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Discovery of a new class of potent, selective, and orally active prostaglandin D_2 receptor antagonists

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Abstract—The process of discovering a series of *N*-(*p*-alkoxy)benzoyl-2-methylindole-4-acetic acid analogs is presented since these compounds represent a new class of potent, selective, and orally active prostaglandin D_2 (PGD_2) receptor antagonists. Most of these compounds exhibit strong PGD_2 receptor binding and PGD_2 receptor antagonism in cAMP formation assays. When given orally, these new antagonists dramatically suppress allergic inflammatory responses, such as the PGD_2-induced or OVA-induced increase of vascular permeability. Structure–activity relationship (SAR) data are also discussed.

1. Introduction

Prostanoids (prostaglandins and thromboxanes) are products of cyclooxygenase that are derived from arachidonic acid. It has been proposed that specific prostanoid receptors exist for each of the primary prostanoids, including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2, which have been designated as the DP, EP, FP, IP, and TP receptors, respectively.[1] In addition, the EP receptor is classified into four subtypes (EP_1, EP_2, EP_3, and EP_4), all of which respond to PGE_2 in different ways.

Prostaglandin D_2 (PGD_2) receptor antagonists might have potential clinical uses because PGD_2 is considered to play an important role in various allergic diseases, including allergic rhinitis,[2] atopic asthma,[3] allergic conjunctivitis,[4] and atopic dermatitis.[5] However, there have been few reports on the efficacy of PGD_2 receptor antagonists in animal models of allergy or human allergic diseases.[6] A selective DP receptor antagonist would be expected to show potential therapeutic value for various allergic disorders.

In preceding papers,[7] we have reported on the discovery of a new class of selective DP receptor antagonists starting from the chemical modification of Indomethacin analogs. Here we report on the identification of hDP receptor antagonists **2r–s**, which exhibited efficacy in animal models, starting from the chemical modification of **1** and **4** (Fig. 1).

2. Chemistry

The synthesis of **5a–i** is outlined in Scheme 1. *N*-Acylation of 2-methylindole-4-acetic acid esters **6** and **7** with acid chlorides **8a–i** afforded **9a–i**, respectively, after which deprotection by conventional methods gave **5a–i**, respectively.

Synthesis of **2a–s** according to a polymer-supported solid phase technique is described in Scheme 2.[8] *N*-Acylation of **7** with *p*-acetyloxybenzoyl chloride provided **10**, catalytic hydrogenation of which led to **11**. The carboxylic acid **11** was supported with a resin to produce **12**, the acetyl moiety of which was removed by aminolysis to give a phenol **13**. Mitsunobu reaction[9] of the polymer-supported phenol **13** with alcohols **15a–s** resulted in **14a–s**, respectively. Removal of the resin from **14a–s** was achieved by a reported method[8] to give **2a–s**, respectively.

