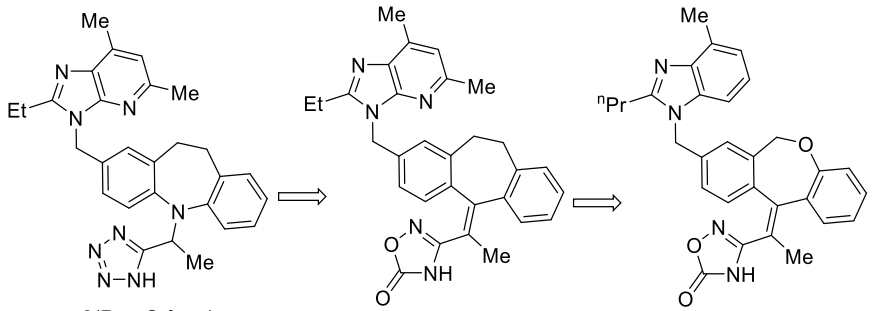


Figure 1. The representative PPARγ ligands.

We previously reported dibenzooxepine derivative **1**,[23] which has a non-TZD structure. Compound **1**, which was derived from high throughput screening hit compound dibenzoazepine derivative **2 (**Figure 2**)**, showed a unique binding mode and potent differentiation-inducing activity of cancer cells. Efficient lead optimization of hit compound **2** was likely to be troublesome due to the asymmetric center adjacent to the nitrogen atom on the azepine ring. To eliminate the chiral center, its bioisosteric scaffolds were designed based on the conformational search of model substrates **(*R*)-2'** and **(*S*)-2'** (Figure 3).[23] As a result, we found that olefinic *E/Z* isomers **4** and **5** effectively mimic the chiral structures of **(*R*)-2'** and **(*S*)-2'**, respectively, and discovered dihydrodibenzocycloheptene derivative **3**, which has potent PPARγ reporter activity comparable to that of **2**. Further optimization of the core structure and the benzimidazole moiety of **2** led to **1**, which has a novel binding mode to PPARγ ligand binding domain (LBD) and potent MKN-45 aggregation activity, an indicator of cancer differentiation-inducing activity.

However, we recognized that the clinical development of **1** as an anticancer agent was difficult due to its very poor metabolic stability. Following the structural optimization of **1**, we successfully developed a promising drug candidate with a good PK profile that showed exerted efficacy in a mouse model of pancreatic cancer. We herein report the structural optimization of **1** resulting in the discovery of a novel class of anticancer PPARγ ligands, with reduced adverse effects of severe fluid retention.



2(R or S-form)
(-)-form 86%ee

3

1

HEK293 reporter EC_{50} (nM)	197	177	2.4
MKN-45 aggregation EC_{50} (nM)	N.T.	N.T.	3.3

Figure 2. Identification of lead compound **1** from HTS hit compound **2**.

down-syn ((*R*)-**2'**) /***down-Z*** (**4**) ***down-anti*** ((*S*)-**2'**) /***down-E*** (**5**)

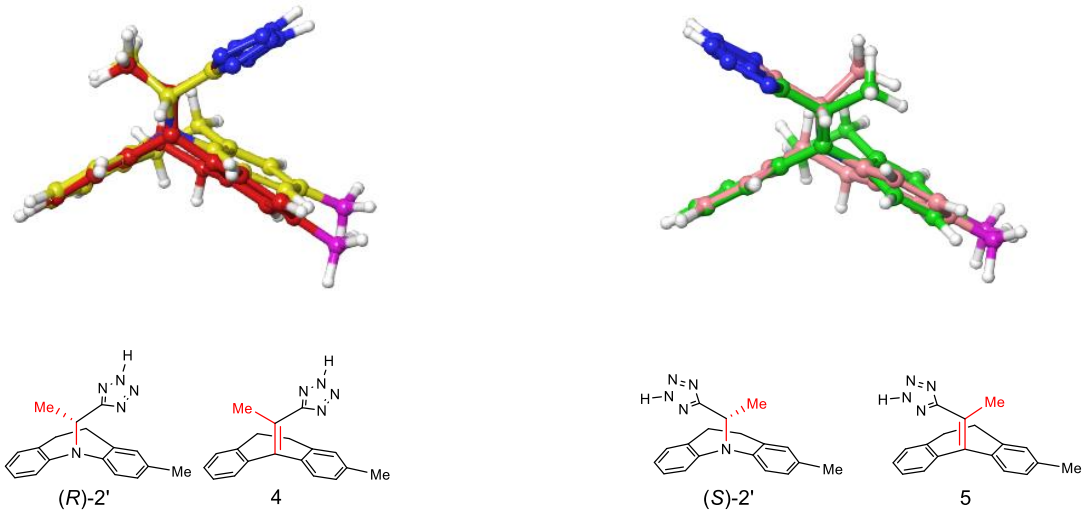


Figure 3. Overlay of the most stable conformations of the chiral model compounds **(*R*)-2'** and **(*S*)-2'** on their non-chiral olefinic bioisosters **4** and **5**, respectively. Global energy minimum conformations of these compounds were calculated by the MacroModel. The hydrogens, nitrogens, and 2-methyl group are colored white, blue and magenta, respectively.

