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Discovery of 6-[5-(4-fluorophenyl)-3-methyl-pyrazol-4-yl]-benzoxazin-3-one derivatives as novel selective nonsteroidal mineralocorticoid receptor antagonists

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ABSTRACT

In the course of our study on selective nonsteroidal mineralocorticoid receptor (MR) antagonists, a series of novel benzoxazine derivatives possessing an azole ring as the core scaffold was designed for the purpose of attenuating the partial agonistic activity of the previously reported dihydropyrrol-2-one derivatives. Screening of alternative azole rings identified 1,3-dimethyl pyrazole **6a** as a lead compound with reduced partial agonistic activity. Subsequent replacement of the 1-methyl group of the pyrazole ring with larger lipophilic side chains or polar side chains targeting Arg817 and Gln776 increased MR binding activity while maintaining the agonistic response at the lower level. Among these compounds, 6-[1-(2,2-difluoro-3-hydroxypropyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (**37a**) showed highly potent in vitro activity, high selectivity versus other steroid hormone receptors, and good pharmacokinetic profiles. Oral administration of **37a** in deoxycorticosterone acetate-salt hypertensive rats showed a significant blood pressure-lowering effect with no signs of antiandrogenic effects.

1. Introduction

The mineralocorticoid receptor (MR) is a ligand-activated transcription factor that belongs to the steroid receptor subfamily within the nuclear receptor superfamily and shares high structural homology with the androgen receptor (AR), progesterone receptor (PR), and glucocorticoid receptor (GR). The classically perceived role of MR has been one of blood pressure control through regulation of body fluid and electrolyte balance by binding with aldosterone, a primary endogenous mineralocorticoid, in the kidney.[1] Recently, it was revealed that MR is expressed in various tissues, including those of the heart, brain, and blood vessels, and abnormal activation of the MR by excessive levels of aldosterone is closely associated with development of congestive heart failure and renal dysfunction as well as hypertension.[2,3] Thus, MR blockade has been shown to be a promising therapeutic option for such cardiovascular diseases. It should be noted that the marketed steroidal MR antagonists, spironolactone and eplerenone, have proven their clinical utility for those diseases.[4] Clinical use of these drugs, however, is limited due to the following issues as well as hyperkalemia. Spironolactone is a potent antagonist, but its low selectivity with respect to AR and PR causes sexual hormone-related side effects, such as impotence, gynecomastia, and menstrual irregularity.[5] On the other hand, eplerenone shows improved selectivity in mitigating these side effects but is less potent than spironolactone.[6] Accordingly, new, potent, and selective MR antagonists have been expected to be a clinically useful drugs. In recent years, there has been a keen interest in the discovery of such compounds.[7]

We have previously reported the benzoxazin-3-one derivatives, **1** and **2**, as novel nonsteroidal MR antagonists (Fig. 1).[8,9] Although compound **1** showed potent MR binding activity (IC_{50} = 41 nM), it still had moderate PR binding activity (PR binding IC_{50} = 1900 nM). On the other hand, compound **2** showed potent MR binding activity (IC_{50} = 43 nM) with improved selectivity for PR (PR binding IC_{50} >10,000 nM). However, in a functional assay using a reporter gene,

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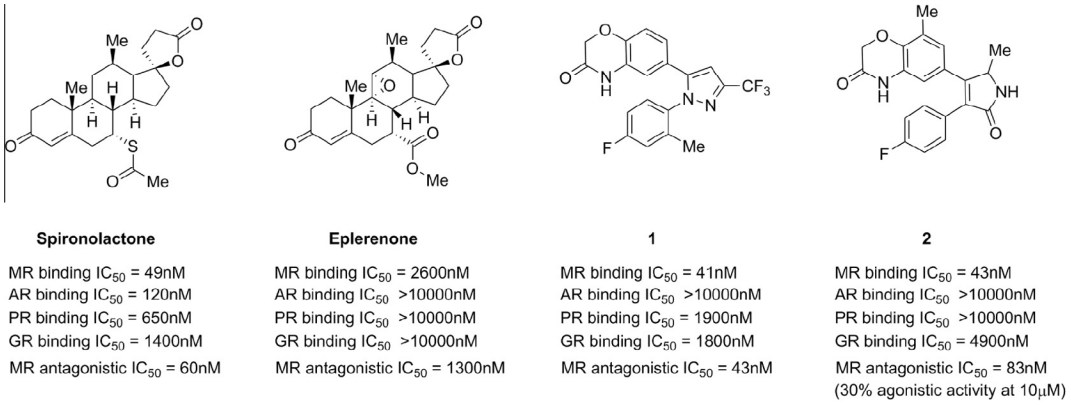


Figure 1. Structures of steroidal and nonsteroidal MR antagonists.

compound **2** showed not only potent MR antagonistic activity (IC_{50} = 83 nM) but also moderate partial agonistic activity at a high concentration (30% activation at 10 μM).[10] It has been reported that strong partial agonistic activity attenuates antimineralocorticoid action in animal models.[11] In addition, the influence of the partial agonistic activity on clinical efficacy and safety remains unclear. Therefore, we decided to identify new compounds with reduced partial agonistic activity. Herein, we describe our efforts to discover compounds with potent binding and attenuated partial agonistic activity toward MR, and high selectivities versus other steroid receptors.

Figure 2 illustrates our compound design. From our previous research on benzoxazin-3-one derivatives, we found that a key pharmacophore for high MR binding affinity consisted of three parts: a benzoxazin-3-one ring, a 4-fluorobenzene ring, and a central cyclic moiety linking these two aromatic rings at the adjacent position. Among these parts, we have generally found that the central ring is the most tolerant to modification without loss of binding activity. To establish a simple structure–partial agonistic activity relationship, we evaluated the functional activities of the related compounds of **1** and **2**. Consequently, most of the dihydropyrrol-2-one derivatives, exemplified by compound **2** showed moderate partial agonistic activity at high concentrations (approximately 20–30% at 10 μM), whereas the pyrazole derivatives, exemplified by compound **1**, showed low agonistic activities. These results suggested that the partial agonistic activity tended to depend on the structure of the central ring. Accordingly, selection of an appropriate central ring followed by overall optimization was expected to produce a compound with potent activity, high selectivity, and a reduction in partial agonistic activity.

Binding mode analyses of compounds **1** and **2** guided our central ring selection. The X-ray co-crystal structure of MR with a dihydrofuran-2-one derivative, a close analog of compound **2** (PDB code: 3WFG),[9] suggested that the dihydropyrrol-2-one derivatives form a water-mediated hydrogen bonding network involving the carbonyl group of the central ring with Arg817 and Gln776. On the other hand, compound **1** and its related derivatives were considered to have no such network. Therefore, it was assumed that the hydrogen bonding network has a key role in the partial agonistic activity and the carbonyl group serves as a 'trigger' moiety. Indeed, several carbonyl-containing dihydrofuran-2-one derivatives also showed partial agonistic activity, although they were less likely to show agonistic activity than the dihydropyrrol-2-one derivatives. This different propensity for agonistic activity might be explained by the weaker hydrogen bonding ability of the carbonyl group. Thus, we selected azole rings for the central ring to avoid formation of such water-mediated hydrogen bonding networks.

As shown in Figure 2, novel azole derivatives (A) were designed. Although positions Y and Z of the azole ring were thought to allow introduction of substituents, the azole derivatives possessing substituents at position Y (exemplified by compound **1**) were unlikely to achieve potent activities and high selectivity simultaneously. In addition, increasing the polarity of position Y appeared to strongly improve selectivity (particularly over PR), based on our earlier findings for compound **2** showing that the PR binding affinity was greatly reduced by polar moieties at the corresponding position. Therefore, we focused on azole derivatives bearing substituents at position Z with unsubstituted heteroatoms (O, N, and NH) for position Y in this study. Both nonpolar and polar side chains

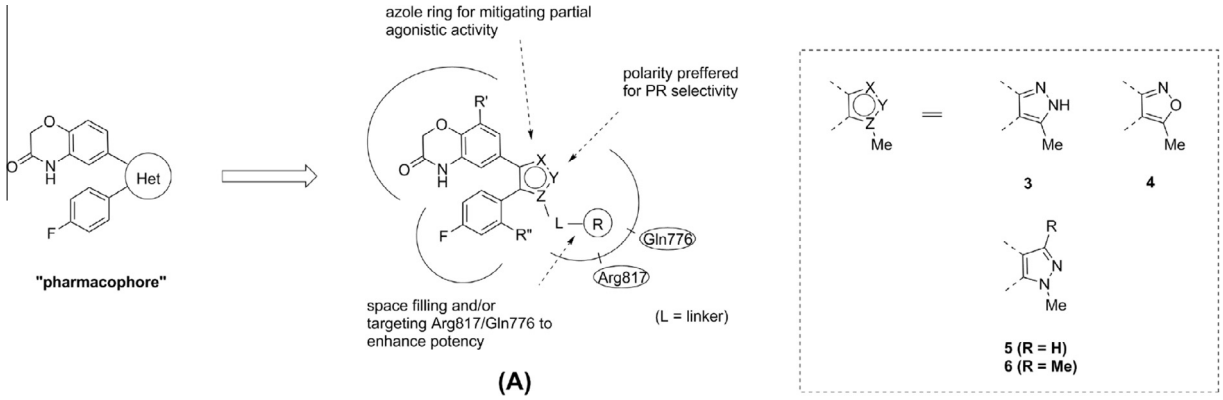
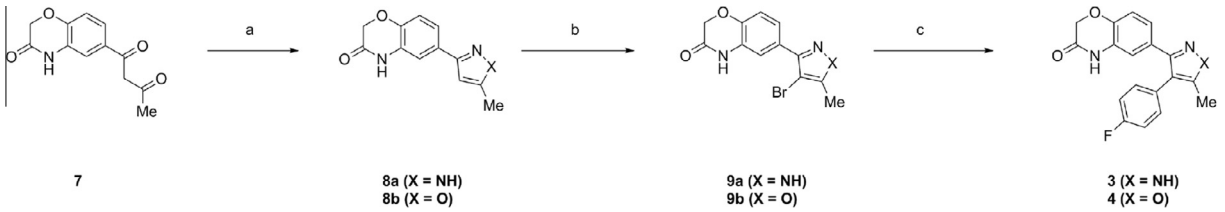


Figure 2. Compound design of novel azole-based MR antagonists.



Scheme 1. Synthesis of compound **3** and **4**. Reagents and conditions: (a) hydrazine or hydroxylamine, 2-propanol, 77% (**8a**) and 95% (**8b**); (b) NBS, DMF, 94% (**9a**) and 98% (**9b**); (c) 4-fluorophenylboronic acid, $Pd(P^tBu_3)_2$, K_3PO_4, 1,4-dioxane, 54% (**3**) and 74% (**4**).

were investigated to assess the impact of hydrophobic or hydrogen bonding interactions with Arg817 and Gln776, respectively. We planned to select a lead scaffold by evaluation of the in vitro potency, selectivity, and partial agonistic activity of compounds **3**–**6** bearing a methyl group at position Z and then optimize the methyl group.

2. Chemistry

The synthesis of 5-methyl pyrazole **3** and 5-methyl isoxazole **4** is described in Scheme 1. Acid-mediated condensation of diketone **7** with hydrazine or hydroxylamine afforded pyrazole **8a** or isoxazole **8b**. Bromination of **8a**,**b** by *N*-bromosuccinimide (NBS) followed by Suzuki coupling reaction with 4-fluorophenylboronic acid gave compounds **3** and **4**, respectively.

Scheme 2 depicts the synthesis of compounds **5**, **6a**–**c**, and **16a**–**j**. Pyrazole ring formation by acid-mediated condensation of ketones **11** and **12** with hydrazines afforded the 1-unsubstituted pyrazole **13b** and the 1-substituted pyrazoles **13a**, **13c**–**o**. Bromination followed by Suzuki coupling reaction with boronic acids **15a**,**b** gave the compounds **5**, **6a**–**c** and **16a**–**f**, **16h**–**j**. Hydrogenolysis of the benzyl ether of **16f** yielded the alcohol **16g**.

Compounds **24a**–**c** were synthesized as shown in Scheme 3. Bromination of **13b** by NBS gave the 1-unsubstituted pyrazole **22**. Subsequent Mitsunobu reaction of **22** with alcohols gave the 1-substituted pyrazoles **23a**–**c** as a mixture of regioisomers. The structure of these regioisomers was confirmed by NMR analysis, and the undesired regioisomer was removed by careful silica gel column chromatography. Suzuki coupling reaction of **23a**–**c** with boronic acid **15a** afforded the pyrazoles **24a**–**c**.

Compounds **27a**,**b** and **30** were synthesized according to Scheme 4. The hydroxyl group of compound **13j** was converted to the azido group, and reduction of the azido group by triphenyl phosphine followed by acetylation and bromination afforded the amide **26a**. Sulfonamide **26b** was synthesized in a similar manner to **26a**. Suzuki coupling reaction of **26a**,**b** with **15a** gave the pyrazoles **27a**,**b**. Bromination of **13j** by NBS, followed by Mitsunobu reaction with acetone cyanohydrin gave the cyanoethyl compound **29**. Suzuki coupling reaction of **29** with **15a** afforded compound **30**.