Keywords: Prostaglandin; DP receptor; Antagonist.
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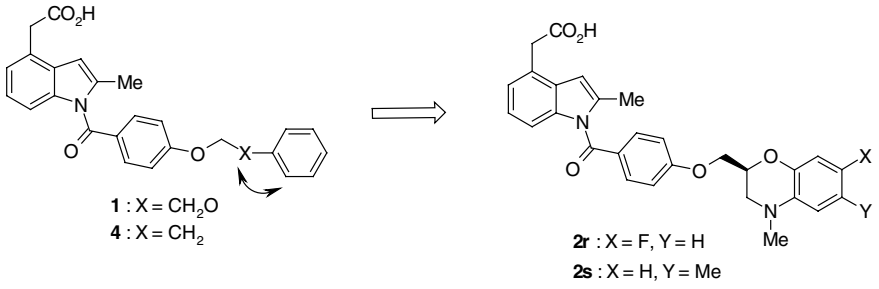
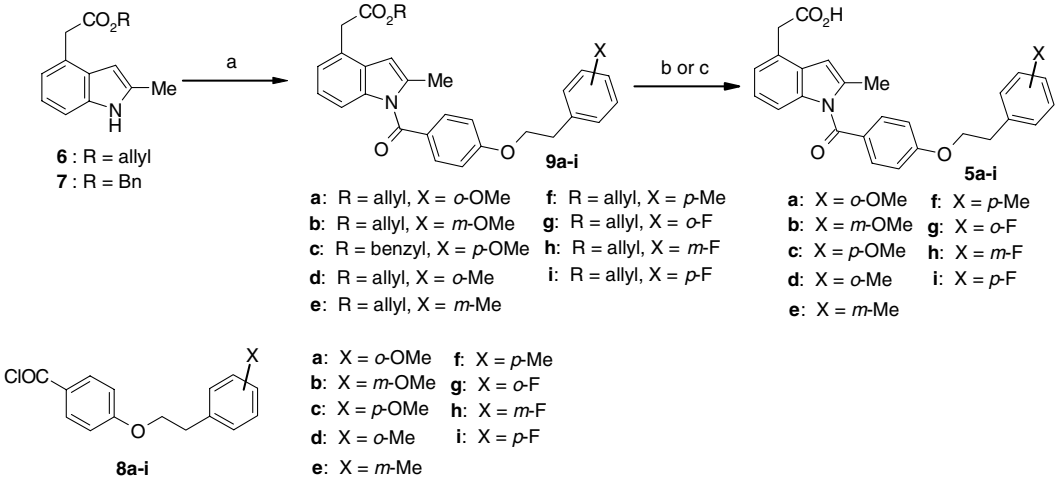


Figure 1.



Scheme 1. Synthesis of **5a**–**i**. Reagents: (a) NaOH (powdered), **8a**–**i**, TBACl, H_2Cl_2; (b) morpholine, $Pd(PPh_3)_4$, THF; (c) H_2, Pd–C, MeOH, EtOAc.

Preparation of **15c**, **15k**, and **15m**–**s** is described in Scheme 3. *O*-Alkylation of a catechol **16** with methyl dichloroacetate in the presence of sodium hydride afforded **17**, after which lithium aluminum hydride reduction led to **15c** (Scheme 3a). *N*-Methylation of **18**,[10] followed by lithium aluminum–hydride reduction, produced **15k**[11] (Scheme 3b). The optically active *N*-methyl benzoxazine **15m** was prepared by *N*-methylation of **19**,[12] followed by catalytic hydrogenolysis (Scheme 3c). The *N*-ethyl benzoxazine **15n** was prepared by *N*-ethylation of **15j** (Scheme 3d). Sodium borohydride reduction of a quinoxaline **20**[13] led to the tetrahydroquinoxaline **21**,[14] after which *N*,*N*′-dimethylation provided **15o** (Scheme 3e). *N*-Methylation of indoline-2-carboxylic acid **22**, followed by lithium aluminum hydride reduction, produced **15p** (Scheme 3f). Lithium aluminum hydride reduction of *N*-^{t}butoxycarbonylindoline **24**[15] resulted in a *N*-methylindoline **15q** (Scheme 3g). Compounds **15r**–**s** were prepared from **25a**–**b**, respectively as outlined in Scheme 3h. *N*-Tosylation of **25a**–**b** with tosyl chloride gave **26a**–**b**, respectively. *N*-Alkylation of **26a**–**b** with (*S*)-(−)-glycidyl triphenylmethyl ether provided **27a**–**b**, respectively. Cyclization reaction of **27a**–**b**, followed by removal of the *N*-tosyl moiety, produced **28a**–**b**, respectively. *N*-Methylation of **28a**–**b** followed by acidic deprotection produced **15r**–**s**, respectively.

