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Discovery of new orally active prostaglandin D_2 receptor antagonists

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ABSTRACT

To identify an orally available drug candidate, a series of 3-benzoylaminophenylacetic acids were synthesized and evaluated as prostaglandin D_2 (PGD_2) receptor antagonists. Some of the compounds tested were found to exhibit excellent inhibitory activity against cAMP accumulation in human platelet rich plasma (hPRP), which is one of the indexes of DP antagonism. The optimization process including improvement of the physicochemical properties such as solubility, which may result in an improved pharmacokinetic (PK) profile, is presented. Optimized compounds were studied for their pharmacokinetics and in vivo potential. A structure–activity relationship study is also presented. Some of the test compounds were found to have in vivo efficacy towards the inhibition of PGD_2-induced and OVA-induced vascular permeability in guinea pig conjunctiva.

1. Introduction

Prostanoids are oxidative metabolites of arachidonic acid and consist of prostaglandins (PGs) and thromboxane (TX). In vivo, they act on the tissues where they were synthesized via specific G-protein coupled receptors named TP, IP, EP, FP and DP. Each of these receptors has been cloned, expressed and characterized.

Characterization of these receptors at the molecular level has resulted in renewed interest in this field, but few selective agonists and antagonists of human prostanoid receptors are available.[1] As a result, the influence of each receptor on various pathologies is still being established by using potent but poorly selective ligands. The situation is similar with regard to DP receptor antagonists. Only a few antagonists such as S-555739 are undergoing clinical trials for the treatment of allergic rhinitis. Recently laropiprant (MK-0524)[2] has proven to be effective in suppressing both subjective and objective manifestations of niacin-induced vasodilatation.

In our previous papers,[3] we reported on the discovery of a new orally active indole-based structure **1b** as a new class of DP receptor antagonists starting from Indomethacin (Fig. 1). We also reported on the discovery of another indole-based antagonist[4a] and 3-benzoylaminophenylacetic acids **2a–b**[4b] as cost-effective DP receptor antagonists. Here we report on the discovery of 3-benzoylaminophenylacetic acids **5e** and **10c** possessing more improved PK profiles.

2. Materials and methods

Test compounds listed in Tables 1–3 were synthesized as outlined in Schemes 1 and 2. Synthesis of methyl α,α-disubstituted phenylacetates, which can be used as the intermediates for the synthesis of **10a–c** and **11a–b**, was carried out as described in Scheme 1a. Conventional nitration of α,α-dimethyl(4-chlorophenyl)acetonitrile **13** and α,α-dimethylene(4-chlorophenyl)acetonitrile **14** with nitric acid-sulfuric acid afforded **15** and **18**, respectively. Acidic hydrolysis of the nitriles **15** and **18** provided carboxylic acids **16** and **19**, respectively. Esterification gave the corresponding methyl esters **17** and **20**, respectively. Catalytic hydrogenation of their nitro groups afforded the corresponding anilines **21** and **22**, respectively.

Synthesis of ethyl α,α-difluoro(3-amino-4-chlorophenyl)acetate **29** is described in Scheme 1b. Treatment of 4-chloro-3-nitrobenzaldehyde **23** with trimethylsilylcyanide in the presence of a catalytic amount of zinc iodide afforded **24**, acidic hydrolysis of which followed by conventional esterification provided **26**. Oxidation of the α-hydroxy ester **26** with sodium hypochlorite afforded α-ketoester **27**, treatment of which with DAST gave ethyl α,α-difluorophenylacetate **28**. Reduction of **28** with iron metal resulted in ethyl α,α-difluoro(3-amino-4-chlorophenyl)acetate **29**. Synthesis of **2d–f**, **3c–e**, **5c–e**, **10a–c**, **11a–b** and **12** is outlined in Scheme 2. O-Alkylation of methyl 4-hydroxybenzoates **30** with the tosylate **31**, preparation of which was reported previously,[4a] provided **32**. Alkaline hydrolysis of **32** followed by acid chloride formation afforded **34a**. Compounds **34b–d** were previously reported.[4a] N-Acylation of methyl 3-aminophenylacetates **35a–c**, **21**, **22** and **29** with

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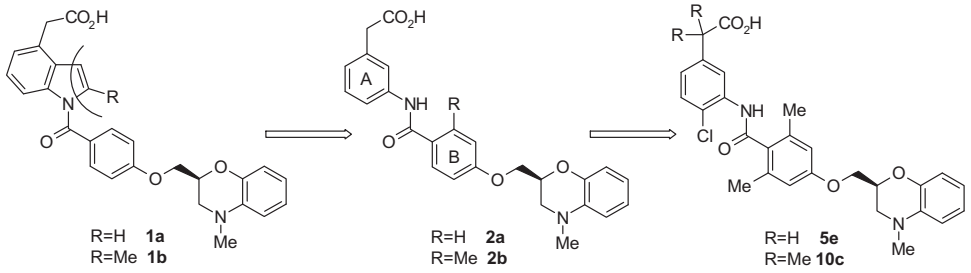
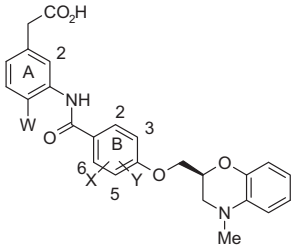


Figure 1. Molecular design of 3-benzoylaminophenylacetic acids **5e** and **10c**.

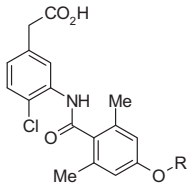
Table 1
Optimization of A and B rings

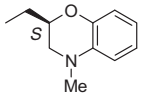
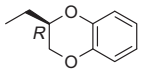
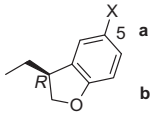
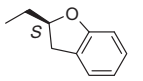
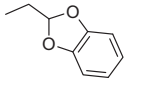


Compd	W	X,Y	Binding K_i (nM) mDP	IC_{50}[a] (nM) mDP	Binding K_i (nM) hIP
1a			1700	NT[b]	2500
1b			16	2.4	37
2a	H	H	310	220	6400
2b	H	2-Me	18	3.3	3600
2c	H	2-Cl	16	20	2700
2d	H	2,3-Me	NT[b]	22	>10,000
2e	H	2,5-Me	NT[b]	28	>10,000
2f	H	2,6-Me	NT[b]	3.5	>10,000
3a	Me	2-Me	31	4.6	2600
3b	Me	2-Cl	26	6.0	1400
3c	Me	2,3-Me	NT[b]	12	5200
3d	Me	2,5-Me	NT[b]	5.7	2600
3e	Me	2,6-Me	NT[b]	4.8	>10,000
4a	F	2-Me	19	1.8	>10,000
4b	F	2-Cl	17	7.4	>10,000
5a	Cl	2-Me	7.7	NT[b]	1800
5b	Cl	2-Cl	13	4.3	1600
5c	Cl	2,3-Me	28	54	3500
5d	Cl	2,5-Me	NT[b]	5.1	1800
5e	Cl	2,6-Me	6.4	7.6	>10,000

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

Table 2
Effects of chemical modifications of the *N*-methyl benzomorpholine moiety on activity profiles



Compd	R		IC_{50}[a] (nM) mDP	Binding K_i (nM) hIP
5e			7.6	>10,000
6			4.3	>10,000
7		**a** X=H	1.5	>10,000
		b X=Me	24	>10,000
8			14	>10,000
9			27	>10,000

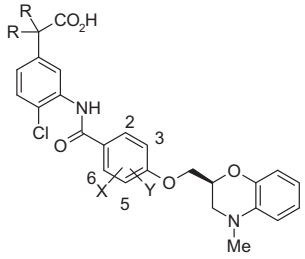
[a] IC_{50} (nM): mDP receptor antagonist activity.

an optional acid chloride among **34a–d** followed by alkaline hydrolysis resulted in **2d–f**, **3c–e**, **5c–e**, **10a–c**, **11a–b** and **12**. Synthesis of building blocks for the chemical modification of the *N*-methyl benzomorpholine moiety of **5e** was carried out as shown in Scheme 3a. O-Alkylation of 2-bromo-4-methylphenol with (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate afforded **37**, which was then treated with *n*-butyllithium followed by sulfonylation with 4-nitrobenzenesulfonyl chloride afforded **39a**. According to the same procedure as described for the preparation of **39a** from **38a**, compounds **39b–e** were prepared from **38b–e**.[4a] Synthesis of **6**, **7a–b**, **8** and **9** is described in Scheme 3b. O-Acetylation of 2,6-dimethyl-4-hydroxybenzoic acid **40** by the conventional procedure followed by the acid chloride formation with oxalyl chloride in the presence of a catalytic amount of dimethyl formamide afforded **42**. N-Acylation of methyl (3-amino-4-chlorophenyl)acetate **35c** with the acid chloride **42** afforded **43**, methanolysis of which with potassium carbonate in methanol afforded **44**. *O*-Alkylation of **44** with the above-described nosylates **39a–e** afforded **6**, **7a–b**, **8** and **9**, respectively. Synthesis of indol-4-yl acetic acid analog **1a** from **45** is outlined in Scheme 4. 2-(1*H*-Indol-4-yl)acetic acid **45** was converted to benzyl ester **46** by the conventional acidic esterification condition. *N*-Acylation of **46** with acid chloride **47**[4a] using the reported reaction conditions[4a] followed by deprotection by the catalytic hydrogenolysis afforded **1a**.

3. Results and discussion

The 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 the 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% of BSA for the discovery of potent antagonists in the protein-rich in vivo animal models. Because of their close homology to human receptors, all

Table 3
Effects of α,α-substitution of the phenylacetic acid moiety on activity profiles



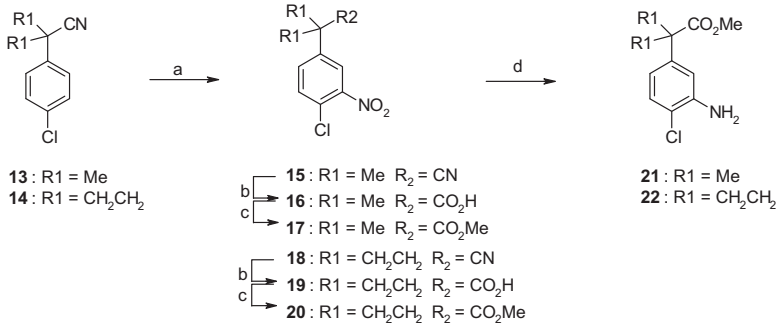
Compd	R	X,Y	IC_{50} (nM)		Binding K_i (nM) hIP
			mDP[a]	hDP[b]	
1b			2.4	57	37
5b	H	2-Cl	4.3	>50	1600
2e	H	2,5-Me	28	NT[c]	>10,000
5e	H	2,6-Me	7.6	4.4	>10,000
10a	Me	2-Cl	7.0	280	3300
10b	Me	2,5-Me	43	32	3300
10c	Me	2,6-Me	0.7	8.1	>10,000
11a	$-CH_2-CH_2-$	2,5-Me	26	19	1700
11b	$-CH_2-CH_2-$	2,6-Me	1.0	6.5	>10,000
12	F	2-Cl	14	NT[c]	520

[a] mDP: mDP receptor antagonist activity.
[b] hDP: hDP PRP assay, inhibition of the accumulation of *c*AMP in human platelet rich plasma challenged with PGD_2.
[c] NT: not tested.

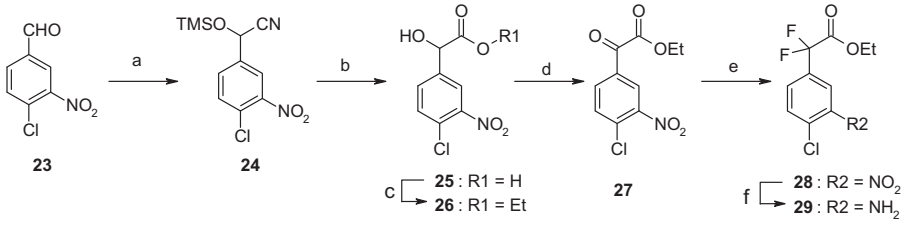
the DP receptor affinities and antagonist activities are assayed using the mouse receptor unless otherwise noted.