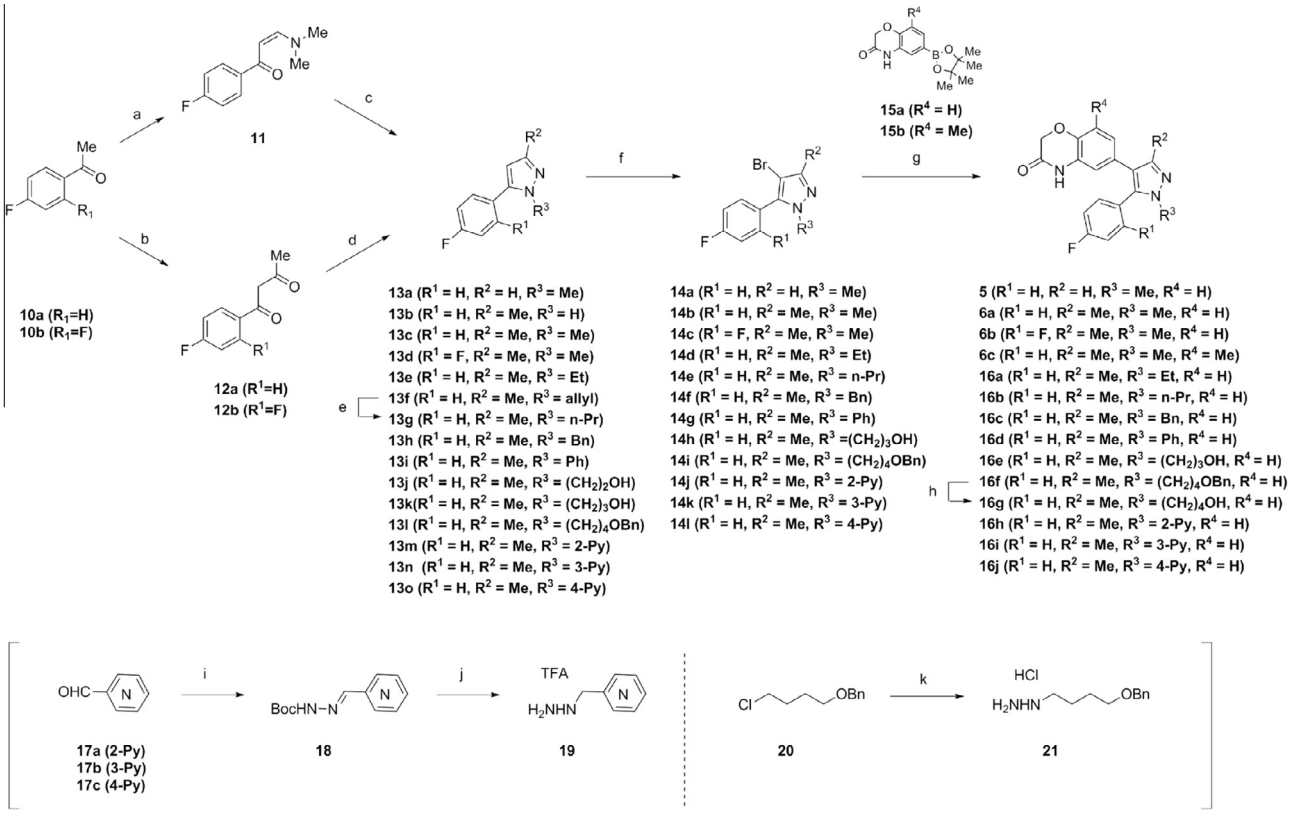
Compounds **36a**, **37a**,**b** were synthesized as shown in Scheme 5. Epoxides **31a**–**c** were converted to the substituted hydrazines, and subsequent acid-mediated pyrazole ring formation afforded compounds **32a**–**c**. Compounds **32a**–**c** were brominated by NBS, and then the hydroxyl groups were oxidized to give the ketones **34a**–**c**. Difluorination of the carbonyl group by using diethylamino sulfur trifluoride (DAST) followed by the Suzuki coupling reaction afforded the difluorides **36a**–**c**. Hydrogenolysis of the benzyl ether of **36b**,**c** yielded the corresponding alcohols **37a**,**b**.

Compounds **41**, **43** were synthesized as illustrated in Scheme 6. Compound **14h** was oxidized by using a pyridine sulfur trioxide complex to give aldehyde **38**. Reaction of **38** with methyl magnesium bromide followed by Dess–Martin oxidation gave the methyl ketone **39**. Difluorination of the carbonyl group of **39** followed by Suzuki coupling reaction with **15a** afforded the fluorinated compound **41**. Methyl ketone **39** was converted to silyl enol ether by reaction with silyl triflate. The resulting silyl enol ether reacted with lead acetate(IV) in the presence of potassium carbonate to give the 1-acetoxymethyl ketone.[12] Difluorination of the 1-acetoxymethyl ketone and the subsequent Suzuki coupling reaction with **15a** yielded difluoride **43**.

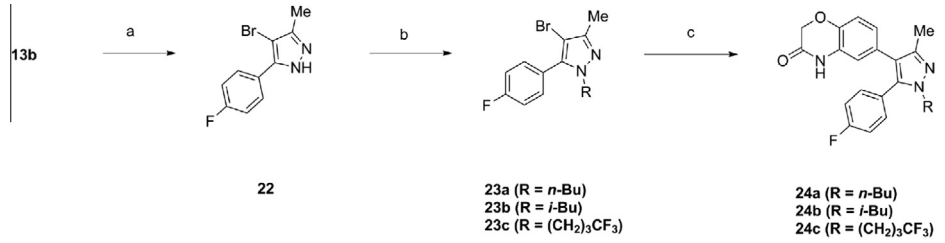
3. Results and discussion

The synthesized compounds were evaluated for their inhibitory activity against the binding of [^{3}H]-aldosterone to MR. The selectivity over the other steroid hormone receptors was determined by radioligand binding assays (AR: [^{3}H]-testosterone; PR: [^{3}H]-progesterone; and GR: [^{3}H]-dexamethasone). MR antagonistic activity and partial agonistic activity were evaluated in reporter gene assays using COS-1 cells by measurement of the inhibitory activity for MR activation induced by aldosterone or the relative transcription activity to the activity of aldosterone, respectively. The results are presented in Tables 1–3 as IC_{50} values for binding activity and antagonistic activity or relative percentage values (%) for agonistic activity.

First, with the aim of identifying a new lead scaffold, a series of azole derivatives bearing a methyl group at position Z were investigated (Table 1). Of the three initial cores prepared (**3**, **4**, **5**), the 1-methyl pyrazole **5** demonstrated the greatest binding activity (IC_{50} = 290 nM) and was selected for further modification. The different binding activities among these cores could be explained by the preference for hydrophobic functionality at position X, which is surrounded by lipophilic amino acid residues. Introduction of additional methyl group into position X of pyrazole **5** increased the binding activity (**6a**, IC_{50} = 110 nM). Importantly, dimethyl compound **6a** showed good antagonistic activity (IC_{50} = 160 nM) and high steroid receptor selectivity (particularly over AR and PR) as well as greatly reduced partial agonistic activity (3% activation at 10 μM). On the basis of these results, a 1,3-dimethylpyrazole ring was selected as a lead scaffold. Subsequently, a fluorine atom and a methyl group were introduced at the 2-position in the 4-fluorobenzene ring and the 5-position in the benzoxazin-3-one ring, respectively (**6b**, **6c**). Although these modifications enhanced the binding affinities in our previous research, there was no significant increase in the potency of the 1,3-dimethylpyrazole compounds. We speculated that the 1-methyl group on the pyrazole ring stabilized the preferred twisted conformation between the 4-fluorobenzene ring and central ring; consequently, an additional effect caused by the fluorine atoms was not observed, and the unfilled space around the 5-position of the benzoxazin-3-one ring was slightly smaller than those of compounds **1** and **2**. Compound **6a** was the best among these compounds and was selected as a lead compound for further optimization. It should be noted that all compounds listed in Table 1 showed significantly weaker affinities for AR and PR and reduced partial agonistic activities as designed, which encouraged us to advance our synthetic study to the next process.

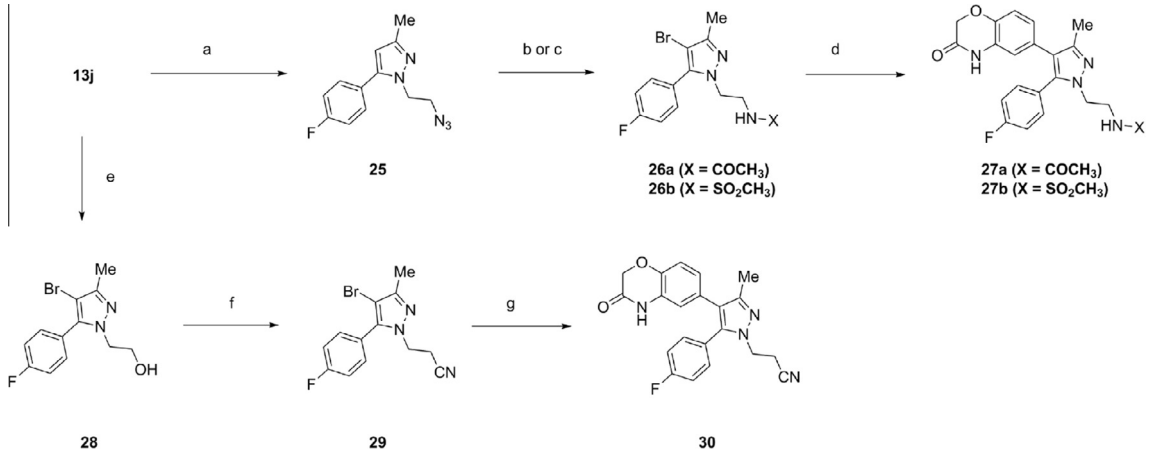


Scheme 2. Synthesis of 1-substituted pyrazole derivatives **5**, **6a–c**, and **16a–i**. Reagents and conditions: (a) DMFDMA, 32%; (b) NaH, EtOAc, THF, 74%; (c) methylhydrazine, EtOH, 81%; (d) R-NHNH$_2$, TFA, TEA, 2-PrOH, 19–97%; (e) H_2, Pd/C, MeOH; (f) NBS, CH_3CN, or DMF, 30–99%; (g) boronic acid **15a** or **15b**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 5–52%; (h) H_2, Pd/C, EtOH, 88%; (i) *tert*-butyl carbazate, EtOH, 55–90%; (j) (1) $NaBH_3CN$, AcOH, (2) TFA; (k) (1) hydrazine hydrate, NaOH, (2) HCl, 83%.

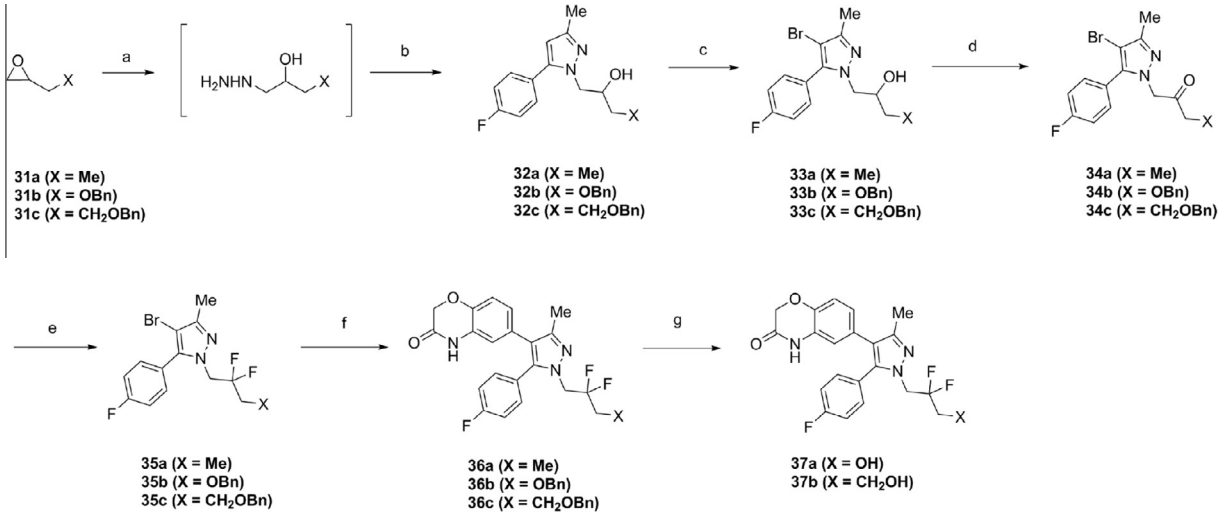


Scheme 3. Synthesis of **24a–c**. Reagents and conditions: (a) NBS, DMF, 98%; (b) R-OH, PPh_3, diisopropyl azodicarboxylate, THF, 11–30%; (c) boronic acid **15a**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 5–43%.

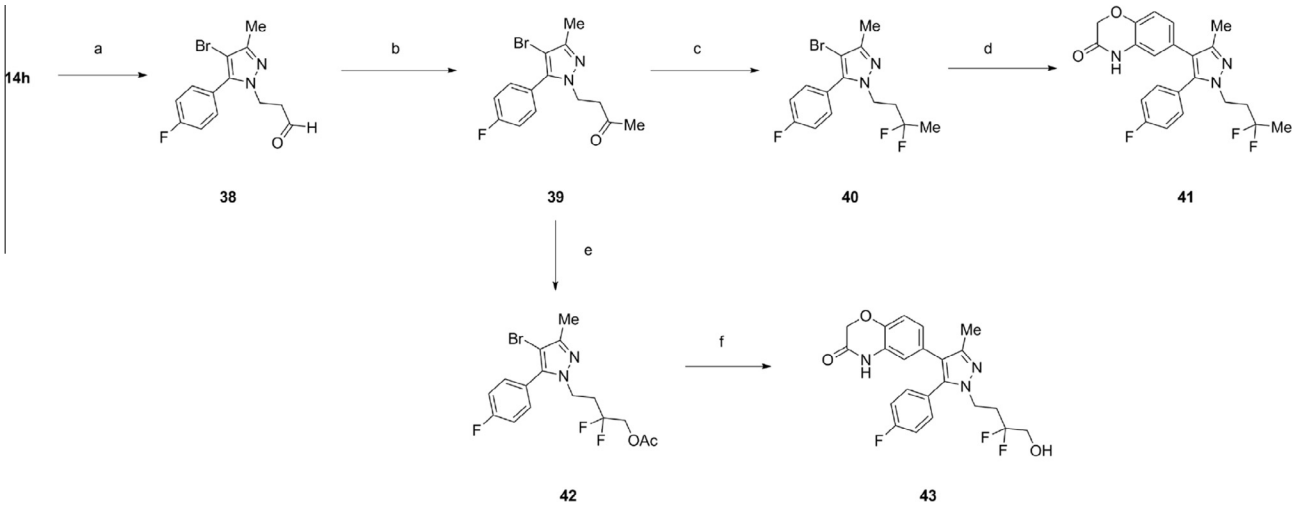
In the second step of our optimization studies, the 1-methyl group of compound **6a** was changed to various nonpolar or polar side chains to increase the binding activity. First, larger lipophilic side chains were investigated in anticipation of the hydrophobic contact (Table 2). Although the ethyl compound **16a** showed decreased binding activity, the *n*-propyl and *n*-butyl compounds showed increased activity (**16b**, **24a**). Particularly, the *n*-butyl compound **24a** had 5-fold more potent activity (IC_{50} = 22 nM) than the methyl compound **6a**. Furthermore, isobutyl compound **24b** possessing a branched-type side chain also showed potent binding activity. These results indicated that there is some space to allow the introduction of various sizes of substituents at the 1-postion of the pyrazole ring. To occupy the space more tightly, benzene ring and fluorine atoms were introduced onto the side chains. Notably, a bulky benzyl group was tolerated, and the compound **16c** showed potent binding activity (IC_{50} = 100 nM). On the other hand, the phenyl compound **16d** had greatly reduced affinity, most likely because of the steric repulsion against the wall of MR. These results suggested that at least more than one methylene linker was needed for incorporation of bulky substituents. Next, fluorine atoms were introduced into the *n*-butyl group of compound **24a**, the most potent compound at this point. These fluorinated compounds **24c**, **41**, and **36a** showed further increased activities. Among them, compounds **24c** and **36a** showed remarkably potent single-digit nanomolar binding activity (**24c**: IC_{50} = 8.4 nM, **36a**: IC_{50} = 5.8 nM). Thus, replacement of the 1-methyl group with a bulkier lipophilic substituent successfully increased the binding activity and, importantly, maintained the partial agonistic activities at the lower levels. Meanwhile, these compounds also showed increased binding activity for the other steroid receptors. In addition, they tended to have high metabolic clearance in rat microsomes (>100 μL/mg/min). We speculated that this metabolic



Scheme 4. Synthesis of **27a**,**b** and **30**. Reagents and conditions: (a) (1) TsCl, pyridine; (2) NaN_3, DMF, 83% in 2 steps; (b) (1) PPh_3, THF, H_2O; (2) Ac_2O, pyridine; (3) NBS, DMF, 94% in three steps; (c) (1) NBS, DMF; (2) PPh_3, THF, H_2O; (3) MsCl, pyridine, 82% in 3 steps; (d) boronic acid **15a**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 44% (**27a**) and 33% (**27b**); (e) NBS, DMF, 69%; (f) acetone cyanohydrin, ADDP, PBu_3, toluene, 76%; (g) boronic acid **15a**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 42%.