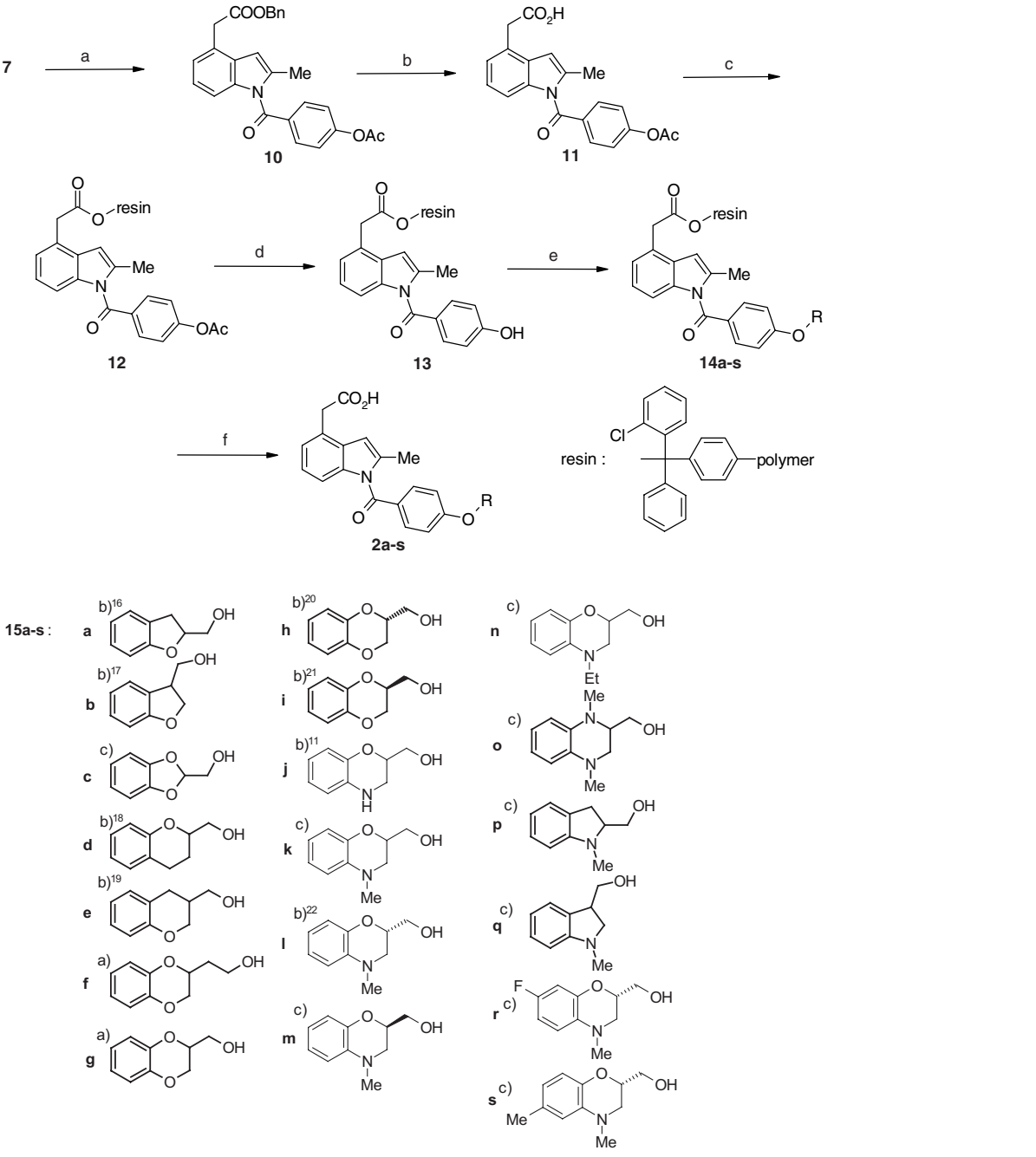
The synthesis of **15a**, **15b**, **15d** and **15e** is reported in Refs. [16–19].