In our previous paper,[4b] we reported on the discovery of a new prostaglandin D_2 (PGD_2) receptor antagonist **2a**. Introduction of a substituent into the 2-position of the B ring was found to be beneficial for increased activities. Introduction of a substituent into the 2-position of the ring A of **2a** resulted in the reduction of the activities while introduction of a substituent into the positions 4, 5 and 6 resulted in the retention of the potent activities. For these reasons, our focus was placed on further optimization of the ring B of **2a**. Results are summarized in Table 1. Introduction of 2-methyl, 2-chloro, 2,3-dimethyl, 2,5-dimethyl and 2,6-dimethyl residues into the ring B of **2a** afforded **2a**–**f**, respectively. Among those tested, **2b**–**f** showed more potent binding affinities and/or antagonist activities relative to **2a**. Above all, **2b** and **2f** exhibited more potent antagonist activities than **2c**–**e**. The same chemical modification as described above in the ring B of the 3-benzoylamino-4-methylphenylacetic acid framework afforded **3a**–**e**, respectively. All of these compounds exhibited nearly equipotent antagonist activity as **2b** and **2f**. Introduction of 2-methyl and 2-chloro residues into the ring B of 3-benzoylamino-4-fluorophenylacetic acid and 3-benzoylamino-4-chlorophenyl acetic acid frameworks afforded **4a**–**b** and **5a**–**b**, respectively with tendency to retain strong antagonist activity. Introduction of another methyl residue into the ring B of **5a** afforded **5c**–**e**, respectively. Among the tested compounds, 2,3-dimethylbenzoyl analog **5c** showed nearly 10-fold less potent antagonist activity relative to **5b** and **5d**–**e**. As described above, 2-methylbenzoylaminophenylacetic acid analogs **2b**, **3a** and **4a** and 2,6-dimethylbenzoylaminophenylacetic acid analogs **2f**, **3e** and **5e** tended to show more potent antagonist activity than the others almost without exception. Especially, these 2,6-dimethylbenzoyl analogs, which showed sufficient selectivity for hIP receptor affinity, were expected to show good PK profiles due to their presumed metabolic stability after oral dosing.

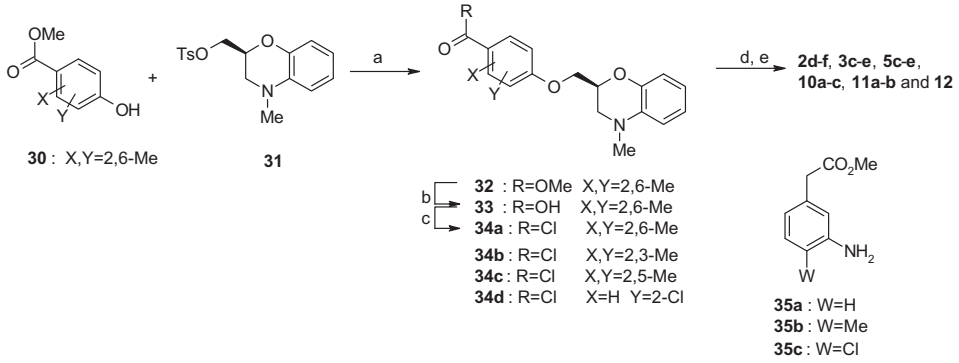
The effects of the chemical modifications of the (2*S*)-*N*-methylbenzmorpholin-2-yl moiety on activity profiles were investigated (Table 2). Replacement of the (2*S*)-*N*-methylbenzmorpholin-2-yl moiety of **5e** with a (2*R*)-2,3-dihydro-1,4-benzodioxin-2-yl, (3*R*)-dihydrobenzofuran-3-yl, (2*S*)-dihydrobenzofuran-2-yl and 1,3-benzodioxol-2-yl moieties afforded **6**–**9**, respectively. Among the tested compounds, **6** and **7a** exhibited more potent antagonist activities than **7b**, **8** and **9** with excellent selectivity for the hIP receptor. Besides, the 2*S*-configuration of **5e**, 2*R*-configuration of **6** and 3*R*-configuration of **7a** were found to be beneficial for the more potent activity relative to their corresponding enantiomers.



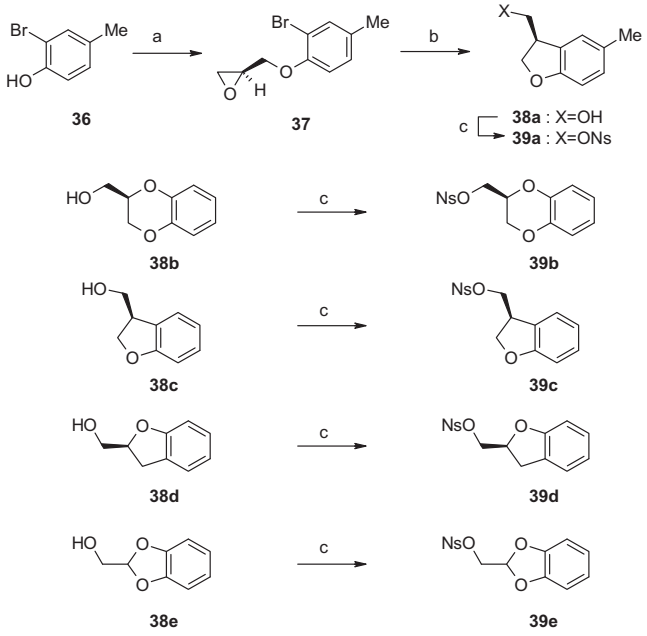
Scheme 1a. Synthesis of methyl 2, 2-disubstituted (3-amino-4-chlorophenyl)acetates. Reagents: (a) HNO_3, H_2SO_4; (b) 50% H_2SO_4; (c) $SOCl_2$, MeOH; (d) H_2, $Pd(OH)_2$–C, EtOAc.



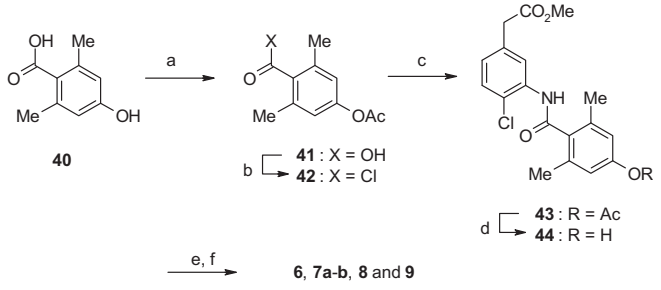
Scheme 1b. Synthesis of ethyl 2,2-difluoro(3-amino-4-chlorophenyl)acetate. Reagents: (a) TMSCN, cat ZnI_2, CH_2Cl_2; (b) concd HCl, AcOH; (c) EtOH, TsOH; (d) NaOCl, AcOH; (e) DAST, CH_2Cl_2; (f) Fe, AcOH–H_2O.



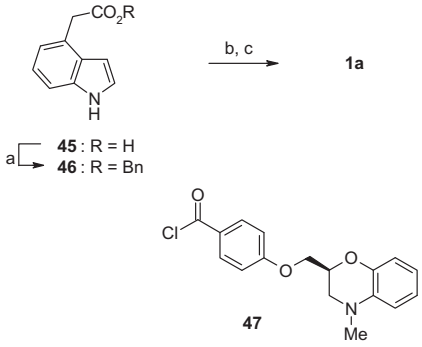
Scheme 2. Synthesis of **2d–f**, **3c–e**, **5c–e**, **10a–c**, **11a–b** and **12**. Reagents: (a) Cs_2CO_3, DMF; (b) NaOH, 2-ethoxyethanol, dioxane; (c) $(COCl)_2$, DMF, DME; (d) **21**, **22**, **29**, and **35a–c**, Py, CH_2Cl_2; (e) NaOH, MeOH, THF.



Scheme 3a. Synthesis of **39a–e**. Reagents: (a) (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate, Cs_2CO_3, DMF; (b) *n*-BuLi, THF; (c) 4-nitrobenzenesulfonyl chloride, Et_3N, DMAP, THF.



Scheme 3b. Synthesis of **6**, **7a–b**, **8** and **9**. Reagents: (a) Ac_2O, Py; (b) $(COCl)_2$, DMF, toluene; (c) **35c**, Py, CH_2Cl_2; (d) K_2CO_3, MeOH; (e) **39a–e**, Cs_2CO_3, DMF; (f) NaOH, MeOH, THF.



Scheme 4. Synthesis of **1a**. Reagents: (a) BnOH, TsCl, pyridine, toluene; (b) **47**, NaOH, TBACl, CH_2Cl_2; (c) H_2, $Pd(OH)_2$–C, EtOAc.

Table 4
Effects of the substituents on the B ring on solubility

Compd	Solubility[a] (μg/mL)
5a	129
5d	17
5e	407
10a	139
10c	490
11b	321
1b	8.2

[a] Solubility: kinetic solubility.

Table 5
Inhibitory effects of **5e** and **10c** on PGD_2-induced vascular permeability in guinea pig conjunctiva ($n = 8$)

Compd	Dose (mg/kg, po)	% Inhibition
1b	0.1	67*
5e	0.1	55*
10c	0.1	58*

**0.01 versus the control as measured with Dunnetts test.
Inhibition of increase in conjunctival vascular permeability caused by topical application of PGD_2 (0.01%, 20 μL/eye) in guinea pigs. All antagonists were administered po 1 h before the challenge.

To avoid the predicted conjugation metabolism of the carboxylic acid function, effect of the α,α-disubstitution of the phenylacetic acid moiety was investigated (Table 3). To look for α,α-disubstituted compounds with an acceptable potency as a DP antagonist, **10a–c**, **11a–b** and **12** were synthesized and evaluated as shown in Table 3.

Introduction of a α,α-dimethyl residue into the phenylacetic acid moiety of **5b**, **2e** and **5e** afforded **10a–c**, respectively. Compounds **10a** and **10c** showed more potent antagonist activity than

Table 6
Inhibitory effects of **5e** on OVA-induced vascular permeability in guinea pig conjunctiva ($n = 7$)

Compd	Dose (mg/kg, po)	% Inhibition
1b	1	23
	3	41*
5e	1	46*
Pyrilamine	1 (mg/kg, iv)	68

Inhibition of increase in conjunctival vascular permeability caused by topical application of OVA (1%, 20 μL/eye) in actively sensitized guinea pigs. All antagonists were administered po 1 h before the antigen challenge.
* 0.1 versus the control as measured by Dunnetts test.

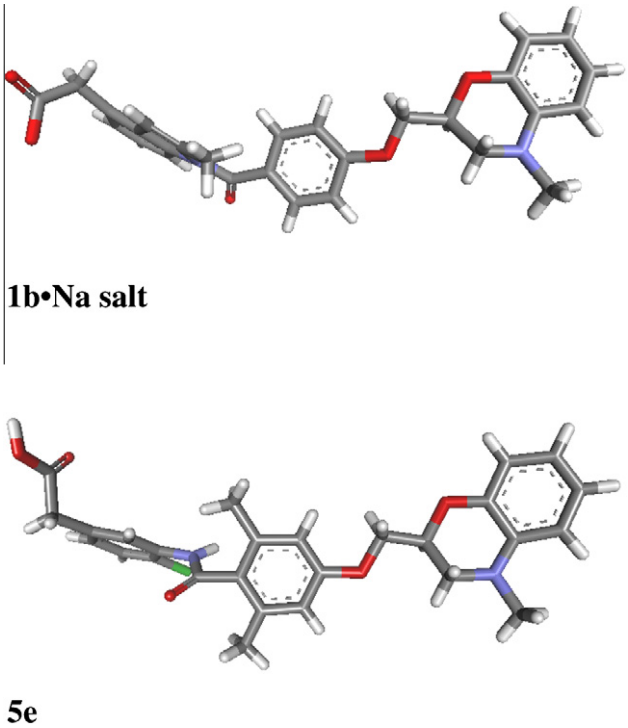


Figure 2. Conformational analysis of **1b** and **5e** using X-ray crystallography.

