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Design and synthesis of new prostaglandin D_2 receptor antagonists

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ABSTRACT

To identify new cost-effective prostaglandin D_2 (DP) receptor antagonists, a series of novel 3-benzoylaminophenylacetic acids were synthesized and biologically evaluated. Among those tested, some representative compounds were found to be orally available. Receptor selectivity and rat PK profiles were also evaluated. The structure–activity relationship (SAR) study is presented.

1. Introduction

Cyclooxygenase metabolizes arachidonic acid to five primary prostanoids PGE_2, $PGF_{2\alpha}$, PGI_2, TXA_2 and PGD_2. Coleman et al.[1] proposed the existence of specific receptors for TX, PGI, PGE, PGF, and PGD named TP, IP, EP, FP, and DP receptors, respectively. Prostaglandin D_2 (PGD_2) is the major prostanoid released from mast cells after challenge with IgE.[2] PGD_2 receptor antagonists might have therapeutic potential for allergic disorders because PGD_2 is considered to play an important role in various allergic diseases such as allergic rhinitis,[3] atopic asthma,[2b] allergic conjunctivitis,[4] and atopic dermatitis.[5]

In our previous work,[6] we reported on the discovery process of *N*-benzoyl-2-methylindole-4-acetic acid analog **1** (Fig. 1) as an optimized DP receptor antagonist starting with chemical modification of indomethacin. Synthetic study of this new scaffold '2-methylindole-4-acetic acid' strongly suggested the necessity of a more cost-effective framework on which to build a drug candidate. As well, the low hIP/DP receptor selectivity of **1** (Table 1) was considered a risk factor that might cause serious side effects such as platelet aggregation. Thus, the molecular design of a new DP receptor antagonist was expanded. As described in Figure 1, 3-aminophenylacetic acid was selected as the new scaffold instead of 2-methylindole-4-acetic acid because of the moderate potency of 3-benzoylaminophenylacetic acid **2a** in terms of both the receptor affinity and the functional activity assays. The optimization process of a new cost-effective chemical lead **2a** is presented in this report.

2. Materials and methods

Compounds listed in Tables 1–3 were synthesized as outlined in Schemes 1–4. Methyl 3-aminophenylacetates **12a–c**, **12f**, **12h** and **12i** were prepared as described in Scheme 1. Compounds **12d–e**, **12g**, **12j**, and **13b** were commercially available. Nucleophilic substitution of 3-nitrophenylmethyl bromide **7** with sodium cyanide afforded **8**, acidic hydrolysis of which followed by esterification with diazomethane provided **10a**. Catalytic hydrogenation of methyl esters **10a–c** afforded the corresponding anilines **12f** and **12h–i**, respectively. Other amino esters **12a–c** were prepared from the corresponding carboxylic acids **11a–c**, respectively. N-Acetylation of **12e** with acetic anhydride in pyridine followed by reduction with diborane-dimethyl sulfide complex afforded methyl 3-ethylaminophenylacetate **13a**. Synthesis of **9b** is outlined in Scheme 1b. Sonogashira cross coupling reaction of **14** and **15** afforded **16**, hydroboration of which followed by the treatment with alkaline hydrogen peroxide afforded a phenylacetic acid **9b**. Synthesis of **2a**, **3a–b**, **4a–b**, **5a–i**, and **6a–g** is described in Scheme 2. N-Acylation of an optional aniline selected from **12a–j** and **13a–b** listed in Scheme 1a with **17**–**19**[7] afforded **2a**, **3a–b**, **4a–b**, **5a–i** and **6a–g**. Synthesis of a sulfonamide analog **2b** was prepared from **12e** as outlined in Scheme 3. N-Sulfonylation of **12e** with 4-acetoxybenzenesulfonyl chloride **22**[8] in the presence of pyridine afforded **20**, methanolysis of which with potassium carbonate in methanol resulted in **21**. O-Alkylation of

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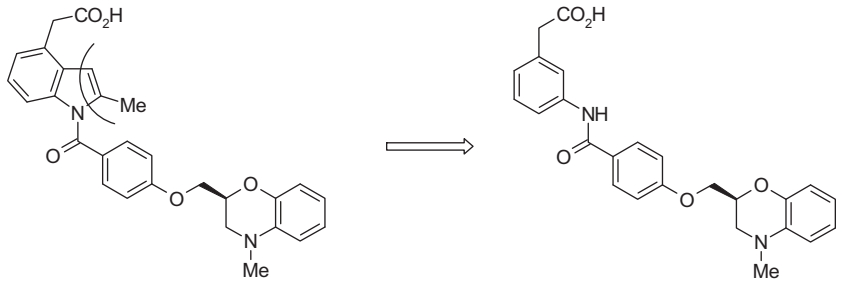
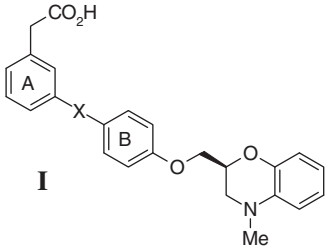


Figure 1. Molecular design of a phenylacetic acid analog **2a**.

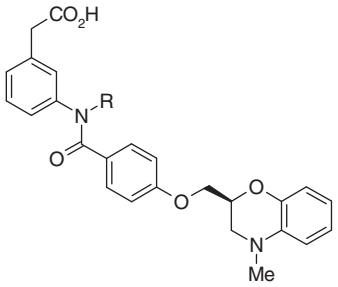
Table 1
Effect of the linker X between rings A and B on activity profiles



Compd	X	Binding K_i (nM)						IC_{50}[a] (nM) mDP
		mEP1	mEP2	mEP3	mEP4	hIP	mDP	
1		6200	99	1700	>10,000	37	16	2.4
2a		2100	520	>10,000	>10,000	6400	310	220
2b		2700	2300	210	140	4600	430	NT[b]
2c		>10,000	3500	>10,000	>10,000	>10,000	5300	NT[b]

[a] IC_{50} (nM): mDP receptor antagonist activity.
[b] NT: not tested.

Table 2
Effect of N-alkylation on the activity profiles

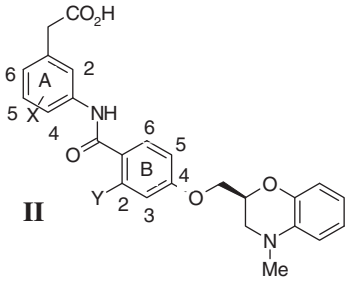


Compd	R	Binding K_i (nM)						IC_{50}[a] (nM) mDP
		mEP1	mEP2	mEP3	mEP4	hIP	mDP	
2a	H	2100	520	>10,000	>10,000	6400	310	220
3a	Me	>10,000	5300	1700	>10,000	>10,000	760	NT[b]
3b	Et	1300	>10,000	700	>10,000	2900	770	NT[b]

[a] IC_{50} (nM): mDP receptor antagonist activity.
[b] NT: not tested.

21 with the tosylate **23**[7] in the presence of cesium carbonate followed by alkaline hydrolysis produced **2b**. Synthesis of **2c** is outlined in Scheme 4. O-Alkylation of the commercially available *p*-nitrophenol **24** with the tosylate **23**[7] in the presence of cesium

Table 3
Effect of substitution of rings A and B on the activity profiles



Compd	X	Y	mDP	
			Binding K_i (nM)	IC_{50}[a] (nM)
2a	H	H	310	220
4a	H	Me	18	3.3
4b	H	Cl	16	20
5a	2-Me	Me	59	28
5b	4-Me	Me	31	4.6
5c	5-Me	Me	8.4	3.4
5d	6-Me	Me	11	6.1
5e	4-Cl	Me	7.7	NT[b]
5f	4-F	Me	19	1.8
5g	5-F	Me	7.4	5.6
5h	6-F	Me	15	1.2
5i	6-OMe	Me	24	20
6a	4-Me	Cl	26	6.0
6b	5-Me	Cl	12	5.4
6c	6-Me	Cl	17	3.9
6d	4-Cl	Cl	13	4.3
6e	4-F	Cl	17	7.4
6f	6-F	Cl	25	50
6g	6-OMe	Cl	36	41

[a] IC_{50} (nM): mDP receptor antagonist activity.
[b] NT: not tested.

carbonate afforded **25**, catalytic hydrogenation of which afforded the corresponding aniline **26**. N-Acylation of **26** with an acid chloride **28**, which was prepared from 3-methoxycarbonylmethylbenzoic acid **27** and oxalyl chloride followed by alkaline hydrolysis resulted in **2c**.

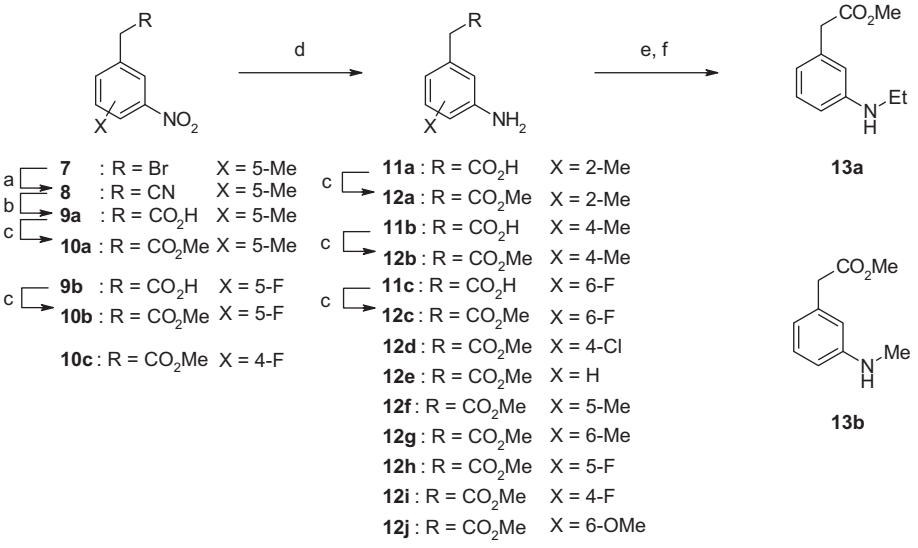
3. Results and discussion

The test compounds listed in Tables 1–3 were all tested for inhibition of the specific binding of a radiolabeled ligand, [^{3}H]PGD_2, to membrane fractions prepared from cells stably expressing mDP receptor. They were also evaluated for their potency to antagonize mDP receptors by measuring PGD_2-stimulated changes in intracellular second messenger cAMP (cyclic adenosine 3', 5'-monophosphate) as an indicator of receptor function. The mDP antagonism was measured in the presence of 0.1% BSA to mimic the protein-rich environment of in vivo animal models. Because of their close homology to human receptors, all the DP receptor affinities and antagonist activities are assayed using the equivalent mouse receptors unless otherwise noted.

