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Design, synthesis and structure–activity relationships of novel benzoxazolone derivatives as 18 kDa translocator protein (TSPO) ligands

Takayuki Fukaya [a,*], Toru Kodo [b], Takeo Ishiyama [a], Hiroyoshi Kakuyama [c], Hiroyuki Nishikawa [c], Satoko Baba [a], Shuji Masumoto [a]

[a] Dainippon Sumitomo Pharma Co., Ltd, Enoki 33-94, Suita, Osaka 564-0053, Japan
[b] Dainippon Sumitomo Pharma Co., Ltd, Doshomachi 2-6-8, Chuo-ku, Osaka 541-0045, Japan
[c] Dainippon Sumitomo Pharma Co., Ltd, Kasugade Naka 3-1-98, Konohana-ku, Osaka 554-0022, Japan

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ABSTRACT

Selective 18 kDa translocator protein (TSPO) ligands are expected to be therapeutic agents with a wide spectrum of action on psychiatric disorders and fewer side effects. We designed novel benzoxazolone derivatives and examined the structure–activity relationship (SAR) of a series of compounds with various substituents at the amide part and C-5 position. Although a number of the synthesized compounds showed high TSPO binding affinity, these compounds had poor drug-like properties. Further optimization of pharmacokinetic properties of these compounds led to discovery of compound **74**, which exhibited anxiolytic effect in the rat Vogel conflict model.

1. Introduction

Benzodiazepines exhibit fast-acting and potent anti-anxiety effect and are frequently prescribed in the treatment of anxiety disorders. However, most of the clinically available agents have side effects, such as dependence, development of tolerance, and withdrawal symptoms that limit their usefulness in long-term treatment.[1] In 1977 Braestrup and Squires reported that Diazepam (**1**), a widely used benzodiazepine drug, binds to a site in peripheral tissues that is not associated with the $GABA_A$ receptor complex.[2] This binding site was previously called the peripheral benzodiazepine receptor (PBR), but was renamed based on its structure and function by Papadopoulous and colleagues in 2006 as 18 kDa translocator protein (TSPO).[3] A subsequent study revealed that TSPO is pharmacologically distinct from the central benzodiazepine receptor (CBR).[4] CBR is a part of the $GABA_A$ receptor complex and its agonists are known to allosterically potentiate the inhibitory action of GABA.[5] On the other hand, TSPO is located mainly in the outer mitochondrial membrane in peripheral tissues and central nervous system (CNS), and is therefore not linked to the $GABA_A$ receptor.[6] TSPO forms a trimeric complex with the 32 kDa voltage-dependent anion channel (VDAC) and the 30 kDa adenine nucleotide transporter (ANT).[7]

Research on the function of TSPO suggests that TSPO in the CNS plays essential roles in the synthesis of neurosteroids.[8] Transport of cholesterol from the outer to the inner mitochondrial membrane is known as the rate-determining step of this synthesis.[9] Thus, specific ligands that bind TSPO have been reported to activate cholesterol transport from the outer to the inner mitochondrial membrane[10] and increase neurosteroids concentration in the CNS.[11]

Since the depressant-like effects as well as the anxiety effects in rodent model of the neurosteroid and TSPO ligands were reported,[12] TSPO ligands are expected to have a wide range of actions on psychiatric disorders. Furthermore, AC-5216 (**7**),[13a] a TSPO ligand, was found to have anti-panic activity in human without sedation or withdrawal symptoms. This clinical finding indicates that TSPO ligands might act as anxiolytics without the side-effects of benzodiazepines. As TSPO is also currently investigated as a biomarker for brain inflammation, various TSPO ligands have been developed as neuroimaging agents.[14]

As shown in Figure 1, several specific TSPO ligands have been reported, including the benzodiazepine derivative Ro5-4864 (**2**),[15] the isoquinoline derivative PK11195 (**3**),[16] the indole FGIN-1-27 (**4**),[17] the phenoxyphenylacetamide derivatives DAA1097 (**5**) and DAA1106 (**6**),[18] and the 8-oxopurine derivative AC-5216 (**7**).[13] Among these ligands, we paid attention to Ro5-4864 (**2**), which differs from Diazepam (**1**) only by a substituent in the *para*-position at the 5-phenyl and shows high selectivity for TSPO over CBR. These properties led us to design new compounds by opening the diazepine ring of Ro5-4864 (**2**) as shown in Figure 2. Based on preliminary structure–activity relationship (SAR) studies of the obtained heteroaryls,[19] the benzoxazolone scaffold proved to

* Corresponding author. Tel.: +81 6 6337 5865; fax: +81 6 6337 6010.
E-mail address: takayuki-fukaya@ds-pharma.co.jp (T. Fukaya).

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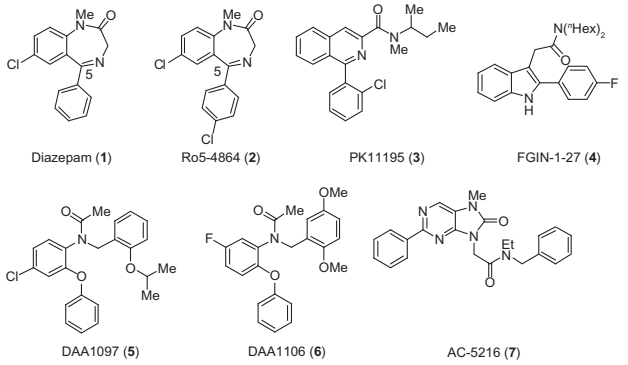


Figure 1. Chemical strucuture of selected TSPO ligands.

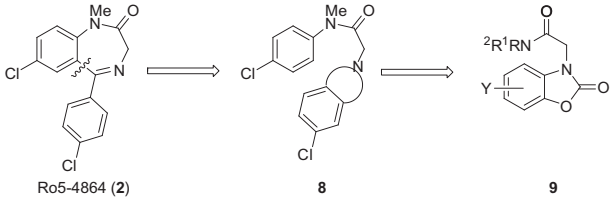
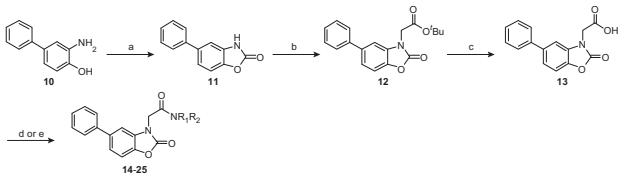


Figure 2. Design of novel TSPO ligands based on Ro5-4864 (**2**).

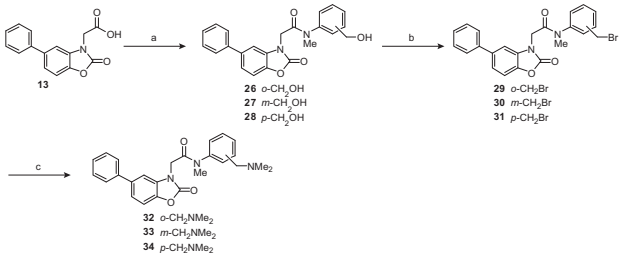
be promising for further optimization. Benzoxazolone derivative's potent activity for TSPO was rationalized in the light of a pharmacophore model made up of three lipophilic pockets and a hydrogen-donor group.[20] Although Ro5-4864 (**2**) binding to TSPO differs among species (30-fold lower affinity for the human TSPO than that for the rat TSPO),[4a] our novel benzoxazolone derivatives exhibited almost similar binding affinity for both rat and human TSPO. Here, we describe in details the SAR of a series of benzoxazolone derivatives as novel TSPO ligands, and show the anxiolytic effect of a selected compound (**74**) in a rodent model.

2. Chemistry

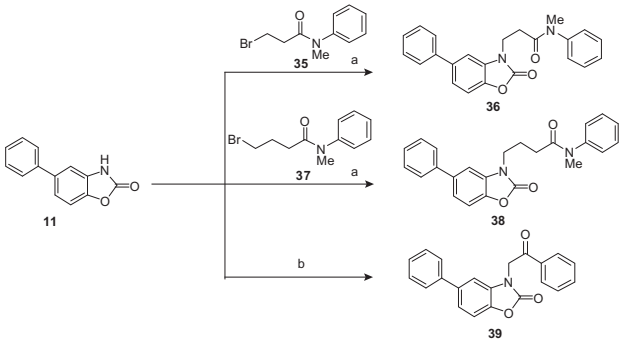
The synthesis of a series of 5-phenyl benzoxazolone derivatives was achieved as illustrated in Schemes 1 and 2. Compound **10** was cyclized by 1,1′-carbonyldiimidazole (CDI) followed by *N*-alkylation to afford the acetate **12**. Deprotection of compound **12** with hydrochloric acid provided the key intermediary acetic acid derivative **13**. Condensation of **13** with appropriate amines was carried out by combination of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC·HCl) and 1-hydroxybenzoriazole (HOBt) or by acid chloride method using oxalyl chloride to afford the desired amide derivatives **14**–**25**. The chemical structures of compounds **14**–**25** are shown in Tables 1 and 3. Compounds **32**–**34** were prepared by transformation of the substituent on the phenyl ring after condensation. Amidation of **13** with anilines gave the



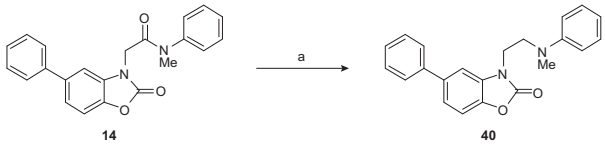
Scheme 1. Synthesis of 5-phenyl benzoxazolone derivatives **14–25**. Reagents and conditions: (a) CDI, THF, reflux; (b) *t*-butyl bromoacetate, K_2CO_3, acetone, rt; (c) HCl/dioxane, AcOH, 50 °C; (d) amine, WSC·HCl, HOBt, DMF, rt; (e) $(COCl)_2$, DMF, CH_2Cl_2, then amine, Et_3N, THF, rt.



Scheme 2. Synthesis of 5-phenyl benzoxazolone derivatives **32–34**. Reagents and conditions: (a) amine, WSC·HCl, DMF, rt; (b) CBr_4, PPh_3, CH_3CN, rt; (c) 50% Me_2NH/H_2O, DMF, rt.



Scheme 3. Synthesis of **36**, **38** and **39**. Reagents and conditions: (a) K_2CO_3, DMF, 60–80 °C; (b) 2-bromoacetophenone, K_2CO_3, DMF, rt.



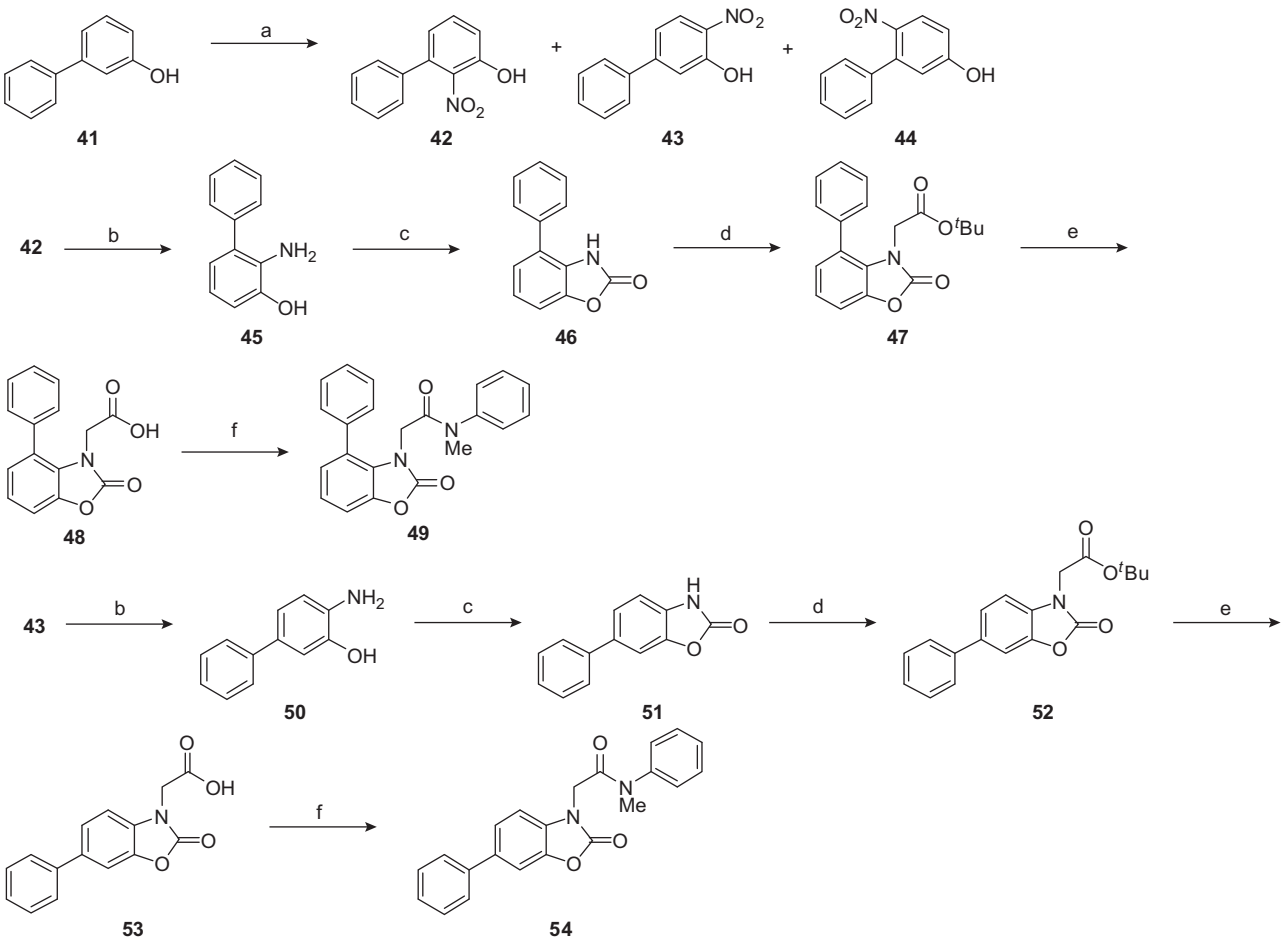
Scheme 4. Synthesis of **40**. Reagents and conditions: (a) BH_3·THF/THF, reflux.

alcohol derivatives **26**–**28**. Treatment of **26**–**28** with CBr_4 in the presence of PPh_3 followed by reaction with dimethylamine produced the targeted compounds **32**–**34**.

