Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 2005–2021

Development of 3,4-dihydro-2*H*-benzo[1,4]oxazine derivatives as dual thromboxane A_2 receptor antagonists and prostacyclin receptor agonists

Michihiro Ohno,* Yoichiro Tanaka, Mitsuko Miyamoto, Takahiro Takeda, Kazuhiro Hoshi, Naohiro Yamada and Atsushi Ohtake

Pharmaceutical Research Laboratories, Toray Industries, Inc. 1,111 Tebiro, Kamakura, Kanagawa 248-8555, Japan

Received 13 September 2005; revised 26 October 2005; accepted 27 October 2005
Available online 16 November 2005

Abstract—We discovered a novel series of 3,4-dihydro-2*H*-benzo[1,4]oxazin-8-yloxyacetic acid derivatives as potent dual-acting agents to block the TXA_2 receptor and to activate the PGI_2 receptor. We report the synthesis, structure–activity relationship, and in vitro, ex vivo, and in vivo pharmacology of this series of compounds. 4-[2-(1,1-Diphenylethylsulfanyl)ethyl]-3,4-dihydro-2*H*-benzo[1,4]oxazin-8-yloxyacetic acid *N*-methyl-D-glucamine salt (**7**) is a promising candidate for a novel treatment in the anti-thrombotic and the cardiovascular fields avoiding hypotensive side effects.

1. Introduction

Thromboxane A_2 (TXA_2) (**1**), discovered by Samuelsson, is an unstable endogenous arachidonic acid metabolite that plays a pivotal role in platelet aggregation and vasoconstriction,[1] and has been implicated as a contributor to cardiovascular, renal, and pulmonary diseases.[2,3] Because of the lack of clinical efficacy with these agents,[4] a combined therapeutics using thromboxane receptor antagonists (TRAs) and thromboxane synthase inhibitors (TSIs) has been developed. This therapy has the advantage that its TSI activity would prevent the biosynthesis of TXA_2, while the accumulated PGH_2 would be redirected to produce beneficial prostaglandin metabolites such as prostacyclin (PGI_2), PGD_2, and PGE_2. However, this conventional TRA/TSI therapy exhibits unsatisfactory clinical effects.[5]

Prostacyclin (**2**), discovered by Vane, is a powerful endogenous inhibitor of platelet aggregation and also plays an important role in biological homeostasis as an endogenous autacoid distributed widely in various tissues.[6] Although these actions attracted notice in the cardiovascular field, the therapeutic application of PGI_2 itself is limited by both chemical and metabolic instability because of its labile enol–ether moiety. Thus, the extensive efforts that have been focused on the synthesis of PGI_2 mimics were directed toward the stabilization of the enol–ether moiety (i.e., **3**).[7,8] Recently, non-prostanoids PGI_2 mimetics with chemical and metabolic instability have been reported (i.e., **4**)[9–14] (Chart 1).

TXA_2 and PGI_2, both synthesized from arachidonic acid, have opposite effects on platelet aggregation. Also the balance between TXA_2 and PGI_2 greatly affects maintenance of the homeostasis of the circulatory system. In the case of ischemic disorders, the TXA_2/PGI_2

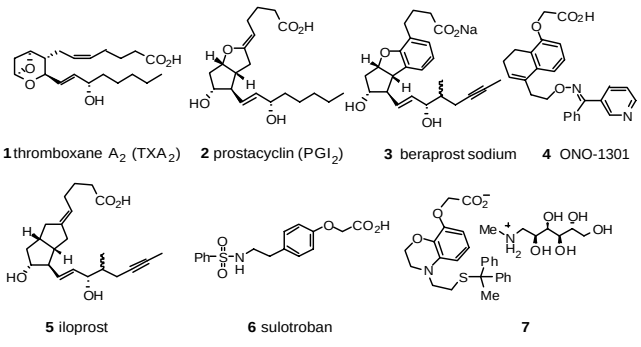


Chart 1.

Keywords: Prostacyclin; Thromboxane A_2 antagonist; Dual prostanoids; Anti-thrombosis.
* Corresponding author. Tel.: +81 467 32 2111; fax: +81 467 32 4791; e-mail: michihiro_ono@nts.toray.co.jp

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.10.050

balance is shifted to the TXA_2 side and phenomena such as platelet activation, subsequent thrombogenesis appear. Thus, it is clinically important to achieve the proper TXA_2/PGI_2 balance. A combination of an agent for inhibiting TXA_2 activity or an agent acting as a PGI_2 receptor agonist is thought to be effective. Moreover, Schering AG reported that PGI_2 mimetic **5** (iloprost) showed strong anti-thrombotic action without vasodilation when it combined with TXA_2/PGH_2 receptor antagonist **6** (sulotroban).[15,16] Therefore, we are interested in developing agents that combine the TXA_2 receptor antagonist activity with prostacyclin receptor agonist activity within a single molecule. Such agents would not only maximize the beneficial effects of each agent but also address the potential clinical problem of using two drugs with different pharmacokinetics. Moreover, one could expect a synergistic effect from the combined therapeutic agent in a single chemical entity to avoid the hypotensive effect of PGI_2.

In a previous publication,[17] we reported dual-acting benzofurans that possess TXA_2 antagonism and PGI_2 agonism. And we demonstrated that a synergistic effect from the combined therapeutic agent in a single chemical entity enables us to avoid the hypotensive effect of PGI_2. In the present study, aimed at further investigating the dual prostanoids possessing TXA_2 antagonism and PGI_2 agonism within a single molecule, we have explored a new scaffold. We describe the design, synthesis, and the biological evaluation of 3,4-dihydro-2*H*-benzo[1,4]oxazine derivatives.

2. Results and discussion

2.1. Chemistry

We designed our compounds from **3** (Chart 2). We picked out benzofuran scaffold, which is regarded as a characteristic structure of **3** and reported a dual-acting benzofuran **8**. We expanded our research to replace the scaffold since benzofuran derivatives are difficult to synthesize. We designed compounds **9a–d** and **10a** replacing the benzofuran ring to nitrogen containing hetero-aromatics. To avoid enantiomeric problems, we attached the ω-side chain through a nitrogen atom. We retained an oxyacetic acid group of compound **8** and its attached position for the following reasons. (1) This derivatization at the 7-position of benzofuran is known to maintain the PGI_2 agonistic properties. (2) This derivatization can avoid ω-oxidation metabolism of the α-chain. (3) It enables us to shorten synthetic steps. We also preserved the terminal 1,1-diphenylethylsulfide in the ω-side chain, which was effective in triggering dual activity for the benzofuran scaffold,[17] and wide range of ω-side chains were screened (**10a–k**) in the following optimization. The product in which the sulfide in compound **10a** was oxidized was also screened. Identification of compounds was confirmed by ^{1}H NMR, IR, and low-resolution mass spectrometry (LRMS), and the purity was demonstrated by elemental analysis.

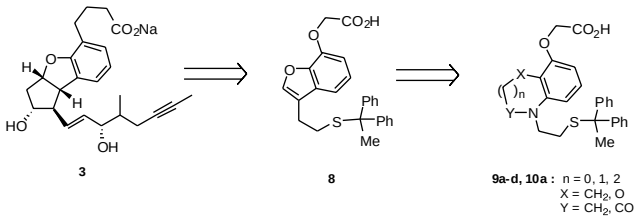
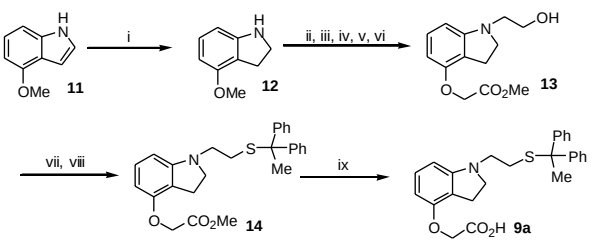


Chart 2.

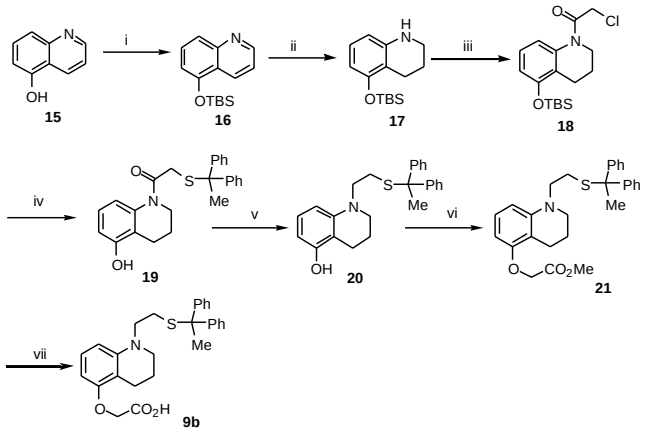
The compounds in Tables 1 and 2 were prepared as described in Schemes 1–7. The exploration of conventional methods for thiol synthesis was the key objective of this project. Nishio reported the single-step conversion from secondary and tertiary alcohols to the corresponding thiols by treatment with Lawesson's reagent.[18,19] We also reported a conventional method for tertiary thiol synthesis, which was improved in the β-elimination side reaction of Nishio's procedure and also enabled the large-scale preparation.[17] Our method became a powerful tool in synthesis of **9a–d** and **10a–h**.

The preparation of indoline derivative **9a** is outlined in Scheme 1. We began the synthesis from commercially available 4-methoxy-1*H*-indole (**11**). Compound **11** was reduced to indoline **12** with BH_3 trimethylamine complex.[20] Indoline **12** was converted into *N*-(2-hydroxyethyl)-indoline **13** in 11% yield in 5 steps. Thus, compound **12** was coupled with 2-(2-bromoethoxy)tetrahydropyran using phenyllithium as base, and tetrahydropyranyl group was removed under mild acidic condition. The methyl protection of the phenolic hydroxyl group was removed using *n*-PrSK,[21,22] and then was treated with methyl bromoacetate to selectively introduce the oxyacetic α-chain moiety at the 4-position of indoline. After coupling compound **13** with 1,1-diphenylethanethiol via the mesylate, compound **9a** was obtained upon hydrolysis of the methyl ester groups of **14**.

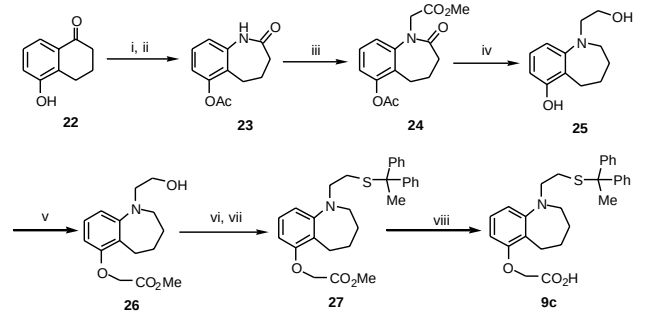
The preparation of tetrahydroquinoline derivative **9b** is outlined in Scheme 2. We began with the protection of hydroxyl group of quinolin-5-ol **15** by *t*-butyldimethylsilyl group. After hydrogenation of compound **16** using Pd/C catalysis, tetrahydroquinoline **17** was sequentially treated with 2-chloroacetyl chloride and sodium 1,1-diphenylethanethiolate, which was accompanied by removal of the *t*-butyldimethylsilyl group. The amide group of compound **19** was reduced by BH_3–tetrahydro-



Scheme 1. Reagents: (i) BH_3–Me_3N, 87%; (ii) PhLi; (iii) $BrCH_2CH_2OTHP$; (iv) HCl, MeOH; (v) *n*-PrSK, DMF; (vi) $BrCH_2CO_2Me$, K_2CO_3, 11% for 5 steps; (vii) MsCl, Et_3N; (viii) $Ph_2MeCSNa$, 99% for 2 steps; (ix) NaOH, 59%.



Scheme 2. Reagents: (i) TBDMS-Cl, imidazole, 100%; (ii) H_2, Pd/C, 82%; (iii) $ClCH_2COCl$, pyridine, 100%; (iv) $Ph_2MeCSNa$, 98%; (v) BH_3, THF, 46%; (vi) $BrCH_2CO_2Me$, K_2CO_3, 72%; (vii) NaOH, 67%.

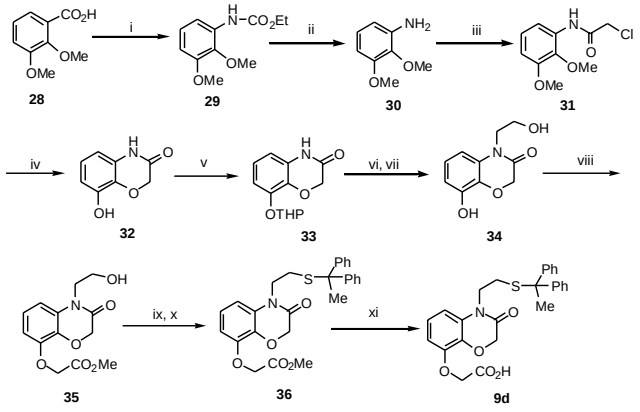


Scheme 3. Reagents: (i) TFA, NaN_3; (ii) Ac_2O, pyridine, 61% for 2 steps; (iii) $BrCH_2CO_2Me$, K_2CO_3, 99%; (iv) $LiAlH_4$, 81%; (v) $BrCH_2CO_2Me$, K_2CO_3, 67%; (vi) CBr_4, Ph_3P; (vii) $Ph_2MeCSNa$, 84% for 2 steps; (vii) NaOH, 62%.

furan complex, and the obtained compound **20** was treated with methyl bromoacetate to introduce the oxyacetic α-chain moiety. Compound **9b** was obtained upon hydrolysis of the methyl ester groups of **21**.