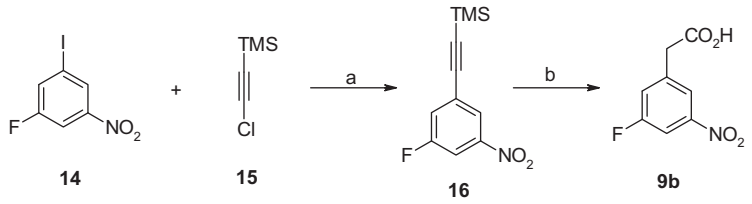
Table 1 describes the SAR of the linkers between the rings A and B of the general formula **I**. 3-Benzoylaminophenylacetic acid analog **2a**, which was designed based on the molecular design described in Figure 1, exhibited moderate potency in both the receptor affinity and the antagonist activity assays while the corresponding retro-amide analog **2c** showed 17-fold less potent affinity in the binding assay. The corresponding sulfonamide analog **2b** showed equipotent binding affinity while it did not show DP receptor selectivity because of its increased affinity for both the EP3 and EP4 receptors.

As shown in Table 2, *N*-alkyl amide analogs of **2a** were prepared and evaluated. N-Methylation and N-ethylation of **2a** resulted in *N*-methyl amide analog **3a** and *N*-ethyl amide analog **3b**, respectively. Both the analogs **3a** and **3b** tended to have slightly less potent receptor affinity for the DP receptor. Among the tested compounds, 3-benzoylaminophenylacetic acid analog **2a** was found to have the most potent antagonist activity. On the basis of the information described above, **2a** was used as a chemical lead for further optimization. Accordingly, the following optimization was carried out using 3-benzoylaminophenylacetic acid as a scaffold.

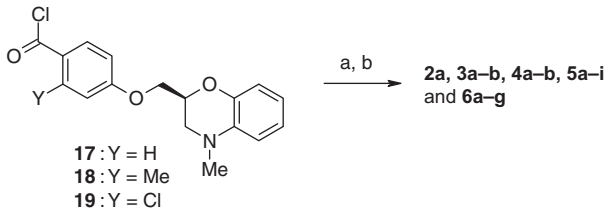
On the basis of the SAR study described in Tables 1 and 2, the sulfonamide and retro-amide moieties were found to show no selectivity for the DP receptor as illustrated by the results for **2b** and **2c**, respectively. The secondary anilide moiety was found to be more beneficial than the tertiary amide moiety as a linker between the rings A and B as illustrated by the results of **2a** and **3a–b**. *N*-Methyl-*N*-phenylbenzamides have been reported to



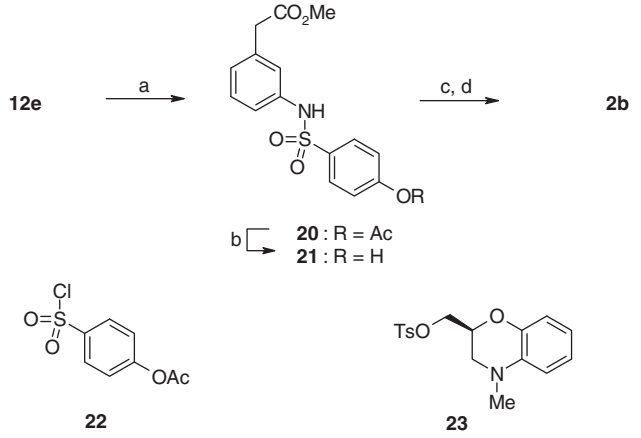
Scheme 1a. Synthesis of methyl (3-aminophenyl)acetates **12a–j** and **13a–b**. Reagents: (a) NaCN, DMSO; (b) H_2SO_4; (c) CH_2N_2, EtOAc; (d) H_2, 10%Pd–C, EtOAc, MeOH; (e) Ac_2O, Py, CH_2Cl_2; (f) BH_3·SMe, THF.



Scheme 1b. Synthesis of (3-fluoro-5-nitrophenyl)acetic acid **9b**. Reagents: (a) $PdCl_2(PPh_3)_2$, CuI, *n*-Bu_4NI, Et_3N, DMF; (b) cyclohexene, BH_3–THF, THF and then 2 M NaOH aq, H_2O_2.



Scheme 2. Synthesis of **2a**, **3a**–**b**, **4a**–**b**, **5a**–**i** and **6a**–**g**. Reagents: (a) **12a**–**j** and **13a**–**b**, Py, CH_2Cl_2; (b) 5 M NaOH aq, MeOH, THF.



Scheme 3. Synthesis of sulfonamide analogs **2b**. Reagents: (a) **22**, Py, CH_2Cl_2; (b) K_2CO_3, MeOH, DME; (c) **23**, Cs_2CO_3, DMF; (d) 5 M NaOH aq, MeOH, THF.

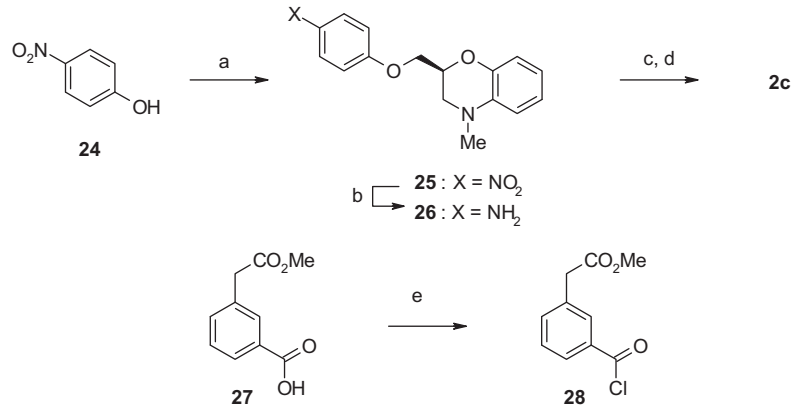
occupy *s-cis* conformation with remarkable higher field shifts of the aromatic protons while *N*-phenylbenzamides have been reported to occupy *s-trans* conformation.[9] Based on the information mentioned above, **3a** was considered to occupy *s-cis* conformation with the observed higher shifts of the aromatic protons (see Section 5.9.3) while **2a** was considered to occupy *s-trans* conformation. More potency of **2a** relative to **3a** strongly suggests that *s-trans* amide conformation is more preferred to the corresponding *s-cis* conformation for DP receptor. More detailed SAR study of our designed and synthesized compounds regarding conformational analysis using X-ray analysis will be reported in the following paper.

Table 3 describes the effect of the substituents on rings A and B of the general formula **II** on the activity profiles. It has been frequently observed that 3-benzoylaminophenylacetic acid analogs such as **2a** are hydrolyzed by metabolic enzymes. Since our objective is to identify an orally active drug candidate, the targeted DP antagonist has to possess tolerable metabolic stability to be systemically effective. To identify a metabolically stable antagonist, we focused on the synthesis and evaluation of 3-(2-methylbenzoyl)aminophenylacetic acid and 3-(2-chlorobenzoyl)aminophenylacetic acid analogs such as **4a** and **4b**. It was fortunate that both the analogs **4a** and **4b** exhibited increased binding affinity and antagonist activity relative to the unsubstituted analog **2a**. Effect of the substitution of ring A on the activity profiles was further investigated for both the series **5a**–**i** and **6a**–**g**. Introduction of 2-methyl, 4-methyl, 5-methyl and 6-methyl residues into ring A of **4a** afforded **5a**–**d**, respectively. 2-Methyl analog **5a** showed reduced potency in both the binding affinity and antagonist activity assays relative to **4a** while the 4-methyl analog **5b** exhibited slightly reduced binding affinity and retained antagonist activity. 5-Methyl analog **5c** and 6-methyl analog **5d** showed slightly increased binding affinity and nearly equipotent antagonist activity. As a result, 4-methyl, 5-methyl and 6-methyl analogs **5b**–**d** tended to show equipotent antagonist activity relative to **4a**. Introduction of a halogen residue into the ring A of **4a** afforded 4-chloro, 4-fluoro, 5-fluoro and 6-fluorophenyl analogs **5e**–**h**, respectively, also with retention of the potency relative to **4a** in binding affinity and antagonist activity assays. Introduction of a 6-methoxy residue as a hydrophilic substituent into the ring A of **4a** afforded **5i** with a tendency for retained receptor affinity and reduced antagonist activity with a decrease in potency compared with **5e**–**h**.