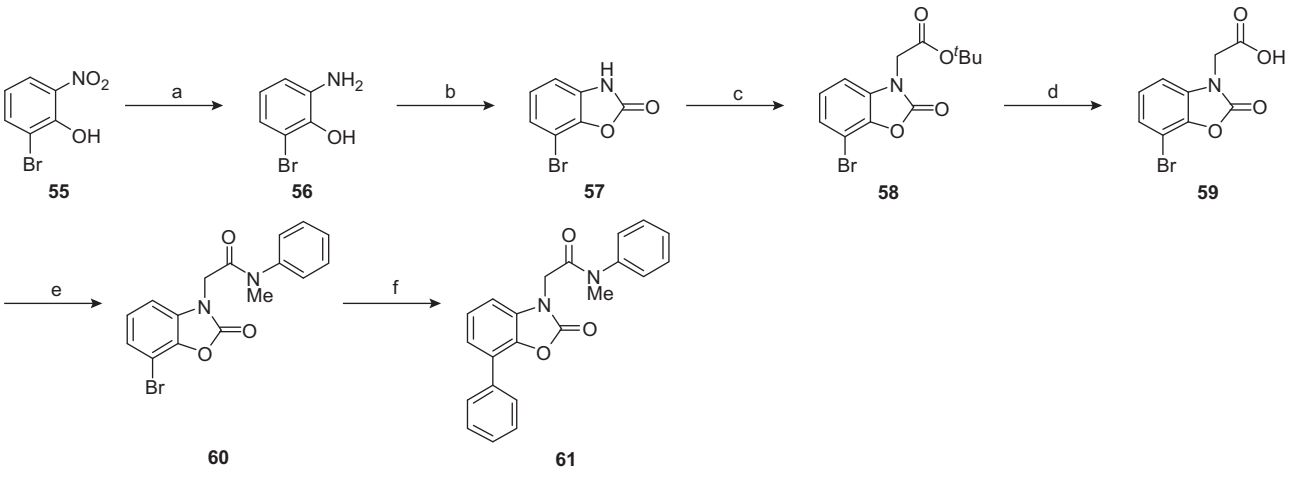
Compounds **36**, **38** and **39** were synthesized as shown in Scheme 3. *N*-alkylation of **11** with **35** and **37**, using potassium carbonate as a base in DMF afforded **36** and **38**, respectively. In a similar way, the ketone **39** was prepared by *N*-alkylation of **11** with bromoacetophenone. The synthesis of compound **40** was accomplished by reduction of the amide moiety of compound **14** as illustrated in Scheme 4.

Scheme 5 depicts the preparation of C-4 and C-6 phenyl derivatives. Nitration[21] of the 3-hydroxybiphenyl **41** with 6 N nitric acid in acetic acid afforded **42**, **43**[22] and **44**[23] in 14%, 26% and 41% yield, respectively, by silica gel column chromatography. The aminophenol **45** was synthesized by palladium-catalyzed hydrogenation of the nitrophenol **42**. In a similar way, compound **50**[24] was prepared from **43**. The amide derivatives **49** and **54** were obtained from **45** and **50**, respectively, by a method similar to that for the synthesis of **14**.

Scheme 6 shows the preparation of C-7 substituted derivatives. Compound **55** was reduced to **56** by treatment with Fe in AcOH at 90 °C. Cyclization of **56** by CDI generated the benzoxazolone derivative **57**. Introduction of the ester moiety was achieved by *N*-alkylation of **57** with *t*-butyl bromoacetate followed by acidic hydrolysis of **58** to give compound **59**. Condensation of **59** with *N*-methylaniline was carried out in the presence of



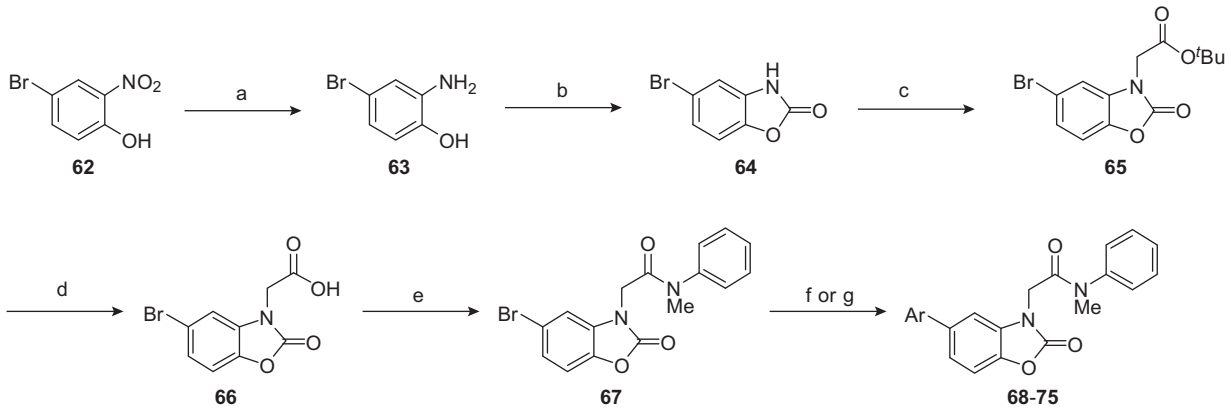
Scheme 5. Synthesis of 4-phenyl benzoxazolone (**49**) and 6-phenyl benzoxazolone (**54**). Reagents and conditions: (a) 6 N HNO_3 aq, AcOH, rt; (b) H_2, 10% Pd-C, MeOH, THF, rt; (c) CDI, THF, rt; (d) *t*-butyl bromoacetate, K_2CO_3, DMF, 60 °C; (e) HCl/dioxane, AcOH, 50 °C; (f) *N*-methylaniline, WSC·HCl, HOBt, DMF, rt.



Scheme 6. Synthesis of 7-phenyl benzoxazolone (**61**). Reagents and conditions: (a) Fe, AcOH, 90 °C; (b) CDI, THF, rt; (c) *t*-butyl bromoacetate, K_2CO_3, DMF, rt; (d) HCl/dioxane, AcOH, 50 °C; (e) *N*-methylaniline, WSC·HCl, HOBt, DMF, rt; (f) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, H_2O, reflux.

WSC·HCl and HOBt in DMF to afford **60**, which was subjected to Suzuki–Miyaura coupling[25] with phenylboronic acid to give the desired compound **61**.

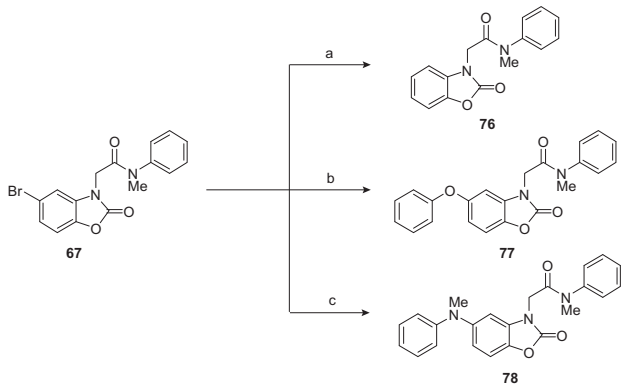
Scheme 7 illustrates the synthesis of a series of benzoxazolone derivatives with a substituent at the C-5 position. Reduction of the nitro group of compound **62** was achieved chemoselectively by hydrogenation in the presence of a catalytic amount of Rh-C[26] in THF to provide compound **63**.[27] Compound **67** was obtained from **63** by multi-step reactions similar to those for the synthesis of **60**. The benzoxazolone derivative **67** served as a versatile interme-



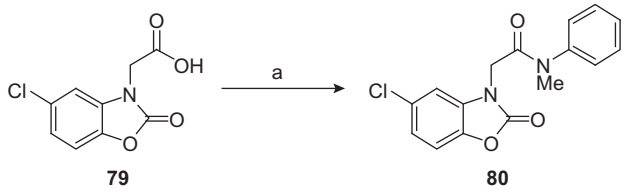
Scheme 7. Synthesis of benzoxazolone derivatives with various substituents at the C-5 position. Reagents and conditions: (a) H_2, 5% Rh-C, THF, rt; (b) CDI, THF, rt; (c) *t*-butyl bromoacetate, K_2CO_3, DMF, rt; (d) HCl/dioxane, AcOH, 50 °C; (e) *N*-methylaniline, WSC·HCl, HOBt, DMF, rt; (f) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, H_2O, reflux; (g) $ArSnBu_3$, $Pd(PPh_3)_4$, toluene, reflux.

diate for derivatives with a substituent at the C-5 position. The targeted compounds **68**–**75** were prepared via Suzuki–Miyaura coupling or Stille coupling[28] of **67** with boronic acid or an organotin reagent. The chemical structures of compounds **68**–**75** are shown in Table 4.

Compounds **76**–**78** were synthesized as shown in Scheme 8. The bromine atom in compound **67** was removed by palladium-catalyzed hydrogenation to give compound **76**. Compound **77** was obtained by Ullmann coupling[29] of **67** with phenol. Synthesis of **78** was achieved by coupling reaction with *N*-methylaniline in the presence of $Pd_2(dba)_3$ and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos) using Cs_2CO_3 as a base in toluene.[30] Compound **80** was prepared by condensation of the commercially available compound **79** with *N*-methylaniline as illustrated in Scheme 9.



Scheme 8. Synthesis of **76**, **77** and **78**. Reagents and conditions: (a) H_2, 10% Pd-C, MeOH, rt; (b) PhOH, CuO, K_2CO_3, pyridine,reflux; (c) *N*-methylaniline, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, toluene, reflux.



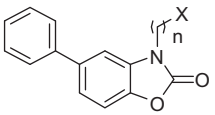
Scheme 9. Synthesis of **80**. Reagents and conditions: (a) *N*-methylaniline, WSC·HCl, HOBt, DMF, rt.

3. Results and discussion

Affinity of the prepared benzoxazolone derivatives for TSPO and CBR was evaluated by measuring each compound's ability to displace [^{3}H]-PK11195 and [^{3}H]-flumazenil from binding to membranes prepared from rat kidney and rat cerebral cortex, respectively. All tested compounds had only negligible affinity for CBR (<50% inhibition at 10,000 nM). The results for TSPO binding are shown in Table.

A preliminary SAR study of the hydrogen bond acceptor part revealed a tertiary acetamide as a key structural moiety for TSPO binding (Table 1). The acetamide **14** exhibited a high affinity for TSPO (98% inhibition at 100 nM, K_i = 1.6 nM). On the other hand, replacing the acetamide moiety with alternative hydrogen bond acceptor groups (**12**, **39**) led to significant loss of TSPO binding. In addition, deletion of the amide moiety in compound **14** to obtain **40** caused a significant decrease in TSPO affinity. Next, we examined the effect of a change in the linker between the amide group and the benzoxazolone ring on TSPO binding. The results obtained with compounds **36** and **38** indicated that elongation of the alkyl linker between the amide group and the benzoxazolone skeleton decreases TSPO binding activity. As expected, the carbamoyl **15** and secondary amide **16** showed low affinity for TSPO with 3% and 19% inhibition, respectively. These findings were consistent

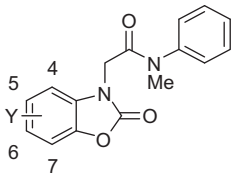
Table 1
Inhibitory activity of benzoxazolone derivatives bearing various hydrogen bond acceptor parts on TSPO



Compd	*n*	X	TSPO inhibition[a] (%)
14	1	CONMePh	98 (K_i[b] = 1.6 nM)
12	1	COO^{t}Bu	0
13	1	COOH	0
39	1	COPh	14
40	1	CH_2NMePh	0
15	1	$CONH_2$	3
16	1	CONHPh	19
36	2	CONMePh	6
38	3	CONMePh	0

[a] Percent inhibition of [^{3}*H*]-PK11195 specific binding at 100 nM of the compound.
[b] K_i value obtained from four concentrations of each compound (experiments run in duplicate).