The preparation of tetrahydro-1*H*-benzazepine derivative **9c** from 5-hydroxy-1-tetralone **22** is outlined in Scheme 3. We performed the ring expansion using Schmidt rearrangement.[23] Thus, compound **22** was treated with sodium azide under acidic condition, and the phenolic hydroxyl group was protected by the acetyl group. N-Alkylation of the amide group of compound **23** was performed by treating it with methyl bromoacetate and potassium carbonate. The amide group and ester group of **24** were simultaneously reduced to corresponding amines and the primary alcohol using $LiAlH_4$, which was accompanied by reductive removal of acetyl group, and the resulting compound **25** was treated with methyl bromoacetate to selectively introduce the oxyacetic α-chain moiety at the 6-position of tetrahydro-1*H*-benzazepine. The alcohol **26** was coupled with 1,1-diphenylethanethiol via bromide. Compound **9c** was obtained upon hydrolysis of the methyl ester groups of **27**.

The preparation of 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine derivative **9d** from 2,3-dimethoxyaniline **30** is

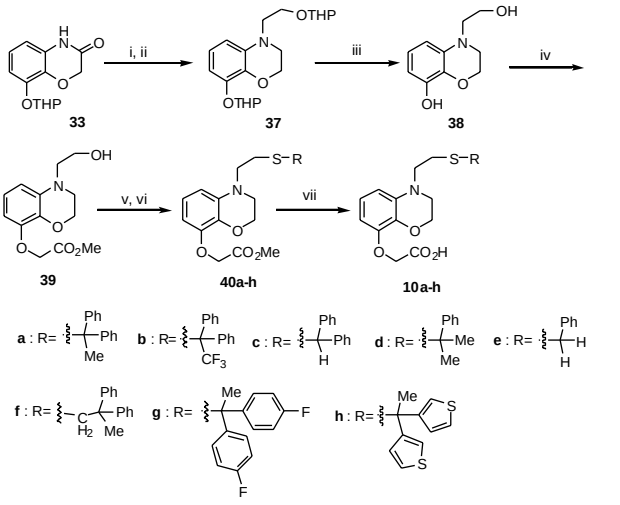


Scheme 4. Reagents: (i) DPPA, Et_3N, EtOH, 78%; (ii) NaOH, 93%; (iii) $(ClCH_2CO)_2O$, 97%; (iv) BBr_3, CH_2Cl_2 then K_2CO_3, DMF, 90%; (v) DHP, PPTS, 80%; (vi) NaH, $BrCH_2CH_2OTHP$; (vii) *p*-TsOH, MeOH, 94% for 2 steps; (viii) $BrCH_2CO_2Me$, K_2CO_3, DMF, 97%; (ix) MsCl, Et_3N; (x) $Ph_2MeCSNa$, 42% for 2 steps; (xi) NaOH, 94%.

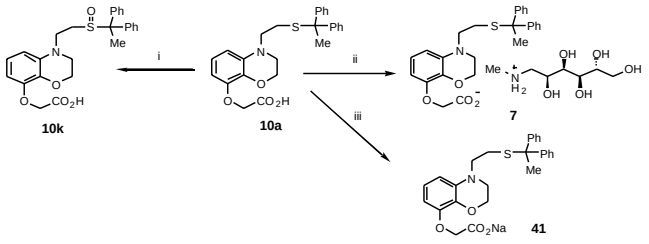
outlined in Scheme 4. Compound **30** was synthesized from 2,3-dimethoxybenzoic acid **28** using Schmidt rearrangement. Thus, compound **28** was treated with diphenylphosphorylazide and Et_3N in ethanol, and the resulting ethyl carbamate **29** was hydrolyzed to obtain compound **30**. After chloroacetylation of compound **30** followed by demethylation using BBr_3, cyclization of 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine was performed by treating it with a base. The phenolic hydroxyl group of compound **32** was protected by tetrahydropyran. In the course of this synthesis, the compounds **32** and **33** were crystallized from ethyl acetate, respectively. This enabled large-scale preparation and storage of the compounds **32** and **33** as common intermediates. Compound **33** was coupled with 2-(2-bromoethoxy)tetrahydropyran and tetrahydropyranyl groups were removed under mild acidic condition. After selective introduction of oxyacetic α-chain by treating with methyl bromoacetate, the resulting compound **35** was coupled with thiol via mesylate. Compound **9d** was obtained upon hydrolysis of the methyl ester groups of **36**.

The preparation of 3,4-dihydro-2*H*-1,4-benzoxazine derivatives **10a**–**h** is summarized in Scheme 5. Common intermediate **33** was coupled with 2-(2-bromoethoxy)-tetrahydropyran, and the amide group was reduced to amine using the BH_3–THF complex, then tetrahydropyranyl groups were removed under mild acidic condition. Compound **38** was treated with methyl bromoacetate to selectively introduce the oxyacetic α-chain. The alcohol **39** was coupled with thiols via mesylate. Compound **10a**–**h** was obtained upon hydrolysis of the methyl ester group of **40a**–**h**, respectively.

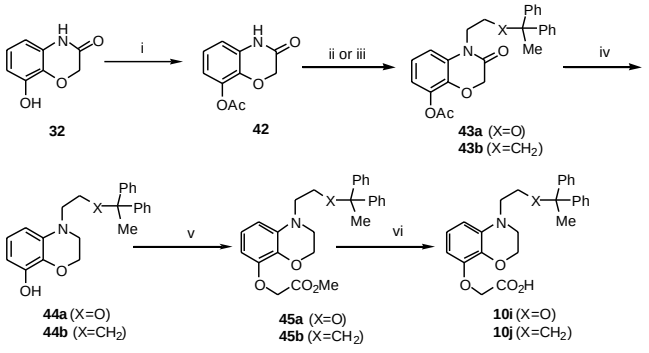
The preparation of compounds **7**, **10k**, and **41** is described in Scheme 6. Compound **7** was obtained in 95% yield by treating **10a** with *N*-methyl-D-glucamine and crystallizing from ethanol. The sulfoxide analogue **10k** was synthesized by direct oxidation of **10a** with *m*-chloroperbenzoic acid. We also tried oxidation of **10a** or **40a** using H_2O_2, *m*-chloroperbenzoic acid, $KMnO_4$, and Oxone®, but failed to obtain the



Scheme 5. Reagents: (i) NaH, $BrCH_2CH_2OTHP$; (ii) BH_3, THF, 93% for 2 steps; (iii) PPTS, MeOH, 97%; (iv) $BrCH_2CO_2Me$, K_2CO_3, DMF, 74%; (v) MsCl, Et_3N; (vi) NaH, thiols, 37–95%; (vii) NaOH, 61–91%.



Scheme 6. Reagents: (i) *m*-CPBA, AcOH, CH_2Cl_2, 81%; (ii) *N*-methyl-D-glucamine, EtOH, 95%; (iii) NaOH, 100%.



Scheme 7. Reagents: (i) Ac_2O, pyridine, 94%; (ii) NaH, *p*-$TsOCH_2CH_2OCPh_2Me$, 57%; (iii) NaH, *p*-$TsO(CH_2)_3CPh_2Me$, 61%; (iv) BH_3, THF, 99% for **a**, 99% for **b**; (v) $BrCH_2CO_2Me$, K_2CO_3, 80% for **a**, 92% for **b**; (vi) NaOH, 65% for **i**, 79% for **j**.

sulfone analogue of **10a**. Compound **41** was obtained by treating **10a** with 1 equivalent NaOH and lyophilizing.

For comparison, we also attached the ether type and carbohydrate type ω-side chain at the 4-position of 3,4-dihydro-2*H*-1,4-benzoxazine. The preparation of compound **10i** and **10j** is described in Scheme 7. The phenolic hydroxyl group of intermediate **32** was protected by acetyl group, and the resulting compound **42** was coupled with 2-(1,1-diphenylethoxy)ethyl-*p*-toluenesulfonate or 4,4-diphenylpentyl-*p*-toluenesulfonate. The amide group of **43a**,**b** was reduced to the amine using the BH_3–tetrahydrofuran complex, which was accompanied by removal of the acetyl group. The resulting compound **44a**,**b** was treated with methyl bromoacetate to selectively introduce as oxyacetic α-chain moiety at the 6-position of **44**. Compound **10i**–**j** was obtained upon hydrolysis of the methyl ester groups of **45a**,**b**, respectively.

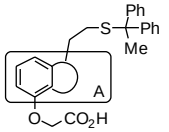
2.2. Biological activity

All compounds synthesized were evaluated as their free acid, sodium salt or *N*-methyl-D-glucamine salt. Compounds synthesized were evaluated in terms of inhibition of aggregation in human platelet-rich plasma (PRP) induced by the P2Y receptor agonist adenosine diphosphate (ADP) or by a stable TXA_2 agonist (U46619). To confirm the mechanistic profile of these compounds, we also performed receptor binding assay in human platelet membrane fraction. These receptor binding assays were carried out by using [^{3}H]SQ-29548 (a selective TXA_2 receptor antagonist) and [^{3}H]APS314d sodium salt (a selective PGI_2 receptor agonist) which is one of the components of **3**. Scatchard analysis of the binding of [^{3}H]SQ-29548 revealed a single binding site (K_d = 10.2 ± 0.51 nM, B_{max} = 5.89 ± 0.62 nM/mg protein). [^{3}H]APS314d sodium salt also had one binding site (K_d = 14.3 ± 0.51 nM, B_{max} = 6.08 ± 0.60 nM/mg protein).

We synthesized indoline analogue (**9a**), which had the same 5–6-fused aromatic system as benzofuran. We screened the scaffold using 1,1-diphenylethylsulfide ω-side chain, which was effective in triggering the PGI_2 agonistic property for the benzofuran scaffold. Also, tetrahydroquinoline derivative (**9b**), having a 6–6-fused aromatic system, and tetrahydro-1*H*-benzazepine derivative (**9c**), having a 6–7-fused aromatic system, were tested. (Table 1) Compound **9a**–**c** showed dual activity, but it was not as potent as compound **8** in both properties. In the next step, we introduced oxygen atom on **9b**, which was the most potent among **9a**–**c**, and designed 3,4-dihydro-2*H*-1,4-benzoxazine derivatives (**9d** and **10a**). Compared to **9b**, compound **10a** was 2.4 times trigger potent in the TXA_2 antagonism. Compound **9d**, which is regarded as the oxidated analogue in the α-carbon of nitrogen of **10a**, was less potent than **10a** in anti-aggregatory activity although it possessed similar dual property as **10a**. And compound **10a** is the best dual prostanoid in this series.

In the following optimization on **10a**, we screened through a wide range of ω-side chains to enhance the TXA_2 antagonism and/or the PGI_2 agonism (Table 2). First, we investigated the influence of alkyl substitution at the end of the ω-side chain and **10b**–**f** were synthesized. Compound **10c** was 10 times less potent than compound **10a** in TXA_2 antagonistic property. And compounds **10b** and **10e** completely lost TXA_2 antago-

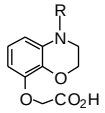
Table 1. Inhibition of platelet aggregation and binding affinities of nitrogen containing hetero-aromatic derivatives

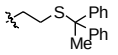
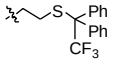
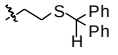
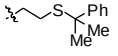
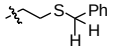
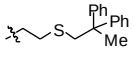
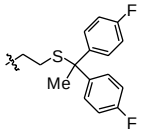
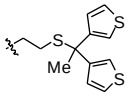
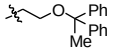
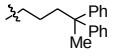
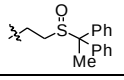


Compound	A	Anti-aggregatory activity IC_{50} (μM)[a]		Receptor affinity K_i (μM)	
		ADP[b]	U46619[c]	IP	TP
8[d]		8.1	0.58	0.57	0.026
9a		4.2	1.6	1.0	0.14
9b		1.3	0.59	0.52	0.12
9c		1.8	1.3	1.3	0.19
9d		15	1.3	1.00	0.038
10a		1.8	0.55	0.43	0.050

[a] IC_{50} represents the concentration that inhibits induced aggregation by 50% in human platelet-rich plasma.
[b] Platelet aggregation was induced by ADP (5 μM).
[c] Platelet aggregation was induced by U46619 (2 μM).
[d] Ref. 17.

Table 2. Inhibition of platelet aggregation and binding affinities of 3,4-dihydro-2*H*-1,4-benzoxazine derivatives



Compound	R	Anti-aggregatory activity IC_{50} (μM)[a]		Receptor affinity K_i (μM)	
		ADP[b]	U46619[c]	IP	TP
10a		1.8	0.55	0.43	0.050
10b		4.7	3.7	0.34	1.2
10c		2.0	1.3	0.39	0.54
10d		44	0.28	6.1	0.046
10e		45	5.6	1.5	2.6
10f		0.90	0.83	0.91	0.29
10g		23	0.54	1.9	0.13
10h		44	1.9	3.3	0.34
10i		16	5.5	4.3	1.0
10j		18	5.9	2.6	2.6
10k		7.9	2.5	0.023	0.97

[a] IC_{50} represents the concentration that inhibits induced aggregation by 50% in human platelet-rich plasma.
[b] Platelet aggregation was induced by ADP (5 μM).
[c] Platelet aggregation was induced by U46619 (2 μM).

nistic property. Only compound **10d** showed excellent potency in TXA_2 antagonistic property, but it completely lost PGI_2 agonistic property. We checked the influence of carbon length of side chain. Compound **10f** showed moderate PGI_2 agonistic property, but it is about 6 times less than compound **10a** in TXA_2 antagonist activity. In second, we checked the influence of aromatic ring. The *p*-fluorophenyl analogue (**10g**) and the thiophene analogue (**10h**) lost both TXA_2 antagonistic and PGI_2 agonistic activities. We checked the influence of the sulfide moiety in the side chain. The ether analogue (**10i**) and the carbohydrate analogue (**10j**) lost both TXA_2 antagonistic property and PGI_2 agonistic property. The sulfide moiety in **10a** is important to trigger both TXA_2 antagonistic and PGI_2 agonistic activities. We also synthesized sulfoxide analogue (**10k**) and investigated the oxidated stage of sulfide in compound **10a**. Although compound **10k** lost TXA_2 antagonistic property, its PGI_2 agonistic property was dramatically improved to be an excellent PGI_2 agonist. The antiplatelet activities of **10k**, however, were less portent than those expected from binding affinity because of its high protein binding.