10b. Especially, **10c** showed nearly 10-fold more potency than **5e**. Introduction of the α,α-dimethylene moiety into **2e** and **5e** afforded **11a**–**b**, respectively. Again, **11b** was found to be more potent than **11a**. Introduction of the α,α-difluoro moiety into **5b** afforded **12** with reduced antagonist activity. All the tested compounds showed good to excellent selectivity for the hIP receptor. Especially, **10c** and **11b** showed very potent antagonist activities in addition to their excellent selectivities for hIP receptor.

To estimate the potency of antagonism of these compounds against the hDP receptor under conditions that are close to the in vivo environment, PGD_2-stimulated cAMP production in human platelet rich plasma (hPRP) was analyzed as an indicator of receptor function. Compounds **1b**, **5b**, **5e**, **10a**–**c**, **11a**–**b** were evaluated for their inhibition of cAMP accumulation in hPRP. Among the tested, **5e**, **10c** and **11b** were found to exhibit excellent inhibitory activities while **10a** and **10b** showed less potency.

In an attempt to improve the PK profiles of the newly identified DP antagonists, enhancement of their physicochemical properties such as solubility is known to be effective. 2,6-Disubstitution and 2-substitution of the *N*-benzoyl moiety may provide effective approaches for such a purpose because of the predicted good solubility due to their non-planar conformations.

To confirm the improvement of the solubility, which is one of the important factors which helps to improve the PK profiles of an orally dosed molecule, solubility of phenylacetic acids **5a** and **5d**–**e**, α,α-dimethylphenylacetic acids **10a** and **10c**, and α,α-dimethylenephenylacetic acid **11b**, which possess 2,6-disubstituted- and 2-substituted-3-benzoylaminophenylacetic acid moieties, were evaluated[5] as shown in Table 4. Among the tested compounds, 3-(2,6-dimethylbenzoylamino)phenylacetic acid analogs **5e**, **10c** and **11b** showed remarkably improved solubility probably because of the predicted change in their conformation based on the complete loss of the planarity of the *N*-benzoylanilide moiety or the loss of the conjugation of the two rings A and B via the anilide moiety. The 2,6-disubstitution of the *N*-benzoyl moiety was proven to be much more effective than the α,α-disubstitution of the phenylacetic acid moiety as illustrated by the differences in solubility of **5e** and **10c**.

Table 7
Rat PK study of **5e** and **10c**

Compd	Route	Dose (mg/kg)	AUC_{inf} (μg h/mL)	C_{max} (μg/mL)	CL_{tot} (mL/min/kg)	$T_{1/2}$ (h)	V_{ss} (mL/kg)	BA (%)
5e	iv	1	11.3	0.93	1.6	6.2	622	
	po	1	8.12			5.8		72
10c	iv	1	9.52		1.8	6.5	669	
	po	1	8.28	1.67		5.6		87

PK study of **5e** and **10c**.

As shown in Table 5, the inhibitory effects of **5e** and **10c**, which showed good antagonist activities and relatively better solubilities among the tested compounds, on PGD_2-induced vascular permeability in guinea pig conjunctiva were investigated. Compound **1b** was included for purposes of comparison. After oral dosing (0.1 mg/kg, po), these three compounds exhibited nearly equipotent efficacy 67%, 55% and 58%, respectively.

The inhibitory effects of **1b** and **5e** on OVA-induced vascular permeability in guinea pig conjunctiva were further investigated as shown in Table 6. The antihistaminic agent pyrilamine was included as a positive control. Compound **5e** demonstrated significant efficacy after its 1 mg/kg of oral dosing while 3 mg/kg of oral dosing of **1b** was required to show equipotent efficacy.

Compounds **5e** and **10c**, which possess potent activities with excellent solubilities, were evaluated for their PK profiles. A single-dose rat PK study after oral dosing (1 mg/kg) and intravenous dosing (1 mg/kg) of each of the compounds was conducted. As shown in Table 7, both the compounds demonstrated excellent C_{max}, $T_{1/2}$, tissue distribution (V_{ss}), oral exposure (AUC) and bioavailability (BA). Especially the value of clearance (CL_{tot}) after iv dosing was found to be much smaller than those of the chemical leads **1b** and **2a**.[4b] As a result, the synthetic approach described above was found to be effective to provide novel compounds with improved PK profiles.

3.1. Conformational analysis of 1b and 5e based on the SAR described above and X-ray crystallography (Fig. 2)

As shown in Table 1, compounds **2f** exhibited nearly the same in vitro potency as **3e** and **5e** while these three compounds showed much more potent in vitro potency relative to **2a**. Thus, the 2,6-dimethyl substituent of the benzoyl moiety was found to be effective for the increased in vitro activity while 4-chloro group of the ring A of **5e** was considered to be effective for the presumed improvement of the PK profile based on the increased in vivo potency in Table 6. On the basis of the data described above, the amide carbonyl moieties of these three compounds were assumed not to be in the same plane as the phenyl ring A. The 2-methyl group of the indole nucleus of **1b** was considered to play the same role as that of the 2,6-dimethyl group of **2f**, **3e** and **5e**.

Further conformational analysis of **1b** and **5e** by X-ray crystallography was carried out. As presented in Figure 2, the benzoyl moiety of **1b** and the 2,6-dimethylbenzoyl moiety of **5e** were found not to be in the same plane as the indole nucleus of **1b** and the phenyl ring A (Table 1) of **5e**, respectively. Both the *N*-benzoyl rings of **1b** and **5e** were found to occupy a nearly orthogonal orientation to the core rings such as the indole nucleus and phenyl ring A, respectively. Based on the SAR presented in Table 1, the above-described conformation of **1b** and **5e** was strongly suggested to be beneficial for the dramatic increase of their activities relative to those of **1a** and **2a**, respectively. Experimental details were described in the 'Section 5' part.

4. Conclusion

A series of 3-benzoylaminophenyl acetic acid analogs were synthesized and evaluated for their mDP receptor affinity and/or antagonist activities. Compounds **1b**, **5b**, **5e**, **10a–c**, **11a–b** were evaluated for their inhibition of cAMP accumulation in hPRP which is one of the indexes of DP antagonism. Among the tested compounds, **5e**, **10c** and **11b** were found to exhibit excellent inhibitory activities while **10a** and **10b** were less potent. The solubilities of compounds **1b**, **5a**, **5d–e**, **10a**, **10c**, **11b** were also tested in an effort to improve their PK profiles. As a result, we succeeded in the discovery of some compounds possessing excellent activities, receptor selectivities and PK profiles. These compounds showed in vivo efficacy towards the inhibition of PGD_2-induced and OVA-induced vascular permeability in guinea pig conjunctiva.

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 (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer, Varian GEMINI-200 or VXR-200s spectrometer using deuterated chloroform ($CDCl_3$) and deuterated methanol (CD_3OD) as the solvent. Fast atom bombardment (FABMS, HRMS) and electron ionization (EI) mass spectra were obtained on a JEOL JMS-DX303HF spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined on a HITACHI MI200H spectrometer. Infrared spectra (IR) were measured in a Perkin-Elmer FT-IR 1760X spectrometer. Melting points and results of elemental analyses were uncorrected. Column chromatography was carried out on silica gel [Merck Silica Gel 60 (0.063–0.200 mm), Wako gel C-200, or Fuji Silysia FL60D]. 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; diethylaminosulfur trifluoride (DAST), trimethylsilyl cyanide (TMSCN), *t*-butyl methyl ether (MTBE), triethylamine (TEA), tetrabutylammonium chloride (TBACl), acetic acid (AcOH), diethyl ether (Et_2O), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), dimethoxyethane (DME), acetonitrile (CH_3CN), 4-(dimethylamino)pyridine (DMAP).

5.2. General procedure for the preparation of 15 and 18

5.2.1. 2-(4-Chloro-3-nitrophenyl)-2-methylpropanenitrile (15)

To conc H_2SO_4 (5.7 mL, 107 mmol) were added successively HNO_3 (5.4 mL, 840 mmol) and then **13** (3.77 g, 210 mmol) at −15 °C. After stirring for 2 h at room temperature, the reaction mixture was poured into ice-water, and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and evaporated to yield **15** (4.6 g, 97% yield) as a pale yellow solid; TLC R_f = 0.30 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (dd, J = 2.4, 0.3 Hz, 1H), 7.68 (dd, J = 8.7, 2.4 Hz, 1H), 7.60 (dd, J = 8.7, 0.3 Hz, 1H), 1.77 (s, 6H).

Compound **18** was prepared as described above.

5.2.2. 1-(4-Chloro-3-nitrophenyl)cyclopropanecarbonitrile (18)

Yield 41%; Pale yellow powder; TLC R_f = 0.43 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J = 2.4 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 8.1, 2.4 Hz, 1H), 1.91–1.84 (m, 2H), 1.52–1.45 (m, 2H).

5.3. General procedure for the preparation of 16 and 19

5.3.1. 2-(4-Chloro-3-nitrophenyl)-2-methylpropanoic acid (16)

To a stirred suspension of **15** (3.0 g, 13.2 mmol) in water (5.0 mL) was added concH_2SO_4 (5.0 mL) at room temperature. After stirring for 5 h under reflux, the reaction mixture was poured into ice-water, 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 recrystallization from EtOAc and *n*-hexane to yield **16** (2.26 g, 70% yield) as a white powder; TLC R_f = 0.47 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, J = 2.1 Hz, 1H), 7.58–7.50 (m, 2H), 1.64 (s, 6H).

Compound **19** was prepared as described above.

5.3.2. 1-(4-Chloro-3-nitrophenyl)cyclopropanecarboxylic acid (19)

Yield 66%; Ivory powder; TLC R_f = 0.43 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88–7.86 (m, 1H), 7.55–7.47 (m, 2H), 1.79–1.74 (m, 2H), 1.33–1.27 (m, 2H).

5.4. General procedure for the preparation of 17 and 20

5.4.1. Methyl 2-(4-chloro-3-nitrophenyl)-2-methylpropanoate (17)

To stirred MeOH (10 mL) were added dropwise thionyl chloride (2.1 mL, 28.7 mmol) at −10 °C and a solution of **16** (3.5 g, 14.4 mmol) in MeOH (10 mL) and DME (2 mL) at 0 °C. After stirring for 1 h while gradually warmed up to room temperature and 3 h at 40 °C, the reaction mixture was cooled to room temperature and concentrated in vacuo to yield **17** (3.8 g, quant), which was used for the next reaction without further purification; TLC R_f = 0.42 (*n*-hexane/EtOAc, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88–7.85 (m, 1H), 7.51–7.47 (m, 2H), 3.68 (s, 3H), 1.61 (s, 6H).

Compound **20** was prepared as described above.

5.4.2. Methyl 1-(4-chloro-3-nitrophenyl)cyclopropanecarboxylate (20)

Pale yellow oil; TLC R_f = 0.62 (*n*-hexane/EtOAc, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87–7.83 (m, 1H), 7.54–7.46 (m, 2H), 3.65 (s, 3H), 1.73–1.66 (m, 2H), 1.25–1.19 (m, 2H).