Table 2
TSPO binding affinity of benzoxazolone derivatives with different substitution sites



Compd	Y	TSPO K_i[a] (nM)
PK11195 (**3**)		1.7
Ro5-4864 (**2**)		6.8
76	H	11
49	4-Ph	120
14	5-Ph	1.6
54	6-Ph	29
61	7-Ph	9.0

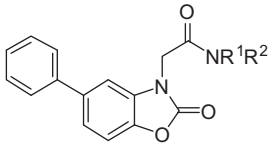
[a] K_i values represent the means of 1–3 separate experiments run in duplicate using four concentrations of each compound.

with those reported by Primofiore and colleagues.[31] Put together, the results above indicate that disubstitution at the acetamide nitrogen is important for interaction with the binding site of TSPO.

As mentioned above, a pharmacophore model study led us to conclude that binding to TSPO requires three hydrophobic groups and a hydrogen bond acceptor group within a ligand molecule. Accordingly, we predicted that a substituent on the benzoxazolone derivative plays an important role in TSPO binding as one of the required hydrophobic groups and examined the effect of changing the substituted position on the benzoxazolone ring. As shown in Table 2, introduction of a phenyl ring at the C-5 position to obtain **14** enhanced the binding affinity by sevenfold compared to that of **76**. Although the 7-Ph derivative **61** retained the affinity for TSPO (K_i = 9.0 nM), compound **49** with a substituent at the C-4 position showed reduced affinity (K_i = 120 nM). Affinity for TSPO of the 6-Ph derivative was threefold lower than that of **76**. Based on these findings we speculated that the position of the substituent on the benzoxazolone ring is important for TSPO binding.

Using the results of the above initial SAR survey, we explored the possibility of finding a novel TSPO ligand using **14** as lead compound. In our investigation of the amide part, we maintained the phenyl at the C-5 position of the benzoxazolone and explored various substituents on the acetamide nitrogen. TSPO affinity and metabolic stability of the resulting compounds are shown in Table 3. First, we converted R^1 and R^2 from an alkyl–phenyl to an alkyl–alkyl and alkyl–benzyl, respectively. Compound **17** ($R^1 = R^2 = {}^n$Pr) and **18** (R^1 = Me, R^2 = Bn) exhibited moderate affinity for TSPO, albeit lower than that of compound **14** (R^1 = Me, R^2 = Ph). We next examined the effect of a substitution at the benzene ring of the amide moiety. Introduction of a methoxy group or a chlorine atom onto the phenyl ring of the amide were favorable, yielding compounds (**19**–**22**) with improved or comparable affinity to that of compound **14**. In case of compounds with a substituent at the *para*-position, there were obvious differences in affinity depending on the kind of substituent (**20**: K_i = 2.2 nM vs **22**: K_i = 0.21 nM). Although a series of compounds with excellent TSPO affinity and selectivity were obtained, these potent compounds suffered from poor solubility (**14**: <0.001 mg/mL, at pH 7.4), which is a drawback for oral bioavailability. We therefore focused on the design of compounds with a hydrophilic group. In an attempt to improve aqueous solubility, we examined the effect of replacing the phenyl ring of the amide part with a pyridine. Although compounds **23**–**25** were found to have at least 10-fold less TSPO affinity than compounds **14**, compounds **23** and **24** exhibited moderate affinity with K_i values of 23 and 28 nM, respectively. As expected, the aqueous solubility of **24** was improved (0.045 mg/mL, at pH 7.4). For the same purpose, we prepared compounds with an amino substituent at the phenyl ring of the amide part of **32**–**34**. The results showed that a substitution at the *ortho*-position was preferred for better affinity, as moving the amino group of compound **32** to the *meta*-position (**33**) or *para*-position (**34**) resulted in a decrease in affinity. Surprisingly, compound **32** exhibited strong TSPO affinity, in spite of bearing a hydrophilic group. These finding suggested that introduction of a hydrophilic group as substituent onto the *ortho*-position of the phenyl ring in the amide part would be tolerated. Unfortunately improvement of metabolic stability of the

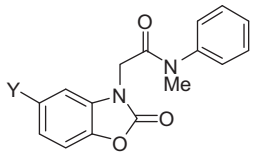
Table 3
TSPO and CBR binding activity, and metabolic stability of benzoxazolone derivatives with various amide moieties



Compd	R^1	R^2	TSPO K_i[a] (nM)	CBR inhibition[b] (%)	Metabolic stability[c] (remaining %)
14	Me	Ph	1.6	5	1
17	*n*-Pr	*n*-Pr	17	2	0[d]
18	Me	Bn	13	36	1
19	Me	*m*-MeO-Ph	0.90	0	0
20	Me	*p*-MeO-Ph	2.2	0	5[d]
21	Me	*m*-Cl-Ph	0.79	0	0
22	Me	*p*-Cl-Ph	0.21	0	0
23	Me	2-Py	23	9	0
24	Me	3-Py	28	29	0
25	Me	4-Py	270	2	N.T.[e]
32	Me	*o*-(Me_2NCH_2)-Ph	2.0	0	0
33	Me	*m*-(Me_2NCH_2)-Ph	12	N.T.	N.T.
34	Me	*p*-(Me_2NCH_2)-Ph	88	N.T.	N.T.

[a] K_i values represent the means of 1–3 separate experiments run in duplicate using four concentrations of each compound.
[b] Percent inhibition of [^3H]-flumazenil specific binding at 10 μM of the compound.
[c] Metabolic stability data refer to percent of compound remaining after incubation with rat S-9 fraction and NADPH for 30 min. The initial concentration of each compound was 1.0 μM.
[d] Examinations were conducted at 10 μM of the compound.
[e] Not tested.

Table 4
TSPO and CBR binding activity, and metabolic stability of benzoxazolone derivatives with various substituents at the 5-position



Compd	Y	TSPO K_i[a] (nM)	CBR inhibition[b] (%)	Metabolic stability[c] (remaining %)
76	H	11	3	49
67	Br	0.18	9	23
80	Cl	0.49	0	24
14	Ph	1.6	5	1
68	*m*-MeO-Ph	0.33	3	9 [d]
69	*p*-MeO-Ph	0.29	0	1
70	*m*-CF_3-Ph	0.48	0	6[d]
71	*p*-CF_3-Ph	0.68	0	26
72	*p*-CF_3O-Ph	0.65	0	67
73	2-Py	1.8	10	0
74	3-Py	11	14	24
75	4-Py	5.3	8	27
77	PhO	3.8	2	1
78	PhNMe	0.36	10	1

[a] K_i values represent the means of 1–3 separate experiments run in duplicate using four concentrations of each compound.
[b] Percent inhibition of [^3H]-flumazenil specific binding at 10 μM of the compound.
[c] Metabolic stability data refer to percent of compound remaining after incubation with rat S-9 fraction and NADPH for 30 min. The initial concentration of each compound was 1.0 μM.
[d] Examinations were conducted at 10 μM of the compound.

compounds that exhibited high affinity for TSPO was not achieved by modification of the amide part. Based on these results, we shifted our attention to not only determining the SAR of the prepared benzoxazolone derivatives, but also improving their PK profile.

To complete our SAR analysis, we examined the effect of a substitution at the C-5 position in the benzoxazolone ring. A range of substituents on the benzene ring at the C-5 position were well tolerated (**68**–**72**). Introduction of a methoxy, a trifluoromethyl, or a trifluoromethoxy group onto the phenyl ring at the C-5 position of **14** tended to increase TSPO binding affinity, leading to potent compounds even at the subnanomolar level. Both of the *meta*-methoxy **68** and the *meta*-trifluoromethy **70** exhibited strong affinity with K_i values of 0.33 and 0.48 nM, respectively, suggesting that electron density on the benzene ring at the C-5 position does not affect interaction between TSPO and the ligand. Moreover subsequent pharmacokinetic evaluation revealed that incorporation of a *para*-electron withdrawing substituent (**71** and **72**) improved metabolic stability, albeit with poor solubility. In particular, *para*-trifluoromethoxy substitution (**72**) reduced metabolism in rat S-9 fraction with 67% remaining after 30 min and increased affinity with a K_i value of 0.65 nM. This finding encouraged us to explore further modifications of the C-5 substituent. Accordingly, we examined the effect of replacing the phenyl ring at the C-5 with a pyridine ring. The 2-pyridyl **73** exhibited high affinity for TSPO (K_i = 1.8 nM), though it did not show improved metabolic stability. In contrast, the 3-pyridyl **74** and 4-pyridyl **75** reduced metabolism in rat S-9 (**74**: 24%, **75**: 27%) with moderate TSPO binding affinity. These findings suggested that position of the basic pyridine nitrogen atom functioned as an important contributor to TSPO affinity and metabolic stability. Further profiling of the 4-pyridyl **75** revealed that this compound at 1 μM significantly inhibits cytochrome P450 in isozymes, such as 2C19 (73%) and 3A4 (50%), which could translate into potential drug–drug interaction. Additionally, we prepared compounds **77** and **78** to examine the effect of inserting a linker between the phenyl ring and the benzoxazolone ring. Introduction of an ether linker (**77**) and an *N*-alkyl linker (**78**) was favorable, albeit with no improvement in metabolic stability. Surprisingly, compound **67** which was prepared as intermediate for the C-5 substituted derivatives exhibited high affinity (K_i = 0.18 nM) and acceptable metabolic stability (23%). These results encouraged us to prepare a derivative with a chlorine atom at the C-5 position. The obtained compound **80** showed high TSPO affinity (K_i = 0.49 nM) and moderate metabolic stability (24%). Although **67** and **80** aqueous solubility was rather poor, a promising series of TSPO ligands are expected by further modification of the amide parts, while keeping a halogen atom at the C-5 position. These findings suggested that a broad range of C-5 substituents at the benzoxazolone ring would be well tolerated and that these substituents play an important role in metabolic stability. Thus, compound **74** showed good TSPO binding affinity with acceptable metabolic stability and aqueous solubility (0.005 mg/mL, at pH 7.4 and 0.440 mg/mL, at pH 2.5) and was selected for further biological evaluation.

The pharmacokinetic properties of **74** were evaluated in rats to determine whether this compound would be suitable for oral administration (Table 5). Although there is room for improvement in bioavailability and plasma clearance, compound **74** showed

Table 5
Pharmacokinetic properties of **74** in rats

Compd		Dose (mg/kg)	AUC (ng h/mL)	T_{max} (h)	C_{max} (ng/mL)	CL (mL/min/kg)	V_{dss} (L/kg)	*F* (%)	B/P[a]
74	iv	1	6042			165.5	4.4		
	po	10	8374	1	50.4			13.9	0.63

[a] B/P means brain/plasma AUC ratio after oral administration (10 mg/kg) of the HCl salt of **74**.

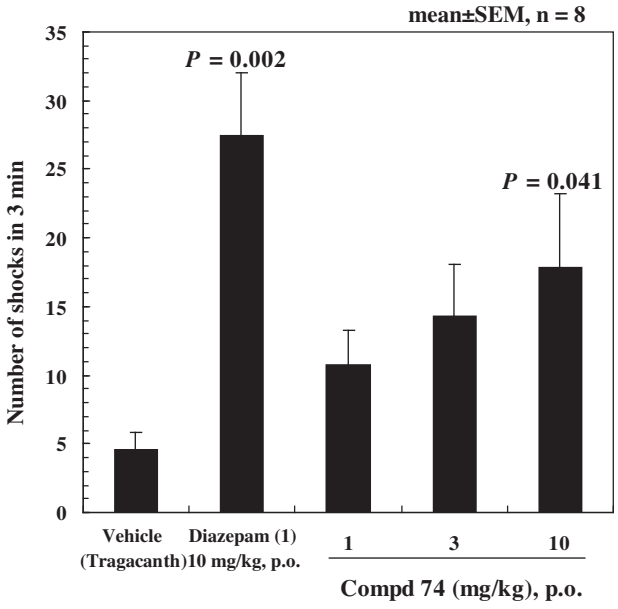


Figure 3. Results of the rat Vogel-type conflict model.

acceptable brain concentrations for in vivo studies. As illustrated in Figure 3, oral administration of **74** (10 mg/kg) resulted in an anxiolytic effect in the rat Vogel conflict model.[32] Furthermore, compound **74** exhibited high affinity for human TSPO with a K_i value of 1.7 nM, and a similar metabolic stability in human liver S-9 and rat live S-9.

4. Conclusion

We have successfully identified a series of benzoxazolone derivatives as a new class of potent TSPO ligands. We examined in this study the SAR of benzoxazolone derivatives with various substituents at the amide part and C-5 position of **14**. Our investigation led to compounds with increased TSPO binding affinity, but poor drug-like properties. Further optimization of the pharmacokinetic properties of these compounds led to the discovery of compound **74** with good TSPO binding affinity and acceptable PK profile. Oral administration of compound **74** (10 mg/kg) produced anxiolytic effect in the rat Vogel conflict model. These results encouraged us to continue our investigation of benzoxazolone derivatives as TSPO ligands with beneficial therapeutic effect on psychiatric disorders.

5. Experimental section

5.1. Chemistry

Melting points were determined on a Stanford Research Systems OptiMelt MPA100 without correction. NMR spectra were recorded at ambient temperature on a JEOL JNM-AL400 FT NMR spectrometer. Chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane as an internal standard, and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). IR spectra were recorded on a JEOL JIR-SPX60 spectrometer as ATR. High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ orbitrap Discovery MS equipment. Elemental analysis was performed on a CE Instruments EA1110 and a Yokokawa analytical system IC7000. In general, reagents and solvents were used as obtained from commercial suppliers without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck silica gel 60 F254 precoated glass plate. Visualization was done with UV light (254 nM) or iodine. Flash column chromatography was conducted using Merck silica gel 60 (70–230 mesh). All reactions were carried out under a nitrogen atmosphere unless otherwise mentioned.