Second, the corresponding SAR was investigated for a series of 3-(2-chlorophenyl)aminoacetic acid analogs. Introduction of a methyl residue into positions 4, 5 and 6 of the ring A of **4b** resulted in **6a**–**c**, respectively, a tendency for retained binding affinity and an increase in the antagonist activity. Introduction of a halogen such as 4-chloro, 4-fluoro and 6-fluoro residues into ring A of **4b** afforded **6d**–**f**, respectively. Compounds **6d**–**e** showed retained binding affinity potency and increased antagonist activity while **6f** had reduced antagonist activity although it retained strong receptor affinity. Introduction of a methoxy residue into ring A of **4b** afforded **6g** with a reduction in the activities of both binding affinity and antagonist activity.

Based on the SAR study described in Table 3, the 2-methyl residue of ring A of **5a** was estimated to cause a deleterious effect on the conformation of both the acetic acid and 3-*N*-benzoyl moieties. Introduction of a substituent into the 6-position of ring A of **4a** and/or **4b**, which affords **5d**, **5h**, **5i**, **6c**, **6f** and **6g**, resulted in complicated results. 3-(2-Methylbenzoyl)aminophenylacetic acid analogs **5d** and **5h** showed retained potency for both the binding affinity and antagonist activity relative to their parent compound **4a** while the corresponding 3-(2-chlorobenzoylamino)phenylacetic acid analogs **6c** and **6f** tended to show increased potency and slightly less potency, respectively, in their antagonist activity with retained receptor affinity. Substitution with the 6-methoxy residue on ring A was found not to be beneficial for further optimization as illustrated by the results of **5i** and **6g**.

Table 4 describes the selectivity profiles of **4a**, **5b**–**c** and **6d**–**e** including those for the mEP, mFP, hTP, hIP and mDP receptors. As shown in Table 4, all the tested compounds were proven to show better selectivity for the human IP receptor compared with the 2-methylindole analog **1** because of their reduced hIP receptor affinities and their retained potent mDP receptor affinities. They showed nearly equipotent mDP antagonist activities with the chemical lead **1**.



Scheme 4. Synthesis of **2c**. Reagents: (a) **23**, Cs_2CO_3, DMF; (b) H_2, 10%Pd–C, MeOH, EtOAc; (c) **28**, Py, CH_2Cl_2; (d) 5 M NaOH aq, MeOH, THF; (e) $(COCl)_2$, DMF, DME.

Table 4
Activity profiles of **1**, **4a**, **5b**, **5c**, **6d** and **6e**

Compd	Binding K_i (nM)								IC_{50}[a] (nM) mDP
	mEP1	mEP2	mEP3	mEP4	mFP	hTP	hIP	mDP	
1	6200	99	1700	>10,000	3100	260	37	16	2.4
4a	>10,000	NT[b]	>10,000	>10,000	>10,000	>10,000	3600	18	3.3
5b	>10,000	170	>10,000	>10,000	>10,000	>10,000	2600	31	4.6
5c	>10,000	62	>10,000	670	>10,000	>10,000	3500	8.4	3.4
6d	6600	44	>10,000	>10,000	>10,000	>10,000	1600	13	4.3
6e	>10,000	22	>10,000	>10,000	>10,000	>10,000	>10,000	17	7.4

[a] IC_{50} (nM): mDP receptor antagonist activity.
[b] NT: not tested.

Table 5
Effect of the pharmacokinetic profiles of the representative compounds in rats

Compd	Route	Dose (mg/kg)	AUC (μg h/mL)	C_{max} (μg/mL)	CL_{tot} (mL/min/kg)	$T_{1/2}$ (h)	V_{ss} (mL/kg)	BA (%)
1	iv	1	7.2[a]		2.4	9.8	1800	
	po	10	24.3[a]	3.5		5.4		34
2a	iv	1	0.67[a]		25	0.27	220	
	po	10	8.0[a]	4.9		1.8		119
5b	iv	1	4.6[b]		3.3	3.1	610	
	po	10	28.8[b]	8.7		5.2		62

[a] AUC_{inf}.
[b] AUC_{last}.

As shown in Table 5, pharmacokinetic (PK) profiles of the representative compounds **1**, **2a** and **5b** were investigated. The newly synthesized antagonist **5b** tended to have improved PK profiles relative to the chemical lead **2a** especially in terms of oral exposure (AUC) and clearance (CL) with good bioavailability (BA). As shown by the PK data of **5b**, concurrent introduction of a 4-methyl residue into the A ring and a 2-methyl residue into the B ring was considered effective to block the enzymatic degradation of **5b**. The tissue availability (V_{ss}) of **5b** was 610 ml/kg, which was found to be between the V_{ss} values of **2a** (220 ml/kg) and **1** (1800 ml/kg).

4. Conclusion

Design and synthesis of new prostaglandin D_2 receptor antagonists starting from 3-benzoylaminophenylacetic acid **2a**, which was designed based on the structural simplification of our original 2-methylindole antagonist **1** (Fig. 1), as a chemical lead led us to discover a new cost-effective antagonist **5b**. Further effort to identify an orally available subtype selective DP antagonist as a drug candidate will be disclosed in due course.

5. Experimental

5.1. General directions

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^1H NMR) were taken on a Varian Mercury 300 spectrometer or Varian GEMINI-200 or VXR-200s spectrometer using deuterated chloroform ($CDCl_3$) or deuterated methanol (CD_3OD) or deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. Fast atom bombardment mass spectra (FAB-MS) and electron ionization (EI) were obtained on a JEOL JMS-DX303HF spectrometer. The matrix assisted laser desorption ionization-time of flight high-resolution mass spectra (MALDI-TOF, HRMS) were obtained on a PerSeptive Voyager Elite spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a HITACHI M1200H spectrometer. Infrared spectra (IR) were measured on a Perkin-Elmer FT-IR 1760X spectrometer. Melting points and results of elemental analyses were uncorrected. Optical rotations were measured in a JASCO

DIP-1000 digital polarimeter. Column chromatography was carried out on silica gel [Merck Silica Gel 60 (0.063–0.200 mm), Wako gel C200 or Fuji Silysia BW235]. Thin layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, Silica Gel 60 F_{254}). The following abbreviations for solvents and reagents are used; tetrahydrofuran (THF), ethyl acetate (EtOAc), dimethylformamide (DMF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (MeOH), triethylamine (TEA), 1,2-dimethoxyethane (DME), dimethylsulfoxide (DMSO), acetic anhydride (Ac_2O), pyridine (Py).

5.2. (3-Methyl-5-nitrophenyl)acetonitrile (8)

To a stirred solution of **7** (782 mg, 3.4 mmol) in DMSO (5 mL) was added NaCN (183 mg, 3.74 mmol) at room temperature. After stirring overnight, the reaction mixture was quenched with water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to yield **8** as a dark brown oil, which was used for the next reaction without further purification; TLC R_f = 0.14 (*n*-hexane/EtOAc, 9:1).

5.3. (3-Methyl-5-nitrophenyl)acetic acid (9a)

To a stirred suspension of **8** (3.4 mmol) in water (2.5 mL) was added concd H_2SO_4 (2.5 mL). After stirring for 1.5 h under reflux, the reaction mixture was cooled to room temperature, poured into water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to yield **9a** (130 mg, 20% in 2 steps) as a pale orange solid, which was used for the next reaction without further purification; TLC R_f = 0.50 ($CHCl_3$/CH_3OH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (s, 2H), 7.44 (s, 1H), 3.74 (s, 2H), 2.47 (s, 3H).

5.4. General procedure for the preparation of methyl nitrophenyl acetates 10a–b and methyl aminophenyl acetates 12a–c

5.4.1. Methyl (3-methyl-5-nitrophenyl)acetate (10a)

To a stirred solution of **9a** (130 mg, 0.7 mmol) in EtOAc (10 mL) was added dropwise a solution of CH_2N_2 in ether until bubbling ceased. The reaction mixture was concentrated in vacuo to yield **10a** (146 mg, quant) as a pale yellow oil; TLC R_f = 0.51 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 2H), 7.43 (s, 1H), 3.73 (s, 3H), 3.70 (s, 2H), 2.46 (s, 3H).

Compounds **10b** and **12a–c** were prepared as described above.

5.4.2. Methyl (3-fluoro-5-nitrophenyl)acetate (10b)

Yield quant; Pale yellow oil; TLC R_f = 0.56 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.86 (dt, *J* = 8.4, 2.4 Hz, 1H), 7.39 (dt, *J* = 8.4, 1.8 Hz, 1H), 3.75 (s, 5H).

5.4.3. Methyl (3-amino-2-methylphenyl)acetate (12a)

Yield quant; Pale yellow powder; TLC R_f = 0.26 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (t, *J* = 7.5 Hz, 1H), 6.69–6.61 (m, 2H), 2.68 (s, 3H) 3.64 (s, 2H), 3.70–3.50 (br, 2H), 2.10 (s, 3H).

5.4.4. Methyl (3-amino-4-methylphenyl)acetate (12b)

Yield quant; Pale pink oil; TLC R_f = 0.29 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (d, *J* = 7.8 Hz, 1H), 6.65–6.59 (m, 2H), 3.68 (s, 3H), 3.70–3.50 (br, 2H), 3.52 (s, 2H), 2.14 (s, 3H).

5.4.5. Methyl (5-amino-2-fluorophenyl)acetate (12c)

Yield quant; Pale yellow oil; TLC R_f = 0.47 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (t, *J* = 9.3 Hz, 1H), 6.60–6.51 (m, 2H), 3.71 (s, 3H), 3.58 (s, 2H), 3.60–3.45 (br, 2H).

5.5. General procedure for the preparation of methyl aminophenyl acetates 12f and 12h–i

5.5.1. Methyl (3-amino-5-methylphenyl)acetate (12f)

To a stirred solution of **10a** (146 mg, 0.7 mmol) in EtOAc (3 mL) and MeOH (3 mL) was added 10% Pd–C (30 mg) at room temperature. The resulting suspension was vigorously stirred for 30 min at room temperature under hydrogen atmosphere. Insoluble substance was removed by filtration. The filtrate was concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **12f** (77 mg, 65%) as a pale yellow oil; TLC R_f = 0.26 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.49 (s, 1H), 6.43 (s, 2H), 3.68 (s, 3H), 3.70–3.50 (br, 2H), 3.49 (s, 2H), 2.24 (s, 3H).