2. Result and Discussion

*2.1. Identification of 3-Fluoro Dibenzooxepine Derivative **9***

Figure 4 shows the very short half-life of **1** when administered orally to mice. To reduce lipophilicity of tricyclic ring and improve metabolic stability, dihydrobenzo[6,7]oxepino[3,4-*b*]pyridine derivatives **6** and **7**, in which either of the benzenes in the core structure was replaced with pyridine in order to reduce the electron density of the aromatic ring and thereby prevent oxidative metabolism, were designed and synthesized. The oxygen atom of **7** in the central seven-membered ring was shifted to the position adjacent to that of **1** for the synthetic convenience.

The synthetic compounds were evaluated *in vitro* via an HEK293 PPARγ reporter gene assay and cell aggregation assay to assess the differentiation-inducing activity against gastric cancer cell line MKN-45, as in our previous study.[23] In addition, compounds were treated with human liver microsome to investigate the metabolic stability.

In the reporter gene assay, the left-pyridine derivative **6** showed an EC_{50} value 86 nM higher than that of **1**, and the activity of the right-pyridine derivative **7** was significantly reduced (EC_{50} = 2194 nM). However, the human liver microsome stability of **7** was significantly better than that of **6** (2.06 L/h/kg vs. >24.9 L/h/kg), suggesting that the right benzene moiety of dibenzooxepine was likely to be the main metabolized site.

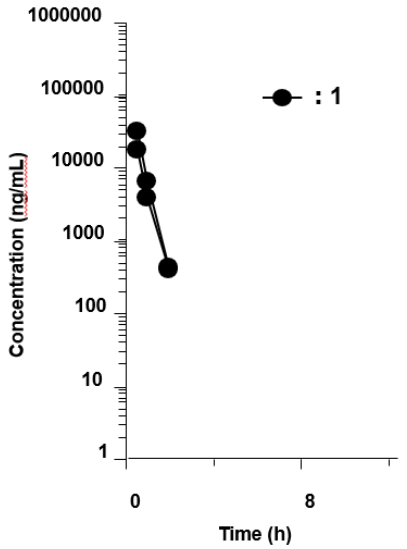


Figure 4. Plasma concentrations of **1** after its oral administration in BALB/c mice (30 mg/kg, *n* = 2).

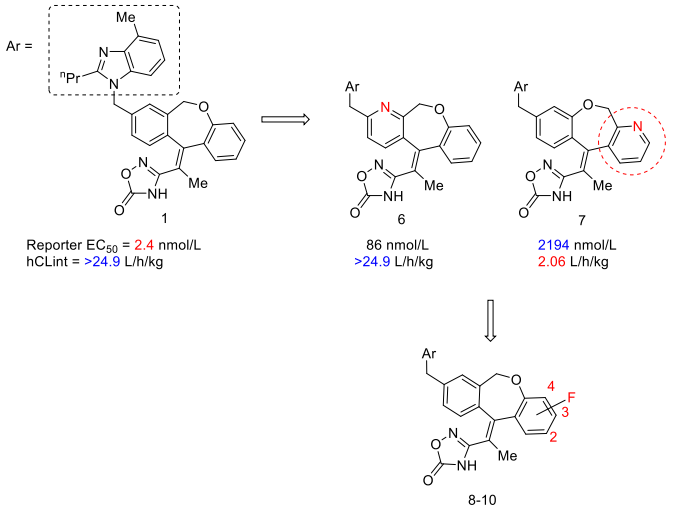


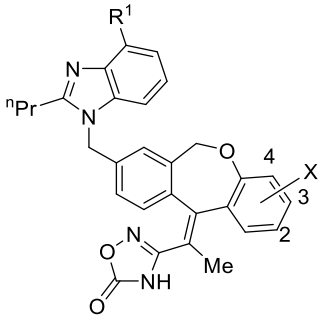
Figure 5. Improvement of the human metabolic stability of lead compound **1** by modification of the tricyclic core-structure.

Based on these results, we attempted to introduce a fluorine atom at the 1-, 2-, 3- or 4-position on the right benzene ring to block the oxidative metabolism, likely by CYPs. All of the derivatives were successfully synthesized except for the 1-fluoro derivative, the preparation of which failed due to regioselectivity in the construction reaction of the dibenzooxepine ring. All of these three fluoro-substituted compounds exerted potent activities both in the reporter gene assay and aggregation assay using MKN-45 cancer cells (Table 1). In the human liver microsome metabolism evaluation, only **9**, the 3-fluoro substituted derivative, was remarkably stable (3.2 L/h/kg) compared to the other derivatives **8** and **10** (>24.9 and 22.6 L/h/kg, respectively). These results suggested that the 3-position of dibenzooxepine was the main metabolic site. This sort of medicinal chemistry approach to preventing oxidative metabolism by introducing a fluorine on benzene ring has been previously reported.[24]

Next, we investigated the *in vivo* mouse pharmacokinetic profile of **9**. The 3-fluoro derivative **9** had a much longer half-life and better AUC than lead compound **1** in mice. Thus, we identified the dibenzooxepine derivative **9** with good pharmacokinetic profiles by the introduction of a fluorine atom at the 3-position.