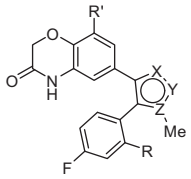


Scheme 5. Synthesis of **36a**, **37a**,**b**, Reagents and conditions: (a) hydrazine hydrate, (b) **12a**, HCl, MeOH, 47–66% in 2 steps; (c) NBS, DMF, 4% in three steps (**31a**), 98% (**33b**) and 98% (**33c**); (d) Dess–Martin periodinane, toluene, 93–99%; (e) DAST, toluene, 39–71%; (f) boronic acid **15a**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 40–61%; (g) H_2, Pd/C, 88% (**37a**), and 70% (**37b**).



Scheme 6. Synthesis of compound **41** and **43**. Reagents and conditions: (a) $SO_3\cdot$Py, DMSO, TEA, 70%; (b) (1) MeMgBr, THF; (2) Dess–Martin periodinane, toluene, 77% in two steps; (c) DAST, toluene, 28%; (d) boronic acid **15a**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 30%; (e) (1) TBSOTf, TEA, toluene; (2) $Pb(OAc)_4$, K_2CO_3, toluene; (3) TBAF, THF; (4) DAST, toluene, 10% in four steps; (f) boronic acid **15a**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 34%.

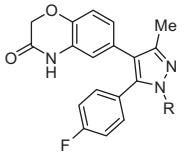
Table 1
Exploration of a central ring as a new template[a]



Compound No.	Azole core	R	R′	MR binding IC_{50} (nM)	Selectivity			MR antagonistic activity IC_{50} (nM) (agonistic activity)[b]
					AR binding IC_{50} (nM)	PR binding IC_{50} (nM)	GR binding IC_{50} (nM)	
3		H	H	3700	>10,000	1600	>10,000	—
4		H	H	710	>10,000	>10,000	>10,000	1400 (3%)
5		H	H	290	>10,000	>10,000	>10,000	170 (1%)
6a		H	H	110	>10,000	9800	3200	160 (3%)
6b		F	H	190	>10,000	9300	2300	600 (2%)
6c		H	Me	150	>10,000	>10,000	2500	350 (8%)

[a] IC_{50} values are shown as the means of duplicate experiments. IC_{50} values are calculated from the concentration–response curves generated by GraphPad Prism.
[b] Agonistic activity at a concentration of 10 μM.

Table 2
Exploration of lipophilic side chains[a]



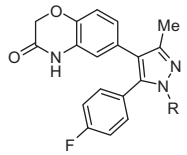
Compound No.	R	MR binding IC_{50} (nM)	Selectivity			MR antagonistic activity IC_{50} (nM) (agonistic activity)[b]
			AR binding IC_{50} (nM)	PR binding IC_{50} (nM)	GR binding IC_{50} (nM)	
6a	Me	110	>10,000	9800	3200	160 (3%)
16a	Et	510	>10,000	>10,000	5600	950 (−1%)
16b	*n*-Pr	81	>10,000	5700	1600	180 (−1%)
24a	*n*-Bu	22	3600	4000	1400	45 (0%)
24b	Isobutyl	60	3700	4300	390	49 (−7%)
16c	Bn	100	5600	2000	630	64 (−9%)
16d	Ph	>10,000	—	—	—	—
24c	$CH_2CH_2CH_2CF_3$	8.4	>10,000	6800	1100	33 (6%)
41	$CH_2CH_2CF_2Me$	10	9200	4000	370	18 (4%)
36a	$CH_2CF_2CH_2Me$	5.8	>10,000	4000	540	27 (11%)

[a] IC_{50} values are shown as the means of duplicate experiments. IC_{50} values are calculated from the concentration–response curves generated by GraphPad Prism.
[b] Agonistic activity at a concentration of 10 μM.

instability was probably due to their high lipophilicity (log*D* >3), and thus we focused on less lipophilic derivatives in the second round of the side chain investigation.[13,14]

To increase the binding activity while avoiding high lipophilicity, the 1-methyl group was changed to less lipophilic side chains substituted with polar groups targeting the two residues Arg817 and Gln776 (Table 3). A range of functional groups including amide, sulfonamide, cyano, hydroxy, and pyridine ring were investigated. All of these compounds displayed greatly reduced log*D* values, and indeed, many of them tended to have improved metabolic clearance in rat microsomes relative to those of the lipophilic compounds described above. Acetamide **27a** showed significantly

Table 3
Exploration of polar side chains targeting for Arg817 and/or Gln776[a]



Compound No.	R	MR binding IC_{50} (nM)	Selectivity			MR antagonistic activity IC_{50} (nM) (agonistic activity)[b]	Log*D* (pH7.4)
			AR binding IC_{50} (nM)	PR binding IC_{50} (nM)	GR binding IC_{50} (nM)		
6a	Me	110	>10,000	9800	3200	160 (3%)	2.51
27a	$CH_2CH_2NHCOMe$	>10,000	—	—	—	—	—
27b	$CH_2CH_2NHSO_2Me$	250	>10,000	>10,000	>10,000	2000 (5%)	1.7
30	CH_2CH_2CN	530	>10,000	5700	4600	490 (−1%)	1.89
16e	$CH_2CH_2CH_2OH$	440	>10,000	>10,000	>10,000	680 (2%)	1.99
16g	$CH_2CH_2CH_2CH_2OH$	75	>10,000	>10,000	5400	130 (8%)	2.16
16h	CH_2-(2-Py)	2800	>10,000	>10,000	>10,000	990 (−14%)	2.45
16i	CH_2-(3-Py)	36	>10,000	>10,000	1700	36 (−4%)	2.22
16j	CH_2-(4-Py)	520	>10,000	>10,000	>10,000	430 (−8%)	2.2
37a	$CH_2CF_2CH_2OH$	51	>10,000	>10,000	2400	71 (1%)	2.05
37b	$CH_2CF_2CH_2CH_2OH$	38	>10,000	>10,000	1300	540 (1%)	2.11
43	$CH_2CH_2CF_2CH_2OH$	95	>10,000	>10,000	2500	180 (3%)	2.34
Spironolactone		49	120	650	1400	60 (4%)	—
Eplerenone		2600	>10,000	>10,000	>10,000	1300 (5%)	—

[a] IC_{50} values are shown as the means of duplicate experiments. IC_{50} values are calculated from the concentration–response curves generated by GraphPad Prism.
[b] Agonistic activity at a concentration of 10 μM.

reduced binding activity, and the methanesulfonamide **27b**, 2-cyanoethyl **30**, and 3-hydroxypropyl **16e** showed moderately decreased activities. On the other hand, the hydroxybutyl compound **16g** showed potent binding activity (IC_{50} = 75 nM). Subsequently, 2-, 3-, and 4-pyridylmethyl compounds were explored (**16h–j**). The position of the nitrogen atom in the pyridine ring was critical for high affinity, and only the 3-pyridylmethyl compound **16i** showed highly potent binding activity among the three tested molecules (IC_{50} = 36 nM). Noticeably, compound **16i** was 3-fold more potent than the benzyl compound **16c**, which indicated that the pyridine nitrogen atom formed a strong hydrogen bond with MR. Thus, it was revealed that replacement of the 1-methyl group with polar side chains could be utilized to increase the binding activity.

Next, we were interested in the incorporation of fluorine atoms into the polar side chains. This combination was expected to provide potent activity with low lipophilicity. 3-Hydroxypropyl and 4-hydroxybutyl groups were selected for the polar side chains, and the fluorine atoms were introduced at their methylene linkers (Table 3, **37a**,**b**, **43**). These compounds showed increased (**37a**,**b**) or retained (**43**) binding activities with little increase of lipophilicity, and compounds **37a** and **37b** showed highly potent binding activities (**37a**: IC_{50} = 51 nM; **37b**: IC_{50} = 38 nM). It is notable that compound **37a** was 8-fold more potent than the nonfluorinated compound **16e**. Consequently, four compounds (**16g**, **16i**, **37a**,**b**) were identified as potent and less lipophilic derivatives. They all showed high selectivity over the other steroid receptors (importantly, with little affinity for AR and PR) and significantly reduced partial agonistic activities. Although the antagonistic activities of compounds **16g** and **37b** were moderate, compounds **16i** and **37a** showed highly potent antagonistic activities comparable to that of spironolactone (**16i**: IC_{50} = 36 nM; **37a**: IC_{50} = 71 nM). Unfortunately, the 3-pyridylmethyl compound **16i** showed rapid metabolic clearance in rat microsomes (>200 μL min^{-1} mg^{-1}) despite its reduced lipophilicity, probably due to the metabolically labile diarylmethane moiety and/or *N*-oxidation on the pyridine ring. On the other hand, the 2,2-difluoro-3-hydroxyl compound **37a** showed acceptable metabolic clearance in rat microsomes (42 μL min^{-1} mg^{-1}) and had good PK profiles (bioavailability in rats = 21%), as shown in Table 4. On the basis of these results, compound **37a** was progressed to in vivo efficacy studies in rats.

Figure 3 shows the X-ray crystal structure of the MR/compound **37a** complex, which shows that compound **37a** binds to the steroid binding domain in MR in a fashion similar to that of the previously reported benzoxazin-3-one derivatives. The amide moiety in the benzoxazin-3-one ring forms hydrogen bonds with Asn770 and Thr945, and the 4-fluorobenzene ring occupies the hydrophobic pocket. The side chain at the 1-position of the pyrazole ring, which was the 2,2-difluoropropyl-3-hydroxy group, orients towards Arg817 and Gln776, and the hydroxyl group directly forms a hydrogen bond to Gln776. The two fluorine atoms fill the space with no specific hydrogen bonding interactions, which suggests that the major effect of these fluorine atoms was the hydrophobic interaction. It was confirmed that compound **37a** has no water-mediated hydrogen bonding network between the MR and the ligand side chain. This finding is consistent with our hypothesis regarding the attenuation of partial agonistic activity in the benzoxazin-3-one derivatives.

The effects of compound **37a** on systolic blood pressure (SBP) and seminal vesicle weight were evaluated in established

Table 4
Pharmacokinetic profiles of compound **37a** in Sprague–Dawley Rats[a]

CL (mL/h/kg)	Vd_{ss} (mL/kg)	$AUC_{0-24\ h,p.o.}$ (ng h/mL)	$MRT_{p.o.}$ (h)	B.A. (%)
990 ± 165	1356 ± 59	662 ± 372	3.77 ± 0.66	21.4 ± 12.6

[a] Rats were administered the drug intravenously at 1 mg/kg and orally at 3 mg/kg (*n* = 3).

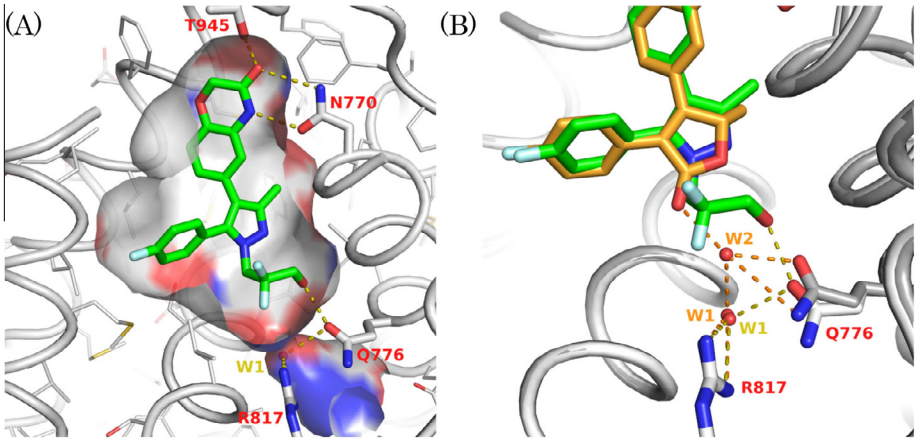


Figure 3. (A) X-ray crystal structure of compound **37a** bound to MR. (B) Overlay of compound **37a** (green) and a dihydrofuran-2-one derivative (orange) in MR. Yellow or orange dotted lines mean hydrogen bonds.