In the next study, we have examined the pharmacological profile of compound **10a** in terms of its anti-platelet effects. Compound **10a** is a novel compound having a potent TXA_2 antagonistic activity together with a moderate PGI_2 agonistic activity. In fact, compound **10a** showed an 8.6-fold higher affinity to thromboxane A_2 receptor (TP) than to prostacyclin receptor (IP), as evidenced with the K_i values determined in binding assays using human platelet membrane. PGI_2 agonistic

Table 3. Solubility of **10a** with *N*-methyl-D-glucamine salt and sodium salt

Salt form of **10a**	dist H_2O (mg/mL)	Saline (mg/mL)	5% xylitol (mg/mL)
N-Methyl-D-glucamine salt (**7**)	>30	<0.1[a]	>30
Sodium salt (**41**)	<0.1	<0.1	<0.1

[a] The compound was precipitated as a sodium salt.

Table 4. Effects of **7**, SQ-29548, **3** (beraprost sodium), and **4** (ONO-1301) on in vitro platelet aggregation in human PRP

Aggregating agent	IC_{50} (nM)			
	7	SQ-29548	**3**	**4**
U-46619	640 ± 110	21 ± 3	7.6 ± 0.7	170 ± 11
ADP	1800 ± 540	> 10000	5.7 ± 1.0	170 ± 3

Platelet aggregation was induced by U-46619 (4 μM) or by ADP (5 μM). Values are means ± SE of three to four determinations.

property of compound **10a** was also confirmed by monitoring intracellular c-AMP concentration using platelet.[24]

2.3. Solubility improvement

To eliminate the effect of dimethyl sulfoxide in pharmacological experiments, we synthesized sodium salt. Compound **10a** possessing two aromatic rings at the end of the side chain, however, is highly lipophilic, so sodium salt does not dissolve even in distilled water. Then we performed a wide range of screening of conjugate amines and found out the *N*-methyl-D-glucamine salt of **10a** (**7**). Compound **7** showed excellent solubility in the 5% xylitol. In saline, compound **7** did not dissolve more than 0.1 mg/mL, since the *N*-methyl-D-glucamine salt turned into sodium salt. In a previous report,[17] we succeeded in obtaining a diethanolamine salt to improve the compound's solubility, but diethanolamine salt of **10a** did not crystallize, which is important for the compound's stability. From these results, we found out that compound **7** with 5% xylitol is the practical formula for pharmacological experiments (Table 3).

2.4. Pharmacological activity

The TXA_2 antagonistic and PGI_2 agonistic activities of compound **7** were examined in in vitro platelet aggregation (Table 4).[24,25] Compound **7** exhibited inhibitory effects on the ADP- and U-46619-induced aggregation. The IC_{50} value of inhibitory effects on the ADP-induced aggregation was about 3-fold less potent than that on the U-46619-induced aggregation. Similar tendency was observed with the TXA_2 receptor antagonist, SQ-29548. On the other hand, the IC_{50} value of inhibitory effects by the selective PGI_2 receptor agonists **3** (beraprost sodium) and **4** (ONO-1301) were almost the same on both ADP- and U-46619-induced platelet aggregation. These results are consistent with the fact that compound **7** has a PGI_2 receptor agonistic activity in addition to the TXA_2 receptor antagonistic activity. This is also supported by the evidence that these results, together with the results of binding assays, indicate that the PGI_2 receptor agonistic activity of compound **7** is relatively less potent than its TXA_2 receptor antagonistic activity.

To confirm the anti-thrombotic character of compound **7**, we tried ex vivo experiment. The inhibitory effects observed with cynomolgus monkey's PRP were $IC_{50} = 2.7 \pm 0.7$ μM (induced by 10 μM ADP) and $IC_{50} = 0.56 \pm 0.02$ μM (induced by 600 μM arachidonic acid). Since these data were quite similar to those observed with human PRP, our further experiments were carried out in cynomolgus monkeys (Table 5). Infusion of PGI_2 receptor agonist **4** showed dose-dependent inhibition of ADP-induced platelet aggregation, but severe vasodilation together with increase of heart rate was observed at the dose of 3 μg/kg/min. The anti-platelet activity of compound **4** is linked to its potent vasodilation. In a similar manner, compound **7** caused dose-dependent inhibitions of the ADP-induced platelet aggregation, which was completely inhibited at the dose of 30 μg/kg/min. And the arachidonic acid-induced aggregation was completely inhibited at the dose of more than 3 μg/kg/min. Furthermore, compound **7** did not show any significant changes in blood pressure and heart rate at the dose less than 10 μg/kg/min. At the dose 30 μg/kg/min, compound **7** showed an increase of heart rate, but no significant change in blood pressure was observed.

The neuroprotective effect of compound **7** on arterial thrombosis was studied in cynomolgus monkey middle cerebral artery (MCA) occlusion–reperfusion model.[26] Via a transorbital approach,[27,28] cynomolgus monkeys underwent a 3-h occlusion of the right middle cerebral artery (MCA), followed by reperfusion and observation for 4 days. Starting 2 h after MCA occlusion, compound **7** was administered. The control animals received drug vehicle bolus and infusion administration after MCA occlusion. Steady-state ^{15}O continuous inhalation was used for assessment of local cerebral blood flow (CBF), cerebral metabolic rate of oxygen ($CMRO_2$), and oxygen extraction fraction (OEF) using high-resolution PET. Five consecutive PET scans (before occlusion, at 2 h after occlusion, and at 2 h, 24 h, and 4 days after reperfusion) were performed on each monkey. The size of the cerebral damage due to ischemia was measured histologically at 4 days after reperfusion. Treatment with compound **7** (15 μg/kg/min infusion for 0.5 h, followed by 7.5 μg/kg/min infusion for 23.5 h), reduces cerebral damage detected by histological observation at 4 days after MCA occlusion. Compound **7** significantly suppressed the $CMRO_2$, which is observed from 2 h to 4 days after the MCA occlusion, but compound **7** did not affect CBF during the experiments. Compound **7** inhibits platelet aggregation induced by ADP by 90% without showing any significant changes in blood pressure and heart rate in this stroke model. These results suggest that the anti-thrombotic effect and the neuronal protective effect of compound **7** are separable from its potent vasodilation.

Table 5. Effects of **7** and **4** (ONO-1301) on blood pressure, heart rate, and ex vivo platelet aggregation in monkey

	7					**4**			
	Dose (μg/kg/min)[a]					Dose (μg/kg/min)[a]			
	Pre	1	3	10	30	Pre	0.3	1	3
Mean blood pressure (mmHg)[b]	92 ± 4	92 ± 17	98 ± 7	96 ± 8	86 ± 8	98 ± 9	96 ± 8	87 ± 8	71 ± 5
Heart rate (beats/min)[b]	165 ± 5	170 ± 8	171 ± 4	172 ± 6	191 ± 3	151 ± 6	155 ± 7	165 ± 8	176 ± 4
Percentage of platelet aggregation									
ADP[c]	67 ± 3	62 ± 5	61 ± 5	36 ± 11*	12 ± 8*	66 ± 6	57 ± 8	32 ± 13	21 ± 19
Arachidonic acid[c]	83 ± 2	28 ± 20*	7 ± 1*	7 ± 1*	8 ± 1*	77 ± 1	74 ± 1	54 ± 22	52 ± 22

[a] Drugs were infused for 30 min at each of the doses in a dose-escalation manner.
[b] Data are expressed as means ± SE of three to seven determinations.
[c] Platelet aggregation was induced by ADP (10 μM) or by arachidonic acid (600 μM). Values are means ± SE of three to seven determinations.
* Significantly different from the vehicle group ($p < 0.01$).

3. Conclusion

In a previous study, we demonstrated the ex vivo experiment of benzofuran-7-oxyacetic acid analogue and illustrated the beneficial properties of PGI_2 stable mimetics in terms of avoiding hypotensive side effects. In this paper, we have expanded the range of structures suitable as dual prostanoids. A variety of 3,4-dihydro-2*H*-benzo[1,4]oxazin-8-yloxyacetic acid analogues were prepared by versatile synthetic route, which allows large-scale preparation. Among the analogues synthesized, we found the dual-acting prostanoid **7** possessing a potent TXA_2 antagonism and a moderate PGI_2 agonism.

In conclusion, our results reported in this paper clearly illustrate the advantage of compound **7** having both PGI_2 receptor agonistic and TXA_2 receptor antagonistic activities. The TXA_2 receptor antagonistic and PGI_2 receptor agonistic activities of compound **7** are demonstrated in in vitro platelet aggregation. The ex vivo and in vivo experiments of compound **7** illustrated the beneficial anti-thrombotic properties in terms of avoiding vasodilation side effect. Remarkably, compound **7** was found to be a promising candidate for a novel medicine in anti-thrombotic and cardiovascular fields. Further experimental evaluations using monkey cerebral artery occlusion–reperfusion model are now in progress on pharmacological properties.

4. Experimental

4.1. Materials

Synthetic reagents were purchased from Aldrich (Milwaukee, WI), Kanto Kagaku Co. (Tokyo, Japan), TCI (Tokyo, Japan), and Sigma Chemical Co. (St. Louis, MO). Anhydrous tetrahydrofuran, methanol, dichloromethane, DMF, and pyridine were purchased from Kanto Kagaku Co. (Tokyo, Japan). All of the reagents and solvents used were of analytical grade from standard commercial source or were purified by standard methods before use. The active isomer of beraprost sodium, [^{3}H]APS-314d sodium, and [^{3}H]SQ-29548 were synthesized at Daiichi Pure Chemicals (Tokyo, Japan). SQ-29548 and U-46619 were purchased from Cayman Chemical (MI, USA), ADP from Sigma (MO, USA), 3.8% sodium citrate from Yamanouchi Pharmaceutical (Tokyo, Japan), and a low molecular weight heparin sodium dalteparin from Kissei Pharmaceutical (Nagano, Japan).

4.2. Chemistry

All melting points were obtained with a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a JASCO FT/IR-410 infrared spectrophotometer. ^{1}H NMR spectra were recorded with a Varian Gemini-2000 spectrometer (300 MHz) with tetramethylsilane as an internal standard. Low mass spectra (MS) or high-resolution mass spectra (HR-MS) were obtained with a JEOL JMS-DX303 mass spectrometer. The fast atom bombardment mass spectra (FAB-MS) were obtained by using glycerol as the matrix. Elemental analysis was performed by Toray Research Center. Thin-layer chromatography was performed on pre-coated TLC plates (silica gel 60 F-254, layer thickness 0.25 mm or DIOL F-254s) manufactured by E. Merck. Silica gel column chromatography was performed on Silica gel 60 (0.063–0.200 mm) manufactured by E. Merck. In general, reactions were carried out in dry solvents under an argon atmosphere unless otherwise mentioned. All reactions that required anhydrous condition were performed under argon or nitrogen, and all glassware was either oven-dried or flame-dried before use.

4.3. (4-(2-(1,1-Diphenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid *N*-methyl-D-glucamine salt (7)

To a solution of (4-(2-(1,1-diphenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid **10a** (1.50 g) in ethanol (150 mL) was added a solution of *N*-methyl-D-glucamine (0.74 g) in ethanol (74 mL) on heating, and the resulting mixture stood at room temperature. The precipitated solid was filtered off to obtain the object compound **7** (2.03 g, 95%). Colorless needles, mp 137.0–141.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.04 (3H, s), 2.45 (3H, s), 2.42–2.52 (2H, m), 2.85 (1H, dd, J = 8.5, 12.5 Hz), 2.94 (1H, dd, J = 3.5, 12.5 Hz), 3.13 (2H, t, J = 7.8 Hz), 3.16 (2H, t, J = 4.3 Hz), 3.36–3.44 (2H, m), 3.46–3.52 (1H, m), 3.58 (1H, dd, J = 3.5, 11.0 Hz), 3.65 (1H, dd, J = 1.5, 5.0 Hz), 3.81–3.87 (1H,

m), 4.05 (2H, t, $J = 4.0$ Hz), 4.16 (2H, s), 5.88 (1H, d, $J = 7.5$ Hz), 6.09 (1H, dd, $J = 1.0$, 8.5 Hz), 6.50 (1H, t, $J = 8.3$ Hz), 7.22–7.28 (2H, m), 7.30–7.42 (8H, m); ^{1}H NMR (300 MHz, CD_3OD) δ 2.13 (3H, s), 2.60 (2H, t, $J = 7.7$ Hz), 2.77 (3H, s), 3.16–3.32 (6H, m), 3.67–3.93 (5H, m), 4.03–4.17 (1H, m), 4.22 (2H, t, $J = 4.4$ Hz), 4.42 (2H, s), 6.05 (1H, d, $J = 8.2$ Hz), 6.32 (1H, d, $J = 8.2$ Hz), 6.64 (1H, t, $J = 8.2$ Hz), 7.26–7.75 (10H, m); Mass (FAB, *m/e*) 448 $(M-H)^+$; Elemental analysis Calcd. for $C_{33}H_{44}N_2O_9S$: C, 61.47; H, 6.88; N, 4.34; S, 4.97. Found: C, 61.22; H, 6.80; N, 4.36; S, 4.99.