However, further structure optimization was needed to achieve a better pharmacokinetic profile and reduce the pharmacologically effective dose. The acidic oxadiazolone moiety of **9** seemed to be essential for binding to PPARγ-LBD and exerting PPARγ agonistic activity as demonstrated in other PPARγ ligands. The tricyclic core structure was also deemed necessary in order to mimic the bioactive mode of lead compound **2**, as we previously reported. We therefore conducted further optimization of the benzimidazole moiety of **9** by referencing the crystal structure of our compound with PPARγ-LBD.

Table 1. 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		hCLint
			EC_{50}(nM)	Efficacy(%)	EC_{50}(nM)	Efficacy(%)	(L/h/kg)
1	Me	H	2.4	9.5±0.6	3.3	100	>24.9
8	H	2-F	12	6.9±1.5	7.0	102	>24.9
9	H	3-F	7.4	11.7±3.6	1.0	104	3.2
10	H	4-F	4.4	8.7±1.6	3.8	101	22.6

The efficacies and EC_{50} values of compounds **1** and **8**−**10** 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 **1** and **8**−**10** for 5 days. The aggregation efficacies were shown as the values relative to the maximum efficacy of **1**. 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).

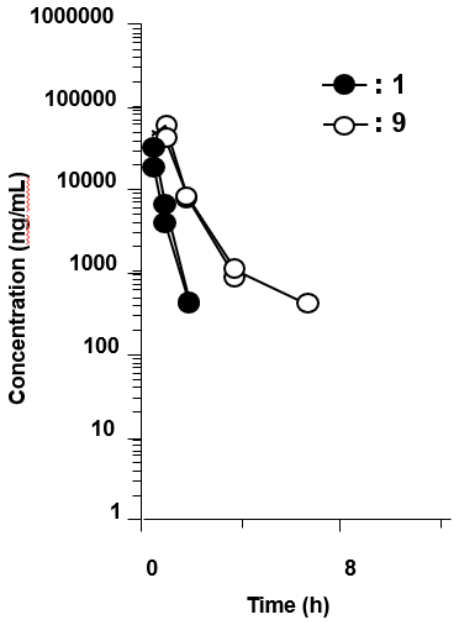


Figure 6. Plasma concentrations of **1** and 3-fluoro-dibenzooxepine derivative **9** in BALB/c mice orally administered (30 mg/kg, n = 2).

*2.2. Discovery of Imidazo[1,2-a]pyridine Derivative **17** and the Binding Mode of **17***

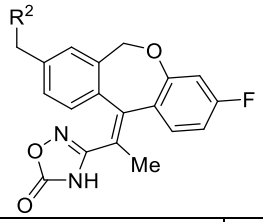
According to the X-ray crystal structure of **1** complex with PPARγ LBD,[23] the benzimidazole moiety of **1** was surrounded by hydrophobic residues of the canonical site, consisting of the residues of Helix 3 (H3) and Helix 5 (H5).[25] This suggested that a bulky and/or polar substituent on the benzimidazole ring seemed to be intolerant. The substitution of carbon for the hydrophilic nitrogen atom in benzene of benzimidazole (i.e. **11** and **12**) resulted in decreased PPARγ reporter activities (EC_{50} = 32 and 24 nM, respectively) compared to lead compound **1** (Table 2). We then attempted to convert the benzimidazole moiety to other hydrophobic structures. As expected, thieno[3,4-*d*]imidazole derivative **13** and imidazo[1,2-*a*]pyridine derivatives **14** showed very potent aggregation activity (EC_{50} = 3.0 and 5.3 nM, respectively) comparable to **9**. Although the mouse AUC of **13** was very low, the AUC of **14** was more than twice that of **9**. The discovery of **14**, which showed the desirable aggregation activity and pharmacokinetic profile, encouraged us to conduct further optimization of the imidazo[1,2-*a*]pyridine moiety.

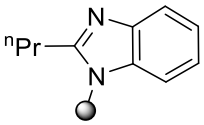
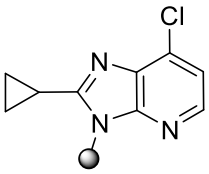
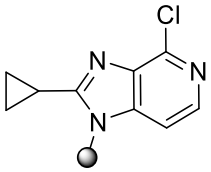
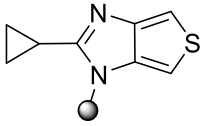
We next designed and synthesized imidazo[1,2-*a*]pyridine derivatives **15-21** (Table 2). Chlorine substituted derivatives **15**, **16** and **17** exerted quite potent MKN-45 aggregation activity, with EC_{50} values of 2.0, 1.4 and 2.5 nM, respectively. The 7-chloro imidazo[1,2-*a*]pyridine derivative **16** showed an excellent mouse PK profile (AUC = 10200 ng·h/mL) compared to **14** (AUC = 3750 ng·h/mL), but its metabolic stability was slightly lower than that of **17**. Although 7-fluoro imidazo[1,2-*a*]pyridine derivative **18** also had potent aggregation activity (EC_{50} = 1.7 nM) and good AUC (AUC = 5510 ng·h/mL) in mice, its human microsome stability was not sufficient (hCLint = 3.9 L/h/kg). Although the introduction of a methoxyethyl group at the 2-position on imidazo[1,2-*a*]pyridine led to potent aggregation EC_{50} values (**19**: 1.3 nM and **20**: 4.5 nM), the mouse PK profiles of these compounds were not sufficient (AUC = 2450 and 1086 ng·h/mL, respectively). In contrast, 8,9-dihydrofuro[3,2-*c*]imidazo[1,2-*a*]pyridine derivative **21** showed an excellent mouse AUC (8760 ng·h/mL); however, the aggregation activity (EC_{50} = 19 nM) was slightly weaker than that of **14** (EC_{50} = 5.3 nM).