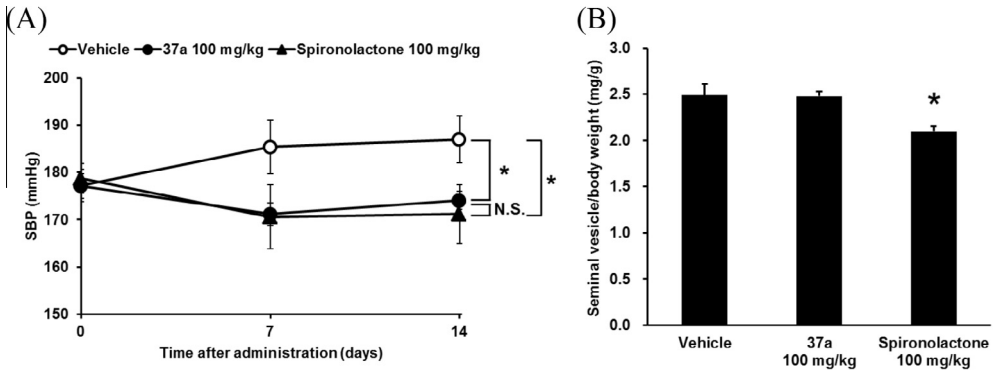


Figure 4. Anti-hypertensive (A), and anti-androgenic (B) effects of compound **37a** in DOCA-salt rats. (A) DOCA-salt hypertensive rats were treated with vehicle, compound **37a**, or spironolactone at a dose of 100 mg/kg for 18 days. SBP and HR were measured by tail-cuff method approximately 24 h after the 7th and 14th administration. Data were shown as mean ± SEM ($n = 8$); $^{*}p \leqslant 0.05$ versus vehicle by repeated-measures ANOVA, which indicated significant group × time interaction but not the main effects of group and time. N.S. means not significant. (B) Seminal vesicle weight was measured by autopsy after the measurement of body weight approximately 24 h after the last administration. Data were shown as mean ± SEM ($n = 7$–8); $^{*}p \leqslant 0.05$ versus vehicle by Aspin–Welch test.

deoxycorticosterone acetate (DOCA)-salt hypertensive rats, and the results are shown in Figures 4A and B. Compound **37a** (100 mg/kg, q.d.) and spironolactone (100 mg/kg, q.d.) were orally administered for 18 days. SBP and heart rate (HR) were measured by the tail-cuff method approximately 24 h after the 7th and 14th administration, and seminal vesicle weight was measured by autopsy after the body weight measurement approximately 24 h after the last administration. Both compound **37a** and spironolactone significantly lowered SBP, and their antihypertensive effects were similar. Meanwhile, these compounds did not affect the HR (data not shown). Furthermore, compound **37a** showed no significant reduction of seminal vesicle weight, a sign of an antiandrogenic effect, probably because of its little binding activity for AR, whereas spironolactone significantly reduced it. Thus, compound **37a** exhibited a potent blood pressure-lowering effect without an antiandrogenic effect. These results indicated that compound **37a** was a new, potent, and selective MR antagonist that appeared to mitigate sexual hormone-related side effects, which have been reported as issues associated with spironolactone treatment in the clinic.

4. Conclusion

We have discovered 6-[5-(4-fluorophenyl)-3-methyl-pyrazol-4-yl]-benzoxazin-3-one derivatives as novel selective nonsteroidal MR antagonists. To reduce the partial agonistic activity, we designed a series of azole derivatives possessing side chains at the position adjacent to the 4-fluorobenzene ring. Screening of azole rings in the central ring moiety identified the 1,3-dimethyl pyrazole **6a** as a lead compound with attenuated partial agonistic activity. Replacement of the 1-methyl group of **6a** with larger lipophilic side chains or polar side chains targeting Arg817 and Gln776 increased the binding activity while maintaining the agonistic response at the lower level. Reduction of the lipophilicity was effective for improvement of metabolic clearance in rat microsomes. Consequently, the combination of fluorine atoms with polar groups achieved potent binding activity with favorable in vitro metabolic profiles. Thus, we discovered 2,2-difluoro-3-hydroxypropyl derivative **37a**, which showed highly potent in vitro activity, high steroidal receptor selectivity, and attenuated partial agonistic activity as well as good PK profiles. Compound **37a** exhibited a significant blood pressure lowering effect in DOCA-salt hypertensive rats with no signs of an antiandrogenic effect. On the basis of these results, compound **37a** was progressed for further pharmacological evaluations.

5. Experimental procedure

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Ultra Shield-300 (300 MHz) or Varian INOVA-400 (400 MHz) instruments. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Peak multiplicities are expressed as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublets of doublet,

br s = broad singlet. Coupling constants (*J* values) are given in hertz (Hz). Elemental analyses were performed by Takeda Analytical Laboratories Ltd. Chemical intermediates were characterized by ^{1}H NMR. The purities of all compounds tested in biological systems were assessed as being >95% using analytical high-performance liquid chromatography (HPLC). The HPLC analyses were performed using a Shimadzu UFLC instrument, equipped with a L-column 2 ODS (3.0 × 50 mm, 2 μm) column, eluting with a gradient of 5–90% solvent B in solvent A (solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in acetonitrile), at a flow rate of 1.2 mL/min, with UV detection at 220 nm. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Chromatographic purification was performed on silica gel columns [(Merck Kieselgel 60, 70–230 mesh size or 230–400 mesh size, Merck) or (Chromatorex NH-DM 1020, 100–200 mesh size)] or on Purif-Pack (SI or NH, particle size: 60 μm, Fuji Silysia Chemical, Ltd). Reagents and solvents were obtained from commercial sources and used without further purification. Abbreviations of solvents are used as follows: $CDCl_3$, deuterated chloroform; DMSO-d_6, dimethyl sulfoxide-d_6; EtOAc, ethyl acetate; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; IPE, diisopropyl ether.

5.1. 6-(5-Methyl-1,2-oxazol-3-yl)-2*H*-1,4-benzoxazin-3(4*H*)-one (8b)

A mixture of hydroxylamine hydrochloride (188 mg, 2.70 mmol) and triethylamine (369 μL, 2.65 mmol) in 2-propanol (15 mL) was stirred at room temperature for 15 min. To the reaction mixture was added 2,2,2-trifluoroacetic acid (423 μL, 5.53 mmol) and the mixture was stirred at room temperature for 15 min. To the resulting mixture was added 1-(3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-6-yl)butane-1,3-dione (600 mg, 2.57 mmol) at room temperature. The reaction mixture was stirred at 60 °C for 12 h, and concentrated under reduced pressure. To the residue was added water, and then 1 N NaOH was added to neutralize the solution. The precipitate was collected by filtration and washed with ether to give **8b** (560 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (3H, s), 4.64 (2H, s), 6.72 (1H, s), 7.05–7.10 (1H, m), 7.30–7.45 (2H, m), 10.90 (1H, br s).

5.2. 6-(5-Methyl-1*H*-pyrazol-3-yl)-2*H*-1,4-benzoxazin-3(4*H*)-one (8a)

The compound **8a** was prepared in a manner similar to that described for **8b**. Yield (77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (3H, s), 4.72 (2H, s), 6.38 (1H, s), 7.00–7.05 (1H, m), 7.30–7.35 (1H, m), 7.67 (1H, s), 10.82 (1H, br s).

5.3. 6-(4-Bromo-5-methyl-1*H*-pyrazol-3-yl)-2*H*-1,4-benzoxazin-3(4*H*)-one (9a)

To a stirred solution of **8a** (390 mg, 1.66 mmol) in DMF (10 mL) was added NBS (295 mg, 1.66 mmol) at room temperature. The mixture was stirred at room temperature for 2 h, and then water was added. The mixture was cooled to 0 °C, and the precipitate was collected by filtration and washed with water and ether to give **9a** (480 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (3H, s), 4.72 (2H, s), 7.05–7.11 (1H, m), 7.42 (1H, s), 7.77–7.81 (1H, m), 10.20 (1H, br s).

5.4. 6-(4-Bromo-5-methyl-1,2-oxazol-3-yl)-2*H*-1,4-benzoxazin-3(4*H*)-one (9b)

The compound **9b** was prepared in a manner similar to that described for **9a**. Yield (98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (3H, s), 4.70 (2H, s), 7.15–7.20 (1H, m), 7.55–7.60 (2H, m), 10.95 (1H, br s).

5.5. 6-[4-(4-Fluorophenyl)-5-methyl-1*H*-pyrazol-3-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (3)

Under nitrogen atmosphere, a mixture of **9a** (158 mg, 0.513 mmol), 4-fluorophenylboronic acid (215 mg, 1.54 mmol), bis(tri-*t*-butylphosphine)palladium (0) (13.1 mg, 0.025 mmol) and tripotassium phosphate (1.05 g, 4.87 mmol) in THF/water (12 mL/2.4 mL) was stirred at 90 °C for 12 h, diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was suspended in hexane and acetonitrile, and the solid was collected by filtration to give **3** (89 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (3H, s), 4.69 (2H, s), 6.72 (1H, dd, *J* = 8.6, 2.0 Hz), 6.78 (1H, d, *J* = 2.0 Hz), 7.02–7.10 (2H, m), 7.16–7.22 (2H, m), 7.26 (1H, s), 7.52 (1H, s), 10.56 (1H, br s). Anal. Calcd for $C_{18}H_{14}N_3O_2F$: C, 66.87; H, 4.36; N, 13.00. Found: C, 66.57; H, 4.52; N, 13.04.

5.6. 6-[4-(4-Fluorophenyl)-5-methyl-1,2-oxazol-3-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (4)

The compound **4** was prepared in a manner similar to that described for **3**. Yield (74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.15 (3H, s), 4.62 (2H, s), 6.95–6.99 (2H, m), 7.07 (1H, s), 7.30–7.35 (2H, m), 7.37–7.42 (2H, m), 10.84 (1H, s). Anal. Calcd for $C_{18}H_{13}N_2O_3F$: C, 66.66; H, 4.04; N, 8.64. Found: C, 66.40; H, 4.16; N, 8.55.

5.7. 3-(Dimethylamino)-1-(4-fluorophenyl)prop-2-en-1-one (11)

A mixture of 1-(4-fluorophenyl)ethanone (2.0 g, 14.5 mmol) and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (4 mL) was refluxed for 2 h, and concentrated under reduced pressure. The residue was suspended in hexane, and the solid was collected by filtration to give **11** (0.89 g, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.92 (3H, s), 3.14 (3H, s), 5.82 (1H, d, *J* = 12.2 Hz), 7.24 (2H, m), 7.71 (1H, d, *J* = 12.2 Hz), 7.96 (2H, dd, *J* = 8.9, 5.7 Hz).

5.8. 1-(4-Fluorophenyl)butane-1,3-dione (12a)

To a suspension of 60% sodium hydride (43.4 g, 1.81 mol) in tetrahydrofuran (1500 mL) was added 4-fluoroacetophenone (50.0 g, 0.361 mol) at room temperature, and the mixture was stirred at room temperature for 30 min. To the mixture was added EtOAc (127.6 g, 1.45 mmol) at room temperature. The mixture was stirred at 40 °C for 3 h, and then poured into 1 N HCl solution. The organic solvent was removed under reduced pressure. The residual mixture was extracted with EtOAc, washed with 1 N HCl solution and saturated brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from hexane to give **12a** (48.3 g, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.19 (3H, s), 6.13 (1H, s), 7.01–7.18 (2H, m), 7.82–7.96 (2H, m), 16.15 (1H, br s).

5.9. 5-(4-Fluorophenyl)-1-methyl-1*H*-pyrazole (13a)

A mixture of **11** (800 mg, 4.14 mmol) and methylhydrazine (257 μL, 4.88 mmol) in ethanol was refluxed for 4 h. The solvent was removed, and the residue was purified by column chromatography on NH-silica gel with hexane/ethyl acetate as an eluent to give **13a** (592 mg, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.83 (3H, s), 6.39 (1H, d, *J* = 1.9 Hz), 7.31–7.40 (2H, m), 7.46 (1H, d, *J* = 1.5 Hz), 7.58 (2H, dd, *J* = 8.9, 5.5 Hz).

5.10. 5-(4-Fluorophenyl)-1,3-dimethyl-1*H*-pyrazole (13c)

A mixture of **12a** (12.6 g, 69.9 mmol), methylhydrazine (6.4 g, 139 mmol), trifluoroacetic acid (10.7 mL, 144 mmol), and triethylamine (19.4 mL, 139 mmol) in 2-propanol (350 mL) was stirred at 80 °C for 1 h, and then concentrated under reduced pressure. The residue was diluted with ethyl acetate, and washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate as an eluent to give **13c** (10.0 g, 75%). ^{1}H NMR (DMSO-d_6) δ 2.16 (3H, s), 3.74 (3H, s), 6.16 (1H, s), 7.27–7.40 (2H, m), 7.54 (2H, dd, J = 8.7, 5.5 Hz).

The compound **13b**, **13e**,**f**, **13h**–**o** were prepared in a manner similar to that described for **13c**.

5.11. 5-(4-Fluorophenyl)-3-methyl-1*H*-pyrazole (13b)

Yield (72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.25 (3H, s), 7.01–7.17 (2H, m), 7.65–7.81 (2H, m), 8.97 (1H, br s).

5.12. 1-Ethyl-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (13e)

Yield (90%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (3H, t, J = 7.0 Hz), 2.17 (3H, s), 3.99 (2H, q, J = 7.0 Hz), 6.11 (1H, s), 7.27–7.37 (2H, m), 7.43–7.54 (2H, m).

5.13. 1-Allyl-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (13f)

Yield (95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 4.57–4.69 (2H, m), 4.86 (1H, dd, J = 17.0, 1.5 Hz), 5.11 (1H, dd, J = 10.5, 1.5 Hz), 5.85–6.01 (1H, m), 6.19 (1H, s), 7.31 (2H, t, J = 9.0 Hz), 7.43–7.53 (2H, m).

5.14. 1-Benzyl-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (13h)

Yield (88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.31 (3H, s), 5.22 (2H, s), 6.09 (1H, s), 6.94–7.07 (4H, m), 7.16–7.33 (5H, m).

5.15. 5-(4-Fluorophenyl)-3-methyl-1-phenyl-1*H*-pyrazole (13i)

Yield (39%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (3H, s), 6.27 (2H, s), 6.91–7.00 (2H, m), 7.13–7.21 (2H, m), 7.21–7.34 (5H, m).

5.16. 2-[5-(4-Fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]ethanol (13j)

Yield (97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 3.75 (2H, q, J = 5.5 Hz), 3.98 (2H, t, J = 5.5 Hz), 4.92 (1H, t, J = 5.0 Hz), 6.12 (1H, s), 7.25–7.40 (2H, m), 7.51–7.67 (2H, m).

5.17. 3-[5-(4-Fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propan-1-ol (13k)

Yield (77%). ^{1}H NMR (DMSO-d_6) δ 1.85 (2H, quin, J = 6.7 Hz), 2.17 (3H, s), 3.25–3.39 (2H, m), 4.03 (2H, t, J = 7.2 Hz), 4.50 (1H, m), 6.11 (1H, s), 7.31 (2H, t, J = 8.7 Hz), 7.49 (2H, t, J = 2.8 Hz).

5.18. 1-[4-(Benzyloxy)butyl]-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (13l)

Yield (49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40–1.65 (2H, m), 1.81–1.93 (2H, m), 2.29 (3H, s), 3.37 (2H, t, J = 6.3 Hz), 4.02 (2H, t, J = 7.2 Hz), 4.41 (2H, s), 6.00 (1H, s), 7.03–7.12 (2H, m), 7.23–7.35 (7H, m).