5.5. General procedure for the preparation of 21 and 22

5.5.1. Methyl 2-(3-amino-4-chlorophenyl)-2-methylpropanoate (21)

To a stirred solution of **17** (1.2 g, 4.66 mmol) in EtOAc (10 mL) and DMSO (16.5 μL, 0.233 mmol) was added $Pd(OH)_2$–C (240 mg) at room temperature. The resulting suspension was vigorously stirred for 5 h at room temperature under hydrogen atmosphere. Insoluble substance was removed by filtration. The filtrate was washed with 0.1 M HCl, water, brine, dried over $MgSO_4$ and

concentrated in vacuo to yield **21** (3.21 g, quant) as a yellow liquid; TLC R_f = 0.43 (*n*-hexane/EtOAc, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 8.7 Hz, 1H), 6.73 (d, J = 2.4 Hz, 1H), 6.66 (dd, J = 8.7, 2.4 Hz, 1H), 4.04 (br s, 2H), 3.65 (s, 3H), 1.53 (s, 6H).

Compound **22** was prepared as described above.

5.5.2. Methyl 1-(3-amino-4-chlorophenyl)cyclopropanecarboxylate (22)

TLC R_f = 0.58 (*n*-hexane/EtOAc, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 2.4 Hz, 1H), 6.68–6.63 (m, 1H), 4.02 (br s, 2H), 1.60–1.52 (m, 2H), 1.17–1.10 (m, 2H).

5.6. (4-Chloro-3-nitrophenyl)[(trimethylsilyl)oxy]acetonitrile (24)

To stirred solution of **23** (3.71 g, 20.1 mmol) and Zn_2I (128 mg, 0.402 mmol) in CH_2Cl_2 (50 mL) was added TMSCN (2.9 mL, 22.1 mmol) at 0 °C under argon atmosphere. After stirring for 1 h, the reaction mixture was poured into 1 M HCl and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo to yield **24** as a dark brown oil, which was used for the next reaction without further purification; TLC R_f = 0.55 (*n*-hexane/EtOAc, 4:1).

5.7. (4-Chloro-3-nitrophenyl)(hydroxy)acetic acid (25)

To a stirred solution of **24** (20.1 mmol) in AcOH (30 mL) was added conc HCl (30 mL). After stirring overnight at 90 °C, the reaction mixture was cooled to room temperature, poured into ice and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo to yield **25** (4.48 g, 96% in 2 steps) as a yellow oil, which was used for the next reaction without further purification; TLC R_f = 0.14 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 3.0 Hz, 1H), 7.67 (dd, J = 8.1, 3.0 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 6.00–4.50 (br, 2H).

5.8. Ethyl (4-chloro-3-nitrophenyl)(hydroxy)acetate (26)

To a stirred solution of **25** (4.48 g, 19.3 mmol) in EtOH (50 mL) was added *p*-toluenesulfonic acid (367 mg, 1.93 mmol). After stirring for 4 h at 70 °C, the reaction mixture was cooled to room temperature, concentrated in vacuo, poured into water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo to yield **26** (4.83 g, 96%) as a yellow oil, which was used for the next reaction without further purification; TLC R_f = 0.37 (*n*-hexane/EtOAc, 2:1)

5.9. Ethyl (4-chloro-3-nitrophenyl)(oxo)acetate (27)

To a stirred solution of **26** (1.59 g, 6.10 mmol) in AcOH (20 mL) was added 10% NaClO aq (13.56 g, 18.32 mmol) at 0 °C. After stirring for 4 h at 70 °C, the reaction mixture was poured into water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **27** (414 mg, 26%) as a yellow oil; TLC R_f = 0.59 (*n*-hexane/EtOAc, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (d, J = 3.0 Hz, 1H), 8.24 (dd, J = 8.1, 3.0 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 4.48 (q, J = 7.5 Hz, 2H), 1.45 (t, J = 7.5 Hz, 3H).

5.10. Ethyl (4-chloro-3-nitrophenyl)(difluoro)acetate (28)

To a stirred solution of **27** (414 mg, 1.61 mmol) in CH_2Cl_2 (5 mL) was added DAST (311 mg, 1.93 mmol) at 0 °C under argon atmosphere. After stirring for 2 h at room temperature and 4 h at 45 °C, the reaction mixture was poured into water and extracted with EtOAc (×2). The combined organic layers were washed with sat $NaHCO_3$ aq, water, brine, dried over $MgSO_4$ and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **28** (278 mg, 62%) as a yellow oil; TLC R_f = 0.57 (*n*-hexane/EtOAc, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, J = 3.0 Hz, 1H), 7.77 (dd, J = 8.1, 3.0 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 4.34 (q, J = 7.2 Hz, 2H), 1.34 (t, J = 7.2 Hz, 3H).

5.11. Ethyl (3-amino-4-chlorophenyl)(difluoro)acetate (29)

To a stirred solution of **28** (253 mg, 0.907 mmol) in AcOH (3 mL) and water (0.3 mL) was added Fe (253 mg, 4.54 mmol) at 80 °C. After stirring for 30 min at 80 °C, the reaction mixture was cooled to room temperature, poured into ice and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo to yield **29** (194 mg, 73%) as a dark brown oil, which was used for the next reaction without further purification; TLC R_f = 0.48 (*n*-hexane/EtOAc, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, J = 3.0 Hz, 1H), 6.99 (dd, J = 8.1, 3.0 Hz, 1H), 6.90 (d, J = 8.1 Hz, 1H), 4.50–3.75 (br, 2H), 4.29 (q, J = 7.2 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H).

5.12. Methyl 2,6-dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoate (32)

To a stirred solution of **30** (2.05 g, 11.4 mmol) in DMF (40 mL) were added Cs_2CO_3 (7.82 g, 24.0 mmol) and **31** (4.00 g, 12.0 mmol). After stirring for 3 h at 65 °C, the reaction mixture was poured into water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield **32** (3.85 g, 99%) as a colorless viscous oil; TLC R_f = 0.70 (*n*-hexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.76 (m, 2H), 6.75–6.63 (m, 2H), 6.60 (s, 2H), 4.73–4.54 (m, 1H), 4.31–4.15 (m, 1H), 4.17–4.03 (m, 1H), 3.88 (s, 3H), 3.37 (dd, J = 10.8, 2.1 Hz, 1H), 3.22 (dd, J = 11.5, 6.4 Hz, 1H), 2.89 (s, 3H), 2.30 (s, 6H).

5.13. 2,6-Dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoic acid (33)

To a stirred solution of **32** (1 g, 2.93 mmol) in 2-ethoxyethanol (10 mL) and dioxane (20 mL) was added 5 M NaOH aq (20 mL). After stirring overnight at 120 °C, the reaction mixture was diluted with water and extracted with MTBE. The aqueous layer was acidified with 5 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated to yield **33** (5.90 g, 61%) as a white solid; TLC R_f = 0.35 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94–6.77 (m, 2H), 6.75–6.67 (m, 2H), 6.64 (s, 2H), 4.73–4.55 (m, 1H), 4.23 (dd, J = 9.9, 5.1 Hz, 1H), 4.11 (dd, J = 9.7, 6.6 Hz, 1H), 3.38 (dd, J = 11.4, 2.7 Hz, 1H), 3.24 (dd, J = 11.7, 6.6 Hz, 1H), 2.91 (s, 3H), 2.42 (s, 6H).

5.14. 2,6-Dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl chloride (34a)

To a stirred solution of **33** (500 mg, 1.53 mmol) in DME (15 mL) were added $(COCl)_2$ (0.38 mL, 4.44 mmol) and DMF (3.4 μL, 0.044 mmol). After stirring for 1 h at 40 °C, the reaction mixture was concentrated in vacuo to yield **34a** (530 mg, 1.53 mmol) as a green viscous oil, which was used for the next reaction without further purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54–7.38 (m, 1H), 7.38–7.20 (m, 1H), 7.15–6.97 (m, 2H), 6.62 (s, 2H), 5.02–4.83

(m, 1H), 4.34 (d, J = 4.0 Hz, 2H), 3.86–3.71 (m, 1H), 3.71–3.57 (m, 1H), 3.25 (d, J = 2.2 Hz, 3H), 2.41 (s, 6H).

5.15. General procedure for the preparation of 2d–f, 3c–e, 5c–e, 10a–c, 11a–b and 12

5.15.1. {4-Chloro-3-[(2,6-dimethyl-4-{[(2S)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5e)

To a stirred solution of **35c** (781 mg, 3.9 mmol) and pyridine (0.95 mL, 11.7 mmol) in CH_2Cl_2 (10 mL) was added a solution of **34a** (1.3 g, 3.9 mmol) in CH_2Cl_2 (10 mL). After stirring overnight at room temperature, the reaction mixture was quenched with water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield methyl ester (1.3 g, 63% in 2 steps) as a pale yellow amorphous powder; TLC R_f = 0.54 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (d, J = 2.2 Hz, 1H), 7.72 (s, 1H), 7.36 (d, J = 8.2 Hz, 1H), 7.04 (dd, J = 8.2, 2.2 Hz, 1H), 6.96–6.76 (m, 2H), 6.75–6.58 (m, 4H), 4.72–4.56 (m, 1H), 4.24 (dd, J = 9.6, 4.5 Hz, 1H), 4.18–4.04 (m, 1H), 3.73 (s, 3H), 3.68 (s, 2H), 3.39 (dd, J = 11.4, 2.4 Hz, 1H), 3.24 (dd, J = 11.7, 6.4 Hz, 1H), 2.91 (s, 3H), 2.39 (s, 6H).

To a stirred solution of methyl ester (1.3 g, 2.6 mmol) described above in MeOH (15 mL) and dioxane (15 mL) was added 5 M NaOH aq (10 mL). After stirring for 2 h at room temperature, the reaction mixture was quenched with 2 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo. The resultant residue was recrystallized from EtOH to yield **5e** (1.1 g, 87%) as a white powder; TLC R_f = 0.28 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, J = 2.2 Hz, 1H), 7.73 (s, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.05 (dd, J = 8.2, 2.2 Hz, 1H), 6.95–6.79 (m, 2H), 6.75–6.58 (m, 4H), 4.71–4.55 (m, 1H), 4.24 (dd, J = 9.6, 4.8 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), 3.24 (dd, J = 11.7, 6.6 Hz, 1H), 2.91 (s, 3H), 2.38 (s, 6H); MS (APCI, Neg, 20 V) m/z 493 (M−H)−; HRMS (FAB) calcd for $C_{27}H_{28}ClN_2O_5$: 495.1687, Found: 495.1671; IR (KBr) 3268, 2936, 1692, 1660, 1604, 1584, 1505, 1465, 1426, 1316, 1293, 1245, 1228, 1168, 1058 cm^{-1}; Optical rotation $[\alpha]_D^{27}$ +14.98 (*c* 1.00, DMF); mp 183.5–185.0 °C.

Compounds **2d–f**, **3c–e**, **5c–d**, **10a–c**, **11a–b** and **12** were prepared as described above.

5.15.2. {3-[(2,3-Dimethyl-4-{[(2S)-4-methyl-3,4-dihydro-2H-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (2d)

Yield 75% in 2 steps; Pale yellow powder; TLC R_f = 0.42 ($CHCl_3$/MeOH/AcOH, 9:1:0.1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.30 (s, 1H), 10.15 (s, 1H), 7.68 (s, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.25 (m, 2H), 6.95 (m, 2H), 6.76 (m, 3H), 6.59 (m, 1H), 4.59 (m, 1H), 4.22 (d, J = 4.8 Hz, 2H), 3.52 (s, 2H), 3.40 (dd, J = 11.7, 2.7 Hz, 1H), 3.22 (dd, J = 11.7, 7.5 Hz, 1H), 2.84 (s, 3H), 2.26 (s, 3H), 2.16 (s, 3H); MS (APCI, Neg, 20 V) m/z 459 (M−H)−; IR (KBr) 3430, 3287, 3045, 2930, 2878, 2363, 1715, 1701, 1649, 1609, 1594, 1579, 1528, 1506, 1490, 1459, 1449, 1439, 1432, 1319, 1300, 1260, 1226, 1193, 1139, 1111, 1041, 991, 910, 892, 827, 811, 776, 759, 740, 705, 670, 607, 558, 529, 521, 503, 494, 485, 453 cm^{-1}; Optical rotation $[\alpha]_D^{23}$ +14.70 (c 0.75, DMSO).