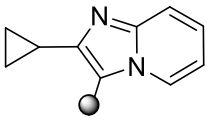
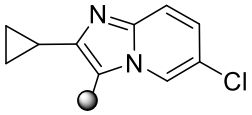
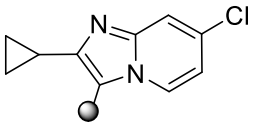
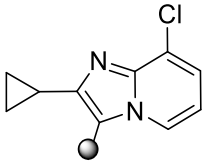
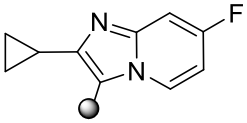
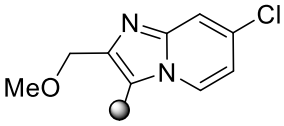
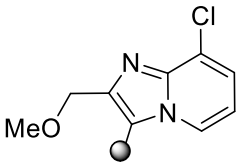
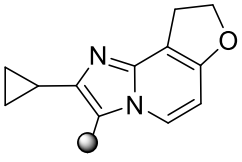
The optimization studies showed that compound **17** exhibited the most promising aggregation activity and PK profile. The binding mode of **17** to PPARγ LBD was next investigated. Figure 7A shows the X-ray analyzed binding mode of **17**, in which the acidic proton of oxadiazolone forms a hydrogen bond with the phenolic oxygen of Tyr473 and this interaction is essential for PPARγ activation.[26] The imidazo[1,2-*a*]pyridine moiety filled the canonical site and the right benzene moiety of the tricyclic core structure was accommodated in the small lipophilic pocket, surrounded by H3 and helix 11 (H11). Figure 7B shows the superimposition of the complex structure of **17** and that of **1**.[23] Even though the placement of atoms at the side aromatic ring differed, the structure of **17** overlapped well with that of **1**.

Therefore, we selected **17**, which had an excellent aggregation activity and PK profile and a unique binding mode, as the candidate for further *in vivo* evaluations.

Table 2. 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)	hCLint (L/h/kg)
		EC_{50} (nM)	Efficacy (%)	EC_{50} (nM)	Efficacy (%)		
9		7.4	11.7±3.6	1.0	104	1480	3.2
11		38	10.3±1.3	32	101	N.T.	2.3
12		59	13.7±2.4	22	105	N.T.	10
13		21	13.7±2.6	3.0	105	196	2.5

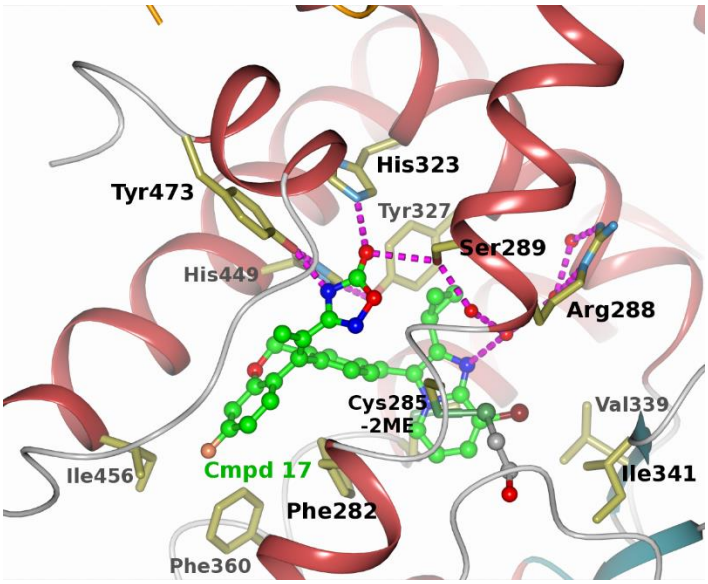
14		19	9.8±3.1	5.3	106	3750	2.4
15		11	15.1±1.5	2.0	102	1710	4.0
16		3.6	12.6±1.7	1.4	101	10200	6.1
17		4.2	10.6±0.4	2.5	103	6890	2.2
18		13	13.5±2.2	1.7	112	5510	3.9
19		5.3	9.0±1.2	1.0	106	2450	2.4
20		7.3	7.4±0.3	4.8	96	1086	1.3
21		23	9.7±0.7	19	110	8760	2.0