5.19. 2-{[5-(4-Fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]methyl} pyridine (13m)

Yield (30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (3H, s), 5.49 (2H, s), 6.19 (1H, s), 6.99–7.10 (3H, m), 7.27–7.37 (3H, m), 7.73–7.82 (1H, m), 8.61 (1H, d, J = 4.9 Hz).

5.20. 3-{[5-(4-Fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]methyl} pyridine (13n)

Yield (19%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (3H, s), 5.34 (2H, s), 6.16 (1H, s), 7.07–7.17 (2H, m), 7.22–7.30 (2H, m), 7.48–7.56 (1H, m), 7.72 (1H, d, J = 8.1 Hz), 8.28–8.40 (1H, m), 8.63 (1H, d, J = 4.0 Hz).

5.21. 4-{[5-(4-Fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]methyl} pyridine (13o)

Yield (33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21 (3H, s), 5.31 (2H, s), 6.26 (1H, s), 6.92–6.94 (2H, m), 7.24–7.43 (4H, m), 8.46–8.48 (2H, m).

5.22. 5-(2,4-Difluorophenyl)-1,3-dimethyl-1*H*-pyrazole (13d)

To a solution of 1-(2,4-difluorophenyl)ethanone (2.0 g, 12.8 mmol) in THF (100 mL) was added dropwise lithium bis(trimethylsilyl)amide in THF (1.1 M, 12.8 mL, 14.1 mmol) at −25 °C. The mixture was stirred for 1 h at −25 °C, and then was cooled to −78 °C. To the mixture was added acetyl chloride (1.19 mL, 16.8 mmol). The mixture was stirred for 3 h, treated with 1 N HCl, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give **12b**. To the crude **12b** was added 2-propanol (100 mL), trifluoroacetic acid (1.99 mL, 26.8 mmol), methyl hydrazine (1.35 mL, 25.6 mmol) and triethylamine (3.61 mL, 25.6 mmol), successively. The mixture was stirred at 80 °C for 30 min, concentrated under reduced pressure, treated with saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate as an eluent to give **13d** (0.63 g, 25% in 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 3.62 (3H, s), 6.17 (1H, s), 7.23 (1H, td, J = 8.4, 2.5 Hz), 7.43–7.60 (2H, m).

5.23. 5-(4-Fluorophenyl)-3-methyl-1-propyl-1*H*-pyrazole (13g)

A suspension of **13f** (2.0 g, 9.24 mmol) and 10 wt% Palladium on carbon (300 mg) in MeOH (50 mL) was stirred at room temperature for 12 h under hydrogen atmosphere. The insoluble material was removed by filtration, and the filtrate was concentrated under reduced pressure to give **13g**. The crude **13g** was used for the next reaction without further purification.

5.24. 4-Bromo-5-(4-fluorophenyl)-1,3-dimethyl-1*H*-pyrazole (14b)

A mixture of **13c** (5 g, 26.3 mmol) and NBS (4.68 g, 26.3 mmol) in acetonitrile (40 mL) was stirred at room temperature for 10 min. The mixture was diluted with EtOAc, and washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on NH-silica gel with hexane/EtOAc as an eluent to give **14b** (2.11 g, 30%). ^{1}H NMR (DMSO-d_6) δ 2.17 (3H, s), 3.69 (3H, s), 7.35–7.40 (2H, m), 7.54 (2H, dd, J = 8.7, 5.7 Hz).

The compound **14a**, **14c–l** were prepared in a manner similar to that described for **14b**.

5.25. 4-Bromo-5-(4-fluorophenyl)-1-methyl-1*H*-pyrazole (14a)

Yield (39%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.76 (3H, s), 7.40 (2H, t, J = 8.9 Hz), 7.56 (2H, dd, J = 8.9, 5.5 Hz), 7.66 (1H, s).

5.26. 4-Bromo-5-(2,4-difluorophenyl)-1,3-dimethyl-1*H*-pyrazole (14c)

Yield (82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 3.63 (3H, s), 7.30 (1H, td, J = 8.5, 1.9 Hz), 7.43–7.62 (2H, m).

5.27. 4-Bromo-1-ethyl-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (14d)

Yield (99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (3H, t, J = 7.0 Hz), 2.18 (3H, s), 3.96 (2H, q, J = 7.0 Hz), 7.34–7.45 (2H, m), 7.45–7.54 (2H, m).

5.28. 4-Bromo-5-(4-fluorophenyl)-3-methyl-1-propyl-1*H*-pyrazole (14e)

Yield (99% in 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.69 (3H, t, J = 7.5 Hz), 1.54–1.70 (2H, m), 2.18 (3 H, s), 3.90 (2H, t, J = 7.0 Hz), 7.39 (2H, t, J = 9.0 Hz), 7.48 (2H, dd, J = 9.0, 5.5 Hz).

5.29. 1-Benzyl-4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (14f)

Yield (80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s), 5.17 (2H, s), 6.97 (2H, dd, J = 5.4, 1.9 Hz), 7.02–7.12 (2H, m), 7.18–7.27 (5H, m).

5.30. 4-Bromo-5-(4-fluorophenyl)-3-methyl-1-phenyl-1*H*-pyrazole (14g)

Yield (88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (3H, s), 6.97–7.07 (2H, m), 7.15–7.33 (7H, m).

5.31. 3-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propan-1-ol (14h)

Yield (95%). ^{1}H NMR (DMSO-d_6) δ 1.80 (2H, quin, J = 6.5 Hz), 2.18 (3H, s), 3.22–3.34 (2H, m), 3.99 (2H, t, J = 7.3 Hz), 4.48 (1H, m), 7.34–7.44 (2H, m), 7.50 (2H, dd, J = 8.7, 5.5 Hz).

5.32. 1-[4-(Benzyloxy)butyl]-4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (14i)

Yield (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42–1.55 (2H, m), 1.75–1.87 (2H, m), 2.28 (3H, s), 3.36 (2H, t, J = 6.3 Hz), 3.90 (2H, t, J = 7.2 Hz), 4.41 (2H, s), 7.09–7.18 (2H, m), 7.23–7.36 (7H, m).

5.33. 2-{[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]methyl}pyridine (14j)

Yield (69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (3H, s), 5.31 (2H, s), 6.93 (1H, d, J = 7.6 Hz), 7.05–7.21 (3H, m), 7.31–7.40 (2H, m), 7.58–7.68 (1H, m), 8.52 (1H, d, J = 4.2 Hz).

5.34. 3-{[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]methyl}pyridine (14k)

Yield (55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.31 (3H, s), 5.26 (2H, s), 7.12–7.22 (2H, m), 7.22–7.31 (2H, m), 7.41–7.50 (1H, m), 7.64 (1H, d, J = 7.9 Hz), 8.36 (1H, s), 8.63 (1H, s).

5.35. 4-{[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]methyl}pyridine (14l)

Yield (43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (3H, s), 5.31 (2H, s), 7.08–7.32 (6H, m), 8.66 (2H, s).

5.36. 6-[5-(4-Fluorophenyl)-1-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (5)

Under argon atmosphere, a mixture of **14a** (500 mg, 1.96 mmol), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2*H*-1,4-benzoxazin-3(4*H*)-one (647 mg, 2.35 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane adduct (160 mg, 0.196 mmol) and cesium carbonate (1.92 g, 5.89 mmol) in THF/H_2O (8 mL/2 mL) was stirred at reflux for 18 h. The reaction mixture was diluted with brine, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on NH-silica gel with hexane/EtOAc as an eluent and crystallized from EtOAc/hexane to give **5** (196 mg, 31%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.68 (3H, s), 4.51 (2H, s), 6.59–6.73 (2H, m), 6.82 (1H, d, J = 8.9 Hz), 7.24–7.49 (4H, m), 7.66 (1H, s), 10.62 (1H, s). Anal. Calcd for $C_{18}H_{14}N_3O_2F$: C, 66.87; H, 4.36; N, 13.00. Found: C, 66.77; H, 4.34; N, 12.93.

The compound **6a–c**, **16a–e**, **16g–i** were prepared in a manner similar to that described for **5**.

5.37. 6-[5-(4-Fluorophenyl)-1,3-dimethyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (6a)

Yield (22%). ^{1}H NMR (DMSO-d_6) δ 2.17 (3H, s), 3.64 (3H, s), 4.53 (2H, s), 6.57 (1H, dd, J = 8.1, 2.1 Hz), 6.65 (1H, d, J = 1.9 Hz), 6.83 (1H, d, J = 8.3 Hz), 7.18–7.42 (4H, m), 10.58 (1H, s). Anal. Calcd for $C_{19}H_{15}N_3O_2F_2$: C, 64.22; H, 4.25; N, 11.83. Found: C, 64.09; H, 4.21; N, 11.75.

5.38. 6-[5-(2,4-Difluorophenyl)-1,3-dimethyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (6b)

Yield (48%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20 (3H, s), 3.59 (3H, s), 4.53 (2H, s), 6.58 (1H, dd, J = 8.1, 2.1 Hz), 6.64 (1H, d, J = 2.3 Hz), 6.83 (1H, d, J = 8.3 Hz), 7.18 (1H, td, J = 8.6, 2.1 Hz), 7.31–7.47 (2H, m), 10.59 (1H, s). Anal. Calcd for $C_{19}H_{15}N_3O_2F_2$: C, 64.22; H, 4.25; N, 11.83. Found: C, 64.09; H, 4.21; N, 11.75.

5.39. 6-[5-(4-Fluorophenyl)-1,3-dimethyl-1*H*-pyrazol-4-yl]-8-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one (6c)

Yield (12%). ^{1}H NMR (300 M Hz, DMSO-d_6) δ 2.06 (3H, s), 2.16 (3H, s), 3.64 (3H, s), 4.54 (2H, s), 6.45 (1H, d, J = 1.9 Hz), 6.51 (1H, d), 7.18–7.41 (4H, m), 10.51 (1H, s). Anal. Calcd for $C_{20}H_{18}N_3O_2F$: C, 68.36; H, 5.16; N, 11.96. Found: C, 68.23; H, 5.16; N, 11.86.

5.40. 6-(1-Ethyl-5-(4-fluorophenyl)-3-methyl-1H-pyrazol-4-yl)-2H-1,4-benzoxazin-3(4H)-one (16a)

Yield (28%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (3H, t, J = 7.0 Hz), 2.19 (3H, s), 3.91 (2H, q, J = 7.0 Hz), 4.53 (2H, s), 6.57 (1H, dd, J = 8.0, 2.0 Hz), 6.65 (1H, d, J = 2.0 Hz), 6.81 (1H, d, J = 8.0 Hz), 7.19–7.36 (4H, m), 10.60 (1H, s). Anal. Calcd for $C_{20}H_{18}N_3O_2F$: C, 68.36; H, 5.16; N, 11.96. Found: C, 68.58; H, 5.39; N, 11.72.

5.41. 6-[5-(4-Fluorophenyl)-3-methyl-1-propyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16b)

Yield (36%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.73 (3H, t, J = 7.5 Hz), 1.57–1.73 (2H, m), 2.19 (3H, s), 3.84 (2H, t, J = 7.5 Hz),

4.53 (2H, s), 6.56 (1H, dd, J = 8.5, 2.0 Hz), 6.65 (1H, d, J = 2.0 Hz), 6.81 (1H, d, J = 8.5 Hz), 7.22–7.35 (4H, m), 10.60 (1H, br. s). Anal. Calcd for $C_{21}H_{20}N_3O_2F$: C, 69.03; H, 5.52; N, 11.50. Found: C, 69.04; H, 5.51; N, 11.50.

5.42. 6-[1-Benzyl-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16c)

Yield (38%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21 (3H, s), 4.53 (2H, s), 5.15 (2H, s), 6.59 (1H, dd, J = 8.3, 2.1 Hz), 6.68 (2H, d, J = 2.1 Hz), 6.82 (1H, d, J = 8.3 Hz), 6.97 (2H, dd, J = 7.9, 1.5 Hz), 7.19–7.32 (7H, m), 10.60 (1H, s). Anal. Calcd for $C_{25}H_{20}N_3O_2F$: C, 72.63; H, 4.88; N, 10.16. Found: C, 72.61; H, 4.94; N, 10.07.

5.43. 6-[5-(4-Fluorophenyl)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16d)

Yield (16%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.26 (3H, s), 4.56 (2H, s), 6.59–6.75 (2H, m), 6.68 (1H, d, J = 8.3 Hz), 7.10–7.23 (6H, m), 7.27–7.39 (3H, m), 10.65 (1H, s). Anal. Calcd for $C_{24}H_{18}N_3O_2F$: C, 72.17; H, 4.54; N, 10.52. Found: C, 72.18; H, 4.58; N, 10.48.

5.44. 6-[5-(4-Fluorophenyl)-1-(3-hydroxypropyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16e)

Yield (17%). ^{1}H NMR (DMSO-d_6) δ 1.73–1.90 (2H, m), 2.18 (3H, s), 3.27–3.40 (2H, m), 3.94 (2H, t, J = 7.2 Hz), 4.47 (1H, t, J = 4.9 Hz), 4.53 (2H, s), 6.56 (1H, dd, J = 8.1, 2.1 Hz), 6.64 (1H, d, J = 1.9 Hz), 6.81 (1H, d, J = 8.0 Hz), 7.17–7.41 (4H, m), 10.60 (1H, s). Anal. Calcd for $C_{21}H_{20}N_3O_3F$: C, 66.13; H, 5.29; N, 11.02. Found: C, 65.89; H, 5.16; N, 10.80.

5.45. 6-{1-[4-(Benzyloxy)butyl]-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl}-2*H*-1,4-benzoxazin-3(4*H*)-one (16f)

Yield (44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47–1.59 (2H, m), 1.81–1.92 (2H, m), 2.30 (3H, s), 3.38 (2H, t, J = 6.3 Hz), 3.99 (2H, t, J = 7.5 Hz), 4.22 (2H, s), 4.57 (2H, s), 6.43 (1H, d, J = 2.1 Hz), 6.65 (1H, dd, J = 8.4, 2.1 Hz), 6.84 (1H, d, J = 8.4 Hz), 6.98–7.08 (2H, m), 7.11–7.18 (2H, m), 7.22–7.36 (5H, m), 7.97 (1H, s).

5.46. 6-[5-(4-Fluorophenyl)-3-methyl-1-(pyridin-2-ylmethyl)-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16h)

Yield (52%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20 (3H, s), 4.54 (2H, s), 5.22 (2H, s), 6.61 (1H, d, J = 8.1 Hz), 6.69 (1H, s), 6.83 (1H, d, J = 8.1 Hz), 7.02 (1H, d, J = 7.7 Hz), 7.16–7.37 (5H, m), 7.68–7.82 (1H, m), 8.49 (1H, d, J = 4.5 Hz), 10.60 (1H, s). Anal. Calcd for $C_{24}H_{19}N_4O_2F$: C, 69.55; H, 4.62; N, 13.52. Found: C, 69.28; H, 4.66; N, 13.13.