5.15.3. {3-[(2,5-Dimethyl-4-{[(2S)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (2e)

Yield 47% in 2 steps; Pale blue powder; TLC R_f = 0.60 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62–7.54 (m, 1H), 7.54–7.46 (m, 1H), 7.46–7.38 (m, 1H), 7.37–7.22 (m, 2H), 7.10–7.06 (m, 1H), 6.92–6.80 (m, 2H), 6.74–6.66 (m, 3H), 4.72–4.62 (m, 1H), 4.27 (dd, J = 9.9, 5.1 Hz, 1H), 4.16 (dd, J = 9.9, 6.6 Hz, 1H), 3.68 (s, 2H), 3.41 (dd, J = 11.7, 3.0 Hz, 1H), 3.28 (dd, J = 11.7, 6.3 Hz, 1H), 2.92 (s, 3H), 2.48 (s, 3H), 2.23 (s, 3H); MS (EI, Pos.) m/z 460 (M+); IR (KBr) 3512, 3505, 3497, 3425, 3257, 3027, 2925, 1787, 1778, 1767, 1754, 1738, 1724, 1712, 1703, 1694, 1679, 1657, 1650, 1643, 1631, 1621, 1610, 1594, 1580, 1574, 1565, 1553, 1546, 1537, 1529, 1510, 1504, 1494, 1485, 1469, 1462, 1452, 1442, 1434, 1415, 1402, 1392, 1382, 1370, 1358, 1345, 1325, 1301, 1257, 1244, 1223, 1178, 1149, 1075, 1040, 998, 989, 955, 921, 910, 877, 858, 845, 828, 809, 796, 773, 741 cm^{-1}; Optical rotation $[\alpha]_D^{23}$ +13.03 (c 0.75, DMSO); mp 200.0–202.0 °C.

5.15.4. {3-[(2,6-Dimethyl-4-{[(2S)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (2f)

Yield 56% in 2 steps; White powder; TLC R_f = 0.32 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (m, 1H), 7.52 (m, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.30 (s, 1H), 7.10 (d, J = 7.5 Hz, 1H), 6.86 (m, 2H), 6.69 (m, 2H), 6.64 (s, 2H), 4.62 (m, 1H), 4.22 (dd, J = 9.9, 5.1 Hz, 1H), 4.10 (dd, J = 9.9, 6.6 Hz, 1H), 3.69 (s, 2H), 3.38 (dd, J = 11.7, 2.7 Hz, 1H), 3.24 (dd, J = 11.7, 6.6 Hz, 1H), 2.90 (s, 3H), 2.36 (s, 6H); MS (APCI, Neg, 20 V) m/z 459 (M−H)−; IR (KBr) 3276, 3039, 2934, 2919, 2882, 2865, 2659, 2555, 1692, 1655, 1628, 1604, 1594, 1523, 1505, 1467, 1439, 1414, 1375, 1362, 1319, 1293, 1243, 1231, 1173, 1162, 1133, 1109, 1086, 1058, 1039, 988, 971, 935, 908, 866, 860, 829, 781, 739, 708, 682, 625 cm^{-1}; Optical rotation $[\alpha]_D^{23}$ +11.89 (c 0.75, DMSO); mp 138.0–140.0 °C.

5.15.5. {3-[(2,3-Dimethyl-4-{[(2S)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-4-methylphenyl}acetic acid (3c)

Yield 85% in 2 steps; White powder; TLC R_f = 0.47 ($CHCl_3$/CH_3OH/AcOH, 9:1:0.1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.28 (s, 1H), 9.59 (s, 1H), 7.36–7.25 (m, 2H), 7.17 (d, J = 7.5 Hz, 1H), 7.06–6.90 (m, 2H), 6.85–6.68 (m, 3H), 6.65–6.54 (m, 1H), 4.67–4.53 (m, 1H), 4.21 (d, J = 5.1 Hz, 2H), 3.52 (s, 2H), 3.45–3.36 (m, 1H), 3.22 (dd, J = 11.6, 7.4 Hz, 1H), 2.85 (s, 3H), 2.32 (s, 3H), 2.22 (s, 3H), 2.16 (s, 3H); MS (APCI, Neg 20 V) m/z 473 (M-H)$^-$; IR (neat) 3430, 3267, 3040, 2917, 2640, 2360, 1856, 1688, 1646, 1609, 1581, 1530, 1505, 1480, 1457, 1451, 1288, 1266, 1256, 1224, 1190, 1137, 1109, 1041, 976, 912, 870, 823, 741, 682, 628, 565, 553, 483, 459 cm^{-1}, Optical rotation $[\alpha]_D^{24}$ +13.73 (*c* 0.75, DMSO).

5.15.6. {3-[(2,5-Dimethyl-4-{[(2S)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-4-methylphenyl}acetic acid (3d)

Yield 76% in 2 steps; White powder; TLC R_f = 0.62 ($CHCl_3$/CH_3OH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.55 (s, 1H), 7.35 (s, 1H), 7.25 (d, J = 1.7 Hz, 1H), 7.16 (d, J = 7.8 Hz, 1H), 7.01 (dd, J = 7.8, 1.7 Hz, 1H), 6.91 (s, 1H), 6.84–6.75 (m, 1H), 6.75–6.68 (m, 2H), 6.64–6.54 (m, 1H), 4.67–4.51 (m, 1H), 4.23 (d, J = 5.1 Hz, 2H), 3.51 (s, 2H), 3.43–3.36 (m, 1H), 3.21 (dd, J = 11.7, 7.2 Hz, 1H), 2.84 (s, 3H), 2.40 (s, 3H), 2.21 (s, 3H), 2.18 (s, 3H); MS (APCI, Neg 20 V) m/z 473 (M-H)$^-$; IR (KBr) 3589, 3569, 3554, 3547, 3538, 3423, 3266, 3027, 2925, 2367, 2345, 1911, 1871, 1847, 1832, 1794, 1774, 1734, 1719, 1709, 1690, 1647, 1630, 1609, 1579, 1561, 1527, 1506, 1476, 1459, 1450, 1389, 1321, 1300, 1259, 1224, 1172, 1153, 1138, 1073, 1040, 995, 894, 816, 781, 742, 698, 680, 670, 638, 568, 535, 526, 503, 467, 456 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +14.52 (*c* 0.75, DMSO); mp 192.0–193.0 °C.

5.15.7. {3-[(2,6-Dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]-4-methylphenyl}acetic acid (3e)

Yield 40% in 2 steps; White powder; TLC R_f = 0.46 ($CHCl_3$/CH_3OH/AcOH, 9:1:0.1); ^1H NMR (300 MHz, DMSO-d_6) δ 9.71 (s, 1H), 7.28 (d, J = 1.5 Hz, 1H), 7.17 (d, J = 7.8 Hz, 1H), 7.02 (dd, J = 7.8, 1.5 Hz, 1H), 6.84–6.68 (m, 5H), 6.64–6.55 (m, 1H), 4.64–4.49 (m, 1H), 4.18 (d, J = 5.1 Hz, 2H), 3.52 (s, 2H), 3.41–3.32 (m, 1H), 3.16 (dd, J = 11.7, 7.2 Hz, 1H), 2.84 (s, 3H), 2.32 (s, 6H), 2.24 (s, 3H); MS (APCI, Neg 20 V) m/z 473 (M−H)−; IR (KBr) 3589, 3569, 3434, 3266, 3038, 2925, 2865, 2651, 2548, 2368, 1899, 1871, 1693, 1651, 1605, 1561, 1505, 1468, 1458, 1452, 1407, 1361, 1317, 1293, 1262, 1244, 1229, 1173, 1159, 1133, 1101, 1059, 1040, 990, 959, 913, 860, 828, 791, 777, 740, 683, 626, 571 cm^{-1}; Optical rotation $[\alpha]_D^{21}$ +12.58 (c 0.75, DMSO); mp 200.0–201.0 °C.

5.15.8. {4-Chloro-3-[(2,3-dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5c)

Yield 58% in 2 steps; Beige powder; TLC R_f = 0.40 (Hex/EtOAc/AcOH, 9:1:0.1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.94 (s, 1H), 7.46–7.32 (m, 2H), 7.02 (dd, J = 8.1, 2.1 Hz, 1H), 6.93–6.76 (m, 3H), 6.75–6.66 (m, 2H), 4.73–4.60 (m, 1H), 4.34–4.23 (m, 1H), 4.16 (dd, J = 9.9, 6.6 Hz, 1H), 3.70 (s, 2H), 3.55–3.37 (m, 1H), 3.29 (dd, J = 11.4, 6.9 Hz, 1H), 2.92 (s, 3H), 2.43 (s, 3H), 2.23 (s, 3H); MS (APCI, Neg, 20 V) m/z 493 (M−H)−; IR (KBr) 3589, 3569, 3414, 3274, 3028, 2925, 2621, 2347, 2278, 1961, 1870, 1794, 1709, 1686, 1655, 1607, 1584, 1561, 1519, 1506, 1485, 1458, 1427, 1298, 1268, 1244, 1225, 1188, 1159, 1138, 1108, 1056, 992, 936, 927, 916, 906, 881, 871, 861, 849, 825, 816, 795, 781, 768, 741, 715, 706, 696, 673, 651, 641, 607, 596, 587, 572, 562, 553, 544, 516, 506, 499, 489, 481, 465, 454 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +13.34 (c 0.75, DMSO).

5.15.9. {4-Chloro-3-[(2,5-dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}acetic acid (5d)

Yield 76% in 2 steps; Pale brown powder; TLC R_f = 0.29 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 12.36 (s, 1H), 9.61 (s, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.38 (s, 1H), 7.12 (dd, J = 8.1, 2.1 Hz, 1H), 6.87 (s, 1H), 6.82–6.73 (m, 1H), 6.73–6.66 (m, 2H), 6.62–6.53 (m, 1H), 4.67–4.52 (m, 1H), 4.28–4.16 (m, 2H), 3.58 (s, 2H), 3.38 (dd, J = 11.7, 2.7 Hz, 1H), 3.22 (dd, J = 11.7, 7.2 Hz, 1H), 2.85 (s, 3H), 2.42 (s, 3H), 2.18 (s, 3H); MS (APCI, Neg, 20 V) m/z 493 (M−H)−; IR (KBr) 3447, 3029, 2940, 2884, 2775, 2625, 2539, 1917, 1877, 1714, 1688, 1607, 1584, 1572, 1530, 1503, 1469, 1454, 1426, 1376, 1317, 1288, 1264, 1245, 1191, 1168, 1138, 1075, 1064, 1044, 984, 911, 879, 849, 831, 807, 786, 765, 750, 743, 732, 663, 651, 606, 574, 556, 513, 468, 456 cm^{-1}; Optical rotation $[\alpha]_D^{21}$ +13.60 (c 0.75, DMSO).

5.15.10. 2-{4-Chloro-3-[(2-chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}-2-methylpropanoic acid (10a)

Yield 53% in 2 steps; White amorphous powder; TLC R_f = 0.26 (Hex/AcOEt, 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.80–8.62 (m, 2H), 7.88 (d, J = 9.3 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.11 (dd, J = 8.4, 2.4 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.96 (dd, J = 8.7, 2.4 Hz, 1H), 6.92–6.81 (m, 2H), 6.74–6.67 (m, 2H), 4.70–4.62 (m, 1H), 4.27 (dd, J = 9.9, 5.4 Hz, 1H), 4.18 (dd, J = 9.9, 6.3 Hz, 1H), 3.38 (dd, J = 11.4, 3.0 Hz, 1H), 3.25 (dd, J = 11.4, 6.3 Hz, 1H), 2.91 (s, 3H), 1.64 (s, 6H); MS (APCI, Neg 40 V) m/z 527 (M−H)−; IR (KBr); 3383, 3068, 2977, 2937, 2871, 2821, 2639, 2548, 1733, 1703, 1672, 1602, 1583, 1526, 1504, 1459, 1414, 1358, 1297, 1242, 1222, 1137, 1106, 1044, 965, 917, 819, 743, 661, 611, 571, 453 cm^{-1}; Optical rotation $[\alpha]_D^{22}$ +12.37 (c 0.75, DMSO); mp 151.0–152.0 °C.