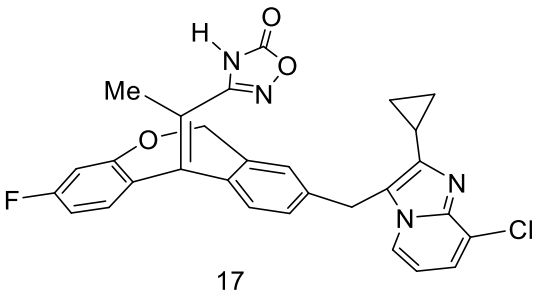
The efficacies and EC_{50} values of compounds **9** and **11**−**21** in human PPARγ/GAL4 transfected HEK293EBNA cells at 24 h after drug treatment. The efficacy of each compound was calculated as the percentage of the maximum activation obtained with pioglitazone at 1000 nM. EC_{50} values were determined 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 **11**−**21** for 5 days. The aggregation efficacies were shown as the values relative to the maximum efficacy of **1**. 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)





17

(B)

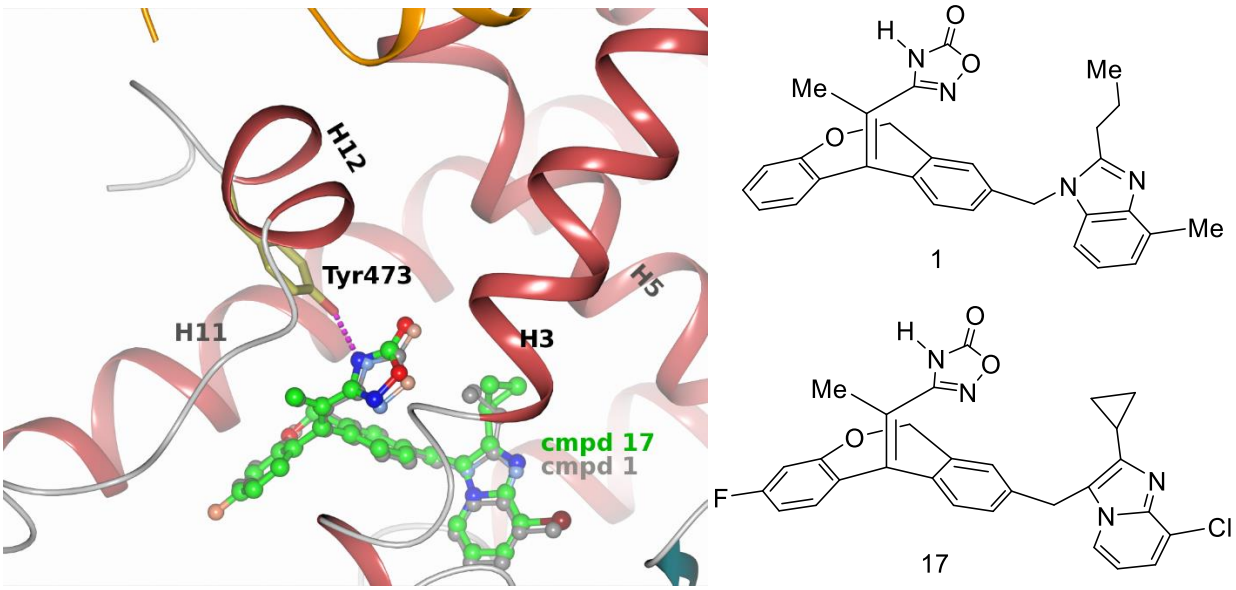


Figure 7. The X-ray crystal structures of ligands complexed with PPARγ(PDB: 6K0T). (A) The binding mode of **17** (ball and stick; green) to the PPARγ LBD. The proton of the oxadiazolone ring of **17** interacted with the phenolic oxygen of Tyr473. 2ME: 2-mercaptoethanol, which was used in the protein purification buffer and found in the ligand binding pocket as a covalent adduct to Cys285. (B) The superimposition of the complex structures of **17** (green) and **1** (gray, PDB: 6AD9).

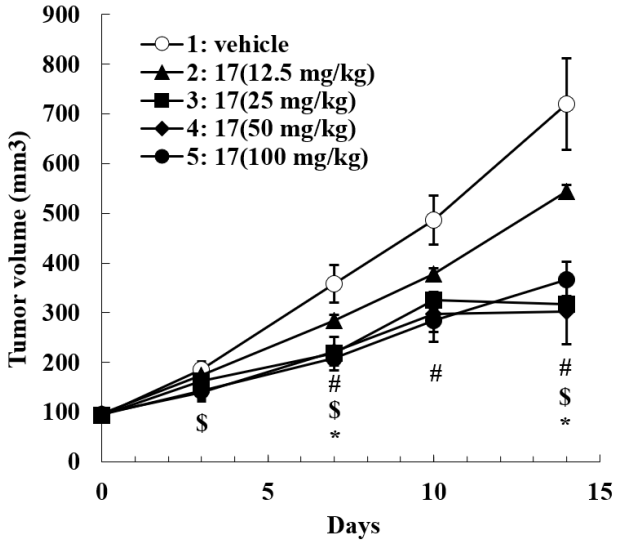
*2.3. Effect of **17** on an AsPC-1 xenograft mouse model*

We evaluated the *in vivo* antitumor activity of **17** using an AsPC-1 pancreatic cancer xenograft mouse model. Because MKN-45 is known as a poorly differentiated cancer cell line, its engraftment was not observed in SCID mice. The differentiation-inducing activity against AsPC-1 cells by PPARγ activation was previously reported in preclinical studies of efatutazone.[8] Compared to the vehicle control, the oral administration of **17** clearly reduced the tumor volume. After 10 days, the tumor growth was significantly reduced at more than 25 mg/kg (oral administration), the point at which the antitumor efficacy in this model was saturated. Therefore, the minimum effective dose was likely to be between 12.5 and 25 mg/kg in this model.