Compounds **12h–i** were prepared as described above.

5.5.2. Methyl (3-amino-5-fluorophenyl)acetate (12h)

Yield quant; Pale yellow oil; TLC R_f = 0.26 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42–6.33 (m, 2H), 6.32–6.24 (m, 1H), 3.85–3.70 (br, 2H), 3.70 (s, 3H), 3.50 (s, 2H).

5.5.3. Methyl (3-amino-4-fluorophenyl)acetate (12i)

Yield quant; Pale yellow powder; TLC R_f = 0.63 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (dd, *J* = 11.1, 8.4 Hz, 1H), 6.71 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.62–6.54 (m, 1H), 3.80–3.60 (br, 2H), 3.68 (s, 3H), 3.50 (s, 2H).

5.6. Methyl [3-(ethylamino)phenyl]acetate (13a)

To a stirred solution of **12e** (820 mg, 4.97 mmol) in CH_2Cl_2 (5 mL) were added pyridine (0.82 mL, 10.1 mmol) and Ac_2O (0.52 mL, 5.50 mmol). After stirring for 30 min at room temperature, the reaction mixture was poured into water and extracted with EtOAc (×2). The combined organic layers were washed with 1 M HCl aq, brine, dried over Na_2SO_4 and concentrated in vacuo to yield the corresponding *N*-acetate as a pale yellow oil, which was used for the next reaction without further purification; TLC R_f = 0.22 (*n*-hexane/EtOAc, 1:1).

To a stirred solution of the *N*-acetate (4.97 mmol) in THF (3 mL) was added a 2 M solution of BH_3SMe_2 in THF (1.9 mL, 3.79 mmol) at 0 °C. After stirring for 1 h without cooling bath, the reaction mixture was additionally stirred for 15 h at 60 °C and then poured into sat $NaHCO_3$ aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield **13a** (320 mg, 33% in 2 steps) as a colorless oil; TLC R_f = 0.49 (*n*-hexane/EtOAc, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.59 (d, *J* = 7.5 Hz, 1H), 6.55–6.48 (m, 2H), 3.68 (s, 3H), 3.54 (s, 2H), 3.15 (q, *J* = 7.2 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H).

5.7. [(3-Fluoro-5-nitrophenyl)ethynyl](trimethyl)silane (16)

To a stirred solution of 1-fluoro-3-iodo-5-nitrobenzene **14** (2 g, 7.5 mmol) in DMF (30 mL) under argon atmosphere were added $Pd(PPh_3)_2Cl_2$ (263 mg, 0.4 mmol), CuI (143 mg, 0.75 mmol), *n*-Bu_4NI (553 mg, 1.5 mmol), triethylamine (1.57 mL, 11.2 mmol) and (trimethylsilyl)acetylene **15** (1.8 mL, 12.7 mmol). After stirring overnight at 70 °C, the reaction mixture was cooled to room temperature, poured into sat NH_4Cl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield **16** (1.31 g, 74%) as a brown oil; TLC R_f = 0.82 (*n*-hexane/EtOAc, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.91–7.84 (m, 1H), 7.50–7.44 (m, 1H), 0.27 (s, 9H).

5.8. (3-Fluoro-5-nitrophenyl)acetic acid (9b)

To a stirred solution of cyclohexene (1.81 g, 22.1 mmol) in THF (15 mL) were added a 1 M solution of BH_3 in THF (11 mL, 11 mmol) and then a solution of **16** (1.31 g, 5.5 mmol) in THF (9 mL) at 0 °C. The reaction mixture was stirred overnight without cooling bath. To the above-described reaction mixture were added slowly 2 M NaOH aq (18 mL) and then 30% H_2O_2 (6.0 mL). After stirring for 15 min, the reaction mixture was diluted with water and extracted with *n*-hexane. The aqueous layer was acidified with concd HCl and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and evaporated to yield **9b** (650 mg, 59%), which was used for the next reaction without further purification; TLC R_f = 0.38 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.88 (dt, *J* = 8.4, 2.4 Hz, 1H), 7.40 (dt, *J* = 8.4, 1.8 Hz, 1H), 3.79 (s, 2H).

5.9. General procedure for the preparation of 2a, 3a–b, 4a–b, 5a–i, and 6a–g

5.9.1. {3-[(2-Methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (4a)

To a stirred solution of **12e** (62 mg, 0.4 mmol) in CH_2Cl_2 (5 mL) were added a solution of **18** (133 mg, 0.4 mmol) in CH_2Cl_2 (3 mL) and then pyridine (0.067 mL, 0.8 mmol). After stirring overnight at room temperature, the reaction mixture was diluted with water and extracted with EtOAc (×2). The combined organic layers were washed with 1 M NaOH aq, water, brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield methyl ester (120 mg, 63%); TLC R_f = 0.14 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60–7.38 (m, 4H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 7.5 Hz, 1H), 6.92–6.77 (m, 4H), 6.74–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, *J* = 9.6, 5.4 Hz, 1H), 4.14 (dd, *J* = 11.4, 6.6 Hz, 1H), 3.71 (s, 3H), 3.65 (s, 2H), 3.39 (dd, *J* = 11.4, 3.0 Hz, 1H), 3.26 (dd, *J* = 11.4, 7.8 Hz, 1H), 2.91 (s, 3H), 2.51 (s, 3H).

To a stirred solution of methyl ester (120 mg, 0.25 mmol) described above in MeOH (4 mL) and THF (4 mL) was added 5 M NaOH aq (2 mL). After stirring for 1 h at room temperature, the reaction mixture was diluted with 2 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to give a crude product, which was washed with EtOAc/*n*-hexane to yield **4a** (85 mg, 73%) as a pale blue powder; TLC R_f = 0.40 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62–7.40 (m, 4H), 7.33 (t, *J* = 8.1 Hz, 1H), 7.10–7.04 (m, 1H), 6.92–6.76 (m, 4H), 6.74–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, *J* = 9.6, 4.8 Hz, 1H), 4.14 (dd, *J* = 9.6, 6.6 Hz, 1H), 3.68 (s, 2H), 3.39 (dd, *J* = 11.4, 2.7 Hz, 1H), 3.25 (dd, *J* = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.50 (s, 3H); MS (APCI, Neg, 20 V) *m*/*z* 445 $(M-H)^-$; IR (KBr) 3256, 3040, 2925, 1698, 1650, 1607, 1572, 1532, 1503, 1442, 1355, 1318, 1299, 1244, 1225, 1176, 1136, 1098, 1041, 994, 975, 912, 868, 825, 773, 739, 707, 607 cm^{-1}, Optical rotation $[\alpha]_D^{24}$ +14.49 (*c* 0.60, DMSO); mp 173–175 °C.

Compounds **2a**, **3a**–**b**, **4b**, **5a**–**i** and **6a**–**g** were prepared as described above.

5.9.2. {3-[(4-{[(2*S*)-4-Methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (2a)

Yield 57% in two steps; Green amorphous powder; TLC R_f = 0.52 (AcOEt/MeOH, 19:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90–7.78 (m, 3H), 7.57 (s, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 7.29 (m, 1H), 7.06–6.95 (m, 3H), 6.91–6.82 (m, 2H), 6.75–6.66 (m, 2H), 4.65 (m, 1H), 4.27 (dd, *J* = 9.6, 4.8 Hz, 1H), 4.16 (dd, *J* = 9.6, 6.6 Hz, 1H), 3.63 (s, 2H), 3.38 (dd, *J* = 11.4, 2.7 Hz, 1H), 3.25 (dd, *J* = 11.4, 6.6 Hz, 1H), 2.90 (s, 3H); MS (APCI, Neg, 20 V) *m*/*z* 431 $(M-H)^-$; IR (neat) 2925, 1712, 1649, 1606, 1544, 1505, 1441, 1304, 1245, 1179, 1042, 909, 844, 735 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +8.86 (*c* 0.75, DMSO).

5.9.3. {3-[Methyl(4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (3a)

Yield 38% in two steps; Green amorphous powder; TLC R_f = 0.63 (AcOEt/MeOH, 19:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29–7.20 (m, 4H), 7.10–7.02 (m, 2H), 6.91–6.69 (m, 6H), 4.55 (m, 1H), 4.28 (dd, *J* = 10.8, 4.8 Hz, 1H), 4.04 (dd, *J* = 10.8, 7.2 Hz, 1H), 3.49 (s, 3H), 3.43 (s, 2H), 3.38 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.09 (dd, *J* = 11.4, 7.2 Hz, 1H), 2.86 (s, 3H); MS (APCI, Neg, 20 V) *m*/*z* 445 $(M-H)^-$; IR (neat) 2939, 1721, 1605, 1504, 1451, 1376, 1301, 1223, 1177, 1042, 906, 841, 738, 703, 600 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +6.65 (*c* 0.75, DMSO).

5.9.4. {3-[Ethyl(4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (3b)

Yield 49% in two steps; Blue amorphous powder; TLC R_f = 0.49 (AcOEt/MeOH, 19:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.18 (m, 3H), 7.12–7.02 (m, 2H), 6.92–6.66 (m, 7H), 4.54 (m, 1H), 4.30 (dd, *J* = 10.8, 4.8 Hz, 1H), 4.03 (dd, *J* = 10.8, 7.5 Hz, 1H), 3.99 (dq, *J* = 2.4, 7.2 Hz, 2H), 3.41 (s, 2H), 3.39 (dd, *J* = 11.7, 2.4 Hz, 1H), 3.07 (dd, *J* = 11.7, 7.8 Hz, 1H), 2.86 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H); MS (APCI, Neg, 20 V) *m*/*z* 459 $(M-H)^-$; IR (neat) 2932, 1726, 1605, 1505, 1450, 1396, 1301, 1245, 1177, 1043, 839, 743 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +4.05 (*c* 0.75, DMSO).