5.1.1. 5-Phenyl-1,3-benzoxazol-2(3*H*)-one (11)

To a solution of 2-amino-4-phenylphenol (6.10 g, 32.9 mmol) in THF (150 mL) was added 1,1′-carbonyldiimidaziole (6.41 g, 39.5 mml) at room temperature. The mixture was stirred at reflux for 2 h and cooled to room temperature. The reaction was then quenched by adding 2 M HCl solution, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo to give **11** (6.89 g, 99%) as a white solid: mp 156–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (1H, br s), 7.56–7.52 (2H, m), 7.48–7.42 (2H, m), 7.40–7.32 (2H, m), 7.30 (1H, d, J = 1.7 Hz), 7.27 (1H, d, J = 8.3 Hz); IR (ATR) 3176, 1763, 1466, 1254, 949 cm^{-1}; HRMS (ESI) m/z calcd for $C_{13}H_8NO_2$ $[M-H]^-$ 210.0561; found 210.0556.

5.1.2. *tert*-Butyl (2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl) acetate (12)

To a suspension of **11** (3.00 g, 14.2 mmol) and K_2CO_3 (2.94 g, 21.3 mmol) in acetone (30 mL) was added *tert*-butyl bromoacetate (2.31 mL, 15.6 mmol) with cooling in an ice bath, and the mixture was stirred at room temperature for 1 day. The reaction mixture was filtrated, and the filtrate was concentrated. The resulting solid was triturated with Et_2O to give **12** (4.46 g, 97%) as a yellow solid: mp 99–100 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.50 (2H, m), 7.45 (2H, dd, J = 7.4, 7.4 Hz), 7.40–7.32 (2H, m), 7.28 (1H, d, J = 8.5 Hz), 7.05 (1H, d, J = 1.7 Hz), 4.50 (2H, s), 1.47 (9H, s); IR (ATR) 1759, 1743, 1485, 1230, 1026 cm^{-1}; HRMS (ESI) m/z calcd for $C_{19}H_{20}NO_4$ $[M+H]^+$ 326.1387; found 326.1395; Anal. Calcd for $C_{19}H_{19}NO_4 \cdot 0.25H_2O$: C, 69.18; H, 5.96; N, 4.25. Found: C, 69.32; H, 5.85; N, 4.64.

5.1.3. (2-Oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetic acid (13)

To a solution of **12** (4.00 g, 12.3 mmol) in AcOH (20 mL) was added 4 N HCl in 1,4-dioxane (12.3 mL). The reaction mixture was stirred at 50 °C for 4 h and cooled to room temperature. The solvent was removed in vacuo, and the resulting solid was triturated with Et_2O to give **13** (3.24 g, 98%) as a beige solid: mp 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.38 (1H, br s), 7.72 (1H, s), 7.68 (2H, dd, J = 8.2, 1.1 Hz), 7.51–7.42 (4H, m), 7.37 (1H, t, J = 7.3 Hz), 4.73 (2H, s); IR (ATR) 2353, 1763, 1728, 1483, 1241 cm^{-1}; HRMS (ESI) m/z calcd for $C_{15}H_{12}NO_4$ $[M+H]^+$ 270.0761; found 270.0756; Anal. Calcd for $C_{15}H_{11}NO_4$: C, 66.91; H, 4.12; N, 5.20. Found: C, 66.77; H, 4.17; N, 5.24.

5.1.4. *N*-Methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylacetamide (14)

To a solution of **13** (500 mg, 1.86 mmol) in DMF (5.0 mL) were added *N*-methylaniline (241 μL, 2.23 mmol), 1-hydroxybenzotriazole (251 mg, 1.86 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (534 mg, 2.79 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h. Water was then added, and the mixture was extracted with a mixture of toluene and EtOAc (1:1). The organic layer was washed with H_2O and brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (1:1, v/v) as eluent. The solvent was removed in vacuo, and the resulting solid was recrystallized from *i*PrOH to give **14** (547 mg, 82%) as a white solid: mp 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.49 (4H, m), 7.48–7.41 (3H, m), 7.39–

7.32 (3H, m), 7.30 (1H, dd, J = 8.3, 1.7 Hz), 7.24 (1H, d, J = 8.3 Hz), 7.04 (1H, d, J = 1.2 Hz), 4.36 (2H, s), 3.32 (3H, s); IR (ATR) 1778, 1662, 1485, 1385, 1120 cm^{-1}; HRMS (ESI) m/z calcd for $C_{22}H_{19}N_2O_3$ $[M+H]^+$ 359.1390; found 359.1381; Anal. Calcd for $C_{22}H_{18}N_2O_3{\cdot}0.25H_2O$: C, 72.81; H, 5.14; N, 7.72. Found: C, 72.85; H, 5.05; N, 7.81.

5.1.5. 2-(2-Oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (15)

To a suspension of **13** (269 mg, 1.00 mmol) in CH_2Cl_2 (3.0 mL) were added oxalyl choride (96.0 μM, 1.10 mmol) and DMF (5.0 μL) with cooling in an ice bath, and then the mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and then the residue was azeotropied with toluene. A solution of acid chloride thus obtained in THF (2.0 mL) was added to a solution of 30% ammonia solution (568 mg, 10.0 mmol) in THF (1.0 mL) at room temperature, and then the mixture was stirred at room temperature for 2 h. The reaction was quenched by adding aqueous saturated $NaHCO_3$, and then the mixture was extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using EtOAc as eluent to give **15** (262 mg, 98%) as a white solid: mp 208–209 °C (*i*PrOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (1H, br s), 7.67 (2H, d, J = 8.0 Hz), 7.56 (1H, s), 7.51–7.41 (4H, m), 7.41–7.34 (2H, m), 4.53 (2H, s); IR (ATR) 1792, 1686, 1483, 1383, 1252 cm^{-1}; HRMS (ESI) m/z calcd for $C_{15}H_{13}N_2O_3$ $[M+H]^+$ 269.0921; found 269.0920; Anal. Calcd for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.25; H, 4.64; N, 10.31.

5.1.6. 2-(2-Oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylacetamide (16)

Compound **16** was prepared from **13** (108 mg, 0.400 mmol) and aniline (43.7 μL, 0.480 mmol) in a manner similar to that described for compound **14** as a white solid (75.7 mg, 55%): mp 231–234 °C (*i*PrOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (1H, s), 7.76–7.64 (2H, m), 7.62–7.56 (2H, m), 7.56–7.39 (6H, m), 7.39–7.27 (2H, m), 7.11–7.00 (1H, m), 4.81 (1H, s), 4.54 (1H, s); IR (ATR) 1755, 1686, 1560, 1481, 1246 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{17}N_2O_3$ $[M+H]^+$ 345.1234; found 345.1228; Anal. Calcd for $C_{21}H_{16}N_2O_3{\cdot}0.25H_2O$: C, 72.30; H, 4.77; N, 8.03. Found: C, 72.54; H, 4.74; N, 8.29.

5.1.7. 2-(2-Oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*,*N*-dipropylacetamide (17)

Compound **17** was prepared from **13** (269 mg, 1.00 mmol) and dipropylamine (137 μL, 1.00 mmol) in a manner similar to that described for compound **14** as a white solid (219 mg, 62%): mp 100–102 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (2H, d, J = 8.3 Hz), 7.42 (2H, m), 7.37–7.29 (2H, m), 7.25 (1H, d, J = 7.3 Hz), 7.13 (1H, s), 4.65 (2H, s), 3.34–3.29 (4H, m), 1.70 (2H, tq, J = 7.4, 7.4 Hz), 1.57 (2H, tq, J = 7.4, 7.4 Hz), 1.01 (3H, t, J = 7.4 Hz), 0.88 (3H, t, J = 7.4 Hz).; IR (ATR) 1790, 1772, 1647, 1485, 1147 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{25}N_2O_3$ $[M+H]^+$ 353.1860; found 353.1859; Anal. Calcd for $C_{21}H_{24}N_2O_3{\cdot}0.25H_2O$: C, 70.66; H, 6.92; N, 7.85. Found: C, 71.04; H, 6.81; N, 7.73.

5.1.8. *N*-Benzyl-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (18)

Compound **18** was prepared from **13** (269 mg, 1.00 mmol) and benzylmethylamine (129 μL, 1.00 mmol) in a manner similar to that described for compound **14** as a white solid (202 mg, 54%): mp 154–156 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (2H, dd, J = 7.2. 7.2 Hz), 7.48–7.20 (10H, m), 7.14 and 7.05 (1H, each d, each J = 1.7 Hz), 4.71 and 4.68 (2H, each s), 4.68 and 4.61 (2H, each s), 3.05 (3H, s); IR (ATR) 1768, 1749, 1649, 1483, 1026 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_3$ $[M+H]^+$ 373.1547; found 373.1544; Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 74.42; H, 5.40; N, 7.69.

5.1.9. *N*-(3-Methoxyphenyl)-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (19)

Compound **19** was prepared from **13** (269 mg, 1.00 mmol) and 3-methoxy-*N*-methylaniline (165 mg, 1.20 mmol) in a manner similar to that described for compound **15** as a white solid (193 mg, 50%): mp 158–160 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (2H, d, J = 8.0 Hz), 7.47–7.33 (4H, m), 7.32–7.21 (2H, m), 7.04 (1H, s), 6.96 (1H, d, J = 7.8 Hz), 6.91 (1H, d, J = 7.8 Hz), 6.86 (1H, s), 4.41 (2H, s), 3.85 (3H, s), 3.31 (3H, s); IR (ATR) 1782, 1674, 1485, 1383, 1039 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M+H]^+$ 389.1496; found 389.1491; Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21. Found: C, 70.93; H, 5.26; N, 7.23.

5.1.10. *N*-(4-Methoxyphenyl)-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (20)

Compound **20** was prepared from **13** (269 mg, 1.00 mmol) and *N*-methyl-*p*-anisidine (165 mg, 1.20 mmol) in a manner similar to that described for compound **14** as a white solid (316 mg, 81%): mp 137–139 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (2H, d, J = 7.3 Hz), 7.44 (2H, dd, J = 7.3, 7.3 Hz), 7.36 (1H, t, J = 7.3 Hz), 7.31–7.22 (4H, m), 7.04–6.98 (3H, m), 4.34 (2H, s), 3.85 (3H, s), 3.28 (3H, s); IR (ATR) 1770, 1670, 1508, 1248, 1022 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M+H]^+$ 389.1496; found 389.1492; Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21. Found: C, 70.99; H, 5.24; N, 7.27.

5.1.11. *N*-(3-Chlorophenyl)-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (21)

Compound **21** was prepared from **13** (269 mg, 1.00 mmol) and 3-chloro-*N*-methylaniline (170 mg, 1.20 mmol) in a manner similar to that described for compound **14** as a white solid (116 mg, 30%): mp 157–159 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.51 (2H, m), 7.47–7.41 (4H, m), 7.39–7.33 (2H, m), 7.31 (1H, dd, J = 8.3, 1.7 Hz), 7.28–7.23 (2H, m), 7.05 (1H, s), 4.38 (2H, s), 3.30 (3H, s); IR (ATR) 1770, 1672, 1481, 1250, 1020 cm^{-1}; HRMS (ESI) m/z calcd for $C_{22}H_{18}ClN_2O_3$ $[M+H]^+$ 393.1000; found 393.0999; Anal. Calcd for $C_{22}H_{17}ClN_2O_3$: C, 67.26; H, 4.36; N, 7.13; Cl, 9.02. Found: C, 67.08; H, 4.39; N, 7.21; Cl, 8.83.

5.1.12. *N*-(4-Chlorophenyl)-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (22)

Compound **22** was prepared from **13** (269 mg, 1.00 mmol) and 4-chloro-*N*-methylaniline (145 μL, 1.20 mmol) in a manner similar to that described for compound **15** as a white solid (283 mg, 72%): mp 83–85 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.41 (6H, m), 7.36 (1H, t, J = 7.3 Hz), 7.32–7.22 (4H, m), 7.03 (1H, s), 4.34 (2H, s), 3.29 (3H, s); IR (ATR) 1768, 1670, 1483, 1250, 1090 cm^{-1}; HRMS (ESI) m/z calcd for $C_{22}H_{18}ClN_2O_3$ $[M+H]^+$ 393.1000; found 393.1001; Anal. Calcd for $C_{22}H_{17}ClN_2O_3$: C, 67.26; H, 4.36; N, 7.13; Cl, 9.02. Found: C, 67.03; H, 4.40; N, 7.22; Cl, 8.81.

5.1.13. *N*-Methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-(pyridin-2-yl)acetamide (23)

Compound **23** was prepared from **13** (539 mg, 2.00 mmol) and 2-(methylamino)pyridine (247 μL, 2.40 mmol) in a manner similar to that described for compound **14** as a white solid (302 mg, 42%): mp 94–96 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53–8.46 (1H, m), 7.87–7.80 (1H, m), 7.57–7.52 (2H, m), 7.43 (2H, dd, J = 7.8, 7.8 Hz), 7.38–7.22 (5H, m), 7.17 (1H, d, J = 1.7 Hz), 4.83 (2H, s), 3.43 (3H, s); IR (ATR) 1772, 1670, 1589, 1252, 1022 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343; found

360.1341; Anal. Calcd for $C_{21}H_{17}N_3O_3 \cdot 0.75H_2O$: C, 67.64; H, 5.00; N, 11.27. Found: C, 68.00; H, 5.10; N, 11.48.