4.4. General procedure of methyl ester hydrolysis; {1-[2-(1,1-Diphenylethylsulfanyl)ethyl]-2,3-dihydro-1*H*-indol-4-yloxy}acetic acid (9a)

To a solution of {1-[2-(1,1-diphenylethylsulfanyl)ethyl]-2,3-dihydro-1*H*-indol-4-yloxy}acetic acid methyl ester (**14**) (333 mg) in methanol (5 mL) and THF (2 mL) was added 1 N sodium hydroxide aqueous solution (1.00 mL), and the mixture was stirred at room temperature for 20 min. The solvent was distilled off under reduced pressure, and the residue was poured into a 5% citric acid aqueous solution, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and concentrated. The residue was recrystallized from ethyl acetate/*n*-hexane to obtain compound **9a** (191 mg, 59%). Colorless prisms, mp 155.5–157.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (3H, s), 2.47–2.54 (2H, m), 2.93 (2H, t, $J = 8.7$ Hz), 2.97–3.04 (2H, m), 3.27 (2H, t, $J = 8.7$ Hz), 4.64 (2H, s), 5.96 (1H, d, $J = 8.1$ Hz), 6.10 (1H, d, $J = 8.1$ Hz), 6.96 (1H, t, $J = 8.1$ Hz), 7.20–7.34 (6H, m), 7.40–7.47 (4H, m); IR (KBr) 1744 cm^{-1} (COOH); Mass (FAB, *m/e*) 433 (M^+); Elemental analysis Calcd. for $C_{26}H_{27}NO_3S$: C, 72.03; H, 6.28; N, 3.23; S, 7.40. Found: C, 71.98; H, 6.32; N, 3.37; S, 7.41.

4.5. (1-(2-(1,1-Diphenylethylthio)ethyl)-1,2,3,4-tetrahydroquinolin-5-yloxy)acetic acid (9b)

By the procedure used in **9a**, compound **9b** (173 mg, 67%) was prepared from 268 mg of **21**. Pale yellow needles; mp 150.5–152.0 °C (recrystallized from methylene chloride/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80–190 (2H, m), 2.06 (3H, s), 2.47–2.55 (2H, m), 2.67 (2H, t, $J = 6.6$ Hz), 3.04–3.19 (4H, m), 4.61 (2H, s), 5.93 (1H, 6, $J = 8.1$ Hz), 6.05 (1H, d, $J = 8.1$ Hz), 6.88 (1H, t, $J = 8.1$ Hz), 7.20–7.34 (6H, m), 7.39–7.46 (4H, m); IR (KBr) 1745 cm^{-1} (COOH); Mass (FAB, *m/e*) 448 $(M+H)^+$; Calcd. for $C_{27}H_{29}NO_3S$: C, 72.45; H, 6.53; N, 3.13; S, 7.16. Found: C, 72.75; H, 6.35; N, 3.26; S, 7.32.

4.6. (1-(2-(1,1-Diphenylethylthio)ethyl)-2,3,4,5-tetrahydro-1*H*-benzazepin-6-yloxy)acetic acid (9c)

By the procedure used in **9a**, compound **9c** (171 mg, 62%) was prepared from 283 mg of **27**. Colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.68–1.52 (4H, m), 2.05 (3H, s), 2.45–2.53 (2H, m), 2.80–2.91 (4H, m), 3.06–3.14 (2H, m), 4.63 (2H, s), 6.39 (2H, d, $J = 8.1$ Hz), 6.97 (1H, t, $J = 8.1$ Hz), 7.16–7.32 (6H, m), 7.36–7.44 (4H, m); IR (liquid film method) 1734 cm^{-1} (COOH); Mass (EI, *m/e*) 461 (M^+); Calcd. for $C_{28}H_{31}NO_3S$: C, 72.85; H, 6.77; N, 3.03; S, 6.95. Found: C, 73.08; H, 6.81; N, 3.00; S, 7.03.

4.7. (4-(2-(1,1-Diphenylethylthio)ethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (9d)

By the procedure used in **9a**, compound **9d** (155 mg, 94%) was prepared from 170 mg of **36**. White powder, mp 106 °C (recrystallized from dichloromethane/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (3H, s), 2.57 (2H, m), 3.81 (2H, m), 4.60 (2H, s), 4.70 (2H, s), 6.16 (1H, dd, $J = 1$, 8 Hz), 6.61 (1H, dd, $J = 1$, 8 Hz), 6.84 (1H, t, $J = 8$ Hz), 7.21–7.34 (6H, m), 7.40–7.45 (4H, m); IR (KBr) 1734 (COOH), 1665 (amide) cm^{-1}; Mass (EI, *m/e*) 463 (M^+); Elemental analysis Calcd. for $C_{26}H_{25}NO_5S$: C, 67.37; H, 5.44; N, 3.02; S, 6.92. Found: C, 67.02; H, 5.46; N, 2.76; S, 6.94.

4.8. (4-(2-(1,1-Diphenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10a)

By the procedure used in **9a**, compound **10a** (15.61 g, 77%) was prepared from 17.29 g of **40a**. Colorless needles, mp 161–161.5 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.06 (3H, s), 2.52 (2H, t, $J = 7.5$ Hz), 3.14 (2H, t, $J = 7.5$ Hz), 3.22 (2H, t, $J = 4.5$ Hz), 4.23 (2H, t, $J = 4.5$ Hz), 4.61 (2H, s), 6.06 (1H, dd, $J = 1$, 8 Hz), 6.31 (1H, dd, $J = 1$, 8 Hz), 6.68 (1H, t, $J = 8$ Hz), 7.20–7.35 (6H, m), 7.39–7.44 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.4, 31.0, 46.8, 50.8, 56.7, 64.7, 68.8, 105.8, 107.4, 121.3, 126.8, 128.0, 128.1, 133.9, 135.5, 146.0, 146.7, 171.1; IR (KBr) 1744 cm^{-1} (COOH); Mass (EI, FAB, *m/e*) 449 (M^+); HR-MS (FAB, *m/e*) Calcd. for $C_{26}H_{27}NO_4S$ is 449.1631, Found 449.1661; Elemental analysis Calcd. for $C_{26}H_{27}NO_4S$: C, 69.46; H, 6.05; N, 3.12; S, 7.13. Found: C, 69.20; H, 6.05; N, 3.21; S, 7.12.

4.9. (4-(2-(1,1-Diphenyl-2,2,2-trifluoroethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10b)

By the procedure used in **9a**, compound **10b** (472 mg, 83%) was prepared from 585 mg of **40b**. Colorless plates, mp 140–140.5 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.56 (2H, t, $J = 7.5$ Hz), 3.19 (4H, m), 4.23 (2H m), 4.61 (2H, s), 5.98 (1H, dd, $J = 1$, 8 Hz), 6.30 (1H, dd, $J = 1$, 8 Hz), 6.65 (1H, t, 1 = 8 Hz), 7.32–7.36 (6H, m), 7.42–7.46 (4H, m); IR (KBr) 1748 cm^{-1} (COOH); Mass (EI, *m/e*) 503 (M^+); Elemental analysis Calcd. for $C_{26}H_{24}F_3NO_4S$: C, 62.02; H, 4.80; N, 2.78; S, 6.37; F, 11.32. Found: C, 61.78; H, 4.85; N, 2.87; S, 6.54; F, 11.37.

4.10. (4-(2-(Diphenylmethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10c)

By the procedure used in **9a**, compound **10c** (381 mg, 90%) was prepared from 439 mg of **40c**. Colorless needles, mp 172.5 °C (recrystallized from ethyl acetate/*n*-

hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.60 (2H, m), 3.27 (2H, t, J = 4 Hz), 3.37 (2H, m), 4.24 (2H, t, J = 4 Hz), 4.61 (2H, s), 5.23 (1H, s), 6.10 (1H, dd, J = 1, 8 Hz), 6.31 (1H, dd, J = 1, 8 Hz), 6.66 (1H, t, J = 8 Hz), 7.22–7.37 (6H, m), 7.42–7.46 (4H, m); IR (KBr) 1743 cm^{-1} (COOH); Mass (EI, *m/e*) 435 (M^+); Elemental analysis Calcd. for $C_{25}H_{25}NO_4S$: C, 68.94; H, 5.79; N, 3.22; S, 7.36. Found: C, 68.85; H, 5.77; N, 3.24; S, 7.25.

4.11. (4-(2-(1-Methyl-1-phenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10d)

By the procedure used in **9a**, compound **10d** (271 mg, 78%) was prepared from 358 mg of **40d**. Colorless needles, mp 114 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72 (6H, s), 2.42 (2H, m), 3.10 (2H, m), 3.19 (2H, t, J = 4.5 Hz), 4.22 (2H, t, J = 4.5 Hz), 4.60 (2H, s), 5.98 (1H, dd, J = 1, 8 Hz), 6.29 (1H, dd, J = 1, 8 Hz), 6.66 (1H, t, J = 8 Hz), 7.24 (1H, m), 7.35 (2H, m), 7.56 (2H, m); IR (KBr) 1744 cm^{-1} (COOH); Mass (EI, *m/e*) 387 (M^+); Elemental analysis Calcd. for $C_{21}H_{25}NO_4S$: C, 65.09; H, 6.50; N, 3.61; S, 8.27. Found: C, 64.72; H, 6.51; N, 3.57; S, 8.03.

4.12. (4-(2-(Benzylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10e)

By the procedure used in **40a**, compound **39** (242 mg) was coupled with phenylmethanethiol and the methyl ester of the obtained sulfide was hydrolyzed by the procedure used in **9a**, to obtain compound **10e** (199 mg, 61%). Colorless needles, mp 127–131 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (2H, t, J = 7.6 Hz), 3.30–3.38 (4H, m), 3.76 (2H, s), 4.27 (2H, t, J = 4.4 Hz), 4.63 (2H, s), 6.25 (1H, dd, J = 8.2, 1.1 Hz), 6.32 (1H, dd, J = 8.2, 1.1 Hz), 6.72 (1H, t, J = 8.2 Hz), 7.25–7.35 (5H, m); IR (KBr) 1742 cm^{-1} (COOH); Mass (EI, *m/e*) 359 (M^+); Elemental analysis Calcd. for $C_{19}H_{21}NO_4S$: C, 63.49; H, 5.89; N, 3.90; S, 8.92. Found: C, 63.20; H, 5.90; N, 3.97; S, 8.78.

4.13. (4-(2-(2,2-Diphenylpropylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10f)

By the procedure used in **9a**, compound **10f** (85 mg, 74%) was prepared from 119 mg of **40f**. Colorless needles, mp 138–139 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (3H, s), 2.35 (2H, t, J = 7.1 Hz), 3.24–3.33 (6H, m), 4.23 (2H, t, J = 4.4 Hz), 4.62 (2H, s), 6.28–6.33 (2H, m), 6.76 (1H, t, J = 8.2 Hz), 7.17–7.31 (10H, m); IR (KBr) 1746 cm^{-1} (COOH); Mass (EI, *m/e*) 463 (M^+); Elemental analysis Calcd. for $C_{27}H_{29}NO_4S$: C, 69.95; H, 6.31; N, 3.02; S, 6.92. Found: C, 69.80; H, 6.22; N, 3.07; S, 6.78.

4.14. (4-(2-(1,1-bis-(4-Fluorophenyl)ethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10g)

By the procedure used in **9a**, compound **10g** (166 mg, 67%) was prepared from 254 mg of **40g**. Colorless needles, mp 142–143 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (3H, s), 2.50 (2H, t, J = 7.6 Hz), 3.21 (2H, t, J = 7.6 Hz), 3.26 (2H, t, J = 7.6 Hz), 4.25 (2H, t, J = 4.4 Hz), 4.62 (2H, s), 6.06 (1H, dd, J = 8.2, 1.1 Hz), 6.31 (1H, dd, J = 8.2, 1.1 Hz), 6.69 (1H, t, J = 8.2 Hz), 6.95–7.03 (4H, m), 7.33–7.40 (4H, m); IR (KBr) 1744 cm^{-1} (COOH); Mass (EI, *m/e*) 485 (M^+); Elemental analysis Calcd. for $C_{26}H_{25}F_2NO_4S$: C, 64.32; H, 5.19; N, 2.88; S, 6.60. Found: C, 64.22; H, 5.13; N, 2.99; S, 6.61.

4.15. (4-(2-(1,1-bis-(3-Thienyl)ethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10h)

By the procedure used in **9a**, compound **10h** (161 mg, 91%) was prepared from 183 mg of **40h**. Colorless granular, mp 149 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (3H, s), 2.54 (2H, t, J = 7.4 Hz), 3.17 (2H, t, J = 7.4 Hz), 3.25 (2H, t, J = 4.4 Hz), 4.25 (2H, t, J = 4.4 Hz), 4.62 (2H, s), 6.11 (1H, dd, J = 8.2, 1.1 Hz), 6.31 (1H, dd, J = 8.2, 1.1 Hz), 6.72 (1H, t, J = 8.2 Hz), 7.12 (2H, dd, J = 3.0, 1.4 Hz), 7.14 (2H, dd, J = 5.2, 1.4 Hz), 7.29 (2H, dd, J = 5.2, 3.0 Hz); IR (KBr) 1744 cm^{-1} (COOH); Mass (EI, *m/e*) 461 (M^+); Elemental analysis Calcd. for $C_{22}H_{23}NO_4S_3$: C, 57.24; H, 5.02; N, 3.03; S, 20.84. Found: C, 56.84; H, 5.04; N, 3.03; S, 20.45.