5.47. 6-[5-(4-Fluorophenyl)-3-methyl-1-(pyridin-3-ylmethyl)-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16i)

Yield (21%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21 (3H, s), 4.53 (2H, s), 5.20 (2H, s), 6.60 (1H, dd, J = 8.3, 1.9 Hz), 6.68 (1H, d, J = 1.9 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.21–7.35 (5H, m), 7.37–7.44 (1H, m), 8.19 (1H, s), 8.45 (1H, d, J = 3.8 Hz), 10.60 (1H, s). Anal. Calcd for $C_{24}H_{19}N_4O_2F$ 0.1(EtOAc): C, 69.24; H, 4.72; N, 13.24. Found: C, 68.92; H, 4.69; N, 13.16.

5.48. 6-[5-(4-Fluorophenyl)-3-methyl-1-(pyridin-4-ylmethyl)-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16j)

Yield (5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.22 (3H, s), 4.54 (2H, s), 5.20 (2H, s), 6.62 (1H, dd, J = 8.3, 2.3 H), 6.69 (1H, d, J = 1.9 Hz), 6.83 (1H, d, J = 8.0 Hz), 6.96 (2H, d, J = 6.1 Hz), 7.20–7.25 (4H, m), 8.47 (2H, d, J = 6.1 Hz), 10.60 (1H, s). Anal. Calcd for $C_{24}H_{19}N_4O_2F$: C, 69.55; H, 4.62; N, 13.52. Found: C, 69.38; H, 4.75; N, 13.25.

5.49. 6-[5-(4-Fluorophenyl)-1-(4-hydroxybutyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (16g)

A mixture of **16f** (0.49 g, 1.01 mmol) and 10 wt% palladium on carbon (0.1 g) in EtOH (30 mL) was stirred at room temperature for 5 h and at 50 °C for 18 h, under hydrogen atmosphere. The insoluble material was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was crystallized from EtOAc/hexane to give **16g** (0.35 g, 88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10–1.37 (2H, m), 1.60–1.73 (2H, m), 2.18 (3H, s), 3.23–3.34 (2H, m), 3.88 (2H, t, J = 7.2 Hz), 4.34 (1H, t, J = 5.1 Hz), 4.52 (2H, s), 6.55 (1H, dd, J = 8.4, 1.8 Hz), 6.64 (1H, d, J = 1.8 Hz), 6.80 (1H, d, J = 8.4 Hz), 7.21–7.32 (4H, m), 10.58 (1H, s). Anal. Calcd for $C_{22}H_{22}N_3O_3F$: C, 66.82; H, 5.61; N, 10.63. Found: C, 66.92; H, 5.71; N, 10.50.

5.50. 8-Methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2*H*-1,4-benzoxazin-3(4*H*)-one (15b)

A mixture of 4-bromo-2-methyl-6-nitrophenol (9.4 g, 40.5 mmol), methyl bromoacetate (6.5 g, 42.5 mmol) and potassium carbonate (8.4 g, 60.8 mmol) in DMSO (50 mL) was stirred at room temperature for 48 h, diluted with water and 10% hydrochloric acid, and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting solid was washed with hexane to give methyl (4-bromo-2-methyl-6-nitrophenoxy)acetate (9.86 g, 80%). A mixture of methyl (4-bromo-2-methyl-6-nitrophenoxy)acetate (10.9 g, 35.8 mmol) and zinc dust (35 g, 535 mmol) in acetic acid (100 mL) and THF (200 mL) was stirred at 45 °C for 30 min and at reflux for 1 h, and filtered. The filtrate was concentrated in vacuo, and the residue was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ solution, water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was washed with hexane, suspended in MeOH, and collected by filtration to give 6-bromo-8-methyl-2*H*-l,4-benzoxazin-3(4*H*)-one (5.8 g, 70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.14 (s, 3H), 4.60 (s, 2H), 6.87 (d, J = 2.3 Hz, 1H), 7.00 (d, J = 2.3 Hz, 1H), 10.72 (s, 1H). A mixture of 6-bromo-8-methyl-2*H*-1,4-benzoxazin-3(4*H*)- one (2.0 g, 8.3 mmol), bis(pinacolato)diboron (2.3 g, 9.1 mmol), potassium acetate (2.9 g, 29.5 mmol) [1,1-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane complex (0.34 g, 0.42 mmol) in 1,4-dioxane (50 mL) was stirred at 90 °C for 12 h under argon atmosphere, diluted with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was suspended in IPE, and collected by filtration to give **15b** (2.36 g, 98%) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (s, 12H), 2.15 (s, 3H), 4.61 (s, 2H), 7.06 (s, 1H), 7.13 (s, 1H), 10.61 (s, 1H).

5.51. *tert*-Butyl-2-(pyridin-2-ylmethylene)hydrazinecarboxylate (18a)

A mixture of pyridine-2-carbaldehyde (5.0 g, 46.7 mmol) and *tert*-butyl carbazate (7.4 g, 56.0 mmol) in EtOH (50 mL) was stirred at room temperature for 3 h, and then concentrated under reduced pressure. The residue was suspended in IPE and collected by filtration to give **18a** (5.6 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (9H, s), 7.21–7.28 (1H, m), 7.65–7.73 (1H, m), 7.94 (1H, s), 8.07 (1H, d, J = 8.1 Hz), 8.36 (1H, s), 8.53–8.59 (1H, m).

The compound **18b**,**c** were prepared in a manner similar to that described for **18a**.

5.52. *tert*-Butyl-2-(pyridin-3-ylmethylene)hydrazinecarboxylate (18b)

Yield (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.54 (9H, s), 7.27–7.34 (1H, m), 7.95 (1H, s), 8.09–8.16 (1H, m), 8.57 (1H, dd, J = 4.9, 1.7 Hz), 8.74 (1H, d, J = 1.9 Hz).

5.53. *tert*-butyl-2-(pyridin-4-ylmethylene)hydrazinecarboxylate (18c)

Yield (62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.54 (9H, s), 7.51–7.57 (2H, m), 7.87 (1H, s), 8.59–8.64 (2H, m), 8.74 (1H, s).

5.54. 2-(Hydrazinomethyl)pyridine trifluoroacetate (19a)

A mixture of **18a** (3.0 g, 13.6 mmol), sodium cyanoborohydride (0.85 g, 13. mmol) and acetic acid/H_2O (15 mL/15 mL) was stirred at room temperature for 2 h, neutralized with 1 N NaOH, and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was diluted with dichloromethane, and then trifluoroacetic acid (10 mL, 136 mmol) was added dropwise. The mixture was stirred at room temperature for 2 h, and concentrated under reduced pressure to give **19a**. The crude **19a** was used for the next reaction.

The compound **19b**,**c** were prepared in a manner similar to that described for **19a**.

5.55. 3-(Hydrazinomethyl)pyridine trifluoroacetate (19b)

The crude product was used for the next reaction.

5.56. 4-(Hydrazinomethyl)pyridine trifluoroacetate (19c)

The crude product was used for the next reaction.

5.57. [4-(Benzyloxy)butyl]hydrazine hydrochloride (21)

To a mixture of hydrazine hydrate (11.6 g, 231 mmol) and sodium hydroxide (2.01 g, 50 mmol) was added [(4-chlorobutoxy)methyl]benzene (10 g, 50 mmol) at 95 °C. The mixture was stirred for 2 h at the temperature, and concentrated under reduced pressure. To the residue was added concentrated HCl (5 mL) and water (120 mL). The mixture was extracted with diethyl ether. The organic layer was concentrated under reduced pressure to give **21** (9.6 g, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50–1.77 (4H, m), 2.86–3.05 (2H, m), 3.43 (2H, t, J = 5.4 Hz), 4.45 (2H, s), 6.60 (3H, br s), 7.23–7.37 (5H, m).

5.58. 4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (22)

The compound **22** were prepared in a manner similar to that described for **14b**. Yield (98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.25 (3H, s), 7.01–7.17 (2H, m), 7.65–7.81 (2H, m), 8.97 (1H, br s).

5.59. 4-Bromo-1-butyl-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (23a)

To a stirred mixture of **22** (600 mg, 3.41 mmol), butan-1-ol (523 mg, 7.06 mmol) and triphenylphosphine (1.8 g, 6.86 mmol) in THF (6 mL) was added dropwise diisopropyl azodicarboxylate in toluene (1.9 M, 3.7 mL, 7.03 mmol) at 60 °C. The mixture was stirred for 30 min, diluted with water, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was suspended in IPE, and the solid was filtered off. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel with hexane/EtOAc as an eluent to give **23a** (211 mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.73 (3H, t, J = 7.2 Hz), 1.03–1.17 (2H, m), 1.52–1.63 (2H, m), 2.18 (3H, s), 3.94 (2H, t, J = 7.0 Hz), 7.33–7.43 (2H, m), 7.45–7.54 (2H, m).

The compound **23b**,**c** were prepared in a manner similar to that described for **23a**.

5.60. 4-Bromo-5-(4-fluorophenyl)-1-isobutyl-3-methyl-1*H*-pyrazole (23b)

Yield (11%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.67 (6H, d, J = 6.8 Hz), 1.87–2.02 (1H, m), 2.19 (3H, s), 3.77 (2H, d, J = 7.3 Hz), 7.31–7.43 (2H, m), 7.43–7.53 (2H, m).

5.61. 4-Bromo-5-(4-fluorophenyl)-3-methyl-1-(4,4,4-trifluorobutyl)-1*H*-pyrazole (23c)

Yield (30%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.86 (2H, quin, J = 7.5 Hz), 2.07–2.18 (2H, m), 2.19 (3H, s), 4.02 (2H, t, J = 7.1 Hz), 7.28–7.45 (2H, m), 7.46–7.56 (2H, m).

5.62. 6-[1-Butyl-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (24a)

The compound **24a** were prepared in a manner similar to that described for **5**. Yield (43%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.75 (3H, t, J = 7.3 Hz), 1.04–1.25 (2H, m), 1.52–1.69 (2H, m), 2.19 (3H, s), 3.88 (2H, t, J = 7.2 Hz), 4.53 (2H, s), 6.56 (1H, dd, J = 8.3, 2.1 Hz), 6.65 (1H, d, J = 2.1 Hz), 6.81 (1H, d, J = 8.3 Hz), 7.21–7.34 (4H, m), 10.60 (1H, s). Anal. Calcd for $C_{22}H_{22}FN_3O_2$: C, 69.64; H, 5.84; N, 11.07. Found: C, 69.63; H, 5.72; N, 11.1.

5.63. 6-[5-(4-Fluorophenyl)-1-isobutyl-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (24b)

The compound **24b** were prepared in a manner similar to that described for **5**. Yield (43%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.70 (6H, d, J = 6.8 Hz), 1.88–2.16 (1H, m), 2.19 (3H, s), 3.71 (2H, d, J = 7.2 Hz), 4.53 (2H, s), 6.56 (1H, dd, J = 8.3, 1.9 Hz), 6.65 (1H, d, J = 1.9 Hz), 6.81 (1H, d, J = 8.3 Hz), 7.24–7.32 (4H, m), 10.60 (1H, s). Anal. Calcd for $C_{22}H_{22}FN_3O_2$: C, 69.64; H, 5.84; N, 11.07. Found: C, 69.45; H, 5.84; N, 11.05.

5.64. 6-[5-(4-Fluorophenyl)-3-methyl-1-(4,4,4-trifluorobutyl)-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (24c)

The compound **24c** were prepared in a manner similar to that described for **5**. Yield (5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.89 (2H, m), 2.10–2.33 (5H, m), 3.96 (2H, t, J = 7.0 Hz), 4.53 (2H, s), 6.58 (1H, dd, J = 8.3, 1.9 Hz), 6.66 (1H, d, J = 1.9 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.22–7.40 (4H, m), 10.60 (1H, s). Anal. Calcd for $C_{22}H_{19}N_3O_2F_4$: C, 60.97; H, 4.42; N, 9.70. Found: C, 60.59; H, 4.16; N, 9.65.

5.65. 1-(2-Azidoethyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (25)

A mixture of **13j** (5.35 g, 24.3 mmol) and *p*-toluenesulfonyl chloride (6.0 g, 31.5 mmol) in pyridine (30 mL) was stirred at room temperature for 12 h, diluted with 1 N HCl, and extracted with EtOAc. The organic layer was washed with 1 N HCl and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. To the residue was added DMF (30 ml) and NaN_3 (2.05 g, 31.5 mmol) at room temperature, successively. The mixture was stirred at 50 °C for 6 h and at room temperature for 2 days, diluted saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic

layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc as an eluent to give **25** (4.94 g, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20 (3H, s), 3.66 (2H, t, J = 5.5 Hz), 4.14 (2H, t, J = 5.5 Hz), 6.18 (1H, s), 7.34 (2H, t, J = 9.0 Hz), 7.51 (2H, dd, J = 9.0, 5.5 Hz).

5.66. *N*-{2-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]ethyl}acetamide (26a)

To a solution of **25** (1.00 g, 4.08 mmol) in THF (25 mL) was added triphenylphosphine (1.28 g, 4.88 mmol) at room temperature. The mixture was stirred at room temperature for 3 h, and then water (360 μL, 20.0 mmol) was added. The resulting mixture was stirred at 50 °C for 12 h, and diluted with 1 N HCl and EtOAc. The aqueous layer was separated, basified with 8 N NaOH, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in pyridine (15 mL), and then acetic anhydride (10 mL), and the mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in DMF (30 mL). To the resulting solution was added NBS (871 mg, 4.89 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min, diluted with aqueous $Na_2S_2O_3$ solution, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc as an eluent to give **26a** (1.30 g, 94% in 3 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65 (3H, s), 2.20 (3H, s), 3.27 (2H, q, J = 6.0 Hz), 3.97 (2H, t, J = 6.0 Hz), 7.37 (2H, t, J = 9.0 Hz), 7.45 (2H, dd, J = 9.0, 5.5 Hz), 7.87 (1H, t, J = 6.0 Hz).