5.1.14. *N*-Methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-(pyridin-3-yl)acetamide (24)

Compound **24** was prepared from **13** (539 mg, 2.00 mmol) and *N*-methy-3-pyridinamine (216 mg, 2.00 mmol) in a manner similar to that described for compound **14** as a white solid (280 mg, 39%): mp 112–114 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (1H, d, J = 4.4 Hz), 8.66 (1H, s), 7.74 (1H, d, J = 8.0 Hz), 7.56–7.40 (5H, m), 7.39–7.22 (3H, m), 7.06 (1H, s), 4.34 (2H, s), 3.34 (3H, s); IR (ATR) 1780, 1670, 1485, 1381, 1097 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343; found 360.1341; Anal. Calcd for $C_{21}H_{17}N_3O_3 \cdot H_2O$: C, 66.83; H, 5.07; N, 11.13. Found: C, 66.71; H, 5.15; N, 11.18.

5.1.15. *N*-Methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-(pyridin-4-yl)acetamide (25)

Compound **25** was prepared from **13** (539 mg, 2.00 mmol) and 4-(methylamino)pyridine (260 mg, 2.40 mmol) in a manner similar to that described for compound **14** as a white solid (280 mg, 39%): mp 148–149 °C (*i*PrOH-Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (2H, d, J = 5.6 Hz), 7.54–7.50 (2H, m), 7.43 (2H, dd, J = 7.6, 7.6 Hz), 7.38–7.29 (4H, m), 7.27–7.24 (1H, m), 7.06 (1H, d, J = 1.7 Hz), 4.51 (2H, s), 3.38 (3H, s); IR (ATR) 1795, 1780, 1664, 1581, 1483 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343; found 360.1342; Anal. Calcd for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69. Found: C, 70.09; H, 4.86; N, 11.68.

5.1.16. *N*-[2-(Hydroxymethyl)phenyl]-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (26)

Compound **26** was prepared from **13** (539 mg, 2.00 mmol) and 2-(methylaminophenyl)methanol (274 mg, 2.00 mmol) in a manner similar to that described for compound **14** as a white solid (393 mg, 51%): mp 93–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (1H, dd, J = 7.2, 1.8 Hz), 7.54–7.38 (6H, m), 7.35–7.25 (3H, m), 7.21 (1H, d, J = 8.3 Hz), 7.17 (1H, d, J = 1.7 Hz), 4.76 (1H, d, J = 12.7 Hz), 4.62 (1H, d, J = 12.7 Hz), 4.38 (1H, d, J = 16.8 Hz), 4.20 (1H, d, J = 16.8 Hz), 3.24 (3H, s); IR (ATR) 3419, 1778, 1664, 1483, 1383 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M+H]^+$ 389.1496; found 389.1486.

5.1.17. *N*-[3-(Hydroxymethyl)phenyl]-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (27)

Compound **27** was prepared from **13** (539 mg, 2.00 mmol) and 3-(methylaminophenyl)methanol (274 mg, 2.00 mmol) in a manner similar to that described for compound **14** as a white solid (495 mg, 64%): mp 140–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (2H, d, J = 7.3 Hz), 7.47–7.41 (3H, m), 7.40–7.33 (3H, m), 7.29 (1H, dd, J = 8.3, 1.7 Hz), 7.20 (1H, d, J = 8.3 Hz), 7.17 (1H, d, J = 7.6 Hz), 7.09 (1H, d, J = 1.7 Hz), 4.74 (2H, s), 4.37 (2H, s), 3.30 (3H, s), 2.54 (1H, s); IR (ATR) 3439, 1767, 1657, 1481, 1397 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M+H]^+$ 389.1496; found 389.1489.

5.1.18. *N*-[4-(Hydroxymethyl)phenyl]-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (28)

Compound **28** was prepared from **13** (241 mg, 0.897 mmol) and 4-(methylaminophenyl)methanol (123 mg, 0.897 mmol) in a manner similar to that described for compound **14** as a white solid (241 mg, 69%): mp 161–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.48 (4H, m), 7.44 (2H, dd, J = 7.6, 7.6 Hz), 7.39–7.28 (4H, m), 7.24 (1H, d, J = 8.3 Hz), 7.04 (1H, s), 4.77 (2H, d, J = 5.6 Hz), 4.36 (2H, s), 3.31 (3H, s), 1.90 (1H, t, J = 5.6 Hz); IR (ATR) 2360, 1778, 1655, 1483, 1244 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M+H]^+$ 389.1496; found 389.1488.

5.1.19. *N*-{2-[(Dimethylamino)methyl]phenyl}-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (32)

To a suspension of **26** (200 mg, 0.515 mmol) and carbon tetrabromide (427 mg, 1.29 mmol) in CH_3CN (7.0 mL) was added triphenylphosphine (338 mg, 1.29 mmol) with cooling in an ice bath, and the mixture was stirred at room temperature for 30 min. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (2:3, v/v) as eluent to give *N*-[2-(Bromomethyl)phenyl]-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (**29**) (226 mg, 97%) as a white solid. Compound **29** (150 mg, 0.332 mmol) was dissolved in DMF (3.0 mL), then 50% methylamine solution (150 mg, 1.66 mmol) was added to the solution with cooling in an ice bath, and the mixture was stirred at room temperature for 30 min. Water was then added, and the mixture was extracted with a mixture of toluene and EtOAc (1:1). The organic layer was washed with H_2O and brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent. The solvent was removed in vacuo, and the resulting solid was recrystallized from *i*PrOH to give **32** (134 mg, 97%) as a white solid: mp 134–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.48 (3H, m), 7.46–7.40 (4H, m), 7.38–7.31 (2H, m), 7.29–7.26 (1H, m), 7.23 (1H, d, J = 8.3 Hz), 7.05 (1H, d, J = 1.7 Hz), 4.43 (1H, d, J = 17.1 Hz), 4.36 (1H, d, J = 17.1 Hz), 3.64 (1H, d, J = 12.9 Hz), 3.28 (3H, s), 3.18 (1H, d, J = 12.9 Hz), 2.27 (6H, s); IR (ATR) 1778, 1662, 1484, 756, 694 cm^{-1}; HRMS (ESI) m/z calcd for $C_{25}H_{26}N_3O_3$ $[M+H]^+$ 416.1969; found 416.1960; Anal. Calcd for $C_{25}H_{25}N_3O_3$: C, 72.27; H, 6.06; N, 10.11. Found: C, 72.26; H, 6.10; N, 10.20.

5.1.20. *N*-{3-[(Dimethylamino)methyl]phenyl}-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (33)

To a suspension of **27** (200 mg, 0.515 mmol) and carbon tetrabromide (427 mg, 1.29 mmol) in CH_3CN (7.0 mL) was added triphenylphosphine (338 mg, 1.29 mmol) with cooling in an ice bath, and the mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (2:3, v/v) as eluent to give *N*-[3-(Bromomethyl)phenyl]-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (**30**) (209 mg, 90%) as a white solid. Compound **30** (150 mg, 0.332 mmol) was dissolved in DMF (3.0 mL), then 50% methylamine solution (150 mg, 1.66 mmol) was added to the solution with cooling in an ice bath, and the mixture was stirred at room temperature for 1 h. Water was then added, and the mixture was extracted with a mixture of toluene and EtOAc (1:1). The organic layer was washed with H_2O and brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent to give **33** (63.7 mg, 46%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (2H, d, J = 7.1 Hz), 7.48–7.42 (3H, m), 7.40–7.20 (6H, m), 7.06 (1H, s), 4.37 (2H, s), 3.48 (2H, s), 3.32 (3H, s), 2.27 (6H, s); IR (ATR) 1772, 1670, 1481, 756, 698 cm^{-1}; HRMS (ESI) m/z calcd for $C_{25}H_{26}N_3O_3$ $[M+H]^+$ 416.1969; found 416.1960; Anal. Calcd for $C_{25}H_{25}N_3O_3 \cdot 0.25H_2O$: C, 71.49; H, 6.12; N, 10.01. Found: C, 71.30; H, 6.04; N, 10.01.

5.1.21. *N*-{4-[(Dimethylamino)methyl]phenyl}-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (34)

To a suspension of **28** (150 mg, 0.386 mmol) and carbon tetrabromide (320 mg, 0.965 mmol) in CH_3CN (5.0 mL) was added triphenylphosphine (253 mg, 0.965 mmol) with cooling in an ice bath, and the mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (2:3,

v/v) as eluent to give *N*-[4-(Bromomethyl)phenyl]-*N*-methyl-2-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetamide (**31**) (151 mg, 87%) as a white solid. Compound **31** (100 mg, 0.222 mmol) was dissolved in DMF (2.0 mL), then 50% methylamine solution (99.9 mg, 1.11 mmol) was added to the solution with cooling in an ice bath, and the mixture was stirred at room temperature for 1 h. Water was then added, and the mixture was extracted with a mixture of toluene and EtOAc (1:1). The organic layer was washed with H_2O and brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent to give **34** (90.2 mg, 98%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (2H, d, J = 7.1 Hz), 7.45 (4H, m), 7.36 (1H, t, J = 6.7 Hz), 7.32–7.26 (3H, m), 7.23 (1H, d, J = 8.3 Hz), 7.03 (1H, s), 4.36 (2H, s), 3.47 (2H, s), 3.31 (3H, s), 2.27 (6H, s); IR (ATR) 1772, 1670, 1481, 1383, 1250 cm^{-1}; HRMS (ESI) m/z calcd for $C_{25}H_{26}N_3O_3$ $[M+H]^+$ 416.1969; found 416.1960; Anal. Calcd for $C_{25}H_{25}N_3O_3{\cdot}0.25H_2O$: C, 71.49; H, 6.12; N, 10.01. Found: C, 71.84; H, 6.12; N, 10.12.

5.1.22. *N*-Methyl-3-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylpropanamide (36)

To a suspension of **11** (106 mg, 0.500 mmol) and K_2CO_3 (117 mg, 0.850 mmol) in DMF (1.0 mL) was added a solution of 3-bromo-*N*-methyl-*N*-phenylpropanamide (72.6 mg, 0.300 mmol) in DMF (1.0 mL) with cooling in an ice bath. The reaction mixture was stirred at 80 °C for 6 h and cooled to room temperature. Water was then added, and the mixture was extracted with a mixture of toluene and EtOAc (1:1). The organic layer was washed with H_2O and brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (3:1, v/v) as eluent to give **36** (116 mg, 62%) as a yellow solid: mp 96–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (2H, d, J = 7.6 Hz), 7.46 (2H, dd, J = 7.6, 7.6 Hz), 7.41–7.34 (3H, m), 7.34–7.28 (3H, m), 7.22 (1H, d, J = 8.3 Hz), 7.06 (2H, d, J = 7.6 Hz), 4.15 (2H, t, J = 7.0 Hz), 3.23 (3H, s), 2.58 (2H, t, J = 7.0 Hz); IR (ATR) 1759, 1655, 1481, 758, 698 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_3$ $[M+H]^+$ 373.1547; found 373.1538; Anal. Calcd for $C_{23}H_{20}N_2O_3{\cdot}0.25H_2O$: C, 73.29; H, 5.48; N, 7.43. Found: C, 73.64; H, 5.39; N, 7.58.

5.1.23. *N*-Methyl-4-(2-oxo-5-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylbutanamide (38)

Compound **38** was prepared from **11** (31.7 mg, 0.150 mmol) and 3-bromo-*N*-methyl-*N*-phenylbutanamide (46.1 mg, 0.180 mmol) in a manner similar to that described for compound **36** as a white solid (13.5 mg, 23%): mp 153–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (2H, d, J = 7.3 Hz), 7.46 (2H, dd, J = 7.3, 7.3 Hz), 7.40–7.20 (7H, m), 7.06–7.01 (2H, m), 3.90 (2H, t, J = 6.6 Hz), 3.21 (3H, s), 2.17–2.03 (4H, m); IR (ATR) 1774, 1651, 1485, 1259, 702 cm^{-1}; HRMS (ESI) m/z calcd for $C_{24}H_{23}N_2O_3$ $[M+H]^+$ 387.1703; found 387.1701; Anal. Calcd for $C_{24}H_{22}N_2O_3{\cdot}0.50H_2O$: C, 72.89; H, 5.86; N, 7.08. Found: C, 73.06; H, 5.77; N, 7.08.

5.1.24. 3-(2-Oxo-2-phenylethyl)-5-phenyl-1,3-benzoxazol-2(3*H*)-one (39)

To a suspension of **11** (500 mg, 2.37 mmol) and K_2CO_3 (491 mg, 3.55 mmol) in DMF (5.0 mL) was added 2-bromoacetophenone (518 mg, 2.60 mmol) at room temperature. The reaction mixture was stirred at room temperature for 15 h. Water was then added, and the mixture was extracted with a mixture of toluene and EtOAc (1:1). The organic layer was washed with H_2O and brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (7:3, v/v) as eluent to give **39** (524 mg, 67%) as a brown solid: mp 54–56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06–8.02 (2H, m), 7.67 (1H, t, J = 7.4 Hz), 7.54 (2H, dd, J = 7.4, 7.4 Hz), 7.51–7.47 (2H, m), 7.43–7.37 (2H, m), 7.36–7.26 (3H, m), 6.99 (1H, d, J = 1.2 Hz), 5.28 (2H, s); IR (ATR) 1772, 1695, 1481, 1344, 1227 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{16}NO_3$ $[M+H]^+$ 330.1125; found 330.1126; Anal. Calcd for $C_{21}H_{15}NO_3{\cdot}0.10H_2O$: C, 76.17; H, 4.63; N, 4.23. Found: C, 76.11; H, 4.70; N, 4.25.