3. Results and discussion

The test compounds listed in Tables 1–4 were biologically evaluated for inhibition of the specific binding of a radiolabeled ligand, [^{3}H]PGD_2, to membrane fractions prepared from cells stably expressing each prostanoid receptor and for inhibition of cAMP formation evoked by PGD_2 in CHO cells in the presence of bovine serum albumin (BSA). Test compounds were also evaluated for binding to all the subtypes of the mouse PGE_2 receptor (mEP1, mEP2, mEP3, and mEP4) and to the human PGI_2 receptor (hIP).

In one of the earlier papers,[7b] we reported on the high mDP receptor affinity and antagonist activity of *N*-benzoyl-2-methylindole-4-acetic acids **3**, **4**, and **1**. However, the hDP receptor affinity and antagonist activity of these compounds was found to be relatively lower than their mDP receptor affinity and mDP antagonist activity, respectively, as described in Table 1.

Based on the results reported in the preceding paper,[7b] the optimum position of the acetic acid residue, which corresponds to the acidic part of PGs, was identified as the position-4 of the *N*-benzoyl-2-methylindole template. Thus, our next focus was placed on further optimization of the *p*-alkoxy moiety, which corresponds to the lipophilic part of PGs.



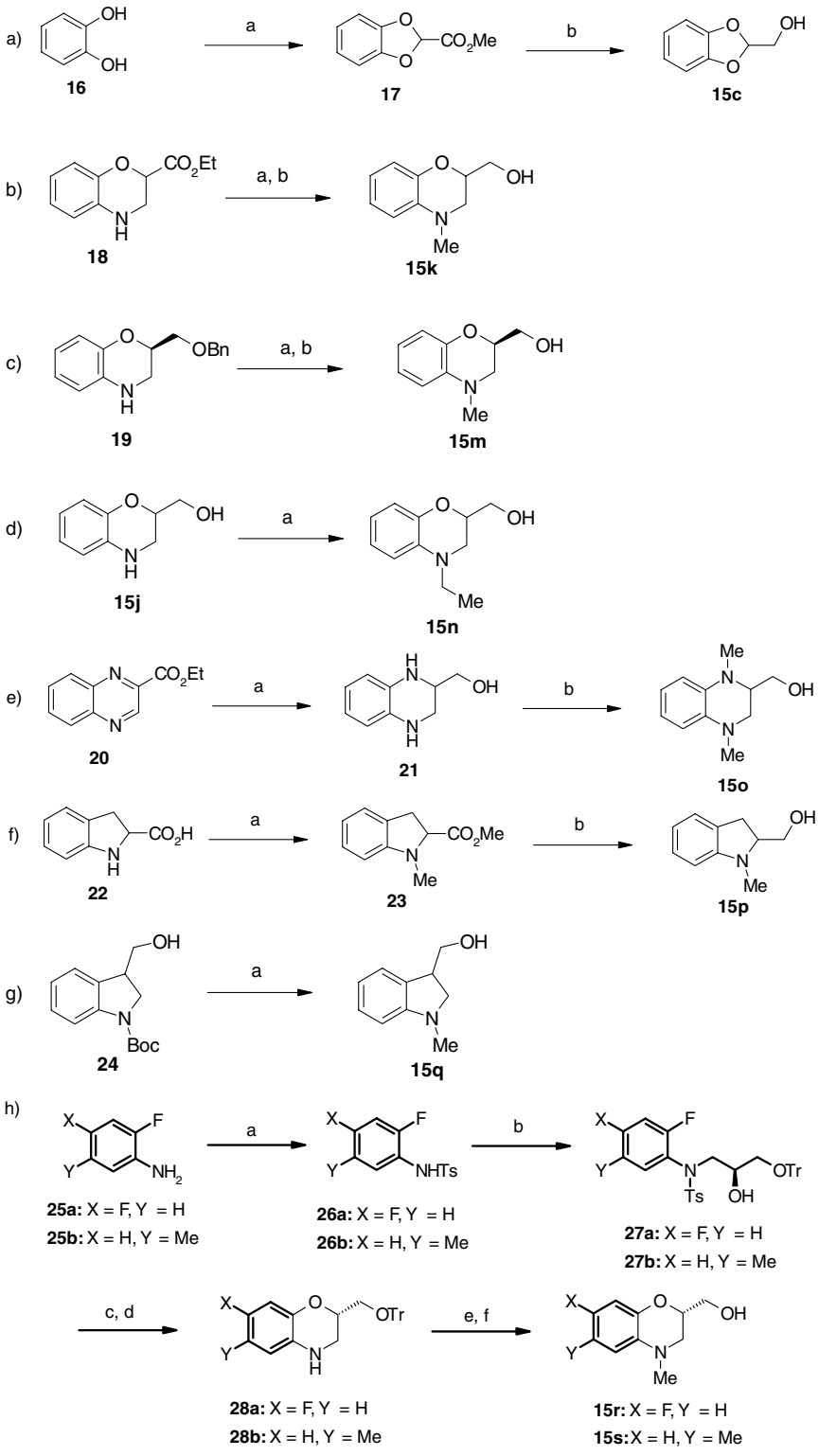
Scheme 2. Synthesis of **2a**–**s**. Reagents: (a) NaOH (powdered), *p*-acetoxybenzoyl chloride, CH_2Cl_2; (b) $Pd(OH)_2$, H_2, EtOAc; (c) resin, diisopropylethylamine; (d) piperidine, CH_2Cl_2; (e) **15a**–**s**, DEAD, PPh_3; (f) AcOH, CF_3CH_2OH, CH_2Cl_2. (a) Commercially available; (b) reported; (c) described in Scheme 3a–h.