5.9.5. {3-[(2-Chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (4b)

Yield 40% in two steps; Ivory powder; TLC R_f = 0.29 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.62 (s, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 1.8 Hz, 1H), 6.98–6.80 (m, 3H), 6.76–6.67 (m, 2H), 4.71–4.61 (m, 1H), 4.26 (dd, *J* = 9.9, 5.1 Hz, 1H), 4.17 (dd, *J* = 9.9, 6.0 Hz, 1H), 3.68 (s, 2H), 3.38 (dd, *J* = 11.7, 3.0 Hz, 1H), 3.25 (dd, *J* = 11.7, 6.0 Hz, 1H), 2.91 (s, 3H); MS (APCI, Neg, 20 V) *m*/*z* 465 $(M-H)^-$; IR (KBr) 3255, 2944, 1699, 1655, 1604, 1531, 1494, 1443, 1295, 1261, 1223, 1174, 1135, 1047, 968, 915, 860, 779, 741, 708, 607 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +13.19 (*c* 0.75, DMSO).

5.9.6. {2-Methyl-3-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5a)

Yield 69% in two steps; Yellow powder; TLC R_f = 0.38 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82–7.68 (m, 1H), 7.54–7.44 (m, 1H), 7.38–7.18 (m, 2H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.92–6.77 (m, 4H), 6.75–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, *J* = 9.9, 5.4 Hz, 1H), 4.14 (dd, *J* = 9.9, 6.3 Hz, 1H), 3.73 (s, 2H), 3.39 (dd, *J* = 11.4, 2.7 Hz, 1H), 3.25 (dd, *J* = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.52 (s, 3H), 2.24 (s, 3H); MS (APCI, Neg, 20 V) *m*/*z* 459 $(M-H)^-$; IR (KBr) 3250, 2925, 1695, 1649, 1605, 1503, 1472, 1449, 1278, 1242, 1223, 1174, 1136, 1042, 939, 822, 785, 737, 692, 607, 479 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +10.14 (*c* 0.75, DMSO).

5.9.7. {4-Methyl-3-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5b)

Yield 68% in two steps; Ivory powder; TLC R_f = 0.35 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04–7.86 (br, 1H), 7.53–7.42 (m, 1H), 7.34–7.22 (m, 1H), 7.19 (d, *J* = 7.8 Hz, 1H),

7.07–6.99 (m, 1H), 6.92–6.76 (m, 4H), 6.75–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.27 (dd, J = 9.9, 4.8 Hz, 1H), 4.15 (dd, J = 9.9, 6.6 Hz, 1H), 3.66 (s, 2H), 3.39 (dd, J = 11.7, 2.7 Hz, 1H), 3.25 (dd, J = 11.7, 6.6 Hz, 1H), 2.91 (s, 3H), 2.52 (s, 3H), 2.28 (s, 3H); MS (APCI, Neg, 20 V) m/z 459 $(M-H)^-$; IR (KBr) 3274, 3033, 2926, 2871, 2824, 2643, 2350, 2304, 2242, 1690, 1650, 1608, 1579, 1529, 1504, 1481, 1449, 1402, 1357, 1311, 1298, 1288, 1270, 1244, 1221, 1176, 1149, 1138, 1101, 1041, 1010, 976, 935, 913, 867, 853, 842, 823, 810, 780, 743, 735, 700, 690, 681, 638, 628, 609, 595, 586, 577, 564, 555, 546, 532, 522, 514, 493, 472, 460 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +12.57 (c 0.75, DMSO); mp 176–177 °C.

5.9.8. {3-Methyl-5-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5c)

Yield 51% in two steps; Ivory powder; TLC R_f = 0.35 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.48–7.28 (m, 4H), 6.92–6.76 (m, 5H), 6.74–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.9, 5.1 Hz, 1H), 4.14 (dd, J = 9.9, 6.6 Hz, 1H), 3.63 (s, 2H), 3.39 (dd, J = 11.4, 2.7 Hz, 1H), 3.25 (dd, J = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.50 (s, 3H), 2.35 (s, 3H); MS (APCI, Neg, 20 V) m/z 459 $(M-H)^-$; IR (KBr) 3262, 3150, 3035, 2924, 2871, 1693, 1650, 1609, 1573, 1534, 1504, 1455, 1414, 1290, 1245, 1223, 1178, 1137, 1041, 947, 909, 867, 847, 824, 735, 689, 654, 609, 569, 522 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +13.59 (c 0.75, DMSO).

5.9.9. {2-Methyl-5-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5d)

Yield 45% in two steps; Ivory powder; TLC R_f = 0.32 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.56–7.34 (m, 4H), 7.18 (d, J = 8.4 Hz, 1H), 6.92–6.76 (m, 4H), 6.75–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.6, 4.5 Hz, 1H), 4.14 (dd, J = 9.6, 6.3 Hz, 1H), 3.69 (s, 2H), 3.39 (dd, J = 11.4, 2.1 Hz, 1H), 3.25 (dd, J = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.50 (s, 3H), 2.30 (s, 3H); MS (APCI, Neg, 20 V) m/z 459 $(M-H)^-$; IR (KBr) 3256, 3042, 2926, 1699, 1647, 1608, 1572, 1505, 1451, 1411, 1356, 1320, 1298, 1244, 1223, 1177, 1148, 1137, 1092, 1052, 997, 975, 913, 864, 825, 790, 775, 737, 704, 664, 633, 600, 570, 545 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +12.31 (c 0.60, DMSO).

5.9.10. {4-Chloro-3-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5e)

Yield 54% in two steps; Yellow powder; TLC R_f = 0.41 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.49 (br s, 1H), 7.99 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 7.8 Hz, 1H), 7.01 (dd, J = 7.8, 1.8 Hz, 1H), 6.93–6.78 (m, 4H), 6.76–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.27 (dd, J = 9.9, 4.8 Hz, 1H), 4.16 (dd, J = 9.9, 6.6 Hz, 1H), 3.70 (s, 2H), 3.40 (dd, J = 11.7, 2.7 Hz, 1H), 3.25 (dd, J = 11.7, 6.6 Hz, 1H), 2.91 (s, 3H), 2.55 (s, 3H); MS (APCI, Neg, 20 V) m/z 479 $(M-H)^-$; IR (KBr) 3275, 3036, 2926, 1687, 1656, 1607, 1583, 1524, 1502, 1459, 1426, 1357, 1290, 1245, 1221, 1177, 1136, 1092, 1051, 978, 914, 864, 822, 782, 741, 655, 504, 479, 469 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +7.96 (c 0.75, DMSO).

5.9.11. {4-Fluoro-3-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5f)

Yield 22% in two steps; Pale orange powder; TLC R_f = 0.31 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, J = 2.7 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.37 (s, 1H), 7.14–6.76 (m, 6H), 6.76–6.68 (m, 2H), 4.70–4.60 (m, 1H), 4.27 (dd, J = 9.3, 5.1 Hz, 1H), 4.15 (dd, J = 9.3, 6.6 Hz, 1H), 3.69 (s, 2H), 3.40 (dd, J = 11.7, 2.7 Hz, 1H), 3.26 (dd, J = 11.7, 6.9 Hz, 1H), 2.92 (s, 3H), 2.53 (s, 3H); MS (APCI, Neg, 20 V) m/z 463 $(M-H)^-$; IR (KBr) 3254, 3141, 3034, 2927, 2866, 2649, 2619, 2345, 1699, 1655, 1608, 1572, 1536, 1503, 1460, 1435, 1408, 1320, 1289, 1264, 1240, 1223, 1177, 1135, 1119, 1051, 995, 977, 911, 865, 824, 785, 771, 734, 679, 665, 613, 566, 523, 499, 484, 454 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +9.03 (c 0.75, DMSO).

5.9.12. {3-Fluoro-5-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5g)

Yield 59% in two steps; Pale blue powder; TLC R_f = 0.41 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.56–7.40 (m, 3H), 7.20 (br s, 1H), 6.93–6.75 (m, 5H), 6.76–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.6, 5.1 Hz, 1H), 4.14 (dd, J = 9.6, 6.6 Hz, 1H), 3.64 (s, 2H), 3.39 (dd, J = 11.7, 2.4 Hz, 1H), 3.25 (dd, J = 11.7, 6.6 Hz, 1H), 2.91 (s, 3H), 2.49 (s, 3H); MS (APCI, Neg, 20 V) m/z 463 $(M-H)^-$; IR (KBr) 3268, 3067, 2927, 1702, 1654, 1623, 1604, 1573, 1535, 1503, 1474, 1430, 1278, 1243, 1225, 1175, 1137, 1096, 1042, 1001, 940, 910, 850, 824, 769, 741, 678, 607, 567, 524 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +12.49 (c 0.75, DMSO).

5.9.13. {2-Fluoro-5-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5h)

Yield 53% in two steps; Ivory powder; TLC R_f = 0.31 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.61 (br s, 1H), 7.52–7.38 (m, 3H), 7.07 (t, J = 8.7 Hz, 1H), 6.92–6.76 (m, 4H), 6.76–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.3, 5.1 Hz, 1H), 4.14 (dd, J = 9.3, 6.3 Hz, 1H), 3.73 (s, 2H), 3.39 (dd, J = 11.7, 2.7 Hz, 1H), 3.25 (dd, J = 11.7, 6.6 Hz, 1H), 2.91 (s, 3H), 2.50 (s, 3H); MS (APCI, Neg, 20 V) m/z 463 $(M-H)^-$; IR (KBr) 3275, 3041, 2929, 2619, 2547, 1700, 1649, 1609, 1573, 1528, 1502, 1455, 1419, 1350, 1321, 1303, 1289, 1233, 1214, 1178, 1136, 1113, 1103, 1065, 1041, 975, 909, 869, 826, 775, 742, 578, 545, 453 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +14.12 (c 0.75, DMSO).