5.15.11. 2-{4-Chloro-3-[(2,5-dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}-2-methylpropanoic acid (10b)

Yield 66% in 2 steps; White amorphous powder; TLC R_f = 0.18 (Hex/AcOEt, 2:1); ^1H NMR (300 MHz, DMSO-d_6) δ 9.69 (s, 1H), 7.57 (d, J = 2.1 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.41 (s, 1H), 7.22 (dd, J = 8.7, 2.1 Hz, 1H), 6.91 (s, 1H), 6.86–6.75 (m, 1H), 6.75–6.69 (m, 2H), 6.65–6.54 (m, 1H), 4.67–4.55 (m, 1H), 4.24 (d, J = 5.1 Hz, 2H), 3.46–3.35 (m, 1H), 3.21 (dd, J = 11.7, 7.2 Hz, 1H), 2.84 (s, 3H), 2.42 (s, 3H), 2.18 (s, 3H), 1.47 (s, 6H); MS (APCI, Neg 20 V) m/z 521 (M−H)−; IR (KBr); 3415, 3041, 2975, 2929, 2872, 2819, 2633, 1732, 1703, 1609, 1581, 1505, 1455, 1414, 1323, 1297, 1259, 1224, 1147, 1105, 1073, 1041, 944, 915, 818, 743, 661, 566, 453 cm^{-1}; Optical rotation $[\alpha]_D^{21}$ +13.58 (c 0.75, DMSO).

5.15.12. 2-{4-Chloro-3-[(2,6-dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}-2-methylpropanoic acid (10c)

Yield 58% in 2 steps; White powder; TLC R_f = 0.51 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, J = 2.4 Hz, 1H), 7.72 (s, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.12 (dd, J = 8.4, 2.4 Hz, 1H), 6.93–6.79 (m, 2H), 6.74–6.68 (m, 2H), 6.66 (s, 2H), 4.69–4.58 (m, 1H), 4.24 (dd, J = 9.6, 4.8 Hz, 1H), 4.12 (dd, J = 9.6, 6.6 Hz, 1H), 3.39 (dd, J = 11.4, 2.7 Hz, 1H), 3.24 (dd, J = 11.4, 6.6 Hz, 1H), 2.91 (s, 3H), 2.39 (s, 6H), 1.66 (s, 6H); MS (APCI, Neg, 20 V) m/z 521 (M−H)−; IR (KBr) 3409, 3198, 2993, 2984, 2925, 2870, 2644, 2551, 1691, 1639, 1605, 1579, 1504, 1474, 1457, 1412, 1402, 1380, 1356, 1321, 1298, 1277, 1266, 1248, 1225, 1171, 1136, 1110, 1078, 1065, 1052, 1041, 940, 912, 893, 853, 823, 745, 723, 672, 558, 522, 459 cm^{-1}; Optical rotation $[\alpha]_D^{26}$ +17.54 (c 1.00, CH_3CN); mp 131.0–133.0 °C.

5.15.13. 1-{4-Chloro-3-[(2,5-dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}cyclopropanecarboxylic acid (11a)

Yield 48% in 2 steps; White amorphous powder; TLC R_f = 0.59 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 9.67 (s, 1H), 7.56 (d, J = 2.1 Hz, 1H), 7.46–7.38 (m, 2H), 7.20 (dd, J = 8.1, 2.1 Hz, 1H), 6.91 (s, 1H), 6.83–6.75 (m, 1H), 6.75–6.68 (m, 2H), 6.64–6.54 (m, 1H), 4.66–4.54 (m, 1H), 4.24 (d, J = 5.1 Hz, 2H), 3.43–3.35 (m, 1H), 3.21 (dd, J = 11.7, 7.2 Hz, 1H), 2.84 (s, 3H), 2.42 (s, 3H), 2.18 (s, 3H), 1.52–1.42 (m, 2H), 1.16–1.10 (m, 2H); MS (APCI, Neg, 20 V) m/z 519 (M−H)−; IR (KBr) 3409, 3198, 2993, 2984, 2925, 2870, 2644, 2551, 1691, 1639, 1605, 1579, 1504, 1474, 1457, 1412, 1402, 1380, 1356, 1321, 1298, 1277, 1266, 1248, 1225, 1171, 1136, 1110, 1078, 1065, 1052, 1041, 940, 912, 893, 853, 823, 745, 723, 672, 558, 522, 459 cm^{-1}; Optical rotation $[\alpha]_D^{22}$ +11.61 (c 0.75, DMSO).

5.15.14. 1-{4-Chloro-3-[(2,6-dimethyl-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}cyclopropanecarboxylic acid (11b)

Yield 48% in 2 steps; White amorphous powder; TLC R_f = 0.59 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.21 (dd, J = 8.4, 2.1 Hz, 1H), 6.84–6.67 (m, 5H), 6.64–6.55 (m, 1H), 4.62–4.50 (m, 1H), 4.18 (d, J = 5.1 Hz, 2H), 3.41–3.33 (m, 1H), 3.22–3.11 (m, 1H), 2.84 (s, 3H), 2.32 (s, 6H), 1.50–1.43 (m, 2H), 1.16–1.10

(m, 2H); MS (APCI, Neg, 20 V) *m*/*z* 519 (M−H)−; IR (KBr) 3565, 3414, 3203, 3037, 2957, 2927, 2863, 2686, 2604, 2360, 2342, 1681, 1639, 1605, 1579, 1557, 1505, 1471, 1456, 1413, 1313, 1298, 1257, 1219, 1206, 1163, 1138, 1105, 1101, 1048, 976, 926, 858, 843, 822, 745, 726, 708, 668, 654, 628, 609, 598, 587, 558, 547, 538, 526, 513, 499, 485, 472, 456 cm^{-1}; Optical rotation $[\alpha]_D^{24}$ +11.74 (*c* 0.75, DMSO); mp 171.8–172.7 °C.

5.15.15. {4-Chloro-3-[(2-chloro-4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)amino]phenyl}(difluoro)acetic acid (12)

Yield 35% in 2 steps; Green powder; TLC R_f = 0.50 ($CHCl_3$/MeOH/H_2O, 80:20:1); ^1H NMR (300 MHz, DMSO-d_6) δ 10.12 (br s, 1H), 7.80 (br s, 1H), 7.58 (m, 2H), 7.40 (m, 1H), 7.21 (d, *J* = 2.1 Hz, 1H), 7.09 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.76 (m, 4H), 6.59 (m, 1H), 4.57 (m, 1H), 4.28 (m, 2H), 3.37 (dd, *J* = 11.7, 2.4 Hz, 1H), 3.17 (dd, *J* = 11.7, 7.5 Hz, 1H), 2.84 (s, 3H); MS (FAB, Pos.) *m*/*z* 537 (M + H)+; IR (KBr) 3381, 1652, 1602, 1589, 1532, 1504, 1458, 1420, 1300, 1243, 1223, 1133, 1099, 1048, 824, 758, 744, 612, 567 cm^{-1}.

5.16. (2*S*)-2-[(2-Bromo-4-methylphenoxy)methyl]oxirane (37)

To a stirred solution of 2-bromo-4-methylphenol **36** (6.8 g, 26.2 mmol) in DMF (50 mL) were added Cs_2CO_3 (12.8 g, 39.3 mmol) and (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate (6.8 g, 26.2 mmol) at room temperature under argon atmosphere, and stirring was continued for 1 h. 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 give **37** (6.1 g, 96%) as a pale yellow oil, which was used for the next reaction without further purification; TLC R_f = 0.50 (*n*-hexane/EtOAc, 2:1).

5.17. [(3*R*)-5-Methyl-2,3-dihydro-1-benzofuran-3-yl]methanol (38a)

To a stirred solution of the above-described crude product **37** (6.0 g, 24.5 mmol) in THF (35 mL) was added dropwise a 1.56 M solution of *n*-butyl lithium in hexane (15.7 mL, 24.5 mmol) at −78 °C. After stirring for 1 h at −78 °C and 1 h while gradually warmed up to room temperature, the reaction mixture was quenched with 1 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **38a** (2.2 g, 55%) as a pale yellow oil; TLC R_f = 0.32 (*n*-hexane/EtOAc, 2:1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.03 (s, 1H), 6.96 (m, 1H), 6.70 (d, *J* = 8.4 Hz, 1H), 4.62 (t, *J* = 9.0 Hz, 1H), 4.45 (dd, *J* = 9.0, 5.1 Hz, 1H), 3.86–3.73 (m, 2H), 3.67–3.53 (m, 1H), 2.29 (s, 3H).

5.18. General procedure for the preparation of 39a–e

5.18.1. [(3*S*)-5-Methyl-2,3-dihydro-1-benzofuran-3-yl]methyl 4-nitrobenzenesulfonate (39a)

To a stirred solution of **38a** (164 mg, 1.0 mmol) in THF (5 mL) were added TEA (0.33 mL, 2.4 mmol), DMAP (12 mg, 0.1 mmol) and 4-nitrobenzenesulfonyl chloride (266 mg, 1.2 mmol) at room temperature. After stirring overnight at room temperature, the reaction mixture was quenched with water and extracted with EtOAc (×2). The combined organic layers were washed with 1 M HCl aq, water, brine, dried over Na_2SO_4 and concentrated in vacuo to give **39a** (349 mg, quant), which was used for the next reaction without further purification; TLC R_f = 0.81 (*n*-hexane/EtOAc, 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, *J* = 8.4 Hz, 2H), 8.03 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.89 (s, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 4.55 (t, *J* = 9.0 Hz, 1H), 4.35 (dd, *J* = 9.0, 4.5 Hz, 1H), 4.28 (dd, *J* = 9.0, 5.4 Hz, 1H), 4.14 (t, *J* = 9.6 Hz, 1H), 3.83–3.73 (m, 1H), 2.23 (s, 3H).

Compounds **39b–e** were prepared as described above.

5.18.2. (2*S*)-2,3-Dihydro-1,4-benzodioxin-2-ylmethyl 4-nitrobenzenesulfonate (39b)

TLC R_f = 0.47 (*n*-hexane/EtOAc, 7:3); ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, *J* = 9.0 Hz, 2H), 8.11 (d, *J* = 9.0 Hz, 2H), 6.84–6.77 (m, 3H), 6.72–6.66 (m, 1H), 4.48–4.41 (m, 1H), 4.37–4.33 (m, 2H), 4.24 (dd, *J* = 11.7, 2.4 Hz, 1H), 4.07 (dd, *J* = 11.7, 6.0 Hz, 1H).

5.18.3. (3*S*)-2,3-Dihydro-1-benzofuran-3-ylmethyl 4-nitrobenzenesulfonate (39c)

TLC R_f = 0.72 (*n*-hexane/EtOAc, 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, *J* = 8.7 Hz, 2H), 8.03 (d, *J* = 8.7 Hz, 2H), 7.20–7.09 (m, 2H), 6.86–6.75 (m, 2H), 4.58 (t, *J* = 9.6 Hz, 1H), 4.37 (dd, *J* = 9.6, 4.5 Hz, 1H), 4.29 (dd, *J* = 9.6, 5.7 Hz, 1H), 4.17 (dd, *J* = 9.6, 8.1 Hz, 1H), 3.86–3.76 (m, 1H).