5.1.25. 3-{2-[Methyl(phenyl)amino]ethyl}-5-phenyl-1,3-benzoxazol-2(3*H*)-one (40)

To a solution of **14** (100 mg, 0.279 mmol) in THF (2.0 mL) was added dropwise 1.0 M boran·THF complex in THF (0.977 mL, 0.977 mmol) with cooling in an ice bath. The reaction mixture was stirred at reflux for 1.5 h and cooled to room temperature. The reaction was then quenched by dropwise addition of MeOH (1.0 mL), and the solvent was removed in vacuo. The residue was dissolve in MeOH (2.0 mL). To a solution thus obtained was added 4 N HCl in 1,4-dioxane (0.279 mM, 1.17 mmol) at room temperature, and heated at reflux for 1 h. The reaction mixture was cooled and a solvent was removed in vacuo. The residue was diluted with toluene, and 1 M NaOH solution was added to the solution. The organic layer was separated, washed with brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (5:1, v/v) as eluent to give **40** (19.2 mg, 20%) as a yellow solid: mp 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.32 (5H, m), 7.31–7.19 (4H, m), 6.87 (1H, d, J = 1.7 Hz), 6.76 (1H, t, J = 7.3 Hz), 6.71 (2H, d, J = 8.0 Hz), 4.07 (2H, t, J = 6.2 Hz), 3.81 (2H, t, J = 6.2 Hz), 2.87 (3H, s); IR (ATR) 1772, 1763, 1541, 1506, 754 cm^{-1}; HRMS (ESI) m/z calcd for $C_{22}H_{21}N_2O_2$ $[M+H]^+$ 345.1598; found 345.1597; Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 76.56; H, 5.89; N, 8.15.

5.1.26. 2-Nitrobiphenyl-3-ol (42), 4-nitrobiphenyl-3-ol (43) and 6-nitrobiphenyl-3-ol (44)

To a solution of 3-phenylphenol (6.00 g, 35.3 mmol) in AcOH (35 mL) was added dropwise 6 N HNO_3 solution (6.00 mL, 36.0 mmol) with cooling in an ice bath, and stirred at room temperature for 30 min. Water was then added, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (10:1 to 1:1, v/v) as eluent to give **43** (1.96 g, 26%) as a yellow solid and a mixture of **42** and **44** as a brown oil. A mixture of **42** and **44** was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent to give **42** (1.08 g, 14%) as a brown solid and **44** (3.11 g, 41%) as a brown oil. **42**: mp 81–83 °C [lit.[22a]: 85–86 °C (benzene)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (1H, s), 7.49 (1H, t, J = 7.9 Hz), 7.42–7.40 (3H, m), 7.27–7.25 (2H, m), 7.15 (1H, dd, J = 7.4, 1.3 Hz), 6.90 (1H, dd, J = 7.4, 1.3 Hz); IR (ATR) 1593, 1525, 1350, 1296, 1211 cm^{-1}; HRMS (ESI) m/z calcd for $C_{12}H_8NO_3$ $[M-H]^-$ 214.0510; found 214.0505; **43**: mp 103–105 °C (EtOH) [lit.[22a]: 104–105 °C (EtOH)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.71 (1H, s), 8.17 (1H, d, J = 8.8 Hz), 7.63–7.62 (2H, m), 7.53–7.42 (3H, m), 7.38–7.35 (1H, m), 7.22 (1H, dd, J = 8.8, 1.7 Hz); IR (ATR) 2362, 616, 1574, 1277, 1169 cm^{-1}; HRMS (ESI) m/z calcd for $C_{12}H_8NO_3$ $[M-H]^-$ 214.0510; found 214.0505; **44**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (1H, d, J = 8.8 Hz), 7.40–7.39 (3H, m), 7.28–7.26 (2H, m), 6.86–6.83 (1H, m), 6.79 (1H, d, J = 2.7 Hz), 6.02 (1H, s); IR (ATR) 3365, 1574, 1508, 1308, 1201 cm^{-1}; HRMS (ESI) m/z calcd for $C_{12}H_8NO_3$ $[M-H]^-$ 214.0510; found 214.0505.

5.1.27. 2-Aminobiphenyl-3-ol (45)

To a solution of **42** (861 mg, 4.00 mmol) in MeOH (20 mL) was added 10% Pd/C (50% wet, 200 mg), and stirred at room temperature for 6 h under hydrogen atmosphere. The reaction mixture was filtered through Celite, and the filtrate was concentrated to give **45** (707 mg, 95%) as a yellow solid: mp 119–120 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (1H, s), 7.46–7.42 (4H, m), 7.35–7.31 (1H, m), 6.77–6.73 (1H, m), 6.63–6.55 (2H, m), 4.95 (2H, br s); IR (ATR) 2922, 1558, 1471, 1296, 1186 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{12}NO$ $[M+H]^+$ 186.0913; found 186.0908.

5.1.28. 4-Phenyl-1,3-benzoxazol-2(3*H*)-one (46)

Compound **46** was prepared from **45** (604 mg, 3.26 mmol) in a manner similar to that described for compound **11** as a brown solid (412 mg, 60%): mp 198–199 °C ($CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.72 (1H, s), 7.57–7.56 (2H, m), 7.50 (2H, dd, *J* = 7.6, 7.6 Hz), 7.42 (1H, t, *J* = 7.6 Hz), 7.29 (1H, dd, *J* = 7.6, 1.5 Hz), 7.24–7.16 (2H, m); IR (ATR) 3178, 1765, 1429, 1259, 1157 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{13}H_8NO_2$ $[M-H]^-$ 210.0561; found 210.0555.

5.1.29. *tert*-Butyl (2-oxo-4-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetate (47)

To a suspension of **46** (250 mg, 1.18 mmol) and K_2CO_3 (245 mg, 1.78 mmol) in DMF (5.0 mL) was added *tert*-butyl bromoacetate (0.192 mL, 1.30 mmol) with cooling in an ice bath. The reaction mixture was stirred at 60 °C for 2 h and cooled to room temperature. Water was then added, and the mixture was extracted with a mixture of toluene and EtOAc (1:1). The organic layer was washed with H_2O and brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was recrystallized from hexane/EtOAc to give **47** (269 mg, 70%) as a white solid: mp 159–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.40 (3H, m), 7.35–7.30 (2H, m), 7.28–7.21 (1H, m), 7.14 (1H, t, *J* = 7.9 Hz), 7.02 (1H, d, *J* = 7.9 Hz), 4.10 (2H, s), 1.32 (9H, s); IR (ATR) 1770, 1734, 1458, 1238, 1153 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{18}NO_4$ $[M-H]^-$ 324.1236; found 324.1233.

5.1.30. (2-Oxo-4-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetic acid (48)

Compound **48** was prepared from **47** (1.24 g, 3.82 mmol) in a manner similar to that described for compound **13** as a pale brown solid (1.01 g, 98%): mp 218–219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (1H, s), 7.46–7.45 (4H, m), 7.36–7.34 (2H, m), 7.23 (1H, t, *J* = 7.9 Hz), 7.06 (1H, d, *J* = 7.9 Hz), 4.07 (2H, s); IR (ATR) 3167, 1747, 1732, 1454, 1190 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{10}NO_4$ $[M-H]^-$ 268.0615; found 268.0610.

5.1.31. *N*-Methyl-2-(2-oxo-4-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylacetamide (49)

Compound **49** was prepared from **48** (108 mg, 0.400 mmol) and *N*-methylaniline (52.0 μL, 0.480 mmol) in a manner similar to that described for compound **14** as a white solid (92.7 mg, 65%): mp 182–184 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (1H, t, *J* = 7.1 Hz), 7.47 (2H, dd, *J* = 7.1, 7.1 Hz), 7.38–7.28 (5H, m), 7.22 (1H, d, *J* = 8.0 Hz), 7.10 (1H, dd, *J* = 8.0, 8.0 Hz), 6.95 (1H, d, *J* = 8.0 Hz), 6.74–6.67 (2H, m), 3.95 (2H, s), 3.10 (3H, s); IR (ATR) 1772, 1676, 1458, 1363, 1255 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{19}N_2O_3$ $[M+H]^+$ 359.1390; found 359.1382; Anal. Calcd for $C_{22}H_{18}N_2O_3$: C, 73.73; H, 5.06; N, 7.82. Found: C, 73.33; H, 5.00; N, 7.84.

5.1.32. 4-Aminobiphenyl-3-ol (50)

Compound **50** was prepared from **43** (1.45 g, 6.74 mmol) in a manner similar to that described for compound **45** as a brown solid (1.22, 98%): mp 179–181 °C (EtOH) [lit.[24]: 182–184 °C (EtOH)]; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (1H, s), 7.47 (2H, d, *J* = 7.3 Hz), 7.36 (2H, dd, *J* = 7.3, 7.3 Hz), 7.20 (1H, t, *J* = 7.3 Hz), 6.96 (1H, d, *J* = 2.0 Hz), 6.89 (1H, dd, *J* = 8.0, 2.0 Hz), 6.66 (1H, d, *J* = 8.0 Hz), 4.67 (2H, s); IR (ATR) 3356, 3282, 1601, 1489, 1431 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{12}NO$ $[M+H]^+$ 186.0913; found 186.0909.

5.1.33. 6-Phenyl-1,3-benzoxazol-2(3*H*)-one (51)

Compound **51** was prepared from **50** (1.02 g, 5.51 mmol) in a manner similar to that described for compound **11** as a brown solid (0.969 g, 83%): mp 250–251 °C ($CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (1H, s), 7.66–7.61 (3H, m), 7.47–7.43 (3H, m), 7.35 (1H, t, *J* = 7.3 Hz), 7.17 (1H, d, *J* = 8.0 Hz); IR (ATR) 3213, 1772, 1724, 1477, 1259 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{13}H_8NO_2$ $[M-H]^-$ 210.0561; found 210.0556.

5.1.34. *tert*-Butyl (2-oxo-6-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetate (52)

Compound **52** was prepared from **51** (700 mg, 3.31 mmol) in a manner similar to that described for compound **47** as a yellow solid (937 mg, 87%): mp 181–183 °C (EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (2H, d, *J* = 7.8 Hz), 7.48–7.32 (5H, m), 6.94 (1H, d, *J* = 8.0 Hz), 4.49 (2H, s), 1.48 (9H, s); IR (ATR) 1770, 1732, 1485, 1356, 1236 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{18}NO_4$ $[M-H]^-$ 324.1236; found 324.1233.

5.1.35. (2-Oxo-6-phenyl-1,3-benzoxazol-3(2*H*)-yl)acetic acid (53)

Compound **53** was prepared from **52** (1.65 g, 5.07 mmol) in a manner similar to that described for compound **13** as a white solid (1.34 g, 98%): mp 224–226 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.42 (1H, s), 7.71–7.67 (3H, m), 7.54 (1H, dd, *J* = 8.2, 1.6 Hz), 7.46 (2H, dd, *J* = 7.7, 7.7 Hz), 7.41–7.34 (2H, m), 4.70 (2H, s); IR (ATR) 1770, 1732, 1485, 1358, 1236 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{10}NO_4$ $[M-H]^-$ 268.0615; found 268.0610.

5.1.36. *N*-Methyl-2-(2-oxo-6-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylacetamide (54)

Compound **54** was prepared from **53** (108 mg, 0.400 mmol) and *N*-methylaniline (52.0 μL, 0.480 mmol) in a manner similar to that described for compound **14** as a white solid (111 mg, 77%): mp 201–203 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.49 (4H, m), 7.48–7.40 (4H, m), 7.40–7.32 (4H, m), 6.94 (1H, d, *J* = 8.0 Hz), 4.35 (2H, s), 3.33 (3H, s); IR (ATR) 1774, 1672, 1485, 1387, 1367 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{19}N_2O_3$ $[M+H]^+$ 359.1390; found 359.1382; Anal. Calcd for $C_{22}H_{18}N_2O_3 \cdot 0.50H_2O$: C, 71.92; H, 5.21; N, 7.62. Found: C, 72.15; H, 4.94; N, 7.69.

5.1.37. 2-Amino-6-bromophenol (56)

To a solution of reduced iron (7.17 g, 128 mmol) in AcOH (30 mL) was added dropwise a solution of 2-bromo-6-nitrophenol (4.00 g, 18.3 mmol) in AcOH (20 mL) at 90 °C. The reaction mixture was stirred at 90 °C for 30 min and cooled to room temperature. The reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was diluted with EtOAc and aqueous saturated $NaHCO_3$. The organic layer was separated, washed with brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (5:1, v/v) as eluent to give **56** (2.84 g, 83%) as a brown solid: mp 84–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.87–6.83 (1H, m), 6.65–6.63 (2H, m), 5.41 (1H, br s), 3.85 (2H, br s); IR (ATR) 3032, 1578, 1473, 1456, 1227 cm^{-1}; HRMS (ESI) *m*/*z* calcd for C_6H_7BrNO $[M+H]^+$ 187.9706; found 187.9704.