4.16. (4-(2-(1,1-Diphenylethoxy)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10i)

By the procedure used in **9a**, compound **10i** (193 mg, 65%) was prepared from 308 mg of **45a**. Colorless granular, mp 108 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.83 (3H, s), 3.40–3.51 (6H, m), 4.27 (2H, t, J = 4.4 Hz), 4.63 (2H, s), 6.30 (1H, dd, J = 8.2, 1.1 Hz), 6.33 (1H, dd, J = 8.2, 1.1 Hz), 6.69 (1H, t, J = 8.2 Hz), 7.17–7.33 (10H, m); IR (KBr) 1746 cm^{-1} (COOH); Mass (EI, *m/e*) 433 (M^+); Elemental analysis Calcd. for $C_{26}H_{27}NO_5$: C, 72.04; H, 6.28; N, 3.23. Found: C, 72.22; H, 5.98; N, 3.40.

4.17. (4-(4,4-Diphenylpentyl)-8-hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10j)

By the procedure used in **9a**, compound **10j** (249 mg, 79%) was prepared from 308 mg of **45b**. Colorless granular, mp 133 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36–1.48 (2H, m), 1.63 (3H, s), 2.08–2.17 (2H, m), 3.16 (2H, t, J = 8.4 Hz), 3.21 (2H, t, J = 4.4 Hz), 4.25 (2H, t, J = 4.4 Hz), 4.62 (2H, s), 6.26 (1H, d, J = 8.5 Hz), 6.30 (1H, d, J = 8.0 Hz), 6.71 (1H, t, J = 8.2 Hz), 7.16–7.22 (6H, m), 7.23–7.31 (4H, m); IR (KBr) 1744 cm^{-1} (COOH); Mass (EI, *m/e*) 431 (M^+); Elemental analysis Calcd. for $C_{27}H_{29}NO_4$: C, 75.15; H, 6.77; N, 3.25. Found: C, 75.36; H, 6.81; N, 3.21.

4.18. (4-(2-(1,1-Diphenylethylsulfinyl)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid (10k)

To a solution of (4-(2-(1,1-diphenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid **10a**

(51 mg) in CH_2Cl_2 (5 mL) was added *m*-CPBA (20 mg), and the mixture was stirred at room temperature for 4 h. The reaction mixture was added to water (20 mL) and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (DIOL type silica gel, solvent: *n*-hexane/ethyl acetate = 1/2) to obtain compound **10k** (43 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97 (3H, s), 2.26–2.46 (2H, m), 3.20–3.37 (2H, m), 3.52–3.60 (2H, m), 4.22–4.27 (2H, m), 4.64 (2H, s), 6.15 (1H, dd, J = 1.2, 8.4 Hz), 6.30 (1H, dd, J = 1.2, 8.4 Hz), 6.65 (1H, t, J = 8.4 Hz), 7.27–7.46 (10H, m); Mass (FAB, *m*/*e*) 466 $(M+H)^+$; Elemental analysis Calcd. for $C_{26}H_{27}NO_5S$: C, 67.08; H, 5.85; N, 3.01; S, 6.89. Found: C, 67.35; H, 5.72; N, 3.07; S, 6.95.

4.19. 4-Methoxy-2,3-dihydro-1*H*-indole (12)

To a solution of 4-methoxy-1*H*-indole (**11**) (3.95 g) in dioxane (50 mL) were added BH_3–Me_3N complex (6.37 g) and 10.5 N HCl (8.0 mL), and the resulting solution was refluxed for 70 min. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was diluted with water (100 mL), neutralized with KOH solution, and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 5/1) to obtain compound **12** (3.48 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.80–3.20 (2H, m), 3.60–3.80 (2H, m), 3.80 (3H, s), 6.28 (1H, d, J = 7.9 Hz), 6.30 (1H, d, J = 7.9 Hz), 6.99 (1H, t, J = 7.9 Hz); Mass (EI, *m*/*e*) 149 (M^+).

4.20. [1-(2-Hydroxyethyl)-2,3-dihydro-1*H*-indol-4-yloxy]acetic acid methyl ester (13)

To a solution of 4-methoxy-2,3-dihydro-1*H*-indole (**12**) (996 mg) in Et_2O (15 mL) was added phenyllithium Et_2O solution (1.08 M, 9.3 mL), and the resulting solution was stirred at room temperature for 15 min. 2-(2-Bromoethoxy)tetrahydropyran (2039 mg) was added to the reaction mixture, and the resulting solution was stirred overnight at room temperature. The reaction mixture was poured into saturated ammonium chloride aqueous solution (30 mL) and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was dissolved in methanol (20 mL) and treated with 1 N HCl (1.5 mL) at room temperature. The reaction mixture was concentrated and the residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 2/1), and the obtained compound was dissolved in DMF (15 mL). *n*-PrSH (3.0 mL) and *t*-BuOK (2.10 g) were added to the solution, and the resulting mixture was refluxed for 3 h. The reaction mixture was poured into water (40 mL) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 1/1). The obtained compound was dissolved in DMF (5 mL) and treated with K_2CO_3 (1.00 g) and methyl bromoacetate (0.30 mL) at room temperature. To the reaction mixture was added a saturated ammonium chloride aqueous solution (30 mL) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 1/1) to obtain compound **13** (187 mg, 11%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05–2.16 (1H, m), 3.03 (2H, t, J = 8.6 Hz), 3.20–3.27 (2H, m), 3.43 (2H, t, J = 8.6 Hz), 3.79 (3H, s), 3.75–3.83 (2H, m), 4.65 (2H, s), 6.16 (1H, d, J = 8.4 Hz), 6.27 (1H, d, J = 8.1 Hz), 6.99–7.06 (1H, m); Mass (EI, *m*/*e*) 251 (M^+).

4.21. {1-[2-(1,1-Diphenylethylsulfanyl)-ethyl]-2,3-dihydro-1*H*-indol-4-yloxy}acetic acid methyl ester (14)

To a solution of [1-(2-hydroxyethyl)-2,3-dihydro-1*H*-indol-4-yloxy]acetic acid methyl ester (**13**) (187 mg) in CH_2Cl_2 (5.0 mL) were added Et_3N (0.18 mL) and methanesulfonyl chloride (0.08 mL), and the mixture was stirred at 0 °C for 1 h. The reaction solution was poured into a 5% citric acid aqueous solution and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated to obtain the mesylate of **13**. Sodium hydride (50 mg) was washed with *n*-hexane and dried under reduced pressure. A solution of 1,1-diphenylethanethiol (327 mg) in DMF (3.0 mL) was added to the sodium hydride at 0 °C, and the mixture was stirred at room temperature for 10 min. A solution of the above mesylate in DMF (5.0 mL) was added to the mixture, and the mixture was stirred at room temperature for 3 h. The solvent was distilled off under reduced pressure, and the residue was poured into a 5% citric acid aqueous solution, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and concentrated. The residue was purified by column chromatography (neutral alumina: *n*-hexane/ethyl acetate = 7/1) to obtain compound **14** (333 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (3H, s), 2.47–2.54 (2H, m), 2.93 (2H, t, J = 8.4 Hz), 2.96–3.04 (2H, m), 3.25 (2H, t, J = 8.4 Hz), 3.78 (3H, s), 4.61 (2H, s), 5.94 (1H, d, J = 8.1 Hz), 6.07 (1H, d, J = 8.1 Hz), 6.93 (1H, t, J = 8.1 Hz), 7.20–7.34 (6H, m), 7.40–7.47 (4H, m); IR (neat) 1765 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 447 (M^+).

4.22. 5-(*t*-Butyldimethylsiloxy)quinoline (16)

To a solution of 5-hydroxyquinoline (**15**) (101 mg) in DMF (5 mL) were added imidazole (113 mg) and *t*-butyldimethylsilyl chloride (162 mg) with stirring overnight at room temperature under argon atmosphere. Water (5 mL) was added to the reaction solution, and the mixture was then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. The residue was purified by column chromatography (silica gel: hexane/ethyl acetate = 9/1) to obtain compound **16** (197 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (6H, s), 1.09 (9H, s), 6.92 (1H, dd, J = 7.8, 0.9 Hz), 7.38 (1H, dd, J = 8.7,

4.5 Hz), 7.56 (1H, dd, J = 8.4, 7.5 Hz), 7.73 (1H, dt, J = 8.4, 0.9 Hz), 8.50 (1H, ddd, J = 8.7, 2.1, 0.9 Hz), 8.89 (1H, dd, J = 4.5, 1.8 Hz); Mass (EI, *m/e*) 259 (M^+).

4.23. 5-(*t*-Butyldimethylsiloxy)-1,2,3,4-tetrahydro-quinoline (17)

To a solution of 5-(*t*-butyldimethylsiloxy)quinoline (**16**) (1715 mg) in EtOH (50 mL) was added 10% Pd–C (100 mg), and the mixture was stirred under hydrogen atmosphere at room temperature. After disappearance of the starting material, the solid catalyst was filtered off, and the filtrate was concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 10/1) to obtain compound **17** (1426 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.22 (6H, d, J = 0.6 Hz), 1.00 (9H, d, J = 0.6 Hz), 1.86–1.97 (2H, m), 2.65 (2H, t, J = 6.6 Hz), 3.21–3.56 (2H, m), 3.80 (1H, br s), 6.13 (1H, d, J = 8.1 Hz), 6.14 (1H, d, J = 8.1 Hz), 6.82 (1H, td, J = 7.8, 0.6 Hz); Mass (EI, *m/e*) 263 (M^+).

4.24. 5-(*t*-Butyldimethylsiloxy)-1-chloroacetyl-1,2,3,4-tetrahydroquinoline (18)

To a solution of 5-(*t*-butyldimethylsiloxy)-1,2,3,4-tetrahydroquinoline (**17**) (1426 mg) in CH_2Cl_2 (50 mL) were added pyridine (1.00 mL) and chloroacetyl chloride (0.70 mL), and the resulting solution was stirred for 5 h at 0 °C under argon atmosphere. The reaction solution was added to water (50 mL) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 5/1) to obtain compound **18** (1839 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.24 (6H, s), 1.02 (9H, s), 1.98 (1H, quint, J = 6.6 Hz), 2.70 (2H, t, J = 6.6 Hz), 3.81 (2H, t, J = 6.6 Hz), 4.25 (2H, s), 6.68 (1H, d, J = 8.4 Hz), 6.75–7.00 (1H, m), 7.08 (1H, t, J = 8.0 Hz); IR (liquid film method) 1667 cm^{-1} (amide); Mass (EI, *m/e*) 339 (M^+).

4.25. 1-(1,1-Diphenylethylthioacetyl)-5-hydroxy-1,2,3,4-tetrahydroquinoline (19)

To a solution of 1,1-diphenylethanethiol (203 mg) in DMF (3 mL) was added sodium hydride (65 mg), and the mixture was stirred at 0 °C for 10 min under argon atmosphere. A solution of 5-(*t*-butyldimethylsiloxy)-1-chloroacetyl-1,2,3,4-tetrahydroquinoline (**18**) (252 mg) in DMF (3 mL) was then added, and the mixture was stirred at room temperature for 3 h. The reaction solution was added to water (30 mL) and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 2/1) to obtain compound **19** (294 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.88–1.98 (2H, m), 2.05 (3H, s), 2.65 (2H, t, J = 7.1 Hz), 3.25 (2H, s), 3.50–3.80 (2H, m), 4.96 (1H, br s), 6.50–6.80 (1H, m), 6.60 (1H, d, J = 8.7 Hz), 6.94 (1H, t, J = 8.1 Hz), 7.14–7.40 (10H, m); IR (KBr) 1622 cm^{-1} (amide); Mass (EI, *m/e*) 403 (M^+).

4.26. 1-(2-(1,1-Diphenylethylthio)ethyl)-5-hydroxy-1,2,3,4-tetrahydroquinoline (20)

To a solution of 1-(1,1-diphenylethylthioacetyl)-5-hydroxy-1,2,3,4-tetrahydroquinoline (**19**) (136 mg) in THF (5 mL) was added BH_3–THF complex (1.0 M, 2.0 mL) at 0 °C, and the resulting solution was stirred at room temperature for 5 h. The reaction solution was added to a saturated ammonium chloride aqueous solution (20 mL) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 5/1) to obtain compound **20** (60 mg, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82–1.92 (2H, m), 2.07 (3H, s), 2.48–2.64 (4H, m), 3.04–3.19 (4H, m), 4.54 (1H, br s), 5.83 (1H, d, J = 8.1 Hz), 6.07 (1H, dd, J = 8.1, 0.9 Hz), 6.81 (1H, t, J = 8.1 Hz), 7.20–7.35 (6H, m), 7.39–7.45 (4H, m); Mass (EI, *m/e*) 389 (M^+).