Next, we measured the mRNA expression of angiopoietin-like 4 (*angptl4*),[27] a PPARγ-regulated

downstream gene, and keratin 20 (*krt20*),[28] a cell-differentiation marker, of AsPC-1/AG1 tumor cells in the xenografts. Compound **17** was orally administered at 100 mg/kg twice a day for 14 days, and at 4 h after the last dose, the quantitative polymerase chain reaction (qPCR) results of the **17**-treated tumor showed that this therapy markedly increased the expression of *angptl4* and *krt20* compared to vehicle control. These data suggested that the tumor differentiation-inducing activity of the **17** *in vivo* model was due to its PPARγ activation potency.

(A)



(B)

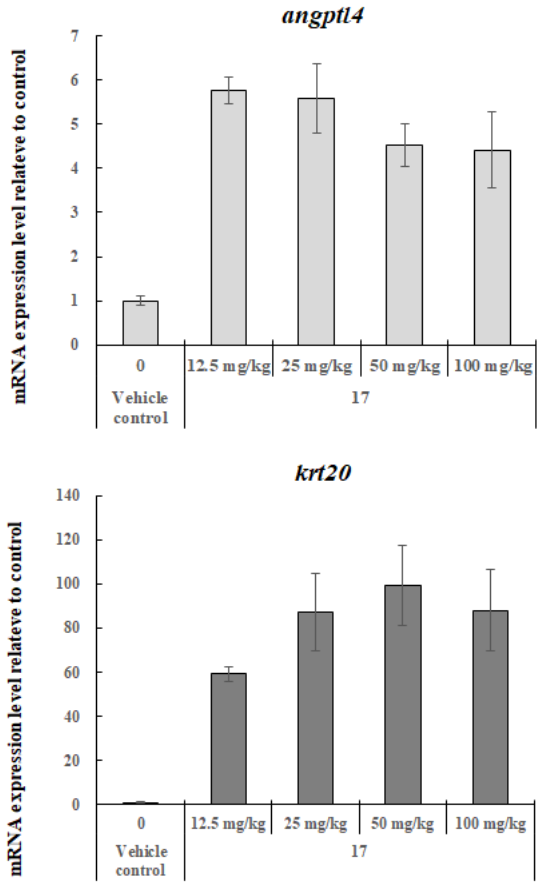


Figure 8. Antitumor effect of **17** on AsPC-1/AG1 xenotransplanted mice. (A) Tumor growth inhibition after the administration of **17** in the xenotransplanted mice. Each plot represents the mean ± SE of the relative tumor volume (n = 5). #: $p<0.05$ between the vehicle-treated group and the **17** 25 mg/kg-treated group. $: $p<0.05$ between the vehicle-treated group and the **17** 50 mg/kg-treated group. *: $p<0.05$ between the vehicle-treated group and the **17** 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 17 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.

2.4. Effect on fluid retention in CD-1 mice

In preclinical and clinical studies, fluid retention due to the administration of PPARγ ligands has been reported.[29,30] This adverse effect is likely to be caused by the TZD structure.[20,22] We therefore investigated whether or not dibenzooxepine derivative **17**, which has a non-TZD structure, caused fluid retention.

To assess fluid retention, the hematocrit values of healthy CD-1 mice treated by **17** were measured. At 50 mg/kg of oral administration of **17**, which was determined to be the effective dose in AsPC-1 xenograft mouse experiments, the hematocrit value was slightly decreased (**17**, 42.9%; vehicle, 44.1%). At 200 mg/kg of oral administration, which was 8 times higher than the lower effective dose, the decrease in the hematocrit value was still only slight (42.0%). There were no significant differences between the vehicle and **17**-treated groups (50 and 200 mg/kg). Compared to the fluid retention induced by pioglitazone administration in mice,[29] that of **17** was considered to be quite moderate.

Thus, severe fluid retention was successfully avoided by lead optimization from the novel scaffold of dibenzooxepine without a TZD moiety.

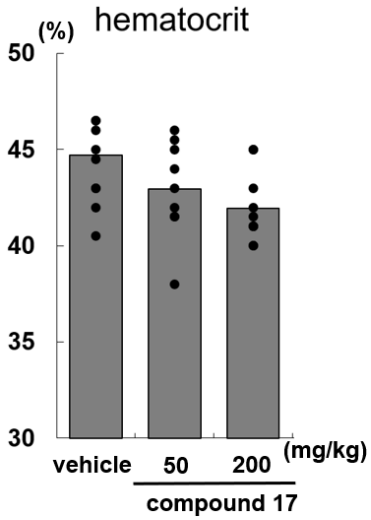


Figure 9. Hematocrit values in CD-1 mice treated orally with **17** twice a daily at 50 and 200 mg/kg. $p = 0.671$ between the vehicle-treated group and the **17** 50 mg/kg-treated group. $p = 0.176$ between the vehicle-treated group and the **17** 200 mg/kg-treated group.