5.67. *N*-{2-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]ethyl}methanesulfonamide (26b)

A mixture of **25** (3.84 g, 15.7 mmol) and NBS (3.35 g, 18.8 mmol) in DMF (50 mL) was stirred at room temperature for 30 min, diluted with aqueous $Na_2S_2O_3$ solution, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc as an eluent to give 1-(2-azidoethyl)-4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (4.94 g, 97%). To a solution of 1-(2-azidoethyl)-4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (800 mg, 2.46 mmol) in THF (20 mL) was added triphenylphosphine (774 mg, 2.95 mmol) at room temperature. The mixture was stirred at room temperature for 3 h, and then water (220 μL, 12.2 mmol) was added. The resulting mixture was stirred at 50 °C for 12 h, diluted with ethyl acetate and 1 N HCl. The aqueous layer was separated, basified with 8 N NaOH, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in pyridine (5 mL), and then methanesulfonyl chloride (285 μL, 3.68 mmol) was added. The mixture was stirred at room temperature for 12 h, diluted with 1 N HCl, and extracted with EtOAc. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc as an eluent to give **26b** (760 mg, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20 (3H, s), 2.79 (3H, s), 3.27 (2H, t, J = 7.0 Hz), 4.02 (2H, t, J = 7.0 Hz), 7.14 (1H, br s.), 7.38 (2H, t, J = 9.0 Hz), 7.51 (2H, dd, J = 9.0, 5.5 Hz).

5.68. *N*-{2-[5-(4-Fluorophenyl)-3-methyl-4-(3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-6-yl)-1*H*-pyrazol-1-yl]ethyl}acetamide (27a)

The compound **27a** were prepared in a manner similar to that described for **5**. Yield (44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.69 (3H, s), 2.20 (3H, s), 3.32 (2H, q, J = 6.0 Hz), 3.90 (2H, t, J = 6.0 Hz), 4.53 (2H, s), 6.56 (1H, dd, J = 8.5, 2.0 Hz), 6.66 (1H, d, J = 2.0 Hz), 6.81 (1H, d, J = 8.5 Hz), 7.20–7.34 (4H, m), 7.94 (1H, t, J = 6.0 Hz), 10.61 (1H, br s). Anal. Calcd for $C_{22}H_{21}N_4O_3F$: C, 64.70; H, 5.18; N, 13.72. Found: C, 65.05; H, 5.53; N, 13.38.

5.69. *N*-{2-[5-(4-Fluorophenyl)-3-methyl-4-(3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-6-yl)-1*H*-pyrazol-1-yl]ethyl}methanesulfonamide (27b)

The compound **27b** were prepared in a manner similar to that described for **5**. Yield (33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20 (3H, s), 2.81 (3H, s), 3.25–3.33 (2H, m), 3.97 (2H, t, J = 6.5 Hz), 4.53 (2H, s), 6.57 (1H, dd, J = 8.5, 2.0 Hz), 6.61–6.68 (1H, m), 6.82 (1H, d, J = 8.5 Hz), 7.18 (1H, t, J = 6.0 Hz), 7.22–7.41 (4H, m), 10.60 (1H, br. s). Anal. Calcd for $C_{21}H_{21}N_4O_4SF$: C, 56.75; H, 4.76; N, 12.61. Found: C, 56.46; H, 4.70; N, 12.29.

5.70. 3-[5-(4-Fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propanenitrile (28)

The compound **28** were prepared in a manner similar to that described for **14b**. Yield (69%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.19 (3H, s), 3.68 (2H, q, J = 5.5 Hz), 3.965 (2H, t, J = 5.5 Hz), 4.89 (1H, t, J = 5.5 Hz), 7.32–7.43 (2H, m), 7.50–7.61 (2H, m).

5.71. 3-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propanenitrile (29)

To a solution of **28** (1.0 g, 3.34 mmol), tributylphosphine (1.25 mL, 5.01 mmol), 90% acetone cyanohydrin (0.44 mL, 4.34 mmol) in toluene (25 mL) was added 1,1′-(azodicarbonyl)dipiperidine (1.3 g, 5.01 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h, diluted with water, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc to give 29 (781 mg, 76%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21 (3H, s), 2.98 (2H, t, J = 6.5 Hz), 4.18 (2H, t, J = 6.5 Hz), 7.37–7.46 (2H, m), 7.47–7.56 (2H, m).

5.72. 3-[5-(4-Fluorophenyl)-3-methyl-4-(3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-6-yl)-1*H*-pyrazol-1-yl]propanenitrile (30)

The compound **30** were prepared in a manner similar to that described for **5**. Yield (43%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21 (3H, s), 3.01 (2H, t, J = 6.5 Hz), 4.12 (2H, t, J = 6.5 Hz), 4.54 (2H, s), 6.59 (1H, dd, J = 8.5, 2.0 Hz), 6.66 (1H, d, J = 2.0 Hz), 6.83 (1H, d, J = 8.5 Hz), 7.23–7.40 (4H, m), 10.60 (1H, br s). Anal. Calcd for $C_{21}H_{17}N_4O_2F$: C, 67.01; H, 4.55; N, 14.89. Found: C, 66.67; H, 4.68; N, 14.49.

5.73. 1-(Benzyloxy)-3-[5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propan-2-ol (32b)

To hydrazine monohydrate (100 g) was added dropwise benzyl glycidyl ether (25.0 g, 152 mmol) at 60 °C. The mixture was stirred at 60 °C for 3 h, and then concentrated under reduced pressure to give 1-(benzyloxy)-3-hydrazinopropan-2-ol (29.6 g). The crude 1-(benzyloxy)-3-hydrazinopropan-2-ol (6.60 g, 33.6 mmol) was added to a solution of **12a** (5.00 g, 27.8 mmol) in methanol

(50 mL) and conc. hydrochloric acid (2.80 mL) under ice-cooling. The mixture was stirred at room temperature for 12 h, and concentrated under reduced pressure. To the residue was added water and EtOAc. The aqueous layer was basified with potassium carbonate, and then organic layer was separated. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc to give **32b** as an oil (6.21 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.29 (3H, s), 3.29–3.38 (1H, m, J = 9.5, 6.7 Hz), 3.48–3.56 (1H, m), 4.06–4.25 (3H, m), 4.45 (2H, s), 4.53 (1H, s), 6.07 (1H, s), 7.00–7.10 (2H, m), 7.15–7.22 (2H, m), 7.25–7.41 (5H, m).

The compound **32a**,**c** were prepared in a manner similar to that described for **32b**.

5.74. 1-(Benzyloxy)-3-[5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propan-2-ol (32a)

The crude product was used for the next reaction.

5.75. 4-(Benzyloxy)-1-[5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-ol (32c)

Yield (47%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.62–1.73 (2H, m), 2.29 (3H, s), 3.53–3.69 (2H, m), 3.91–4.01 (1H, m), 4.10 (1H, dd, J = 14.0, 3.0 Hz), 4.15–4.26 (1H, m), 4.45 (2H, s), 4.48 (1H, d, J = 3.4 Hz), 6.06 (1H, s), 7.03–7.14 (2H, m), 7.22–7.41 (7H, m).

The compound **33a**–**c** were prepared in a manner similar to that described for **14b**.

5.76. 1-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-ol (33a)

Yield (4% in 2 steps).^{1}H NMR (300 MHz, DMSO-d_6) δ 0.79 (3H, t, J = 7.3 Hz), 1.11–1.41 (2H, m), 2.19 (3H, s), 3.69–3.88 (3H, m), 4.87 (1H, d, J = 5.1 Hz), 7.37 (2H, t, J = 8.9 Hz), 7.56 (2H, dd, J = 8.9, 5.6 Hz).

5.77. 1-(Benzyloxy)-3-[4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propan-2-ol (33b)

Yield (98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28 (3H, s), 3.33–3.42 (1H, m), 3.45–3.53 (1H, m), 3.90–4.22 (4H, m), 4.46 (2H, s), 7.04–7.44 (9H, m).

5.78. 4-(Benzyloxy)-1-[4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-ol (33c)

Yield (97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62–1.72 (2H, m), 2.28 (3H, s), 3.53–3.68 (2H, m), 3.88–3.98 (1H, m), 4.03 (1H, dd, J = 13.8, 3.2 Hz), 4.14–4.26 (1H, m), 4.44 (2H, s), 7.09–7.19 (2H, m), 7.21–7.44 (7H, m).

5.79. 1-(Benzyloxy)-3-[4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]acetone (34b)

To a solution of **33b** (1.50 g, 3.58 mmol) in toluene (30 mL) was added Dess–Martin periodinane (2.00 g, 4.72 mmol) under ice-cooling. The mixture was stirred at room temperature for 12 h, diluted with EtOAc and a solution of sodium thiosulfate pentahydrate (6.90 g) in saturated $NaHCO_3$, and then stirred for 30 min. The organic layer was separated, washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc to give **34b** (1.39 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.29 (3H, s), 4.05 (2H, s), 4.51 (2H, s), 4.98 (2H, s), 7.08–7.17 (2H, m), 7.22–7.43 (7H, m).

The compound **34a**,**c** were prepared in a manner similar to that described for **34b**.

5.80. 1-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-one (34a)

Yield (94%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (3H, t, J = 7.2 Hz), 2.18 (3H, s), 2.37 (2H, q, J = 7.2 Hz), 4.99 (2H, s), 7.31–7.47 (4H, m).

5.81. 4-(Benzyloxy)-1-[4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-one (34c)

Yield (99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s), 2.61 (2H, t, J = 6.0 Hz), 3.69 (2H, t, J = 6.0 Hz), 4.43 (2H, s), 4.82 (2H, s), 6.99–7.10 (2H, m), 7.19–7.38 (7H, m).

5.82. 1-[3-(Benzyloxy)-2,2-difluoropropyl]-4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (35b)

To a solution of **34b** (890 mg, 2.13 mmol) in toluene (20 mL) was added dropwise diethylamino sulfur trifluoride (700 mg, 4.34 mmol) under ice-cooling. The mixture was stirred at 40 °C for 6 h, and then diethylamino sulfur trifluoride (350 mg, 2.17 mmol) was further added. The mixture was stirred at 40 °C for 24 h, treated with saturated $NaHCO_3$ solution with ice-cooling, and extracted with EtOAc. The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc as an elutant to give **35b** (622 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s), 3.68 (2H, t, J = 12.5 Hz), 4.44 (2H, t, J = 12.7 Hz), 4.58 (2H, s), 7.09–7.19 (2H, m), 7.23–7.40 (7H, m).

The compound **35a**,**c** were prepared in a manner similar to that described for **35b**.

5.83. 4-Bromo-1-(2,2-difluorobutyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (35a)

Yield (71%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84 (3H, t, J = 7.4 Hz), 1.79 (2H, tt, J = 17.7, 7.5 Hz), 2.21 (3H, s), 4.45 (2H, t, J = 13.4 Hz), 7.34–7.43 (2H, m), 7.44–7.51 (2H, m).

5.84. 1-[4-(Benzyloxy)-2,2-difluorobutyl]-4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (35c)

Yield (39%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (2H, tt, J = 16.3, 6.3 Hz), 2.30 (3H, s), 3.60 (2H, t, J = 6.3 Hz), 4.31–4.47 (4H, m), 7.09–7.20 (2H, m), 7.22–7.39 (7H, m).

5.85. 6-{1-[3-(Benzyloxy)-2,2-difluoropropyl]-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl}-2*H*-1,4-benzoxazin-3(4*H*)-one (36b)

A mixture of **35b** (600 mg, 1.37 mmol), **15a** (490 mg, 1.78 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane adduct (220 mg, 0.27 mmol), cesium carbonate (1.20 g, 3.69 mmol) in THF/water (25 mL/5 mL) was stirred at reflux for 24 h under argon atmosphere, diluted with water and EtOAc, and then filtered. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc as an elutant, and crystallized from IPE/hexane to give **36b** (420 mg, 61%). ^{1}H

NMR (300 MHz, DMSO-d_6) δ 2.20 (3H, s), 3.74 (2H, t, J = 13.3 Hz), 4.41–4.57 (6H, m), 6.58 (1H, dd, J = 8.2, 2.0 Hz), 6.66 (1H, d, J = 2.0 Hz), 6.82 (1H, d, J = 8.2 Hz), 7.18–7.40 (9H, m), 10.61 (1H, s).

The compound **36a**,**c** were prepared in a manner similar to that described for **36b**.

5.86. 6-[1-(2,2-Difluorobutyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (36a)

Yield (40%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (3H, t, J = 7.4 Hz), 1.84 (2H, m), 2.20 (3H, s), 4.38 (2H, t, J = 13.3 Hz), 4.53 (2H, s), 6.58 (1H, dd, J = 8.1, 2.1 Hz), 6.65 (1H, d, J = 1.9 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.21–7.35 (4H, m), 10.61 (1H, s). Anal. Calcd for $C_{22}H_{20}N_3O_2F_3$: C, 63.61; H, 4.85; N, 10.12. Found: C, 63.44; H, 4.83; N, 10.03.

5.87. 6-{1-[4-(Benzyloxy)-2,2-difluorobutyl]-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl}-2*H*-1,4-benzoxazin-3(4*H*)-one (36c)

Yield (55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.11–2.31 (5H, m), 3.51 (2H, t, J = 6.4 Hz), 4.36–4.56 (6H, m), 6.58 (1H, dd, J = 8.3, 1.9 Hz), 6.65 (1H, d, J = 1.9 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.18–7.39 (9H, m), 10.61 (1H, s).

5.88. 6-[1-(2,2-Difluoro-3-hydroxypropyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (37a)

A mixture of **36b** (380 mg, 0.75 mmol), 10wt% palladium on carbon (400 mg) in MeOH/THF (8 mL/8 mL) was stirred at 50 °C for 12 h under hydrogen atmosphere, and then filtered. The filtrate was concentrated under reduced pressure, and the residue was suspended in IPE. The crystals were collected by filtration to give **37a** (275 mg, 88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20 (3H, s), 3.60 (2H, td, J = 13.9, 6.3 Hz), 4.42 (2H, t, J = 14.0 Hz), 4.53 (2H, s), 5.53 (1H, t, J = 6.3 Hz), 6.58 (1H, dd, J = 8.3, 2.1 Hz), 6.65 (1H, d, J = 2.1 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.21–7.35 (4H, m), 10.60 (1H, s). Anal. Calcd for $C_{21}H_{18}N_3O_3F_3$: C, 60.43; H, 4.35; N, 10.07. Found: C, 60.51; H, 4.32; N, 10.02,

5.89. 6-[1-(2,2-Difluoro-4-hydroxybutyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (37b)

The compound **37b** were prepared in a manner similar to that described for **37a**. Yield (70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.93–2.14 (2H, m), 2.21 (3H, s), 3.45–3.55 (2H, m), 4.43 (2H, t, J = 14.2 Hz), 4.53 (2H, s), 4.69 (1H, t, J = 5.1 Hz), 6.58 (1H, dd, J = 8.2, 2.2 Hz), 6.66 (1H, d, J = 2.2 Hz), 6.82 (1H, d, J = 8.2 Hz), 7.21–7.33 (4H, m), 10.61 (1H, s). Anal. Calcd for $C_{22}H_{20}N_3O_3F_3$: C, 61.25; H, 4.67; N, 9.74. Found: C, 61.34; H, 4.64; N, 9.62.