Because more diverse chemical modification of the terminal phenyl moiety relative to **3** was expected to be possible, compounds **4** and **1** were selected as the chemical leads for further optimizations.

Introduction of a substituent into the terminal phenyl moiety of **4** was followed by biological evaluation, as shown in Table 2. Introduction of a methoxy moiety at the terminal phenyl moiety of **4** afforded **5a**–**c**. The *o*-methoxy derivative **5a** showed slightly weaker mDP receptor affinity, nearly equal hDP receptor affinity, and slightly lower hDP antagonist activity relative to **4**.

The *m*-methoxy derivative **5b** showed more than 25-fold weaker mDP receptor affinity, nearly 2-fold weaker hDP receptor affinity, and 10-fold higher hDP antagonist activity compared with **4**.

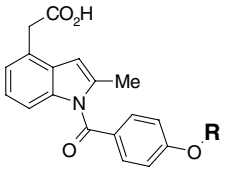
The *p*-methoxy derivative **5c** demonstrated nearly 3-fold weaker mDP receptor affinity, slightly weaker hDP

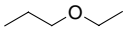
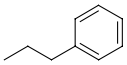
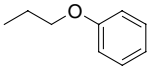


Scheme 3. Synthesis of **15c**, **15k**, and **15m**–**s**. (a) Reagents: (a) NaH, methyl dichloroacetate, DMF; (b) $LiAlH_4$, THF. (b) Reagents: (a) K_2CO_3, MeI, acetone; (b) $LiAlH_4$, THF. (c) Reagents: (a) K_2CO_3, MeI, acetone; (b) H_2, Pd–C, HCl, MeOH. (d) Reagents: (a) K_2CO_3, EtI, DMF. (e) Reagents: (a) $NaBH_4$, MeOH, THF; (b) K_2CO_3, MeI, acetone. (f) Reagents: (a) K_2CO_3, MeI, acetone; (b) $LiAlH_4$, THF. (g) Reagents: (a) $LiAlH_4$, ether. (h) Reagents: (a) TsCl, Pyridine, CH_2Cl_2; (b) (*S*)-(−)-glycidyl triphenylmethyl ether, K_2CO_3, $BnEt_3NCl$, 1,4-dioxane; (c) ^{t}BuOK, THF; (d) Na, naphthalene, DME; (e) MeI, K_2CO_3, acetone; (f) AcOH, THF, H_2O.