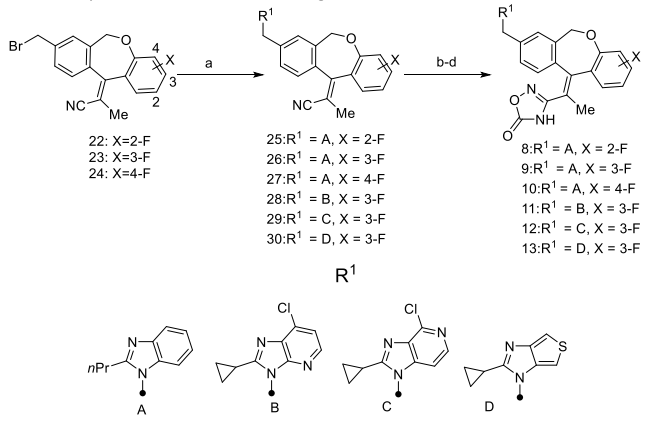
2.5. Chemistry

Compounds **8**-**13** were synthesized from 8-(bromomethyl)-dibenzo[*b*,*e*]oxepin intermediates **22-24** (supporting information) as summarized in Scheme 1. Treatment of imidazole compounds with K_2CO_3 at room temperature, followed by addition of **22-24**, afforded **25**-**30**. The addition of hydroxylamine to the cyano group of **25**-**30**, followed by a ring-closure reaction using ethyl chloroformate and *tert*-BuOK afforded the target oxadiazolone compounds **8**-**13**.

Synthesis of imidazo[1,2-*a*]pyridine derivatives **14-18** were referred to the synthesis of Cu-catalyzed three component coupling reaction, which was reported as one-pot synthesis of alipidem as shown in Scheme 2.[31] The reaction in the presence of $Cu(OTf)_2$, 2-aminopyridine, aldehyde and 8-ethynyl-3-fluorodibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile **31** (supporting information), produced **32**-**36**. Key intermediate **31** was the mixture of *E/Z* isomers, and accordingly this coupling reaction provided an *E/Z* mixture, which was separated by silica column chromatography to afford **32**-**36** as (*E*)-isomers. Target oxadiazolones **14**-**18** were prepared by the cyclization reaction described above.

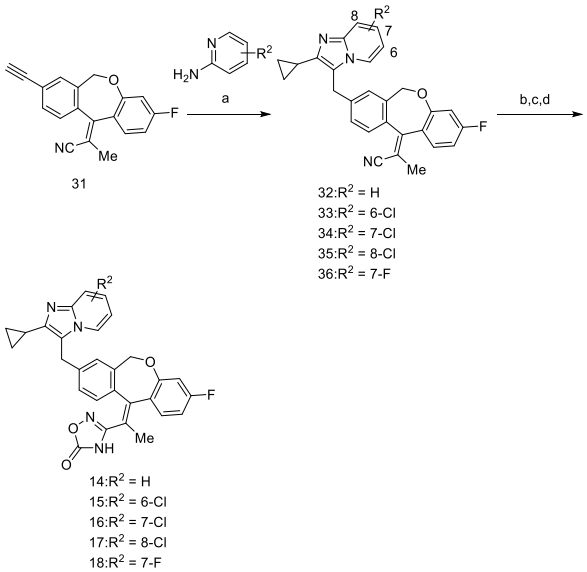
The introduction of an imidazo[1,2-*a*]pyridine ring using (*E*)-2-(3-fluoro-8-formyldibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)propanenitrile **37** (supporting information) is shown in Scheme 3. A coupling reaction with Grignard reagents and iodoaryl intermediates gave hydroxybenzyl compounds, which were reduced with silyl reduction reagents to generate **38**-**40**. The synthesis of oxadiazolone using **38**-**40** provided target compounds **19**-**21**.

Scheme 1. Synthesis of benzimidazole analogues [a]



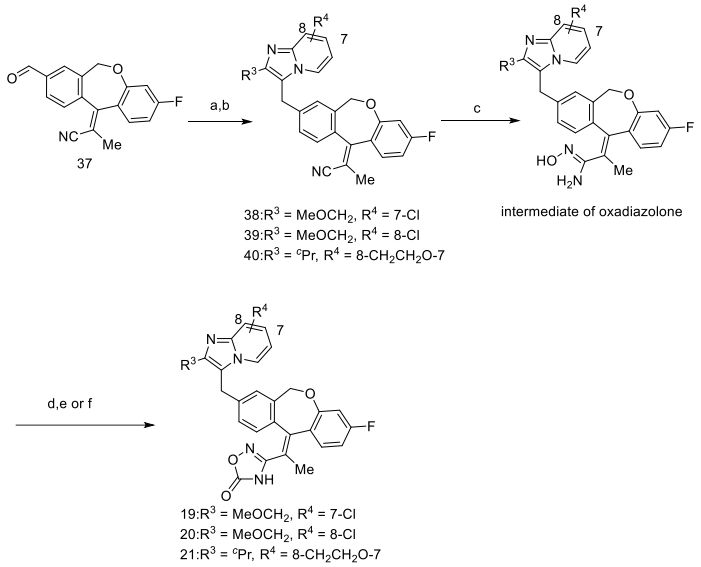
[a]Reagents: a) R^1H, K_2CO_3, DMF; b) 50% H_2NOH aq., EtOH, reflux; c) $ClCO_2Et$, Et_3N, CH_2Cl_2; d) *tert*-BuOK, toluene, THF.