4.27. Methyl(1-(2-(1,1-diphenylethylthio)ethyl)-1,2,3,4-tetrahydroquinolin-5-yloxy)acetate (21)

To a solution of 1-(2-(1,1-diphenylethylthio)ethyl)-5-hydroxy-1,2,3,4-tetrahydroquinoline **20** (60 mg) in DMF (5 mL) were added K_2CO_3 (100 mg) and methyl bromoacetate (0.050 mL), and the resulting mixture was stirred at room temperature for 4 h. The reaction solution was added to a saturated ammonium chloride aqueous solution (15 mL) and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 9/1) to obtain compound **21** (51 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78–1.89 (2H, m), 2.06 (3H, s), 2.47–2.55 (2H, m), 2.69 (2H, t, J = 6.6 Hz), 3.04–3.18 (4H, m), 3.78 (3H, s), 4.58 (2H, s), 5.91 (1H, d, J = 8.4 Hz), 6.02 (1H, d, J = 8.4 Hz), 6.86 (1H, t, J = 1, 8.4 Hz), 7.20–7.35 (6H, m), 7.38–7.47 (4H, m); IR (liquid film method) 1763 cm^{-1} (COOMe); Mass (EI, *m/e*) 461 (M^+).

4.28. 6-Acetoxy-2-oxo-2,3,4,5-tetrahydro-1*H*-benzazepine (23)

To a solution of 5-hydroxy-1-tetralone (**22**) (506 mg) in trifluoroacetic acid (10 mL) was added sodium azide (254 mg), and the mixture was heated under reflux. After disappearance of the starting material, the reaction solution was poured into water (20 mL), neutralized with a sodium bicarbonate aqueous solution, and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated to obtain a residue, which was dissolved in CH_2Cl_2 (5 mL) and pyridine (2 mL). Ac_2O (0.45 mL) was added to the solution, and the mixture was stirred at room temperature for 6 h. The reaction solution was added to saturated ammonium chloride aqueous solution (30 mL) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 1/1) to obtain compound **23** (416 mg, 61%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99–2.07 (2H, m), 2.14 (2H, t, J = 6.9 Hz), 2.32 (3 H, s), 2.56 (2H, t, J = 7.2 Hz), 6.89 (2H, d, J = 8.1 Hz), 7.24 (1H, t, J = 8.1 Hz), 9.66 (1H, s); IR (KBr) 1758 (OAc), 1676 (CONH) cm^{-1}; Mass (EI, *m*/*e*) 219 (M^+).

4.29. Methyl(6-acetoxy-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)acetate (24)

By the procedure used in **21**, compound **24** (333 mg, 99%) was prepared from 252 mg of **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95–2.30 (2H, m), 2.30–2.41 (2H, m), 2.35 (3H, s), 2.76–2.90 (2H, m), 3.75 (3H, s), 4.51 (2H, s), 6.96 (1H, dd, J = 8.1, 0.9 Hz), 7.06 (1H, dd, J = 8.1, 0.9 Hz), 7.27 (1H, t, J = 8.1 Hz); IR (liquid film method) 1750 (COOMe, OAc), 1661(amide) cm^{-1}; Mass (EI, *m*/*e*) 291 (M^+).

4.30. 1-(2-Hydroxyethyl)-6-hydroxy-2,3,4,5-tetrahydro-1*H*-1-benzazepine (25)

To a solution of methyl(6-acetoxy-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)acetate (**24**) (1080 mg) in THF (15 mL) was added $LiAlH_4$ (355 mg), and the mixture was stirred at 0 °C for 3 h. The reaction solution was added to water (100 mL) and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 2/1) to obtain compound **25** (624 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55–1.66 (2H, m), 1.68–1.81 (2H, m), 2.63 (1H, br s), 2.82–2.90 (2H, m), 2.90–2.97 (2H, m), 3.31 (2H, t, J = 5.3 Hz), 3.71 (2H, t, J = 5.3 Hz), 5.31 (1H, br s), 6.47 (1H, dd, J = 8.1, 1.2 Hz), 6.57 (1H, d, J = 8.1 Hz), 6.98 (1H, t, J = 8.1 Hz); Mass (EI, *m*/*e*) 207 (M^+).

4.31. Methyl(1-(2-hydroxyethyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepin-6-yloxy)acetate (26)

By the procedure used in **21**, compound **26** (47 mg, 67%) was prepared from 52 mg of **25**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55–1.67 (2H, m), 1.68–1.80 (2H, m), 2.51 (1H, br s), 2.93–2.98 (4H, m), 3.31 (2H, t, J = 5.1 Hz), 3.69 (2H, t, J = 5.1 Hz), 3.80 (3H, s), 4.62 (2H, s), 6.46 (1H, dd, J = 8.1, 0.9 Hz), 6.67 (1H, dd, J = 8.1, 0.9 Hz), 7.06 (1H, t, J = 8.1 Hz); IR (liquid film method) 1760 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 279 (M^+).

4.32. Methyl(1-(2-(1,1-diphenylethylthio)ethyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepin-6-yloxy)acetate (27)

To a solution of methyl(1-(2-hydroxyethyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepin-6-yloxy)acetate (**26**) (370 mg) in CH_2Cl_2 (15 mL) were added triphenylphosphine (1085 mg) and carbon tetrabromide (922 mg), and the mixture was stirred at 0 °C for 3.5 h. A saturated sodium bicarbonate aqueous solution (15 mL) was added to the reaction solution, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated to obtain the bromide of **24**. To a solution of 1,1-diphenylethanethiol (428 mg) in DMF (10 mL) was added sodium hydride (65 mg), and the mixture was stirred at 0 °C for 5 min. A solution of the bromide of **24** in DMF (3 mL) was added, and the reaction mixture was stirred at 0 °C for 3 h. The reaction solution was added to a saturated ammonium chloride aqueous solution (30 mL) and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 8/1) to obtain compound **27** (532 mg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50–1.68 (4H, m), 2.05 (3H, s), 2.45–2.53 (2H, m), 2.78–2.95 (4H, m), 3.00–3.15 (2H, m), 3.78 (3H, s), 4.59 (2H, s), 6.35 (2H, d, J = 8.1 Hz), 6.95(1H, t, J = 8.1 Hz), 7.17–7.36 (6H, m), 7.38–7.44 (4H, m); IR (liquid film method) 1765 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 475 (M^+).

4.33. 2,3-Dimethoxyphenylcarbamic acid ethyl ester (29)

To a solution of 2,3-dimethoxybenzoic acid (**28**) (1.11 g) in dioxane (25 mL) and Et_3N (10 mL) was added diphenylphosphorylazide (1.70 g), and the mixture was stirred at 100 °C for 7.5 h. The solvent was removed under reduced pressure. The residue was added to 5% citric acid aqueous solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 3/1) to obtain compound **29** (1.08 g, 78%). Colorless plates, mp 45 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (3H, t, J = 7.0 Hz), 3.86 (3H, s), 3.87 (3H, s), 4.23 (2H, q, J = 7.0 Hz), 6.62 (1H, dd, J = 1.5, 8.0 Hz), 7.03 (1H, t, J = 8.0 Hz), 7.28 (1H, br s), 7.74 (1H, dd, J = 1.5, 8.5 Hz); Mass (EI, *m*/*e*) 225 (M^+).

4.34. 2,3-Dimethoxyaniline (30)

To a solution of 2,3-dimethoxyphenylcarbamic acid ethyl ester (**29**) (385 mg) in EtOH (10 mL) was added 5.48 N NaOH (4.0 mL), and the mixture was stirred at 100 °C for 9 h. The solvent was removed under reduced pressure. The residue was added to 5% citric acid aqueous solution and then extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel: *n*-hexane/ethyl acetate = 3/1) to obtain compound **30** (244 mg, 93%). Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (3H, s), 3.84 (3H, s), 6.34 (1H, dd, J = 1.0, 8.0 Hz), 6.39 (1H, dd, J = 1.0, 8.0 Hz), 6.854 (1H, t, J = 8.0 Hz); Mass (EI, *m*/*e*) 153 (M^+).

4.35. 2,3-Dimethoxy-α-chloroacetoanilide (31)

To a solution of 2,3-dimethoxyaniline (**30**) (1.00 g) in THF (5 mL) was added chloroacetic anhydride (1.26 g), and the resulting solution was stirred at room temperature for 4 h. The solvent was removed under reduced pressure, and the reaction solution was poured into a saturated sodium bicarbonate solution, and then extracted with ethyl acetate. The organic layer was

washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by column chromatography (silica gel; ethyl acetate/*n*-hexane = 1/2) to obtain compound **31** (1.64 g, 97%). Colorless flakes, mp 58–59 °C (recrystallized from dichloromethane/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88 (3H, s), 3.91 (3H, s), 4.20 (2H, s), 6.73 (1H, dd, J = 1.5, 8.5 Hz), 7.06 (1H, t, J = 8.5 Hz), 7.96 (1H, dd, J = 1.5, 8.5 Hz), 9.05 (1H, br s); Mass (EI, *m*/*e*) 229, 231 (M^+) (peak height is 3:1).

4.36. 8-Hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (32)

To a solution of 2,3-dimethoxy-α-chloroacetoanilide (**31**) (1.43 g) in dichloromethane (30 mL) cooled to −78 °C was added BBr_3 in CH_2Cl_2 (1.0 M, 13.0 mL). The mixture was stirred at 0 °C for 2 h, poured into water, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was dissolved in DMF (30 mL), and potassium carbonate (1.03 g) was added. The mixture was stirred for 2.5 h and the solvent was removed under reduced pressure. The residue was poured into 5% citric acid and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was recrystallized from ethyl acetate to obtain compound **32** (922 mg, 90%). Colorless plates, mp 226 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.49 (2H, s), 6.34 (1H, dd, J = 1, 8 Hz), 6.45 (1H, dd, J = 1, 8 Hz), 6.71 (1H, t, J = 8 Hz), 9.37 (1H, br s), 10.57 (1H, br s); IR (KBr) 1682 cm^{-1} (CONH); Mass (EI, *m*/*e*) 165 (M^+).

4.37. 3-Oxo-8-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-1,4-benzoxazine (33)

To a solution of 8-hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (**32**) (740 mg) in DMF (1.5 mL) were added dihydropyran (1.0 g) and pyridinium *p*-toluenesulfonate (360 mg), and the mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure, and the residue was poured into saturated sodium bicarbonate aqueous solution, and then extracted with ethyl acetate. The organic layer was washed with water, 5% citric acid, water, and brine, dried over magnesium sulfate, and then concentrated. The residue was recrystallized from ethyl acetate to obtain compound **33** (890 mg, 80%). Colorless plates, mp 186 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54 (3H, m), 1.76 (3H, m), 3.52 (1H, m), 3.79 (1H, m), 4.54 (2H s), 5.40 (1H, t, J = 4 Hz), 6.54 (1H, dd, J = 1.5, 8 Hz), 6.76 (1H, dd, J = 1.5, 8 Hz), 6.83 (1H, t, J = 8 Hz), 10.67 (1H, br s); IR (KBr) 1684 cm^{-1} (CONH); Mass (EI, *m*/*e*) 249 (M^+).

4.38. 4-(2-Hydroxyethyl)-8-hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (34)

A solution of 3-oxo-(8-tetrahydropyrane-2-yloxy)-3,4-dihydro-2*H*-1,4-benzoxazine (**33**) (529 mg) in DMF (15 mL) was added to sodium hydride (127 mg), which was washed with *n*-hexane and dried under reduced pressure, and the mixture was stirred at room temperature for 10 min. A solution of 2-(2-bromoethoxy)tetrahydropyran (935 mg) in DMF (3.0 mL) was added, and the mixture was stirred at room temperature for 5.5 h. The solvent was distilled off under reduced pressure, and the residue was poured into a 5% citric acid aqueous solution, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was dissolved in MeOH (50 mL), and *p*-toluenesulfonic acid hydrate (160 mg) was added. The mixture was stirred at room temperature for 2 h and the solvent was distilled off under reduced pressure. The residue was poured into 5% citric acid and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was recrystallized from ethyl acetate/*n*-hexane to obtain compound **34** (415 mg, 94%). Colorless plates, mp 166 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.54 (2H, m), 3.90 (2H, t, J = 6 Hz), 4.55 (2H, s), 4.85 (1H, br), 6.55 (1H, dd, J = 1, 8 Hz), 6.70 (1H, dd, J = 1, 8 Hz), 6.70 (1H, dd, J = 1, 8 Hz), 6.82 (1H, t, J = 8 Hz), 9.42 (1H, br s); IR (KBr) 1669 cm^{-1} (amide); Mass (EI, *m*/*e*) 209 (M^+).

4.39. Methyl(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (35)

By the procedure used in **21**, the compound **35** (280 mg, 97%) was prepared from 216 mg of **34**. Colorless plates, mp 106 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13 (1H, br s), 3.80 (3H, s), 3.94 (2H, bt, J = 5.5 Hz), 4.13 (2H, t, J = 5.5 Hz), 4.70 (2H, s), 4.72 (2H, s), 6.62 (1H, dd, J = 1, 8 Hz), 6.80 (1H, dd, J = 1, 8 Hz), 6.96 (1H, t, J = 8 Hz); IR (KBr) 1763 (COOMe), 1663 (amide) cm^{-1}; Mass (EI, *m*/*e*) 281 (M^+).

4.40. Methyl(4-(2-(1,1-diphenylethylthio)ethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (36)

By the procedure used in **14**, compound **36** (172.5 mg, 42%) was prepared from 187 mg of **35**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (3H, s), 2.57 (2H, m), 3.79 (3H, s), 3.81 (2H, m), 4.59 (2H, s), 4.69 (2H, s), 6.13(1H, dd, J = 1, 8 Hz), 6.56 (1H, dd, J = 1, 8 Hz), 6.81 (1H, t, J = 8 Hz), 7.21–7.34 (6H, m), 7.40–7.45 (4H, m); IR (liquid film method) 1763 (COOMe), 1686 (amide) cm^{-1}; Mass (EI, *m*/*e*) 477 (M^+).