5.18.4. (2*S*)-2,3-Dihydro-1-benzofuran-2-ylmethyl 4-nitrobenzenesulfonate (39d)

TLC R_f = 0.78 (*n*-hexane/EtOAc, 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, *J* = 9.3 Hz, 2H), 8.06 (d, *J* = 9.3 Hz, 2H), 7.17–7.04 (m, 2H), 6.84 (td, *J* = 7.5, 0.9 Hz, 1H), 6.58 (d, *J* = 8.1 Hz, 1H), 5.02–4.92 (m, 1H), 4.40 (dd, *J* = 10.8, 3.3 Hz, 1H), 4.30 (dd, *J* = 10.8, 5.7 Hz, 1H), 3.32 (dd, *J* = 15.9, 9.9 Hz, 1H), 3.01 (dd, *J* = 15.9, 6.9 Hz, 1H).

5.18.5. 1,3-Benzodioxol-2-ylmethyl 4-nitrobenzenesulfonate (39e)

TLC R_f = 0.80 (*n*-hexane/EtOAc, 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, *J* = 9.0 Hz, 2H), 8.06 (d, *J* = 9.0 Hz, 2H), 6.80 (dd, *J* = 5.7, 3.3 Hz, 2H), 6.68 (dd, *J* = 5.7, 3.3 Hz, 2H), 6.25 (t, *J* = 3.3 Hz, 1H), 4.44 (d, *J* = 3.3 Hz, 2H).

5.19. 4-(Acetyloxy)-2,6-dimethylbenzoic acid (41)

To a stirred solution of 4-hydroxy-2,6-dimethylbenzoic acid **40** (20 g, 121 mmol) in pyridine (50 mL) was added Ac_2O (50 mL). After stirring for 2 h at room temperature, the reaction mixture was diluted with EtOAc and added water at 0 °C. After stirring for 2 min at 0 °C, the reaction mixture was extracted with EtOAc (×2). The combined organic layers were washed with 2 M HCl aq, water and brine, dried over $MgSO_4$ and concentrated in *vacuo* to give a crude product, which was washed with EtOAc/*n*-hexane to yield **41** (15 g, 60%) as an ivory powder; TLC R_f = 0.33 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 6.82 (s, 2H), 2.44 (s, 6H), 2.30 (s, 3H).

5.20. 4-(Chlorocarbonyl)-3,5-dimethylphenyl acetate (42)

To a stirred solution of **41** (2.0 g, 9.6 mmol) in toluene (15 mL) were added $(COCl)_2$ (1.7 mL, 19.2 mmol) and DMF (4 μL, 0.05 mmol). After stirring for 1 h at 40 °C, the reaction mixture was concentrated in vacuo to yield **42** (2.2 g, 9.6 mmol), which was used for the next reaction without further purification; ^1H NMR (300 MHz, $CDCl_3$) δ 6.82 (s, 2H), 2.41 (s, 6H), 2.30 (s, 3H).

5.21. Methyl (3-{[4-(acetyloxy)-2,6-dimethylbenzoyl]amino}-4-chlorophenyl)acetate (43)

To a stirred solution of **35c** (958 mg, 4.8 mmol) and pyridine (1.16 mL, 14.4 mmol) in CH_2Cl_2 (10 mL) was added a solution of **42** (1.1 g, 4.8 mmol) in CH_2Cl_2 (10 mL). After stirring overnight at 40 °C, the reaction mixture was quenched with water and extracted with EtOAc (×2). The combined organic layers were washed with 2 M HCl aq, water and brine, dried over $MgSO_4$ and

concentrated in vacuo to yield **43** as a ivory powder, which was used for the next reaction without further purification; TLC R_f = 0.54 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (d, *J* = 2.1 Hz, 1H), 7.76 (s, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.06 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.83 (s, 2H), 3.73 (s, 3H), 3.69 (s, 2H), 2.41 (s, 6H), 2.31 (s, 3H).

5.22. Methyl {4-chloro-3-[(4-hydroxy-2,6-dimethylbenzoyl)amino]phenyl}acetate (44)

To a stirred solution of the above-described crude product **43** (4.8 mmol) in MeOH (20 mL) and THF (20 mL) was added K_2CO_3 (2.0 g, 14.4 mmol) at room temperature. After stirring for 2 h at room temperature, insoluble substance was removed by filtration. The filtrate was diluted with EtOAc, washed with 2 M HCl aq, water and brine, dried over $MgSO_4$ and concentrated in vacuo to give a crude product, which was washed with EtOAc/*n*-hexane to yield **44** (800 mg, 48% in 3 steps) as a beige powder; TLC R_f = 0.40 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (d, *J* = 2.1 Hz, 1H), 7.74 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.04 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.53 (s, 2H), 5.16 (s, 1H), 3.73 (s, 3H), 3.68 (s, 2H), 2.35 (s, 6H).

5.23. General procedure for the preparation of 6, 7a–b, 8 and 9

5.23.1. [4-Chloro-3-({4-[(2*R*)-2,3-dihydro-1,4-benzodioxin-2-ylmethoxy]-2,6-dimethylbenzoyl}amino)phenyl]acetic acid (6)

To stirred solution of **44** (194 mg, 0.56 mmol) and **39b** (197 mg, 0.56 mmol) in DMF (3 mL) was added Cs_2CO_3 (365 mg, 1.12 mmol). After stirring for 1 h at rt, the reaction mixture was 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. The resulting residue was purified by column chromatography on silica gel to yield methyl ester (120 mg, 43%) as a pale yellow viscous oil; TLC R_f = 0.68 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50–8.47 (m, 1H), 7.72 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.07–7.02 (m, 1H), 6.95–6.80 (m, 4H), 6.66 (s, 2H), 4.60–4.54 (m, 1H), 4.44–4.36 (m, 1H), 4.33–4.10 (m, 3H), 3.73 (s, 3H) 3.68 (s, 2H), 2.39 (s, 6H).

To a stirred solution of methyl ester (120 mg, 0.24 mmol) described above in MeOH (3 mL) and THF (3 mL) was added 5 M NaOH aq (2 mL). After stirring for 2 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 **6** (60 mg, 51%) as a white powder; TLC R_f = 0.46 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, *J* = 2.1 Hz, 1H), 7.73 (s, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.06 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.88 (m, 4H), 6.66 (s, 2H), 4.57 (m, 1H), 4.40 (dd, *J* = 11.4, 2.4 Hz, 1H), 4.21 (m, 3H), 3.73 (s, 2H) 2.39 (s, 6H); MS (APCI, Neg, 40 V) *m*/*z* 480 (M−H)−; IR (KBr) 3266, 3045, 3018, 2955, 2911, 2883, 1735, 1692, 1659, 1630, 1602, 1593, 1585, 1519, 1493, 1467, 1425, 1406, 1317, 1305, 1283, 1269, 1249, 1226, 1176, 1166, 1144, 1108, 1062, 1052, 1046, 926, 913, 887, 867, 850, 828, 789, 750 cm^{-1}; mp 174.0–176.0 °C; Optical rotation $[\alpha]_D^{25}$ +4.85 (*c* 0.84, $CHCl_3$).

Compounds **7a**–**b**, **8** and **9** were prepared as described above.

5.23.2. [4-Chloro-3-({4-[(3*R*)-2,3-dihydro-1-benzofuran-3-ylmethoxy]-2,6-dimethylbenzoyl}amino)phenyl]acetic acid (7a)

Yield 6% in 2 steps; Ivory powder; TLC R_f = 0.39 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, *J* = 1.8 Hz, 1H), 7.73 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 6.6 Hz, 1H), 7.18 (m, 1H), 7.05 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.89 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 8.1 Hz, 1H), 6.63 (s, 2H), 4.71 (t, *J* = 9 Hz, 1H), 4.51 (dd, *J* = 9.3, 5.1 Hz, 1H), 4.16 (dd, *J* = 9, 5.1 Hz, 1H), 4.02 (t, *J* = 8.4 Hz, 1H), 3.92 (m, 1H), 3.72 (s, 2H) 2.38 (s, 6H); MS (APCI, Neg, 40 V) *m*/*z* 464 (M−H)−; IR (KBr) 3569, 3547, 3504, 3496, 3449, 3413, 3239, 3033, 2958, 2926, 2737, 1734, 1703, 1656, 1625, 1601, 1583, 1521, 1483, 1460, 1426, 1381, 1319, 1292, 1242, 1227, 1173, 1161, 1108, 1100, 1052, 963, 954, 940, 868, 859, 849, 837, 748, 457 cm^{-1}; mp 147.0–149.0 °C; Optical rotation $[\alpha]_D^{25}$ +15.48 (*c* 0.57, $CHCl_3$).

5.23.3. {4-Chloro-3-[(2,6-dimethyl-4-{[(3*R*)-5-methyl-2,3-dihydro-1-benzofuran-3-yl]methoxy}benzoyl)amino]phenyl}acetic acid (7b)

Yield 24% in 2 steps; White powder; TLC R_f = 0.39 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, *J* = 2.1 Hz, 1H), 7.73 (s, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.09 (m, 1H), 7.05 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.98 (m, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.63 (s, 2H), 4.69 (t, *J* = 9.00 Hz, 1H), 4.50 (dd, *J* = 9.00, 5.1 Hz, 1H), 4.16 (dd, *J* = 8.4, 5.1 Hz, 1H), 3.99 (t, *J* = 9.00 Hz, 1H), 3.88 (m, 1H), 3.73 (s, 2H) 2.38 (s, 6H), 2.30 (s, 3H); MS (APCI, Neg, 40 V) *m*/*z* 478 (M−H)−; IR (KBr) 3570, 3562, 3547, 3413, 3255, 3013, 2923, 2863, 2737, 1734, 1692, 1658, 1606, 1583, 1519, 1491, 1464, 1426, 1406, 1376, 1316, 1283, 1245, 1226, 1215, 1175, 1162, 1140, 1104, 1064, 1054, 966, 807 cm^{-1}; mp 168.0–169.0 °C; Optical rotation $[\alpha]_D^{25}$ −18.92 (*c* 0.76, $CHCl_3$).

5.23.4. [4-Chloro-3-({4-[(2*S*)-2,3-dihydro-1-benzofuran-2-ylmethoxy]-2,6-dimethylbenzoyl}amino)phenyl]acetic acid (8)

Yield 31% in 2 steps; White powder; TLC R_f = 0.40 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, *J* = 2.1 Hz, 1H), 7.73 (s, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.17 (m, 2H), 7.05 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.85 (m, 2H), 6.65 (s, 2H), 5.15 (m, 1H), 4.22 (dd, *J* = 9.9, 6.3 Hz, 1H), 4.10 (dd, *J* = 9.9, 4.8 Hz, 1H), 3.73 (s, 2H) 3.40 (dd, *J* = 15.3, 9.3 Hz, 1H), 3.13 (dd, *J* = 15.3, 6.3 Hz, 1H), 2.38 (s, 6H); MS (APCI, Neg, 40 V.) *m*/*z* 464 (M−H)−; IR (KBr) 3407, 3238, 3030, 2930, 1693, 1656, 1603, 1584, 1520, 1482, 1464, 1425, 1409, 1378, 1321, 1288, 1233, 1176, 1163, 1109, 1054, 1017, 984, 954, 914, 865, 827, 789, 744, 689, 661 cm^{-1}; mp 160.0–161.0 °C; Optical rotation $[\alpha]_D^{25}$ +41.89 (*c* 0.68, $CHCl_3$).

5.23.5. (3-{[4-(1,3-Benzodioxol-2-ylmethoxy)-2,6-dimethylbenzoyl]amino}-4-chlorophenyl)acetic acid (9)

Yield 34% in 2 steps; White powder; TLC R_f = 0.39 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, *J* = 1.8 Hz, 1H), 7.73 (s, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.05 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.85 (m, 4H), 6.66 (s, 2H), 6.44 (t, *J* = 4.2 Hz, 1H), 4.27 (d, *J* = 4.2 Hz, 2H), 3.72 (s, 2H) 2.38 (s, 6H); MS (APCI, Neg, 40 V) *m*/*z* 466 (M−H)−; IR (KBr) 3405, 3246, 3064, 2956, 2920, 2870, 2765, 1693, 1658, 1631, 1605, 1584, 1520, 1484, 1467, 1454, 1426, 1407, 1378, 1353, 1319, 1305, 1284, 1235, 1177, 1169, 1109, 1101, 1089, 1066, 1054, 1005, 983, 956, 926, 913, 897, 869, 861, 827, 800, 789, 766, 736, 689, 662, 654, 560, cm^{-1}; mp 165.0–168.0 °C.