5.9.14. {2-Methoxy-5-[(2-methyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5i)

Yield 52% in two steps; Gray powder; TLC R_f = 0.36 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.58–7.39 (m, 3H), 7.34 (br s, 1H), 6.93–6.76 (m, 5H), 6.74–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.3, 4.8 Hz, 1H), 4.14 (dd, J = 9.3, 6.3 Hz, 1H), 3.85 (s, 3H), 3.69 (s, 2H), 3.39 (dd, J = 11.4, 2.7 Hz, 1H), 3.25 (dd, J = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.50 (s, 3H); MS (APCI, Neg, 20 V) m/z 475 $(M-H)^-$; IR (KBr) 3262, 3131, 3044, 2929, 2838, 2646, 2553, 1699, 1645, 1607, 1573, 1504, 1459, 1428, 1300, 1287, 1260, 1239, 1227, 1176, 1147, 1135, 1103, 1092, 1063, 1050, 1026, 975, 939, 865, 817, 807, 775, 744, 633, 598, 574, 545, 455 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +9.44 (c 0.75, DMSO).

5.9.15. {3-[(2-Chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-4-methylphenyl} acetic acid (6a)

Yield 50% in two steps; Ivory powder; TLC R_f = 0.42 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.02 (s, 2H), 7.90 (d, J = 8.7 Hz, 1H), 7.19 (d, J = 8.1 Hz, 1H), 7.09–7.00 (m, 2H), 7.00–6.93 (m, 1H), 6.93–6.81 (m, 2H), 6.76–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.27 (dd, J = 9.6, 5.1 Hz, 1H), 4.18 (dd, J = 9.6, 6.3 Hz, 1H), 3.68 (s, 2H), 3.39 (dd, J = 11.7, 2.7 Hz, 1H), 3.25 (dd, J = 11.7, 6.3 Hz, 1H), 2.91 (s, 3H), 2.32 (s, 3H); MS (APCI, Neg, 20 V) m/z 479 $(M-H)^-$; IR (KBr) 3257, 3147, 3040, 2928, 2823, 2557, 1698, 1657, 1639, 1604, 1581, 1534, 1503, 1450, 1426, 1407, 1358, 1291, 1264, 1245, 1225, 1138, 1046, 993, 962, 918, 846, 827, 785, 740, 718, 688, 615, 586, 566, 557, 536, 526, 506, 495, 472, 462 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +17.39 (c 0.75, DMSO).

5.9.16. {3-[(2-Chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-5-methylphenyl}acetic acid (6b)

Yield 57% in two steps; Ivory powder; TLC R_f = 0.39 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.41 (s, 2H), 7.01 (d, J = 2.4 Hz, 1H), 6.98–6.81 (m, 4H), 6.75–6.67 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.9, 5.1 Hz, 1H), 4.17 (dd, J = 9.9, 6.3 Hz, 1H), 3.64 (s, 2H), 3.38 (dd, J = 11.4, 2.7 Hz, 1H), 3.25 (dd, J = 11.4, 6.3 Hz, 1H), 2.91 (s, 3H), 2.36 (s, 3H); MS (APCI, Neg, 20 V) m/z 479 $(M-H)^-$; IR (KBr) 3236, 3066, 2927, 2868, 2821, 2664, 2593, 1701, 1648, 1608, 1602, 1545, 1505, 1494, 1456, 1355, 1302, 1276, 1257, 1220, 1177, 1134, 1047, 967, 919, 851, 790, 740, 732, 688, 637, 614, 565, 522 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +11.90 (c 0.75, DMSO).

5.9.17. {5-[(2-Chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-2-methylphenyl}acetic acid (6c)

Yield 36% in two steps; Ivory powder; TLC R_f = 0.47 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.57–7.52 (m, 1H), 7.49–7.42 (m, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 2.4 Hz, 1H), 6.98–6.81 (m, 3H), 6.76–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.9, 5.1 Hz, 1H), 4.17 (dd, J = 9.9, 6.3 Hz, 1H), 3.69 (s, 2H), 3.38 (dd, J = 11.4, 2.7 Hz, 1H), 3.25 (dd, J = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.31 (s, 3H); MS (APCI, Neg, 20 V) m/z 479 $(M-H)^-$; IR (KBr) 3238, 3045, 2942, 2865, 2639, 1699, 1652, 1604, 1561, 1525, 1505, 1474, 1452, 1411, 1320, 1294, 1275, 1259, 1217, 1173, 1161, 1134, 1102, 1064, 1050, 997, 968, 912, 859, 824, 819, 790, 749, 735, 694, 632, 568, 546 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +13.67 (c 0.75, DMSO).

5.9.18. {4-Chloro-3-[(2-chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (6d)

Yield 44% in two steps; Ivory powder; TLC R_f = 0.41 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (br s, 1H), 8.56 (br s, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.07–7.00 (m, 2H), 6.96 (dd, J = 9.0, 2.4 Hz, 1H), 6.93–6.80 (m, 2H), 6.76–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.28 (dd, J = 9.9, 5.4 Hz, 1H), 4.18 (dd, J = 9.9, 6.3 Hz, 1H), 3.71 (s, 2H), 3.39 (dd, J = 11.7, 2.7 Hz, 1H), 3.25 (dd, J = 11.7, 6.3 Hz, 1H), 2.91 (s, 3H); MS (APCI, Neg, 20 V) m/z 499 $(M-H)^-$; IR (KBr) 3370, 3228, 3042, 2943, 2888, 2821, 2657, 2558, 1931, 1891, 1711, 1664, 1600, 1586, 1540, 1497, 1467, 1425, 1412, 1347, 1291, 1242, 1223, 1199, 1172, 1150, 1134, 1085, 1054, 984, 939, 915, 856, 825, 815, 775, 752, 728, 709, 693, 659, 606, 600, 577, 558, 475 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +13.25 (c 0.75, DMSO).

5.9.19. {3-[(2-Chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-4-fluorophenyl}acetic acid (6e)

Yield 10% in two steps; White powder; TLC R_f = 0.43 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51–8.42 (m, 1H), 7.88 (d, J = 9.6 Hz, 1H), 7.15–6.80 (m, 7H), 6.76–6.66 (m, 2H), 4.71–4.61 (m, 1H), 4.26 (dd, J = 9.6, 5.4 Hz, 1H), 4.18 (dd, J = 9.6, 6.3 Hz, 1H), 3.70 (s, 2H), 3.40 (dd, J = 12.0, 3.3 Hz, 1H), 3.25 (dd, J = 12.0, 6.6 Hz, 1H), 2.91 (s, 3H); MS (APCI, Neg, 20 V) m/z 483 $(M-H)^-$; IR (KBr) 3397, 3244, 3039, 2942, 2888, 2817, 2644, 2552, 2379, 2345, 2279, 2251, 2219, 2174, 2041, 1994, 1922, 1870, 1847, 1803, 1793, 1773, 1717, 1668, 1649, 1638, 1615, 1599, 1548, 1498, 1483, 1468, 1436, 1406, 1375, 1348, 1317, 1290, 1258, 1241, 1224, 1187, 1138, 1098, 1084, 1052, 1010, 969, 938, 916, 908, 897, 876, 859, 845, 824, 815, 792, 776, 752, 707, 681, 647, 616, 602, 589, 579, 542, 532, 515, 503, 486, 474 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +14.73 (c 0.75, DMSO).

5.9.20. {5-[(2-Chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-2-fluorophenyl}acetic acid (6f)

Yield 50% in two steps; Pale gray powder; TLC R_f = 0.41 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.67–7.60 (m, 1H), 7.56–7.47 (m, 1H), 7.08 (t, J = 9.0 Hz, 1H), 7.02 (d, J = 2.1 Hz, 1H), 6.99–6.81 (m, 3H), 6.76–6.67 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.9, 5.4 Hz, 1H), 4.17 (dd, J = 9.9, 6.0 Hz, 1H), 3.74 (s, 2H), 3.38 (dd, J = 11.7, 2.7 Hz, 1H), 3.25 (dd, J = 11.7, 6.3 Hz, 1H), 2.91 (s, 3H); MS (APCI, Neg, 20 V) m/z 483 $(M-H)^-$; IR (KBr) 3266, 3068, 2930, 2826, 2653, 2545, 2319, 1701, 1655, 1625, 1605, 1561, 1530, 1502, 1420, 1321, 1300, 1272, 1255, 1212, 1107, 1064, 1041, 969, 943, 920, 860, 838, 825, 790, 768, 742, 620, 574, 534, 489 cm^{-1}; Optical rotation $[\alpha]^{21}{}_D$ +11.99 (c 0.75, DMSO).