4.41. 4-(2-(Tetrahydropyran-2-yloxy)ethyl)-8-(tetrahydro-pyran-2-yloxy)-3,4-dihydro-2*H*-1,4-benzoxazine (37)

To a solution of 3-oxo-8-tetrahydropyranyloxy-3,4-dihydro-2*H*-1,4-benzoxazine (**33**) (6.04 g) in anhydrous DMF (100 mL) was added sodium hydride (1.02 g), and the mixture was stirred at room temperature for 1 h. 2-(2-Bromoethoxy)tetrahydropyrane (7.60 g) was

added to the reaction mixture, and the resulting mixture was stirred at room temperature for 17.5 h. The solvent was removed under reduced pressure, and the residue was poured into a 5% citric acid aqueous solution, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was dissolved in THF (150 mL), and a 1.0 M BH_3–THF solution (60 mL) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was added to saturated sodium bicarbonate aqueous solution and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was purified by column chromatography (silica gel; ethyl acetate/*n*-hexane = 1/2) to obtain compound **37** (7.01 g, 93%). Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50–2.10 (12H, m), 3.47 (5H, m), 3.62 (2H, m), 3.82 (1H, m), 3.91 (1H, m), 4.03 (1H, m), 4.25 (2H, t, J = 4.5 Hz), 4.59 (1H, t, J = 3 Hz), 5.36 (1H, t, J = 3 Hz), 6.41 (1H, dd, J = 1, 8 Hz), 6.52 (1H, dd, J = 1, 8 Hz), 6.72 (1H, t, J = 8 Hz); Mass (EI, *m*/*e*) 363 (M^+).

4.42. 4-(2-Hydroxyethyl)-8-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine (38)

To a solution of 4-(2-(tetrahydropyran-2-yloxy)ethyl)-8-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-1,4-benzoxazine (**37**) (3.89 g) in methanol (80 mL) was added pyridinium *p*-toluenesulfonate (520 mg), and the mixture was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure, and the residue was poured into 5% citric acid, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was recrystallized from ethyl acetate/*n*-hexane to obtain compound **38** (2.00 g, 97%). Colorless plates, mp 105.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.26 (1H, t, J = 5.5 Hz), 3.36 (2H, t, J = 4 Hz), 3.54 (2H, q, J = 5.5 Hz), 4.10 (2H, t, J = 4 Hz), 4.65 (1H, t, J = 5.5 Hz), 6.07 (1H, dd, J = 1, 8 Hz), 6.15 (1H, dd, J = 1, 8 Hz), 6.49 (1H, t, J = 8 Hz), 8.49 (1H, s); Mass (EI, *m*/*e*) 195 (M^+).

4.43. Methyl(4-(2-hydroxyethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (39)

To a solution of 4-(2-hydroxyethyl)-8-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazine (**38**) (17.3 g) in DMF (200 mL) were added anhydrous potassium carbonate (30.6 g) and methyl bromoacetate (24.1 g), and the mixture was stirred at room temperature for 5.5 h. The solvent was distilled off, and the residue was poured into 5% citric acid, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and then concentrated. The residue was recrystallized from ethyl acetate/*n*-hexane to obtain compound **39** (17.6 g, 74%). Colorless plates, mp 64–66 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (1H, t, J = 5.5 Hz), 3.42 (4H, m), 3.79 (3H, s), 3.83 (2H, q, J = 5.5 Hz), 4.31 (2H, m), 4.68 (2H, s), 6.26 (1H, dd, J = 1, 8 Hz), 6.47 (1H, dd, J = 1, 8 Hz), 6.73 (1H, t, J = 8 Hz); IR (KBr) 1719 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 267 (M^+).

4.44. General procedure for coupling with thiols; methyl(4-(2-(1,1-diphenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (40a)

To a solution of methyl(4-(2-hydroxyethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (**39**) (15.7 g) in CH_2Cl_2 (360 mL) were added Et_3N (29.5 mL) and methanesulfonyl chloride (14.6 mL), and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into a 5% citric acid aqueous solution and then extracted with ethyl acetate. The organic layer was washed with water, saturated sodium bicarbonate water, and water and brine, dried over magnesium sulfate, and then concentrated to obtain mesylate of **39** (17.8 g). To sodium hydride (2.82 g), which was washed with *n*-hexane, was added a solution of 1,1-diphenylethanethiol (15.1 g) in DMF (1000 mL) and the mixture was stirred at room temperature for 1 h. A solution of the mesylate of **39** above (17.8 g) in DMF (100 mL) was added to the reaction mixture, and the resulting mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure, and the residue was poured into a 5% citric acid aqueous solution, and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and concentrated. The residue was purified by column chromatography (neutral alumina: methyl acetate/*n*-hexane = 1/3) to obtain compound **40a** (19.7 g, 83%). Colorless prisms, mp 101 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.06 (3H, s), 2.51 (2H, t, J = 8.0 Hz), 3.13 (2H, t, J = 8.0 Hz), 3.19 (2H, t, J = 4.5 Hz), 3.78 (3H, s), 4.22 (2H, t, J = 4.5 Hz), 4.65 (2H, s), 6.01 (1H, dd, J = 1.0, 8.0 Hz), 6.19 (1H, dd, J = 1.0, 8.0 Hz), 6.62 (1H, t, J = 8.0 Hz), 7.21–7.44 (10H, m); IR (KBr) 1765 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 463 (M^+).

4.45. Methyl(4-(2-(1,1-diphenyl-2,2,2-trifluoroethylthio)-ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (40b)

By the procedure used in **40a**, compound **40b** (614 mg, 85%) was prepared from 375 mg of **39**. Pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.55 (2H, t, J = 7 Hz), 3.17 (4H, m), 3.78 (3H, s), 4.21 (2H), 4.64 (2H, s), 5.94 (1H, dd, J = 1, 8 Hz), 6.19 (1H, dd, J = 1, 8 Hz), 6.60 (1H, t, J = 8 Hz), 7.31–7.36 (6H, m), 7.42–7.46 (4H, m); IR (liquid film method) 1765 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 517 (M^+).

4.46. Methyl(4-(2-(diphenylmethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (40c)

By the procedure used in **40a**, compound **40c** (476 mg, 81%) was prepared from 347 mg of **39**. Colorless plates, mp 94–95 °C (recrystallized from ethyl acetate/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (2H, m), 3.26 (2H, m), 3.36 (2H, m), 3.78 (3H, s), 4.24 (2H, m), 4.65 (2H, s), 5.24 (1H, s), 6.06 (1H, dd, J = 1, 8 Hz), 6.20 (1H, dd, J = 1, 8 Hz), 6.61 (1H, t, J = 8 Hz), 7.26–7.31 (6H, m), 7.41–7.46 (4H, m); IR (KBr) 1763 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 449 (M^+).

4.47. Methyl(4-(2-(1-methyl-1-phenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (40d)

By the procedure used in **40a**, compound **40d** (483 mg, 94%) was prepared from 343 mg of **39**. Pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72 (6H, s), 2.42 (2H, t, J = 8 Hz), 3.09 (2H, t, J = 8 Hz), 3.17 (2H, t, J = 4.5 Hz), 3.78 (3H, s), 4.20 (2H, t, J = 4.5 Hz), 4.64 (2H, s), 5.94(1H, dd, J = 1, 8 Hz), 6.17 (1H, dd, J = 1, 8 Hz), 6.60 (1H, t, J = 8 Hz), 7.24 (1H, m), 7.34 (2H, m), 7.54 (2H, m); IR (liquid film method) 1765 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 401 (M^+).

4.48. Methyl(4-(2-(2,2-diphenylpropylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (40f)

By the procedure used in **40a**, compound **40f** (134 mg, 37%) was prepared from 200 mg of **39**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (3H, s), 2.36 (2H, t, J = 7.6 Hz), 3.24–3.35 (6H, m), 3.79 (3H, s), 4.24 (2H, t, J = 4.4 Hz), 4.66 (2H, s), 6.22(1H, d, J = 8.5 Hz), 6.27 (1H, d, J = 8.5 Hz), 6.70 (1H, t, J = 8.5 Hz), 7.17–7.32 (10H, m); IR (liquid film method) 1765 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 477 (M^+).

4.49. Methyl(4-(2-(1,1-bis-(4-fluorophenyl)ethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (40g)

By the procedure used in **40a**, compound **40g** (311 mg, 83%) was prepared from 200 mg of **39**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (3H, s), 2.50 (2H, t, J = 7.6 Hz), 3.21–3.35 (6H, m), 3.79 (3H, s), 4.24 (2H, t, J = 4.4 Hz), 4.66 (2H, s), 6.22 (1H, d, J = 8.5 Hz), 6.27 (1H, d, J = 8.5 Hz), 6.70 (1H, t, J = 8.5 Hz), 7.17–7.32 (10H, m); IR (liquid film method) 1763 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 499 (M^+).

4.50. Methyl(4-(2-(1,1-bis-(3-thienyl)ethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (40h)

By the procedure used in **40a**, compound **40g** (253 mg, 95%) was prepared from 150 mg of **39**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (3H, s), 2.51–2.58 (2H, m), 3.16 (2H, t, J = 7.7 Hz), 3.23 (2H, t, J = 4.4 Hz), 3.79 (3H, s), 4.23 (2H, t, J = 4.4 Hz), 4.65 (2H, s), 6.07(1H, dd, J = 8.2, 1.1 Hz), 6.20 (1H, dd, J = 8.2, 1.1 Hz), 6.66 (1H, t, J = 8.2 Hz), 7.11 (2H, dd, J = 3.0, 1.4 Hz), 7.14 (2H, dd, J = 5.2, 1.4 Hz), 7.29 (2H, dd, J = 5.2, 3.0 Hz); IR (liquid film method) 1767 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 475 (M^+).

4.51. (4-(2-(1,1-Diphenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid sodium salt (41)

(4-(2-(1,1-Diphenylethylthio)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetic acid **10a** (510 mg) was dissolved in distilled water (10 mL) and sodium hydroxide aqueous solution (0.0978 N, 11.54 mL) with heating, and the solution was filtered with a membrane filter. The filtrate was lyophilized to obtain the object compound (535 mg, 100%). Colorless amorphous, mp 110 °C; ^{1}H NMR (300 MHz, D_2O, 30 °C) δ 1.79 (3H, s), 2.24 (2H, m), 2.75 (4H, m), 3.86 (2H, m), 4.23 (2H, s), 5.66 (1H, br), 6.08 (1H, br), 6.37 (1H, br), 7.00 (6H, m), 7.23 (4H, m); Mass (FAB-Pos, *m*/*e*) 449 $(M+H)^+$, 472 $(M+H+Na)^+$; Elemental analysis Calcd. for $C_{26}H_{26}NNaO_4S + 1.5H_2O$: C, 62.64; H, 5.86; N, 2.81; S, 6.43. Found: C, 62.35; H, 5.77; N, 3.02; S, 6.59.

4.52. 8-Acetoxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (42)

To a solution of 8-hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (**32**) (10.56 g) in toluene (30 mL) and pyridine (6 mL) was added acetic anhydride (7.09 mL), and the mixture was stirred for 24 h at room temperature. The reaction solution was poured into a 1 N hydrochloric acid aqueous solution and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and then concentrated. The residue was recrystallized from ethyl acetate/*n*-hexane to obtain compound **42** (12.5 g, 94%). Colorless needles, mp 227 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (3H, s), 4.62 (2H, s), 6.70 (1H, dd, J = 8.0, 1.5 Hz), 6.77 (1H, dd, J = 8.0, 1.5 Hz), 6.96 (1H, t, J = 8.0 Hz), 7.89 (1H, br s); IR (KBr) 1767 (OAc), 1693 (CONH) cm^{-1}; Mass (EI, *m*/*e*) 207 (M^+).

4.53. 8-Acetoxy-4-(2-(1,1-diphenylethoxy)ethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (43a)

To a solution of 2-(1,1-diphenylethoxy)ethanol (606 mg) in THF (10 mL) was added *n*-butyllithium hexane solution (1.47 M, 2.6 mL), and the mixture was stirred for 10 min at −40 °C. *p*-Toluenesulfonyl chloride (715 mg) was added to the solution, and the mixture was stirred for 1.5 h at −40 °C. The reaction mixture was poured into water and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and concentrated to obtain tosylate of 2-(1,1-diphenyl-ethoxy)ethanol. To a suspension of sodium hydride (84 mg) in DMF (5 mL) was added a solution of 8-acetoxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (**42**) (363 mg) in DMF (5 mL), and the mixture was stirred at room temperature for 30 min. The tosylate above was added, and the mixture was stirred overnight at room temperature. The reaction solution was poured into a 5% citric acid aqueous solution and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and then concentrated. The residue was purified by column chromatography (solvent: ethyl acetate/cyclohexane = 1/4) to obtain compound **43a** (429 mg, 57%). Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81 (3H, s), 2.32 (3H, s), 3.52 (2H, t, J = 5.8 Hz), 4.12 (2H, t, J = 5.8 Hz), 4.53 (2H, s), 6.77 (1H, dd, J = 8.2, 1.4 Hz), 6.94 (1H, t, J = 8.2 Hz), 7.04 (1H, dd, J = 8.2, 1.4 Hz), 7.18–7.29 (10H, m); IR (KBr) 1773 (OAc), 1688 (amide) cm^{-1}; Mass (EI, *m*/*e*) 431 (M^+).