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



[a]Reagents: a) ^cPrCHO, CuCl, $Cu(OTf)_2$, toluene, 120 ^oC; b) 50% H_2NOH aq., EtOH, reflux; c) $ClCO_2Et$, Et_3N, CH_2Cl_2; d) *tert*-BuOK, toluene, THF.

Scheme 3 Synthesis of the side chain structure from 8-formyldihydrodibenzooxepine [a]



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

3. Conclusion

Based on the results of the metabolite analysis of **1** and modification of both benzene rings of the core structure, we designed fluorine-substituted derivatives. 3-fluoro dibenzooxepine derivative **9** was metabolically stable, and accordingly exerted better PK than **1** in mice. The further optimization of the benzimidazole moiety led to imidazo[1,2-*a*]pyridine **17**, which not only showed better mouse PK than **1**, but also maintained potent MKN-45 aggregation activity, probably derived from the unique binding mode to PPARγ. Furthermore, compound **17** exerted tumor growth inhibition in AsPC-1/AG1 pancreatic cancer xenografted mice. Although TZD derivatives have been concerned severe fluid retention, the hematocrit value of **17** was only slightly decreased, even when administered at a much higher dose than its effective dose. This result suggested that treatment with **17** would not induce the severe fluid retention.

In conclusion, we successfully developed potential anticancer agent **17** with promising properties. Further research using this compound for clinical studies will be expected to offer novel options for cancer therapy.

4. EXPERIMENTAL SECTION

4.1 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). Microwave reaction was conducted using Biotage Initiator 2.5.

Chemical shifts in ^{1}H NMR spectra were reported in δ values (ppm) relative to tetramethylsilane. 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).

4.2. Chemistry

(*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 (8). To a stirred solution of **25** (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 **8** (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 (9). To a stirred solution of **26** (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 **9** (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 (10). To a stirred solution of **27** (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 **10** (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 (11). To a stirred solution of **28** (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 **11** (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 (12). To a stirred solution of **29** (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 **12** (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 (13). To a stirred solution of **30** (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 **13** (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 (14). To a stirred solution of **32** (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 **14** (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 (15). To a stirred solution of **33** (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 **15** (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 (16). To a stirred solution of **34** (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 **16** (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 (17). To a stirred solution of **35** (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 **17** (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 (18). To a stirred solution of **36** (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 **18** (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 (19). To a stirred solution of **39** (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 **19** (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 (20). To a stirred solution of **40** (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 **20** (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 (21). To a stirred solution of **41** (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 **21** (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 (25). To a stirred solution of **22** (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 **25** (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 (26). To a stirred solution of **23** (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 **26** (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 (27). To a stirred solution of **24** (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 **27** (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 (28). To a stirred solution of **23** (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 **28** (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 (29). To a stirred solution of **23** (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 **29** (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 (30). 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 **23** (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 **30** (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 (32). 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 **31** (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 **32** (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 (33). 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 **31** (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 **33** (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 (34). 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 **31** (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 **34** (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 (35). 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 **31** (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 **35** (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 (36). 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 **31** (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 **36** (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 (38). 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 **37** (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 **38** (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 (39). 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 **37** (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 **39** (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 (40). 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 **37** (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 **40** (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) 478.

4.3. Biological Methods.

4.3.1 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 construct into HEK293EBNA cells, cells were seeded at 1x 10^5 cells/ml in a 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 set at 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.

4.3.2. MKN45 cell aggregation assay.

MKN45 cells were aggregated when the 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 °C 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 Hoechst 33342 (SIGMA), and the area of nuclei was calculated. An area exceeding 1,015.58 μm^2 was defined as an aggregated cell cluster. The ratio of the area of the aggregated cell clusters to the total cell area was calculated and set as the formation rate of aggregation. The cell aggregation-inducing activity was normalized to that of **1**, with the maximum activity of **1** set as 100%. The maximum activity of each compound was then calculated as the percentage of the maximum activity of **1**. EC_{50} values were determined as the concentration achieving 50% of the maximum activity using the XLFit.

4.3.3 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 Hakko Kirin.

4.3.4 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.

4.3.5 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.

4.3.6. Xenograft Study.

AsPC-1/AG1 cells (3×10^7) suspended in 0.1mL PBS were inoculated subcutaneously into SCID mice. **17** 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 **17** (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 **17** (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.

4.3.7. 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).

4.3.8. 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 **17** 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.

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Notes

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