5.1.38. 7-Bromo-1,3-benzoxazol-2(3*H*)-one (57)

Compound **57** was prepared from **56** (2.74 g, 14.6 mmol) in a manner similar to that described for compound **11** as an orange so-

lid (3.04 g, 97%): mp 244–245 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (1H, s), 7.28 (1H, dd, J = 7.0, 2.8 Hz), 7.11–7.09 (2H, m); IR (ATR) 3101, 1716, 1616, 1448, 1398 cm^{-1}; HRMS (ESI) m/z calcd for $C_7H_3BrNO_2$ $[M-H]^-$ 211.9353; found 211.9350.

5.1.39. *tert*-Butyl (7-bromo-2-oxo-1,3-benzoxazol-3(2*H*)-yl)acetate (58)

Compound **58** was prepared from **57** (1.00 g, 4.67 mmol) in a manner similar to that described for compound **47** as a white solid (1.38 g, 90%): mp 99–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (1H, d, J = 8.3 Hz), 7.07 (1H, dd, J = 8.3, 8.3 Hz), 6.82 (1H, d, J = 8.3 Hz), 4.45 (2H, s), 1.47 (9H, s); IR (ATR) 1772, 1718, 1616, 1468, 1369 cm^{-1}; HRMS (ESI) m/z calcd for $C_{13}H_{13}BrNO_4$ $[M-H]^-$ 326.0033; found 326.0032.

5.1.40. (7-Bromo-2-oxo-1,3-benzoxazol-3(2*H*)-yl)acetic acid (59)

Compound **59** was prepared from **58** (1.32 g, 4.02 mmol) in a manner similar to that described for compound **13** as a white solid (1.07 g, 98%): mp 235–237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (1H, s), 7.39–7.33 (2H, m), 7.19 (1H, t, J = 8.0 Hz), 4.67 (2H, s); IR (ATR) 1770, 1730, 1616, 1467, 1252 cm^{-1}; HRMS (ESI) m/z calcd for $C_9H_5BrNO_4$ $[M-H]^-$ 269.9407; found 269.9402.

5.1.41. 2-(7-Bromo-2-oxo-1,3-benzoxazol-3(2*H*)-yl)-*N*-methyl-*N*-phenylacetamide (60)

Compound **60** was prepared from **59** (20.0 mg, 73.5 μmol) and *N*-methylaniline (7.96 μL, 73.5 μmol) in a manner similar to that described for compound **14** as a white solid (9.40 mg, 35%): mp 170–172 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (2H, dd, J = 7.6, 7.6 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.32 (2H, d, J = 7.6 Hz), 7.26–7.23 (1H, m), 7.04 (1H, dd, J = 8.0, 8.0 Hz), 6.82 (1H, d, J = 8.0 Hz), 4.31 (2H, s), 3.32 (3H, s); IR (ATR) 1782, 1670, 1466, 1254, 1016 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{14}BrN_2O_3$ $[M+H]^+$ 361.0182; found 361.0182.

5.1.42. *N*-Methyl-2-(2-oxo-7-phenyl-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylacetamide (61)

To a suspension of **60** (50 mg, 138 μmol) and phenylboronic acid (20.3 mg, 166 μmol) in 1 M K_2CO_3 solution and 1,4-dioxane (2.0 mL) was added $Pd(PPh_3)_4$ (8.00 mg, 6.92 μmol) in room temperature. The reaction mixture was stirred at reflux for 2 h and cooled to room temperature. Water was then added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (1:1, v/v) as eluent to give **61** (45.3 mg, 92%) as a brown solid: mp 126–128 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77–7.72 (2H, m), 7.55–7.42 (5H, m), 7.41–7.28 (4H, m), 7.23 (1H, t, J = 7.8 Hz), 6.84 (1H, d, J = 6.6 Hz), 4.36 (2H, s), 3.33 (3H, s); IR (ATR) 1774, 1664, 1470, 1435, 748 cm^{-1}; HRMS (ESI) m/z calcd for $C_{22}H_{19}N_2O_3$ $[M+H]^+$ 359.1390; found 359.1385; Anal. Calcd for $C_{22}H_{18}N_2O_3$: C, 73.73; H, 5.06; N, 7.82. Found: C, 73.38; H, 5.08; N, 7.84.

5.1.43. 2-Amino-4-bromophenol (63)

To a solution of 4-bromo-2-nitrophenol (50.7 g, 233 mmol) in THF (500 mL) was added 5% Rh/C (5.00 g), and stirred at room temperature for 11 h under hydrogen atmosphere. The reaction mixture was filtered through Celite, and the filtrate was concentrated to give **63** (43.3 g, 99%) as a brown solid: mp 133–135 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (1H, s), 6.72 (1H, d, J = 2.4 Hz), 6.56 (1H, d, J = 8.3 Hz), 6.50 (1H, dd, J = 8.3, 2.4 Hz), 4.91 (2H, br s); IR (ATR) 3062, 1497, 1444, 1437, 1279 cm^{-1}; HRMS (ESI) m/z calcd for C_6H_7BrNO $[M+H]^+$ 187.9706; found 187.9704.

5.1.44. 5-Bromo-1,3-benzoxazol-2(3*H*)-one (64)

Compound **64** was prepared from **63** (49.0 g, 261 mmol) in a manner similar to that described for compound **11** as a brown solid (53.2 g, 95%): mp 206–208 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (1H, s), 7.27–7.26 (3H, m); IR (ATR) 2359, 1751, 1622, 1473, 1254 cm^{-1}; HRMS (ESI) m/z calcd for $C_7H_3BrNO_2$ $[M-H]^-$ 211.9353; found 211.9350.

5.1.45. *tert*-Butyl (5-bromo-2-oxo-1,3-benzoxazol-3(2*H*)-yl)acetate (65)

Compound **65** was prepared from **64** (53.0 g, 248 mmol) in a manner similar to that described for compound **47** as a beige solid (75.2 g, 92%): mp 144–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (1H, dd, J=8.5, 1.9 Hz), 7.10 (1H, d, J = 8.5 Hz), 7.03 (1H, d, J = 1.9 Hz), 4.43 (2H, s), 1.48 (9H, s); IR (ATR) 1781, 1736, 1608, 1485, 1387 cm^{-1}; HRMS (ESI) m/z calcd for $C_{13}H_{15}BrNO_4$ $[M+H]^+$ 328.0179; found 328.0181.

5.1.46. (5-Bromo-2-oxo-1,3-benzoxazol-3(2*H*)-yl)acetic acid (66)

Compound **66** was prepared from **65** (170 mg, 0.518 mmol) in a manner similar to that described for compound **13** as a white solid (132 mg, 94%): mp 204–206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (1H, br s), 7.68 (1H, d, J = 1.7 Hz), 7.37–7.33 (2H, m), 4.66 (2H, s); IR (ATR) 2953, 1736, 2701, 1483, 1227 cm^{-1}; HRMS (ESI) m/z calcd for $C_9H_5BrNO_4$ $[M-H]^-$ 269.9407; found 269.9406.

5.1.47. 2-(5-Bromo-2-oxo-1,3-benzoxazol-3(2*H*)-yl)-*N*-methyl-*N*-phenylacetamide (67)

Compound **67** was prepared from **66** (10.0 g, 36.8 mmol) and *N*-methylaniline (4.78 mL, 44.1 mmol) in a manner similar to that described for compound **14** as a white solid (7.16 g, 54%): mp 122–124 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (2H, dd, J = 7.6, 7.6 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.34 (2H, d, J = 7.6 Hz), 7.23 (1H, dd, J = 8.3, 1.7 Hz), 7.06 (1H, d, J = 8.3 Hz), 7.01 (1H, d, J = 1.7 Hz), 4.28 (2H, s), 3.33 (3H, s); IR (ATR) 1772, 1666, 1483, 1377, 1244 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{14}BrN_2O_3$ $[M+H]^+$ 361.0182; found 361.0177; Anal. Calcd for $C_{16}H_{13}BrN_2O_3$: C, 53.21; H, 3.63; N, 7.76; Br, 22.12. Found: C, 53.15; H, 3.68; N, 7.84; Br, 21.90.

5.1.48. 2-[5-(3-Methoxyphenyl)-2-oxo-1,3-benzoxazol-3(2*H*)-yl]-*N*-methyl-*N*-phenylacetamide (68)

Compound **68** was prepared from **67** (90.3 mg, 0.250 mmol) and 3-methoxyphenylboronic acid (49.4 mg, 0.325 mmol) in a manner similar to that described for compound **61** as a yellow solid (51.9 mg, 53%): mp 182–184 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (2H, dd, J = 7.6, 7.6 Hz), 7.44 (1H, t, J = 7.6 Hz), 7.39–7.32 (3H, m), 7.29 (1H, dd, J = 8.2, 1.6 Hz), 7.23 (1H, d, J = 8.2 Hz), 7.12 (1H, d, J = 7.6 Hz), 7.06 (1H, s), 7.02 (1H, s), 6.91 (1H, dd, J = 8.4, 2.8 Hz), 4.35 (2H, s), 3.88 (3H, s), 3.32 (3H, s); IR (ATR) 1778, 1670, 1483, 1379, 1242 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M+H]^+$ 389.1496; found 389.1487; Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21. Found: C, 70.77; H, 5.17; N, 7.25.

5.1.49. 2-[5-(4-Methoxyphenyl)-2-oxo-1,3-benzoxazol-3(2*H*)-yl]-*N*-methyl-*N*-phenylacetamide (69)

Compound **69** was prepared from **67** (90.3 mg, 0.250 mmol) and 4-methoxyphenylboronic acid (49.4 mg, 0.325 mmol) in a manner similar to that described for compound **61** as a white solid (77.7 mg, 80%): mp 168–170 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (2H, dd, J = 7.8, 7.8 Hz), 7.48–7.43 (3H, m), 7.34 (2H, d, J = 8.0 Hz), 7.27–7.19 (2H, m), 7.00–6.95 (3H, m), 4.35 (2H, s), 3.86 (3H, s), 3.32 (3H, s); IR (ATR) 1772, 1670, 1489, 1387, 1248 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M+H]^+$ 389.1496; found 389.1487; Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21. Found: C, 70.82; H, 5.32; N, 7.13.

5.1.50. *N*-Methyl-2-{2-oxo-5-[3-(trifluoromethyl)phenyl]-1,3-benzoxazol-3(2*H*)-yl}-*N*-phenylacetamide (70)

Compound **70** was prepared from **67** (90.3 mg, 0.250 mmol) and 3-(trifluoromethyl)phenylboronic acid (61.7 mg, 0.325 mmol) in a manner similar to that described for compound **61** as a yellow solid (77.7 mg, 73%): mp 193–195 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (1H, s), 7.71 (1H, d, J = 7.6 Hz), 7.62 (1H, d, J = 7.6 Hz), 7.59–7.49 (3H, m), 7.45 (1H, t, J = 7.3 Hz), 7.36 (2H, d, J = 7.3 Hz), 7.33–7.27 (2H, m), 7.04 (1H, s), 4.37 (2H, s), 3.33 (3H, s); IR (ATR) 1788, 1651, 1489, 1381, 1329 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{18}F_3N_2O_3$ $[M+H]^+$ 427.1264; found 427.1252; Anal. Calcd for $C_{23}H_{17}F_3N_2O_3{\cdot}0.50H_2O$: C, 63.45; H, 4.17; N, 6.43; F, 13.09. Found: C, 63.47; H, 4.04; N, 6.29; F, 13.21.

5.1.51. *N*-Methyl-2-{2-oxo-5-[4-(trifluoromethyl)phenyl]-1,3-benzoxazol-3(2*H*)-yl}-*N*-phenylacetamide (71)

Compound **71** was prepared from **67** (500 mg, 1.38 mmol) and 4-(trifluoromethyl)phenylboronic acid (316 mg, 1.66 mmol) in a manner similar to that described for compound **61** as a white solid (342 mg, 58%): mp 218–220 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (2H, d, J = 8.3 Hz), 7.64 (2H, d, J = 8.3 Hz), 7.53 (2H, dd, J = 7.4, 7.4 Hz), 7.45 (1H, t, J = 7.4 Hz), 7.35 (2H, d, J = 7.4 Hz), 7.33–7.27 (2H, m), 7.05 (1H, d, J = 1.5 Hz), 4.37 (2H, s), 3.33 (3H, s); IR (ATR) 1784, 1772, 1684, 1676, 1489 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{18}F_3N_2O_3$ $[M+H]^+$ 427.1264; found 427.1251; Anal. Calcd for $C_{23}H_{17}F_3N_2O_3{\cdot}0.75H_2O$: C, 62.80; H, 4.24; N, 6.37; F, 12.96. Found: C, 62.47; H, 4.02; N, 6.35; F, 12.60.

5.1.52. *N*-Methyl-2-{2-oxo-5-[4-(trifluoromethoxy)phenyl]-1,3-benzoxazol-3(2*H*)-yl}-*N*-phenylacetamide (72)

Compound **72** was prepared from **67** (50.0 mg, 0.138 mmol) and 4-(trifluoromethoxy)phenylboronic acid (34.2 mg, 0.166 mmol) in a manner similar to that described for compound **61** as an orange solid (40.0 mg, 66%): mp 164–166 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.49 (4H, m), 7.45 (1H, t, J = 7.3 Hz), 7.35 (2H, d, J = 7.6 Hz), 7.29 (2H, d, J = 8.5 Hz), 7.26–7.24 (2H, m), 7.01 (1H, s), 4.35 (2H, s), 3.32 (3H, s); IR (ATR) 1786, 1774, 1662, 1489, 1385 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{18}F_3N_2O_4$ $[M+H]^+$ 443.1213; found 443.1199; Anal. Calcd for $C_{23}H_{17}F_3N_2O_4$: C, 62.44; H, 3.87; N, 6.33; F, 12.88. Found: C, 62.32; H, 3.93; N, 6.45; F, 12.84.