5.24. Benzyl 1*H*-indol-4-ylacetate (46)

To a stirred solution of 2-(1*H*-indol-4-yl)acetic acid **45** (11.0 g, 62.9 mmol) in pyridine (45 mL) were added benzyl alcohol (6.9 mL, 66.0 mmol) and a solution of *p*-toluenesulfonyl chloride (13.2 g, 69.2 mmol) in toluene (38 mL) at 0 °C. After stirring vigorously for 2 h at room temperature, the reaction mixture was diluted with water and extracted with MTBE (×2). The combined organic layers were washed with $NaHCO_3$ aq, water and brine, dried over Na_2SO_4 and concentrated in vacuo to give **46** as a dark brown oil, which was used for the next reaction without further purification; TLC R_f = 0.45 (*n*-hexane/EtOAc, 2:1).

5.25. [1-(4-{[(2S)-4-Methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)-1*H*-indol-4-yl]acetic acid (1a)

To a stirred solution of **46** (1 g, 3.69 mmol) and **47** (1.76 g, 5.54 mmol) in CH_2Cl_2 (17 mL) were added 20 M NaOH aq (0.92 mL, 18.5 mmol) and TBACl (83 mg, 0.30 mmol). After stirring for 30 min at room temperature, the reaction mixture was quenched with water and extracted with EtOAc (×2). The combined organic layers were washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel to yield benzyl ester (1.47 g, 73%) as a pale yellow oil; TLC R_f = 0.22 (*n*-hexane /EtOAc, 3:1).

To a stirred solution of benzyl ester (0.80 g, 1.46 mmol) in EtOAc (10 mL) was added 20% $Pd(OH)_2/C$ (100 mg) at room temperature. The resulting suspension was vigorously stirred for 30 min at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo to give a crude product, which was washed with EtOAc/*n*-hexane to yield **1a** (0.39 g, 59%) as a white powder; TLC R_f = 0.33 (EtOAc/*n*-hexane, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.1 Hz, 2H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.37 (d, *J* = 3.9 Hz, 1H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 6.6 Hz, 1H), 7.05 (d, *J* = 8.7 Hz, 2H), 6.93–6.80 (m, 2H), 6.77–6.63 (m, 3H), 4.73–4.63 (m, 1H), 4.31 (dd, *J* = 9.9, 5.1 Hz, 1H), 4.21 (dd, *J* = 9.9, 6.0 Hz, 1H), 3.90 (s, 2H) 3.40 (dd, *J* = 11.7, 2.7 Hz, 1H), 3.28 (dd, *J* = 11.7, 6.6 Hz, 1H), 2.92 (s, 3H); MS (EI, Pos.) *m*/*z* 456 (M+); IR (KBr) 3422, 2935, 1702, 1669, 1606, 1509, 1489, 1461,1431, 1385, 1338, 1306, 1253, 1227, 1209, 1178, 1160, 1138, 1042, 994, 955, 891, 848, 814, 757, 683 cm^{-1}.

6. Biological assay method

6.1. Prostanoid mDP, 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, mDP, and hIP.

Membranes from CHO cells expressing prostanoid receptors were incubated with radioligands (2.5 nM of [^{3}H]PGD_2 for mDP; 5.0 nM of [^{3}H]Iloprost for hIP) and the test compounds at various concentrations in assay buffer (25 mM HEPES–NaOH buffer containing 1 mM EDTA, 5 mM $MgCl_2$ and 10 mM $MnCl_2$, pH 7.4 for mDP; 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 20 min for mDP and 30 min for hIP. The incubation was terminated by filtration through Whatman GF/B filters. The filters were washed with ice-cold buffer (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 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 PGD_2 (for mDP) 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.

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

[L]: Concentration of radioligand; 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 enzyme immunoassay 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.

6.3. Measurement of DP receptor antagonistic activity using human platelet-rich plasma (PRP)

To estimate the potency of antagonism of test compounds against the human prostanoid DP receptor in the conditions that may be close to the *in vivo* environment, a functional assay was performed by using PGD_2-stimulated cAMP production in human platelet rich plasma (PRP) as an indicator of receptor function.

For the assessment of the antagonist activity of test compounds, blood was collected from the cubital vein of a healthy adult who gave written informed consent, using a syringe filled with a 3.8 percent sodium citrate solution of about one ninth of predetermined volume. The collected blood was subjected to centrifugal separation at 100 G, at room temperature for 15 minutes to obtain PRP in the upper layer. EDTA was added to the obtained PRP so that the final concentration thereof to be about 10 mmol/L. The PRP was subjected to centrifugal separation at 1500 G, at room temperature for 15 min to obtain supernatant platelet poor plasma (PPP). After the obtained platelet pellets were suspended, the suspension was diluted with PPP so that the platelet density to be adjusted to 5.0×10^5/μL. To the obtained platelet suspension, 3-isobutyl-1-methylxanthine and prostanoid EP3 receptor antagonist were added so that the final concentrations thereof to be 8 mmol/L and 1 μmol/L, respectively. 297 μL of the prepared PRP was dispensed to each test tube, followed by subjecting to incubation at 37 °C for 5 min. After adding 1.5 μL of DMSO or a variety of concentrations of the compound of the present invention, 10 min of incubation was carried out at 37 °C. 1.5 μL of DMSO or PGD_2 (final concentration: 3 μmol/L) was added thereto to initiate the reaction. After 15 min of incubation was carried out at 37 °C, 300 μmol/L of ice-cooled 10 percent trichloroacetic acid (TCA) was added thereto to terminate the reaction. The TCA-treated sample was subjected to centrifugal separation at 15,000*g* for 3 min at

4 °C. The concentration of thus obtained supernatant cAMP was measured by enzyme immunoassay using cAMP EIA system (Amersham plc). 300 μL of the supernatant obtained above was mixed with 600 μL of a solution of 0.5 mol/L tri-*n*-octylamine in chloroform. After extracting TCA in the organic layer, the cAMP content in the water layer sample was measured according to the method described in cAMP assay kit. The strength of DP receptor antagonistic activity of the test compounds was represented by IC_{50} (concentration of the test compounds required to inhibit by 50% the production of cAMP in the absence of the test compounds) calculated from an inhibition ratio against a cAMP production amount which increases by 3 μmol/L PGD_2 stimulation.

7. Single dose rat pharmacokinetic study of 5e and 10c

Single dose pharmacokinetics of **5e** and **10c** were studied in rats. Formulation for intravenous injection was prepared using 30% HP-β-CD containing 5% DMSO (1 mg/mL/kg) and $NaHCO_3$ (1eq). Formulation for oral dosing was prepared using 0.5% MC (1 mg/5 mL/kg). Test compounds (1 mg/kg) were dosed intravenously to the fasted male rats ($n = 3$). Test compounds (1 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, 5, 15 and 30 min, 1, 2, 4, 6, 8 and 24 h; po: pre-dosing, 15 and 30 min 1, 2, 4, 6, 8 and 24 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:

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

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

8. Inhibitory effects of selected compounds on PGD2-induced vascular permeability in guinea pig conjunctiva

Topical application of PGD_2 to the eye of guinea pigs is known to cause plasma exudation in the conjunctiva via DP receptor. We assessed the PGD_2 receptor antagonism of test compounds in conjunctival vascular permeability induced by PGD_2. For the assessment of the antagonist activity of the test compounds, male Hartley guinea pigs were challenged by instillation of PGD_2 (0.01%, 20 μL/eye) to the eye, and then 5% Evans blue dye (1 mL/kg, iv) was immediately injected as a marker of plasma exudation. All antagonists were orally administered 1 h before the challenge. After 30 min, the guinea pigs were exsanguinated, and the eye tissue including conjunctiva were extracted. Isolated conjunctiva were incubated in DMF (1 mL) at 37 °C to extract the extravasated dye, and the incubation mixture was centrifuged. The absorption of the supernatant at 620 nm was determined, and the amount of Evans blue dye leaked into the tissues was quantified by interpolation on the standard curve.

9. Inhibitory effects of 5e on ovalbumin-induced vascular permeability in guinea pig conjunctiva

Male Hartley guinea pigs were sensitized by intraperitoneal injection of a mixture of ovalbumin (OVA) (1 mg) and inactivated *Bordetella pertussis* (5×10^9). Two weeks later, animals were challenged by topical application of OVA (1%, 20 μL/eye) to the eye, and then Evans blue dye (20 mg/kg, iv) was immediately injected as a marker of plasma exudation. All antagonists were orally administered 1 h before the antigen challenge. After 30 min, the guinea pigs were exsanguinated, and the eye tissue including conjunctiva were extracted. Isolated conjunctiva was incubated in DMF (1 mL) at 37 °C to extract the extravasated dye, and the incubation mixture was centrifuged. The absorption of the supernatant at 620 nm was determined, and the amount of Evans blue dye leaked into the tissues was quantified by interpolation on the standard curve.

10. X-ray crystallography of 1b·Na salt and 5e

The crystals of **1b**·Na salt were obtained from MeCN/H_2O (95:5) solution. The crystals of approximately $0.4 \times 0.4 \times 0.01$ mm were selected for the data collection and mounted in a sealed capillary tube with a small amount of the mother liquid to prevent decomposition in air.

All measurements were made on a RIGAKU AFC5R diffractometer with graphite-monochromated Cu-Kα radiation and RU-200 rotating anode X-ray generator. Of 4615 reflections that were collected, 3930 were unique. Equivalent reflections were merged. An empirical absorption correction was applied.

The program package of teXan (1) was used for analysis. The position of all the non-H atoms were determined by the program SHELXL86 (2). Hydrogen atoms were placed at calculated positions and confirmed by the difference Fourier synthesis.

Crystallographic data for **1b**: monoclinic $P2_1$(#4), $a = 8.98(1)$, $b = 7.74(2)$, $c = 38.53(4)$ Å, $\beta = 93.8(1)°$, $V = 2671(6)$ Å^3, $Z = 4$, $\lambda = 1.54178$ Å, R; $R_w = 0.106, 0.114$.

(1) teXan:Molecular Structure Corporation; 1993.
(2) Sheldrick, G. M.; Kruger, C.; Goddard R., Eds.; Oxford University Press, 1985; pp 175–189.

The crystals of **5e** were obtained by recrystallization from EtOH/H_2O solution. A pale yellow platelet crystal ($0.34 \times 0.40 \times 0.04$ mm) was mounted in a cryoloop and frozen at 93 K in a liquid nitrogen stream.

Data was collected on Rigaku R-AXIS RAPID with graphite-monochromated Cu-Kα radiation at 93 K. A total of 25894 reflections were collected, of which 8541 were unique. Equivalent reflections were merged.

The program package of Crystal Structure (1) was used for analysis. The position of all the non-H atoms were determined by the program SHELXL 97 (2) and DIRDIF (3), and refined anisotropically. Hydrogen atoms were placed at calculated positions and refined using a riding model. Crystallographic data for **5e**: monoclinic $P2_1$(#4), $a = 13.986(2)$, $b = 4.8040(5)$, $c = 35.232(4)$ Å, $\beta = 95.485(7)°$, $V = 2356.4(1)$ Å^3, $Z = 4$, $\lambda = 1.54178$ Å, R; R_w ($I > 3.00\sigma(I)$) = 0.0938, 0.0973.

These data have submitted to the Cambridge Structure Database with reference number CCDC 843498.

(3) Crystal Structure, version 3.6.0, 2000–2004, Crystal Structure Analysis Package, Rigaku and Rigaku /MSC.
(4) Sheldrick, G. M.; SHELX97; University of Göttingen, Germany 1997.
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