5.9.21. {5-[(2-Chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-2-methoxyphenyl}acetic acid (6g)

Yield 44% in two steps; White powder; TLC R_f = 0.41 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.57 (dd, J = 8.4, 2.7 Hz, 1H), 7.48 (d, J = 2.7 Hz, 1H), 7.01 (d, J = 2.7 Hz, 1H), 6.97–6.81 (m, 4H), 6.75–6.66 (m, 2H), 4.70–4.60 (m, 1H), 4.26 (dd, J = 9.9, 5.4 Hz, 1H), 4.16 (dd, J = 9.9, 6.3 Hz, 1H), 3.86 (s, 3H), 3.70 (s, 2H), 3.38 (dd, J = 11.7, 3.0 Hz, 1H), 3.25 (dd, J = 11.7, 6.6 Hz, 1H), 2.91 (s, 3H); MS (APCI, Neg, 20 V) m/z 495 $(M-H)^-$; IR (KBr) 3404, 3247, 3132, 3068, 2931, 2838, 2644, 2558, 1701, 1651, 1604, 1560, 1529, 1504, 1459, 1418, 1279, 1221, 1136, 1119, 1040, 968, 917, 863, 810, 740, 572 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +8.66 (c 0.75, DMSO).

5.10. Methyl [3-({[4-(acetyloxy)phenyl]sulfonyl}amino)phenyl]acetate (20)

To a stirred solution of **12e** (300 mg, 1.8 mmol) and pyridine (0.29 mL, 3.6 mmol) in CH_2Cl_2 (5.0 mL) was added dropwise a solution of **22** (426 mg, 1.8 mmol) in CH_2Cl_2 (3.0 mL) dropwise. After stirring for 1 h at room temperature, the reaction mixture was diluted with 2 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to yield **20**, which was used for the next reaction without further purification; TLC R_f = 0.11 (n-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82–7.74 (m, 2H), 7.24–7.15 (m, 3H), 7.08–6.94 (m, 3H), 6.56 (s, 1H), 3.68 (s, 3H), 3.56 (s, 2H), 2.30 (s, 3H).

5.11. Methyl (3-{[(4-hydroxyphenyl)sulfonyl]amino}phenyl)acetate (21)

To a stirred solution of **20** (1.8 mmol) in MeOH (10 mL) and DME (5 mL) was added K_2CO_3 (354 mg, 2.6 mmol). After stirring for 30 min at room temperature, the resulting insoluble substance was removed by filtration. The filtrate was concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **21** (370 mg, 68% in 2 steps); TLC R_f = 0.22 (n-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68–7.58 (m, 2H), 7.19 (t, J = 8.1 Hz, 1H), 7.06–6.92 (m, 3H), 6.86–6.74 (m, 2H), 6.70–6.40 (br, 1H), 3.68 (s, 3H), 3.56 (s, 2H).

5.12. (3-{[(4-{[(2*S*)-4-Methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}phenyl)sulfonyl]amino}phenyl)acetic acid (2b)

To a stirred solution of **21** (370 mg, 1.1 mmol) in DMF (15 mL) were added Cs_2CO_3 (750 mg, 2.3 mmol) and **23** (384 mg, 1.1 mmol). After stirring for 2 h at 60 °C, the reaction mixture was poured into water and extracted with EtOAc (×2). The com-

bined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield methyl ester (282 mg, 51%); TLC R_f = 0.46 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 9.3 Hz, 2H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.06–6.78 (m, 7H), 6.74–6.65 (m, 2H), 6.36 (s, 1H), 4.67–4.57 (m, 1H), 4.23 (dd, *J* = 9.6, 5.4 Hz, 1H), 4.13 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.67 (s, 3H), 3.55 (s, 2H), 3.36 (dd, *J* = 11.7, 2.7 Hz, 1H), 3.22 (dd, *J* = 11.7, 6.6 Hz, 1H), 2.89 (s, 3H).

To a stirred solution of methyl ester described above (282 mg, 0.56 mmol) in MeOH (3 mL) and THF (3 mL) was added 5 M NaOH aq (1.5 mL). After stirring for 1 h at room temperature, the reaction mixture was poured into 1 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield **2b** (90 mg, 84%) as a pale blue amorphous powder; TLC R_f = 0.33 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.3 Hz, 2H), 7.24–7.17 (m, 1H), 7.13–7.06 (m, 1H), 7.04–6.97 (m, 1H), 6.94–6.70 (m, 8H), 4.67–4.57 (m, 1H), 4.27 (dd, *J* = 10.2, 5.1 Hz, 1H), 4.14 (dd, *J* = 10.2, 5.7 Hz, 1H), 3.53 (s, 2H), 3.37 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.17 (dd, *J* = 11.4, 7.2 Hz, 1H), 2.88 (s, 3H); MS (APCI, Neg, 20 V) *m*/*z* 467 $(M-H)^-$; IR (KBr) 3254, 2944, 1711, 1594, 1501, 1460, 1414, 1334, 1303, 1248, 1223, 1153, 1093, 1041, 981, 913, 834, 746, 703, 646, 584, 554 cm^{-1}; Optical rotation $[\alpha]_D^{25}$ +9.27 (*c* 0.75, DMSO).

5.13. (2*S*)-4-Methyl-2-[(4-nitrophenoxy)methyl]-3,4-dihydro-2*H*-1,4-benzoxazin (25)

To a stirred solution of 4-nitrophenol **24** (1 g, 7.2 mmol) in DMF (20 mL) were added Cs_2CO_3 (4.73 g, 14.4 mmol) and **23** (2.4 g, 7.2 mmol). After stirring overnight at 60 °C, the reaction mixture was diluted with water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to yield **25**, which was used for the next reaction without further purification; TLC R_f = 0.52 (*n*-hexane/EtOAc, 7:3).

5.14. 4-{(2*S*)-4-Methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}aniline (26)

To a stirred solution of **25** (7.2 mmol) in EtOAc (15 mL) and MeOH (15 mL) was added 10% Pd–C (500 mg) at room temperature under argon atmosphere. The resulting suspension was vigorously stirred for 3.5 h at room temperature under hydrogen atmosphere. Insoluble substance was removed by filtration. The filtrate was concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **26** (1.2 g, 62% in 2 steps); TLC R_f = 0.12 (toluene/EtOAc, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90–6.75 (m, 4H), 6.72–6.61 (m, 4H), 4.64–4.54 (m, 1H), 4.16 (dd, *J* = 9.6, 5.1 Hz, 1H), 4.02 (dd, *J* = 9.6, 6.9 Hz, 1H), 3.60–3.30 (br, 2H), 3.38 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.22 (dd, *J* = 11.4, 6.6 Hz, 1H), 2.89 (s, 3H).

5.15. Methyl [3-(chlorocarbonyl)phenyl]acetate (28)

To a stirred solution of 3-(2-methoxy-2-oxoethyl)benzoic acid **27** (300 mg, 1.5 mmol) in DME (10 mL) were added oxalyl chloride (0.34 mL, 3.9 mmol) and DMF (1.2 L, 0.015 mmol). After stirring for 30 min at 40 °C, the reaction mixture was concentrated in vacuo to give a crude product, which was used for the next reaction without further purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10–8.00 (m, 2H), 7.62 (d, *J* = 7.5 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 3.73 (s, 5H).

5.16. (3-{[(4-{[(2S)-4-Methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}phenyl)amino]carbonyl}phenyl)acetic acid (2c)

To a stirred solution of **26** (365 mg, 1.4 mmol) in CH_2Cl_2 (7 mL) were added a solution of the above-described crude product **28** (1.5 mmol) in CH_2Cl_2 (3 mL) and then pyridine (0.24 mL, 3.0 mmol). After stirring for 2 h at room temperature, the reaction mixture was diluted with water and extracted with EtOAc (×2). The combined organic layers were washed with 1 M NaOH aq, water, brine, dried over Na_2SO_4 and concentrated in vacuo to give a crude product, which was used for the next reaction without further purification; TLC R_f = 0.49 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.72 (m, 3H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.50–7.44 (m, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 6.91–6.81 (m, 2H), 6.75–6.65 (m, 2H), 4.70–4.60 (m, 1H), 4.24 (dd, *J* = 9.6, 5.1 Hz, 1H), 4.14 (dd, *J* = 9.6, 6.6 Hz, 1H), 3.72 (s, 2H), 3.40 (dd, *J* = 11.4, 2.7 Hz, 1H), 3.25 (dd, *J* = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.05 (s, 3H).

To a stirred solution of the above-described crude product (1.4 mmol) in MeOH (5 mL) and THF (5 mL) was added 5 M NaOH aq (3 mL). After stirring for 3 h at room temperature, the reaction mixture was diluted with 2 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to give a crude product, which was washed with EtOAc/*n*-hexane to yield **2c** (330 mg, 51% in 2 steps) as a pale blue amorphous powder; TLC R_f = 0.34 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86–7.70 (m, 3H), 7.58–7.48 (m, 2H), 7.48–7.40 (m, 2H), 6.98–6.88 (m, 2H), 6.88–6.80 (m, 2H), 6.75–6.64 (m, 2H), 4.68–4.58 (m, 1H), 4.23 (dd, *J* = 9.6, 4.5 Hz, 1H), 4.11 (dd, *J* = 9.6, 6.6 Hz, 1H), 3.72 (s, 2H), 3.39 (dd, *J* = 11.7, 2.7 Hz, 1H), 3.25 (dd, *J* = 11.7, 6.9 Hz, 1H), 2.90 (s, 3H); MS (APCI, Neg, 20 V) *m*/*z* 431 $(M-H)^-$; IR (KBr) 3277, 3042, 2923, 1699, 1648, 1608, 1584, 1510, 1456, 1411, 1301, 1221, 1176, 1136, 1042, 990, 957, 915, 823, 740, 711, 607, 520, 459 cm^{-1}; Optical rotation $[\alpha]_D^{25}$ +6.23 (*c* 0.75, DMSO).

6. Biological assay method

6.1. Prostanoid mEP1–4, mDP, hTP, mFP and hIP receptor binding assay

Competitive binding studies were conducted using radiolabeled ligands and membrane fractions prepared from Chinese hamster ovary (CHO) cells stably expressing the respective prostanoid receptors, mEP1, mEP2, mEP3α, mEP4, mDP, hTP mFP, and hIP.