5.1.53. *N*-Methyl-2-[2-oxo-5-(pyridin-2-yl)-1,3-benzoxazol-3(2*H*)-yl]-*N*-phenylacetamide (73)

To a solution of **67** (181 mg, 500 μmol) and 2-(tributylstannyl)pyridine (0.192 mL, 600 μmol) in toluene (3.0 mL) was added $Pd(PPh_3)_4$ (28.9 mg, 25.0 μmol) in room temperature. The reaction mixture was stirred at reflux for 7 h and cooled to room temperature. Water was then added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (1:3, v/v) as eluent to give **73** (131 mg, 73%) as a beige solid: mp 166–168 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (1H, d, J = 4.9 Hz), 7.79–7.73 (1H, m), 7.70 (1H, d, J = 7.8 Hz), 7.68–7.62 (2H, m), 7.53 (2H, dd, J = 7.3, 7.3 Hz), 7.45 (1H, t, J = 7.3 Hz), 7.37 (2H, d, J = 7.3 Hz), 7.29–7.23 (2H, m), 4.39 (2H, s), 3.31 (3H, s); IR (ATR) 1786, 1660, 1587, 1471, 1464 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343; found 360.1341; Anal. Calcd for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69. Found: C, 70.05; H, 4.83; N, 11.67.

5.1.54. *N*-Methyl-2-[2-oxo-5-(pyridin-3-yl)-1,3-benzoxazol-3(2*H*)-yl]-*N*-phenylacetamide (74)

Compound **74** was prepared from **67** (1.81 g, 5.00 mmol) and 3-pyridineboronic acid (0.738 g, 6.00 mmol) in a manner similar to that described for compound **61** as a pale brown solid (1.44 g, 80%): mp 163–165 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.80 (1H, d, J = 2.0 Hz), 8.61 (1H, dd, J = 5.0, 2.0 Hz), 7.86–7.82 (1H, m), 7.53 (2H, dd, J = 7.6, 7.6 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.40–7.33 (3H, m), 7.30–7.26 (2H, m), 7.04 (1H, s), 4.37 (2H, s), 3.33 (3H, s); IR (ATR) 1780, 1657, 1483, 1425, 1385 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343; found 360.1341; Anal. Calcd for $C_{21}H_{17}N_3O_3{\cdot}0.25H_2O$: C, 69.32; H, 4.85; N, 11.55. Found: C, 68.97; H, 4.75; N, 11.18.

5.1.55. *N*-Methyl-2-[2-oxo-5-(pyridin-4-yl)-1,3-benzoxazol-3(2*H*)-yl]-*N*-phenylacetamide (75)

Compound **75** was prepared from **67** (2.00 g, 5.54 mmol) and 4-pyridineboronic acid (0.817 g, 6.64 mmol) in a manner similar to that described for compound **61** as a grey solid (1.35 g, 68%): mp 213–214 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (2H, d, J = 4.9 Hz), 7.53 (2H, dd, J = 7.4, 7.4 Hz), 7.49–7.43 (3H, m), 7.39–7.34 (3H, m), 7.29 (1H, d, J = 8.3 Hz), 7.10 (1H, d, J = 1.5 Hz), 4.38 (2H, s), 3.33 (3H, s); IR (ATR) 1780, 1770, 1668, 1597, 1485 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343; found 360.1340; Anal. Calcd for $C_{21}H_{17}N_3O_3{\cdot}0.25H_2O$: C, 69.32; H, 4.85; N, 11.55. Found: C, 69.58; H, 4.74; N, 11.69.

5.1.56. *N*-Methyl-2-(2-oxo-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylacetamide (76)

To a solution of **67** (542 mg, 1.50 mmol) in MeOH (50 mL) was added 10% Pd/C (50% wet, 271 mg), and stirred at room temperature for 2.5 h under hydrogen atmosphere. The reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by silica gel column chromatography using $CHCl_3$ as eluent to give **76** (407 mg, 96%) as a white solid: mp 141–143 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (2H, dd, J = 7.6, 7.6 Hz), 7.44 (1H, t, J = 7.6 Hz), 7.33 (2H, d, J = 7.6 Hz), 7.20–7.06 (3H, m), 6.88 (1H, d, J = 7.1 Hz), 4.32 (2H, s), 3.32 (3H, s); IR (ATR) 1767, 1670, 1489, 1369, 1240 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{15}N_2O_3$ $[M+H]^+$ 283.1077; found 283.1071; Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 68.13; H, 4.99; N, 10.04.

5.1.57. *N*-Methyl-2-(2-oxo-5-phenoxy-1,3-benzoxazol-3(2*H*)-yl)-*N*-phenylacetamide (77)

A mixture of **67** (722 mg, 2.00 mmol), phenol (753 mg, 8.00 mmol), CuO (796 mg, 10.0 mmol) and K_2CO_3 (1.66 g, 12.0 mmol) in pyridine (10 mL) was heated at reflux for 18 h and cooled to room temperature. The reaction mixture was filtered through Celite, and the filtrate was diluted with $CHCl_3$ and 2 M HCl solution. The organic layer was separated, washed with brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (3:1, v/v) as eluent. The solvent was removed in vacuo, and the resulting solid was triturated with Et_2O to give **77** (269 mg, 36%) as a white solid: mp 133–135 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (2H, dd, J = 7.3, 7.3 Hz), 7.42 (1H, t, J = 7.3 Hz), 7.35 (2H, dd, J = 7.8, 7.8 Hz), 7.28 (2H, d, J = 7.3 Hz), 7.14–7.10 (2H, m), 6.99 (2H, d, J = 7.8 Hz), 6.72 (1H, dd, J = 8.5, 2.2 Hz), 6.58 (1H, d, J = 2.2 Hz), 4.25 (2H, s), 3.29 (3H, s); IR (ATR) 1778, 1664, 1487, 1387, 1217 cm^{-1}; HRMS (ESI) m/z calcd for $C_{22}H_{19}N_2O_4$ $[M+H]^+$ 375.1339; found 375.1337; Anal. Calcd for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.32; H, 4.98; N, 7.44.

5.1.58. *N*-Methyl-2-{5-[methyl(phenyl)amino]-2-oxo-1,3-benzoxazol-3(2*H*)-yl}-*N*-phenylacetamide (78)

A mixture of **67** (181 mg, 0.500 mmol), *N*-methylaniline (81.3 μL, 0.750 mmol), $Pd_2(dba)_3$ (22.9 mg, 0.0250 mmol), Xantphos (43.4 mg, 0.0750 mmol) and Cs_2CO_3 (228 mg, 0.700 mmol)

in toluene (4.0 mL) was heated at reflux for 7 h and cooled to room temperature. The reaction was quenched by adding aqueous saturated $NaHCO_3$, and then the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using hexane/EtOAc (3:1, v/v) as eluent to give **78** (60.6 mg, 31%) as a white solid: mp 123–125 °C (*i*PrOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (2H, dd, J = 7.3, 7.3 Hz), 7.41 (1H, t, J = 7.3 Hz), 7.30–7.22 (4H, m), 7.11 (1H, d, J = 8.5 Hz), 6.95–6.88 (3H, m), 6.80 (1H, dd, J = 8.5, 2.0 Hz), 6.58 (1H, d, J = 2.0 Hz), 4.23 (2H, s), 3.30 (3H, s), 3.28 (3H, s); IR (ATR) 1768, 1655, 1495, 1489, 1392 cm^{-1}; HRMS (ESI) m/z calcd for $C_{23}H_{22}N_3O_3$ $[M+H]^+$ 388.1656; found 388.1652; Anal. Calcd for $C_{23}H_{21}N_3O_3{\cdot}0.50H_2O$: C, 69.68; H, 5.59; N, 10.60. Found: C, 69.84; H, 5.41; N, 10.54.

5.1.59. 2-(5-Chloro-2-oxo-1,3-benzoxazol-3(2*H*)-yl)-*N*-methyl-*N*-phenylacetamide (80)

Compound **80** was prepared from (5-chloro-2-oxo-1,3-benzoxazol-3(2*H*)-yl)acetic acid (455 mg, 2.00 mmol) and *N*-methylaniline (0.260 mL, 2.40 mmol) in a manner similar to that described for compound **14** as a white solid (292 mg, 46%): mp 132–133 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (2H, dd, J = 7.6, 7.6 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.34 (2H, d, J = 7.6 Hz), 7.12–7.05 (2H, m), 6.87 (1H, d, J = 2.0 Hz), 4.28 (2H, s), 3.33 (3H, s); IR (ATR) 1788, 1774, 1655, 1487, 1381 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{14}ClN_2O_3$ $[M+H]^+$ 317.0687; found 317.0689; Anal. Calcd for $C_{16}H_{13}ClN_2O_3$: C, 60.67; H, 4.14; N, 8.84; Cl, 11.19. Found: C, 60.61; H, 4.20; N, 8.84; Cl, 11.02.

5.2. Biology

5.2.1. TSPO-binding assay

Male SD rats (Japan Charles River) were decapitated, and the kidney was dissected. The kidney was homogenized in 5 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.6) with a microhomogenizer (Physcotron, Niti-on Inc.). The homogenate was centrifuged at 20,000*g* and 4 °C for 10 min, and the resulting pellet was resuspended in the same volume of fresh buffer and recentrifuged. These resuspension and centrifugation procedures were repeated once more, and the obtained pellet was resuspended in 50 mM Tris–HCl buffer (pH 7.6) at the protein concentration of 2.63 mg/mL and stored frozen at −80 °C until required. The crude mitochondrial preparation (0.895 mL) was incubated with [^3H]-PK11195 (final concentration 1.0 nM) and various concentration of the test compounds in a total volume of 1.0 mL for 1 h at 4 °C. The reaction terminated by rapid fitration through a GF/B glass filter presoaked with 0.3% polyetyleneimine. The filters were immediately washed with ice-cold 50 mM Tris–HCl buffer (pH 7.6), and the filter-bound radioactivity was quantified using a liquid scintillation analyzer (Tri Carb 2700TR, Packard). Nonspecific binding was determined in the presence of 10 μM PK11195. All assays were carried out in duplicate except the total binding and nonspecific binding, which were in quadruplicate. The specific binding was determined by subtracting nonspecific from total binding. The IC_{50} values for each test compound were determined according to a nonlinear least-square curve-fitting method using the SAS® system (SAS Institute Inc.). In the assay with rat kidney TSPOs, K_i values were calculated according to the following formula: $K_i = IC_{50}/(1+[L]/K_D)$ where [L] and K_D are the concentration of [^3H]-PK11195 and the dissociation constant of PK11195 calculated by Scatchard analysis, respectively.

5.2.2. CBR-binding assay

Male SD rats (Japan Charles River) were decapitated, and the cerebral cortex was dissected. The cerebral cortex was homogenized in 10 volumes of ice-cold potassium phosphate buffer (200 mM KCl, 20 mM KOH, 20 mM KH_2PO_4, pH 7.4) with a microhomogenizer (Physcotron, Niti-on Inc.). The homogenate was centrifuged at 32,500 g and 4 °C for 15 min, and the resulting pellet was resuspended in the same volume of fresh buffer and recentrifuged. These resuspension and centrifugation procedures were repeated once more, and the obtained pellet was resuspended in potassium phosphate buffer (200 mM KCl, 20 mM KOH, 20 mM KH_2PO_4, pH 7.4) at the protein concentration of 2.63 mg/mL and stored frozen at −80 °C until required. The crude cerebral cortex preparation (0.895 mL) was incubated with [^3H]-flumazenil (final concentration 1.0 nM) and 10 μM of the test compounds in a total volume of 1.0 mL for 1 h at 25 °C. The reaction terminated by rapid fitration through a GF/B glass filter presoaked with 0.3% polyetyleneimine. The filters were immediately washed with ice-cold potassium phosphate buffer (200 mM KCl, 20 mM KOH, 20 mM KH_2PO_4, pH 7.4), and the filter-bound radioactivity was quantified using a liquid scintillation analyzer (Tri Carb 2700TR, Packard). Nonspecific binding was determined in the presence of 10 μM [^3H]-flumazenil. All assays were done in duplicate. The specific binding was determined by subtracting nonspecific from total binding.

5.2.3. Vogel-type conflict test in rats

For this test, the method of Vogel and colleagues[32] with a minor modification was used in male SD rats. For the test operant, behavior boxes ($26 \times 25 \times 16.5$ cm^3) with a stainless steel grid floor (Ohara Co., Ltd) were used. A water bottle with a metal drinking tube was fitted from the outside to the box so that only the drinking tube extended into the box. Electric shocks (0.30 mA, 0.5 s) were administered to each rat by automatically switching the connections to the drinking tube and the grid floor from the drinkometer to an electric stimulator. After 48 h of water deprivation, the rats were individually placed in the test chamber. The number of shocks that each rat received after every 20 licks was recorded for 3 min. Test compounds were orally administered 1 h before the test session.

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Supplementary data

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