4.54. 8-Acetoxy-4-(4,4-diphenylpentyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (43b)

By the procedure used in **43a**, compound **43b** (428 mg, 61%) was prepared from 391 mg of 4,4-diphenylpentane-1-ol and 507 mg of **42**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42–1.55 (4H, m), 1.61 (3H, s),

2.14–2.24 (2H, m), 2.31 (3H, s), 3.83 (2H, t, $J = 7.7$ Hz), 4.55 (2H, s), 6.46 (1H, dd, $J = 8.2$, 1.4 Hz), 6.74 (1H, dd, $J = 8.2$, 1.4 Hz), 6.87 (1H, t, $J = 8.2$ Hz), 7.13–7.21 (6H, m), 7.21–7.30 (4H, m); Mass (EI, *m*/*e*) 429 (M^+).

4.55. 4-(2-(1,1-Diphenylethoxy)ethyl)-8-hydroxy-3,4-dihyro-2*H*-1,4-benzoxazine (44a)

To a solution of 8-acetoxy-3,4-(2-(1,1-diphenylethoxy)-ethyl)-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (**43a**) (386 mg) in THF (10 mL) was added BH_3–THF solution (1.0 M, 3 mL), and the resulting mixture was stirred at room temperature for 4 h. The reaction solution was poured into water and extracted with ethyl acetate. The organic layer was washed with water, sodium bicarbonate water, and water and brine, dried over sodium sulfate, and then concentrated. The obtained oily residue was dissolved in THF (1 mL) and MeOH (10 mL), and anhydrous potassium carbonate (170 mg) was added. The mixture was stirred at room temperature for 1 h and the solvent was removed under reduced pressure. The residue was poured into 5% citric acid and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography (solvent: ethyl acetate/cyclohexane = 1/5) to obtain compound **44a** (339 mg, 99%). Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.83 (3H, s), 3.40–3.52 (6H, m), 4.25 (2H, t, $J = 4.5$ Hz), 5.38 (1H, s), 6.11 (1H, dd, $J = 8.1$, 1.4 Hz), 6.30 (1H, dd, $J = 8.1$, 1.4 Hz), 6.63 (1H, t, $J = 8.1$ Hz), 7.17–7.36 (10H, m); Mass (EI, *m*/*e*) 375 (M^+).

4.56. 4-(4,4-Diphenylpentyl)-8-hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine (44b)

By the procedure used in **44a**, compound **44b** (391 mg, 99%) was prepared from 424 mg of **43b**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36–1.48 (2H, m), 1.63 (3H, s), 2.08–2.16 (2H, m), 3.15 (2H, t, $J = 7.3$ Hz), 3.19 (2H, t, $J = 4.4$ Hz), 4.22 (2H, t, $J = 4.4$ Hz), 5.36 (1H, s), 6.10 (1H, dd, $J = 8.1$, 1.1 Hz), 6.31 (1H, dd, $J = 8.1$, 1.1 Hz), 6.66 (1H, t, $J = 8.1$ Hz), 7.14–7.21 (6H, m), 7.23–7.29 (4H, m); Mass (EI, *m*/*e*) 373 (M^+).

4.57. Methyl(4-(2-(1,1-diphenylethoxy)ethyl)-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (45a)

By the procedure used in **39**, compound **45a** (229 mg, 80%) was prepared from 239 mg of **44a**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.83 (3H, s), 3.40–3.51 (6H, m), 3.79 (3H, s), 4.27 (2H, t, $J = 4.4$ Hz), 4.67 (2H, s), 6.19 (1H, dd, $J = 8.2$, 1.1 Hz), 6.27 (1H, dd, $J = 8.2$, 1.1 Hz), 6.64 (1H, t, $J = 8.2$ Hz), 7.17–7.34 (10H, m); IR (liquid film method) 1763 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 447 (M^+).

4.58. Methyl(4-(4,4-diphenylpentyl)-8-hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-8-yloxy)acetate (45b)

By the procedure used in **39**, compound **45b** (386 mg, 92%) was prepared from 353 mg of **44b**. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35–1.47 (2H, m), 1.63 (3H, s), 2.08–2.16 (2H, m), 3.15 (2H, t, $J = 7.4$ Hz), 3.19 (2H, t, $J = 4.4$ Hz), 3.79 (3H, a), 4.24 (2H, t, $J = 4.4$ Hz), 4.66 (2H, s), 6.19 (1H, dd, $J = 8.2$, 1.4 Hz), 6.24 (1H, dd, $J = 8.2$, 1.4 Hz), 6.66 (1H, t, $J = 8.2$ Hz), 7.14–7.21 (6H, m), 7.23–7.30 (4H, m); IR (liquid film method) 1763 cm^{-1} (COOMe); Mass (EI, *m*/*e*) 445 (M^+).

4.59. Blood samples

Blood samples were collected from healthy human volunteers under the approval by the Institutional Ethics Committee of the Pharmaceutical Research Laboratories, Toray Industries, Inc. Written informed consent was obtained from each of the volunteers. The volunteers did not take any drugs at least within 2 weeks before their participation in this study. Blood samples were also collected from male cynomolgus monkeys (Japan SLC, Shizuoka, Japan) in accordance with the Guidelines for the Animal Care and Use established at the Pharmaceutical Research Laboratories, Toray Industries, Inc.

4.60. Platelet aggregation in PRP

Nine volumes of blood collected from human volunteers were mixed with one volume of 3.8% sodium citrate in a tube. The citrated blood samples were immediately centrifuged at 90–140*g* for 10 min at room temperature. The resulting supernatant was used as the PRP fraction. The remaining blood was further centrifuged at 1400*g* for 10 min. The resulting supernatant was used as the platelet-poor plasma fraction. Human PRP were pretreated with compound at various concentrations for 1 min before the addition of U-46619 (2 μM), arachidonic acid (600 μM), collagen (1 μg/mL) or ADP (5 μM). The platelet stimulation with ADP was carried out in the presence or absence of SQ-29548 (10 μM). Platelet aggregation was monitored by recording transmittance on a four-channel light transmission aggregometer (NBS Hematracer® 601, MC Medical, Japan) for 5 min after the addition of a platelet-stimulating agent. For evaluating the effect of the test drugs, percent inhibitions of platelet aggregation were calculated from the increases in transmittance observed with the test drugs ($N = 3$), on the assumption that no inhibition was observed in the control incubations of PRP with vehicle alone. And the optical density of platelet-poor plasma was taken to represent 100% aggregation.

4.61. Binding assay for TP and IP receptor in human platelet membrane

Platelet-rich plasma (PRP), prepared above, was washed twice in washing buffer, pH 6.5, containing 115 mM NaCl, 4.3 mM K_2HPO_4, 24.4 mM Na_2HPO_4, 5 mM glucose, 1 mM EDTA-2Na, and 0.01 mM indomethacin, and resuspended in 10 mM Tris buffer, pH 7.4, containing 5 mM $MgCl_2$ and 2 mM EDTA-2Na. The platelets were alternately frozen and thawed three times and then centrifuged at 40,000*g* for 20 min at 4 °C. The membrane preparation was resuspended at 4 °C in assay buff-

er, pH 7.4, containing 50 mM Tris and 5 mM $MgCl_2$, and stored at −80 °C. For TP receptor binding assay, human platelet membrane (10 μg protein) was incubated in assay buffer in the presence of the selective TP receptor antagonist, [^{3}H]SQ-29548, and **7**, for 30 min at 25 °C. For IP receptor binding assay, human platelet membrane (10 μg protein) was incubated in assay buffer in the presence of the selective IP receptor agonist, [^{3}H]APS-314d sodium, and **7**, for 60 min at 4 °C. The reaction mixture was separated bound and free radiolabeled ligand by rapid filtration through GF/C filters presoaked in 10 mM Tris–HCl buffer. Filters were washed and the residual [^{3}H]SQ-29548 or [^{3}H]APS-314d sodium bound to the filter was determined by liquid scintillation counting. Specific binding was defined as the difference between total binding and non-specific binding, which was determined in the presence of 10 μM SQ-29548 or 10 μM APS-314d sodium. K_i was calculated using the equation $K_i = 1C_{50}/(1 + L/K_d)$, where L is the concentration of ligand.

4.62. Blood pressure, heart rate, and ex vivo platelet aggregation in monkeys

Cynomolgus monkeys were anesthetized with sodium pentobarbital (35 mg/kg iv) and given compound **7** at doses of 1, 3, 10, and 30 μg/kg/min or compound **4** at doses of 0.3, 1, and 3 μg/kg/min both in a manner of dose escalation by infusion for 30 min for each dose via the catheter inserted into the forearm or saphenous vein. Arterial blood pressure and heart rate were monitored with a polygraph system through a femoral catheter. Arterial blood was collected to examine ex vivo platelet aggregation. The blood samples were processed to prepare PRP for determining by the light transmission method, as described above.

4.63. Statistics

The data are shown as means ± SE. Statistical comparisons between means were performed by one-way ANOVA and Dunnett's test at a significance level of $p < 0.05$ (JMP 5.01J, SAS Institute Inc.).

Acknowledgments

We dedicate this publication to Dr. Kiyotaka Ohno (Toray, Kamakura). We thank Dr. Katsuhiko Iseki (Toray, Kamakura) for helpful discussion. We thank Prof. K. Umemura (Hamamatsu University School of Medicine, Hamamatsu) and Dr. H. Tsukada (Hamamatsu Photnics K. K., Hamakita) for providing us with the pharmacological data employing positron emission tomography. We thank Dr. Masafumi Isogaya and Mika Nukaya for providing us with the biological data. We thank Dr. K. A. Jacobson (NIDDK, NIH, MD) for helpful discussion and proofreading.

References and notes

1. Hamberg, M.; Svensson, J.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2994.
2. Hall, S. E. *Med. Res. Rev.* **1991**, *11*, 503.
3. Rote, W. E.; Mu, D. X.; Lucchesi, B. R. *Stroke* **1993**, *24*, 820.
4. Serruys, P. W.; Rutsch, W.; Heyndrickx, G. R.; Danchin, N.; Mast, E. G.; Wijns W.; Rensing, B. J.; Vos, J.; Stibbe, J.; CARPORT study group. *Circulation* **1991**, *84*, 1568.
5. The RAPT Investigators (Van de Werf, F.). *Circulation* **1994**, *89*, 588.
6. Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature* **1976**, *263*, 663.
7. Collins, P. W.; Djuric, S. W. *Chem. Rev.* **1993**, *93*, 1533.
8. Narita, S.; Takahashi, A.; Aoki, T.; Sato, H.; Satoh, S.; Yamada, S.; Kudo, M.; Yamaguchi, T.; Kogi, K.; Shibasaki, M. *Bioorg. Med. Chem.* **1993**, *1*, 77.
9. Meanwell, N. A.; Romine, J. L.; Seiler, S. M. *Drugs Future* **1994**, *19*, 361.
10. Wise, H.; Jones, R. L. *Trends Phramacol. Sci.* **1996**, *17*, 17.
11. Hayashi, K.; Nagamatsu, T.; Oka, T.; Suzuki, Y. *Jpn. J. Pharmacol.* **1997**, *73*, 73.
12. Minamoto, K. *Nippon Kyobu Geka Gakkai Zasshi* **1997**, *45*, 1931.
13. Kondo, K.; Hamanaka, N. *Folia Pharmacol. Jpn.* **1995**, *106*, 181.
14. Tsubaki, K.; Taniguchi, K.; Tabuchi, S.; Okitsu, O.; Hattori, K.; Seki, J.; Sakane, K.; Tanaka, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2787.
15. Sturzebecher, S.; Witt, W. *Prostaglandins* **1988**, *36*, 751.
16. Klar, U.; Pletsch, A.; Rehwinkel, H.; Schreyer, R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1215.
17. Ohno, M.; Miyamoto, M.; Hoshi, K.; Takeda, T.; Yamada, N.; Ohtake, A. *J. Med. Chem.* **2005**, *48*, 5279.
18. Nishio, T. *J. Chem. Soc., Chem. Commun.* **1989**, 205.
19. Nishio, T. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1113.
20. Berger, J. G. *Synthesis* **1974**, 508.
21. Shenvi, A. B.; Ciganek, E. *J. Org. Chem.* **1984**, *49*, 2942.
22. Nagase, H.; Kawai, K.; Hayakawa, J.; Wakita, H.; Mizusuna, A.; Matsuura, H.; Tajima, C.; Takezawa, Y.; Endoh, T. *Chem. Pharm. Bull.* **1998**, *46*, 1695.
23. Valderrama, J. A.; Pessoa-Mahana, H.; Tapia, R. *Synth. Commun.* **1992**, *22*, 629.
24. Yamada, N.; Miyamoto, M.; Isogaya, M.; Suzuki, M.; Ikezawa, S.; Ohno, M.; Otake, A.; Umemura, K. *J. Thromb. Haemost.* **2003**, *1*, 1813.
25. Miyamoto, M.; Yamada, N.; Ikezawa, S.; Ohno, M.; Otake, A.; Umemura, K.; Matsushita, T. *Br. J. Pharmacol.* **2003**, *140*, 889.
26. Umemura, K.; Tsukada, H.; Kakiuchi, T.; Yamada, N.; Matsuura, H. *J. Nucl. Med.* **2005**, *46*, 1931.
27. Takamatsu, H.; Tsukada, H.; Kakiuchi, T.; Nishiyama, S.; Noda, A.; Umemura, K. *J. Nucl. Med.* **2000**, *41*, 1409.
28. Takamatsu, H.; Tsukada, H.; Noda, A.; Kakiuchi, T.; Nishiyama, S.; Nishimura, S.; Umemura, K. *J. Nucl. Med.* **2001**, *42*, 1833.