Membranes from CHO cells expressing prostanoid receptors were incubated with radioligands (2.5 nM of [^{3}H]PGE_2 for mEP1–4; 2.5 nM of [^{3}H]PGD_2 for mDP; 5.0 nM [^{3}H]-SQ29548 for hTP;2.5 nM [^{3}H]PGF2α for mFP; 5.0 nM of [^{3}H]Iloprost for hIP) and the test compounds at various concentrations in assay buffer (10 mM KH_2PO_4–KOH buffer containing 1 mM EDTA, 10 mM $MgCl_2$, and 100 mM NaCl, pH 6.0 for mEP1–4 and mFP; 25 mM HEPES–NaOH buffer containing 1 mM EDTA, 5 mM $MgCl_2$ and 10 mM $MnCl_2$, pH 7.4 for mDP; 10 mM Tris–HCl buffer containing 100 mM NaCl, pH 7.5, for hTP; 50 mM Tris–HCl buffer containing 1 mM EDTA and 10 mM $MgCl_2$, pH 7.5, for hIP). Incubation was carried out at room temperature for 60 min except for mEP1, and mDP (20 min), and hTP and hIP (30 min). The incubation was terminated by filtration through Whatman GF/B filters. The filters were washed with ice-cold buffer (10 mM KH_2PO_4–KOH buffer containing 100 mM NaCl, pH 6.0 for mEP1–4 and mFP; 10 mM Tris–HCl buffer containing 100 mM NaCl and 0.01w/v% BSA, pH 7.4 for mDP; 10 mM Tris–HCl buffer containing 100 mM NaCl, pH 7.5, for hTP and hIP), dried for 60 min at 60 °C and the radioactivity on the filter was measured in 6 mL of liquid scintillation (ACSII) mixture with a liquid scintillation counter. Nonspecific binding

was achieved by adding excess amounts of unlabeled PGE_2 (for mEP1–4), unlabeled PGD_2 (for mDP), unlabeled SQ29548 (for hTP), unlabeled PGF2α (for mFP) or unlabeled Iloprost (for IP) with assay buffer. The concentrations of the test substance required to inhibit the amounts of the specific binding in the vehicle group by 50% (IC_{50} value) were estimated from the regression curve. The K_i value (M) was calculated according to the following equation.

$$K_i = IC_{50}/(1 + [L]/K_d)$$

where [L]: concentration of radioligand and K_d: dissociation constant of radiolabeled ligand towards the prostanoid receptors.

6.2. Measurement of the mDP receptor antagonist activity

To confirm that test compounds antagonize the mDP receptor and to estimate potencies of antagonism for the mDP receptor, a functional assay was performed by measuring PGD2-stimulated changes in intracellular second messenger cAMP as an indicator of receptor function.

For the assessment of the antagonist activity of test compounds, a suspension of CHO cells expressing mDP receptor was seeded at a cell density of 1×10^5 cells per well and cultivated for 2 days. The cells in each well were rinsed with minimum essential medium (MEM), and MEM containing 2 μM of Diclofenac was added to each well. The cells were incubated for approximately 10 min at 37 °C and the culture medium was removed. The assay medium (MEM containing 0.1% BSA, 1 mM IBMX and 2 μM Diclofenac) was added to each well and the cells were incubated for approximately 10 min at 37 °C. The assay medium, assay medium containing 10 nM of PGD_2, or assay medium containing various concentrations of test compounds and 10 nM of PGD_2 was added to each well and the cells were further incubated for 10 min at 37 °C. The reaction was terminated by the addition of ice-cold trichloroacetic acid (TCA; 10 w/v%).

After centrifugation of the reaction mixture, TCA was extracted by adding a mixture of tri-*n*-octylamine and chloroform (5:18 v/v) to the resultant supernatant, mixing and re-centrifugation. The cAMP level in the resultant aqueous layer (upper layer) was determined by enzymeimmunoassay using a cAMP assay kit (GE Healthcare UK Ltd). The relative responsiveness (%) of cAMP production was calculated relative to the maximum increase in cAMP that occurred in the absence of test compound (100%) to estimate of the IC_{50} values.

7. Single dose rat pharmacokinetic study of 2a, 5b and 1

Single dose pharmacokinetics of **2a**, **5b** and **1** were studied in rats. Formulation for intravenous injection was prepared using 30% HP-β-CD containing 5% DMSO (1 mg/ml/kg). Formulation for oral dosing was prepared using 0.5% MC (10 mg/5 ml/kg). Test compounds (1 mg/kg) were dosed intravenously to the fasted male rats ($n = 3$). Test compounds (10 mg/kg) were dosed orally to the fasted male rats ($n = 3$). After dosing, blood samples (250 μl) were collected from the jugular vein using a heparinized syringe at the selected time points (iv: pre-dosing, 2, 5, 15 and 30 min, 1, 2, 4, 6 and 8 h; po: pre-dosing, 5, 15 and 30 min 1, 2, 4, 6 and 8 h, respectively). The blood samples were ice-chilled and then centrifuged at 12,000 rpm for 2 min at 4 °C to obtain plasma, which was preserved at −80 °C in a freezer. The AUC, C_{max}, T_{max}, $T_{1/2}$, V_{ss} and CL were obtained by measuring the time course of the plasma concentration of the test compounds. Bioavailability (BA) was calculated according to the following equation:

$$BA(\%) = (AUC_{po}/D_{po})/AUC_{iv}/D_{iv}) \times 100$$

where AUC_{po}: AUC after oral dosing; AUC_{iv}: AUC after intravenous dosing; D_{po}: dosage of oral administration; D_{iv}: dosage of intravenous administration.

References and notes

1. (a) Coleman, R. A. Prostanoids Receptors. In *The IUPHAR Compendium of Receptor Characterization and Classification*; Girdlestne, D., Ed.; Burlington Press: Cambridge, **1998**; pp 229–244; (b) Coleman, R. A.; Smith, W. L.; Narumiya, S. *Pharmacol. Rev.* **1994**, *46*, 205.
2. (a) Lewis, R. A.; Soter, N. A.; Diamond, P. T.; Austen, K. F.; Oates, J. A.; Roverts, L. J., II *J. Immunol.* **1982**, *129*, 1627; (b) Matsuoka, T.; Hirata, M.; Tanaka, H.; Takahashi, Y.; Murata, T.; Kabashima, K.; Sugimoto, Y.; Kobayashi, T.; Ushikubi, F.; Aze, Y.; Yoshida, N.; Honda, Y.; Nagai, H.; Narumiya, S. *Science* **2000**, *287*, 2013.
3. (a) Doyle, W. J.; Boehm, S.; Skoner, D. P. *J. Allergy Clin. Immunol.* **1990**, *86*, 924; (b) Johnston, S. L.; Smith, S.; Harrison, J.; Ritter, W.; Hawarth, P. H. *J. Allergy Clin. Immunol.* **1993**, *91*, 903; (c) Alving, K.; Matran, R.; Lundberg, J. M. *Acta Physiol. Scand.* **1991**, *143*, 93.
4. (a) Woodward, D. F.; Hawley, S. B.; Williams, L. S.; Ralston, T. R.; Protzman, C. E.; Spada, C. S.; Nieves, A. L. *Invest. Ophthalmol. Vis. Sci.* **1990**, *31*, 138; (b) Woodward, D. F.; Nieves, A. L.; Friedlaender, J. *Pharmacol. Exp. Ther.* **1996**, *279*, 137.
5. (a) Soter, N. A.; Lewis, R. A.; Corey, E. J.; Austen, K. F. *J. Invest. Dermatol.* **1983**, *80*, 115; (b) Flower, R. J.; Harvey, E. A.; Kingston, W. P. *Br. J. Pharmacol.* **1976**, *56*, 229.
6. (a) Torisu, K.; Kobayashi, K.; Iwahashi, M.; Egashira, H.; Nakai, Y.; Okada, Y.; Nanbu, F.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4557; (b) Torisu, K.; Kobayashi, K.; Iwahashi, M.; Nakai, Y.; Onoda, T.; Nagase, T.; Sugimoto, I.; Okada, Y.; Matsumoto, R.; Nanbu, F.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4891; (c) Torisu, K.; Kobayashi, K.; Iwahashi, M.; Nakai, Y.; Onoda, T.; Nagase, T.; Sugimoto, I.; Okada, Y.; Matsumoto, R.; Nanbu, F.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2004**, *12*, 4685; (d) Torisu, K.; Kobayashi, K.; Iwahashi, M.; Nakai, Y.; Onoda, T.; Nagase, T.; Sugimoto, I.; Okada, Y.; Matsumoto, R.; Nanbu, F.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2004**, *12*, 5361.
7. Iwahashi, M.; Shimabukuro, A.; Onoda, T.; Matsunaga, Y.; Okada, Y.; Matsumoto, R.; Nambu, F.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2011**, *19*, 4574.
8. Iwahashi, M.; Kobayashi, K.; Nambu, F. WO03078409, **2003**.
9. (a) Kagechika, H.; Himi, T.; Kawachi, E.; Shudo, K. *J. Med. Chem.* **1989**, *32*, 2292; (b) Itai, A.; Toriumi, Y.; Tomioka, N.; Kagechika, H.; Azumaya, I.; Shudo, K. *Tetrahedron Lett.* **1989**, *30*, 6177; (c) Toriumi, Y.; Kasuya, A.; Itai, A. *J. Org. Chem.* **1990**, *55*, 259; (d) Yamaguchi, K.; Matsumura, G.; Kagechika, H.; Azumaya, I.; Ito, Y.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1991**, *113*, 5474; (e) Itai, A.; Toriumi, Y.; Saito, S.; Kagechika, H.; Shudo, K. *J. Am. Chem. Soc.* **1992**, *114*, 10649.