5.90. 3-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]propanal (38)

To a solution of **14h** (4.20 g, 13.4 mmol) in DMSO (42 mL) was added triethylamine (30 mL) at room temperature, and then pyridine sulfur trioxide complex (17.1 g, 107 mmol) was added to the mixture at room temperature. The mixture was stirred at room temperature for 1 h, poured into ice water, basified with potassium carbonate, and then extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydride Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc as an elutant to give **38** (2.95 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.26 (3H, s), 2.99 (2H, td, J = 6.7, 0.9 Hz), 4.27 (2H, t, J = 6.7 Hz), 7.15–7.25 (2H, m), 7.35–7.44 (2H, m), 9.74 (1H, t, J = 0.9 Hz).

5.91. 4-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-one (39)

To a solution of **38** (1.00 g, 3.21 mmol) in THF (10 mL) was added dropwise methylmagnesium bromide in diethyl ether (3 M, 1.60 mL, 4.8 mmol) at room temperature. The mixture was stirred at room temperature for 30 min, and then pored into the ice water. The aqueous layer was treated with conc. HCl, and then basified with 28% ammonia solution. The mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give 4-[4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-ol. To a solution of the crude 4-[4-bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]butan-2-ol in toluene (20 mL) was added Dess–Martin periodinane (1.80 g, 4.24 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h, diluted with EtOAc, treated with a solution of sodium thiosulfate pentahydrate (5.80 g) in saturated $NaHCO_3$ solution, and then stirred at room temperature for 30 min. The organic layer was separated, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/EtOAc to give **39** (0.8 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13 (3H, s), 2.27 (3H, s), 3.00 (2H, t, J = 6.9 Hz), 4.19 (2H, t, J = 6.9 Hz), 7.14–7.24 (2H, m), 7.36–7.44 (2H, m).

5.92. 4-Bromo-1-(3,3-difluorobutyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazole (40)

The compound **40** were prepared in a manner similar to that described for **35**. Yield (28%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.51 (3H, t, J = 19.2 Hz), 2.19 (3H, s), 2.26–2.46 (2H, m), 4.10 (2H, dd, J = 8.4, 6.7 Hz), 7.40 (2H, t, J = 8.9 Hz), 7.46–7.56 (2H, m).

5.93. 6-[1-(3,3-Difluorobutyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (41)

The compound **41** were prepared in a manner similar to that described for **36b**. Yield (30%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53 (3H, t, J = 19.2 Hz), 2.19 (3H, s), 2.38 (2H, m), 4.00–4.11 (2H, m), 4.53 (2H, s), 6.57 (1H, dd, J = 8.3, 2.1 Hz), 6.65 (1H, d, J = 2.1 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.24–7.39 (4H, m), 10.59 (1H, s). Anal. Calcd for $C_{22}H_{20}N_3O_2F_3$: C, 63.61; H, 4.85; N, 10.12. Found: C, 63.49; H, 4.77; N, 9.96.

5.94. 4-[4-Bromo-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-1-yl]-2,2-difluorobutyl acetate (42)

To a mixture of **39** (470 mg, 1.45 mmol) and triethylamine (4 mL) in toluene (4 mL) was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (535 mg, 2.02 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, diluted with saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. To the residue was added toluene (4 mL), lead(IV) acetate (962 mg, 2.96 mmol) and potassium bicarbonate (579 mg, 5.78 mmol) at room temperature, successively. The mixture was stirred at room temperature for 3 h, diluted with saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in THF (4 mL), and then *N*,*N*,*N*-tributylbutan-1-aminium fluoride in THF (1 M, 2.9 mL, 2.90 mmol) was added. The mixture was stirred at room temperature for 1 h, diluted with saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. To the residue was added toluene (3 mL) and

diethylamino sulfur trifluoride (436 mg, 2.70 mmol) at room temperature, successively. The mixture was stirred at 40 °C for 24 h, poured into iced-water, and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was by column chromatography on silica gel with hexane/EtOAc to give **42** (60 mg, 10% in 4 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.05 (3H, s), 2.19 (3H, s), 2.33–2.49 (2H, m), 4.13 (2H, t, J = 7.3 Hz), 4.23 (2H, t, J = 13.8 Hz), 7.35–7.46 (2H, m), 7.47–7.56 (2H, m).

5.95. 6-[1-(3,3-Difluoro-4-hydroxybutyl)-5-(4-fluorophenyl)-3-methyl-1*H*-pyrazol-4-yl]-2*H*-1,4-benzoxazin-3(4*H*)-one (43)

The compound **43** were prepared in a manner similar to that described for **36b**. Yield (34%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.19 (3H, s), 2.25–2.48 (2H, m), 3.51 (2H, td, J = 13.7, 6.3 Hz), 4.00–4.14 (2H, m), 4.53 (2H, s), 5.51 (1H, t, J = 6.2 Hz), 6.57 (1H, dd, J = 8.3, 2.1 Hz), 6.65 (1H, d, J = 2.1 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.22–7.42 (4H, m), 10.60 (1H, s). Anal. Calcd for $C_{22}H_{20}N_3O_3F_3$: C, 61.25; H, 4.67; N, 9.74. Found: C, 61.40; H, 4.65; N, 9.55.

5.96. Radioligand Binding Assay

Binding displacement assays were carried out in 96-well v-bottom polypropylene plates with a final volume of 50.5 μL of TEGM buffer (10 mM Tris–HCl (pH 7.2), 1 mM EDTA, 10% glycerol, 1 mM DTT, 1 mM 2-mercaptoethanol, 10 mM sodium molybdate, Protease inhibitor Cocktail (Roche)) containing [^{3}H]-Aldosterone (final concentration, 10 nM), serially diluted test compounds, and 0.75–1.5 mg/mL of cytosolic protein prepared from human MR transiently transfected FreeStyle™ 293 cells (Invitrogen). Each concentration was run in duplicate. The cytosols were incubated for 16 h at 4 °C. Unbound radioactivity was removed by the addition of 35 μL of dextran/gelatin coated charcoal suspension (5% charcoal, 0.5% dextran T-70 (GE Healthcare UK Ltd), 0.1% gelatin (Sigma–Aldrich Co.), and 10 mM Tris–HCl (pH 7.2), 1 mM EDTA). The mixture was incubated for 10 min at 4 °C and centrifuged at 910 × g for 10 min at 4 °C. Then 30 μL of the supernatant from each well was transferred to a 96-well white plate with 150 μL of scintillation fluid and the radioactivity was measured by TopCount™ (PerkinElmer Inc.). For the determination of non-specific binding, cold aldosterone instead of drug was added to reaction mixture at 100 μM. Specific binding was determined by subtracting the value of non-specific binding component from the total binding value. The raw data for the specifically bound counts were normalized between 0% and 100% activity and nonlinear fitted to a sigmoidal equation to calculate the IC_{50} values using PRISM 3.0 (GraphPad Software Inc.). Other steroid receptor (GR, AR, and PR) binding assays were carried out by a method similar to MR binding assay except for ligands. [^{3}H]-Dexamethasone, [^{3}H]-Testosterone or [^{3}H]-Progesterone was used as a ligand in GR, AR or PR binding assay, respectively. In the case of PR binding assay, ligand concentration was 5 nM.

5.97. MR agonist/antagonist assay (luciferase reporter gene assay)

COS-1 cells were inoculated at 5 × 10^6 cells/F150 in D-MEM (low glucose) supplemented with 10% FBS and 50 mg/mL gentamicin and then cultured at 37 °C in 5% CO_2 for 1 day. To prepare DNA:transfection reagent complexes, a solution of 2.5 mL Opti-MEM, 100μL Plus Reagent (Invitrogen), 9 μg pMCMYneo-hMR, 5 μg pMAM-Luc and 1 μg pRL-TK was mixed with a solution of 2.5 mL Opti-MEM and 125 μL Lipofectamine Reagent (Invitrogen). The mixture was maintained at room temperature for 15 min. After substitution of culture media with Opti-MEM, the mixture was added to the cells. After 3 h incubation, 25 mL D-MEM (low glucose) supplemented with 0.1% BSA and 50 μg/mL gentamicin was added and then the cells were incubated at 37 °C in 5% CO_2 for 1 day. The transfected cells were harvested and re-suspended at 3.3 × 10^5 cells/mL in D-MEM (low glucose) supplemented with 0.1% BSA and 50 μg/mL gentamicin. Then, in agonist assay, 45 μL of the cell suspension was transferred in 96-well plate (corning#3688). After incubation at 37 °C in 5% CO_2 for 3 h, 5 μL/well of test compound at various concentrations was added to the cells. In antagonist assay, 40 μL of the cell suspension was transferred in 96-well plate (corning#3688). After incubation, 5 μL/well of test compound at various concentrations and 5 μL/well of aldosterone (final concentration: 1 nM) were added to the cells. After 1 day incubation at 37 °C in 5% CO_2, the medium was removed. 20 μL/well of two-fold diluted pikkagene (NIPPON GENE CO., Ltd) solution with HBSS was added to each well and the luciferase activity was measured. The data were nonlinear fitted to a sigmoidal equation to calculate the IC_{50} and its 95% confidence interval (CI).

5.98. Metabolic stability assay

In vitro oxidative metabolic studies of tested compounds were carried out using hepatic microsomes obtained from rats. The reaction mixture with a final volume of 0.1 mL consists of 0.2 mg/mL of hepatic microsome in 50 mmol/L KH_2PO_4-K_2HPO_4 phosphate buffer (pH 7.4) and 1 μmol/L test compound. After 5-min preincubation at 37 °C, the reaction was initiated by addition of an NADPH-generating system containing 50 mmol/L $MgCl_2$, 50 mmol/L gulucose-6-phosphate, 5 mmol/L β-NADP+ and 15 unit/mL glucose-6-phosphate dehydrogenase at 10% volume of reaction mixture. After the addition of NADPH-generating system, the mixture was incubated at 37C for 0 and 60 min. The reaction was terminated by addition of equivalent volume of acetonitrile. After the samples were mixed and centrifuged, the supernatant fractions were subjected to high performance liquid chromatography equipped with UV detector. Test compound in the reaction mixture was measured by HPLC system equipped with a UV detector. For metabolic stability determinations, chromatograms were analyzed for parent compound disappearance rate from the reaction mixtures. All incubations were made in duplicate.

5.99. Pharmacokinetic analysis in rats

Compounds **37a** was administered to rats (non-fasted, 8-week old) intravenously at 1 mg/kg and orally at 3 mg/kg. After intravenous and oral administration, blood samples were collected at designated time points. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.01 mol/L ammonium acetate. The compound concentrations were measured by a liquid chromatography equipped with tandem mass spectrometry.

5.100. Anti-hypertensive effect and anti-androgenic effect in DOCA-salt hypertensive rats

Male 5-week-old Wistar rats were obtained from CLEA Japan, Inc. (Tokyo, Japan). At 6 weeks of age, rats were anesthetized by injection of pentobarbiturate (50 mg/kg body wt, i.p.) for subcutaneous implantation of a 25 mg pellet of DOCA. After recovery from anesthesia, rats were housed in standard cages and maintained on standard chow and ad libitum access to both tap water and a 1% NaCl drinking solution. After 3 weeks, SBP and HR were measured by a tail-cuff method (Softron BP-98A, Softron Co.) and development of hypertension was confirmed. Hypertensive rats were

Table 5
Data collection and refinement statistics

Crystal	MR-LBD/compound **37a**
Data collection	
Space group	$P2_12_12_1$
Unit cell dimensions	
a, *b*, *c* (Å)	58.1, 66.4, 74.9
Resolution range (Å)	50–1.1 (1.14–1.10)
Observed reflections	514,047
Unique reflections	111,992
Redundancy	4.6 (2.1)
Completeness (%)	95.1 (73.6)
I/σ	12.3 (2.1)
R_{sym}[a]	0.052 (0.377)
Molecules in ASU	1
Refinement	
Resolution (Å)	40–1.10 (1.13–1.10)
Reflections	106,388
R_{work}/R_{free}[b]	0.154/0.177 (0.342/0.326)
Number of atoms	
Protein	2118
Ligand/ion	62
Water	224
Average *B* value (Å^2)	18.2
Rms deviation from ideal geometry	
Bond lengths (Å)	0.009
Bond angles (°)	1.379
Ramachandran[c] plot (%)	
Preferred regions	97.4
Allowed regions	2.6
Outliers	0
PDB code	4PF3

Values in parentheses are for the highest resolution shell.
[a] $R_{sym} = \Sigma_h\Sigma_i|I(h)_i - \langle I(h)\rangle|/\Sigma_h\Sigma_i\langle I(h)\rangle$, where $\langle I(h)\rangle$ is the mean intensity of symmetry-related reflections.
[b] $R_{work} = \Sigma||F_{obs}|_i - |F_{calc}||/\Sigma|F_{obs}|$. R_{free} was calculated for randomly chosen 5% of reflections excluded from refinement.
[c] Calculated with Coot.

divided into groups with equalization of body weight, SBP and HR. The groups of rats were treated with vehicle (0.5 w/v % methylcellulose, $n = 8$), compound **37a** ($n = 8$) and spironolactone ($n = 8$) at a dose of 100 mg/kg. Drugs were administrated orally once a day. After 7 days and 14 days of treatment, SBP and HR were measured approximately 24 h after the last dosing. After 18 days of treatment, rats were sacrificed under anesthesia and seminal vesicle weight was measured by autopsy after the measurement of body weight approximately 24 h after the last administration. Data are shown as mean ± SEM. The care and use of the animals and the experimental protocols used in the studies were reviewed and approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company (Osaka, Japan).

5.101. Crystallization and structure determination

The human MR-LBD triple mutant, 712–984 (C808S/S810L/A976V), with compound **37a** was prepared as described previously.[8] Crystals of the MR-LBD/compound **37a** complex were obtained at 20 °C by sitting-drop vapor diffusion method with the reservoir solution contained 0.1 M Tris pH 8.0, 23% ethanol. The crystals were immersed in the reservoir solution containing 25% ethylene glycol and flash-frozen with liquid nitrogen. Diffraction data were collected at the SPring-8 beamline BL41XU and processed using d*trek.[15] The structure was refined with Refmac[16] and Coot[17] using the MR-LBD structure (PDB code: 3WFG) as a start model. The dictionary file for compound **37a** was prepared using AFITT (OpenEye Scientific Software, USA). The final model was validated using Molprobity.[18] All structural figures were generated using PyMOL (Schrödinger, USA). Crystallographic processing and refinement statistics are summarized in Table 5. The coordinates and structure factors have been deposited in PDB with accession codes 4PF3.

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