receptor affinity, and nearly 3-fold lower hDP receptor antagonist activity compared with **4**. Introduction of a methyl moiety at the terminal phenyl moiety of **4** afforded **5d**–**f**. The *o*-methyl derivative **5d** showed more than 10-fold weaker mDP receptor affinity, nearly equal hDP receptor affinity, and 5-fold higher hDP antagonist

Table 1. New chemical leads for a DP receptor antagonist



Compd	R	Binding K_i (μM)		IC_{50} (μM)[a]	
		mDP	hDP	mDP	hDP
3		0.020	0.18	0.074	0.20
4		0.0018	0.087	0.12	0.15
1		0.017	0.29	0.053	0.25

[a] IC_{50} (μM): hDP receptor antagonist activity.

activity compared with **4**. The *m*-methyl derivative **5e** showed nearly 7-fold weaker mDP receptor affinity, nearly equal hDP receptor affinity and nearly 10-fold higher hDP receptor antagonist activity relative to **4**. The *p*-methyl derivative demonstrated nearly 10-fold weaker mDP receptor affinity, nearly 3-fold weaker hDP receptor affinity, and nearly 2-fold lower hDP receptor antagonist activity relative to **4**. Introduction of a fluoro residue at the terminal phenyl moiety of **4** provided **5g**–**i**.

The *o*-fluoro derivative **5g** showed nearly equal mDP receptor affinity, nearly 2-fold stronger hDP receptor affinity, and equal hDP receptor antagonist activity relative to **4**. The *m*-fluoro derivative **5h** showed nearly 10-fold weaker mDP receptor affinity, nearly 3-fold weaker hDP receptor affinity, and nearly 2-fold lower hDP receptor antagonist activity relative to **4**. The *p*-fluoro derivative **5i** demonstrated nearly 4.5-fold weaker mDP receptor affinity, nearly equal hDP receptor affinity, and nearly 3-fold lower hDP receptor antagonist activity relative to **4**. The subtype selectivity of the compounds listed in Table 2 was not improved relative to that of **4**. hDP Receptor antagonist activity was increased for the *m*-methoxy and *m*-methyl derivatives **5b** and **5e** relative to their *o*-and *p*-isomers, while it was not always improved for the *m*-fluoro derivative **5h**.

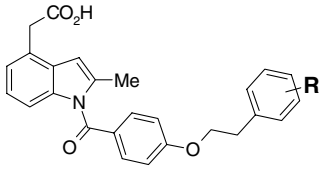
As a result, it was concluded that the increase of hDP receptor affinity was not so great as that of mDP receptor affinity in the above-described chemical modification of **4**. Thus structural transformation of the terminal phenoxy moiety of **1** to a fused bicyclic structures as described in Figure 1 instead of simply introducing a substituent was carried out as another approach for the optimization.

Synthesis and evaluation of the dihydrobenzofuran, 1,2-methylenedioxybenzene, dihydrobenzopyran, and benzodioxane derivatives listed in Table 3 was carried out.

As shown in Table 3, dihydrobenzofuran derivative **2a** demonstrated slightly weaker mDP receptor affinity and 10-fold stronger hDP receptor affinity relative to **1**, while it showed nearly 7-fold higher hDP receptor antagonist activity. The regio isomer **2b** exhibited higher hDP receptor antagonist activity, although its mDP and hDP receptor affinity was weaker than that of **2a**.

Introduction of another oxygen into the fused dihydrofuran of **2a** afforded **2c**, which showed nearly 2-fold stronger mDP receptor affinity, slightly weaker hDP receptor affinity, and slightly lower hDP receptor

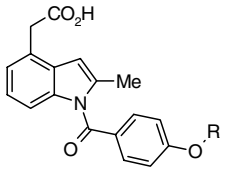
Table 2. Effect of the terminal phenyl moiety on the activity profiles

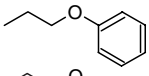
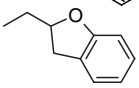
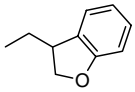
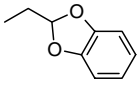
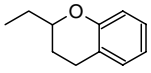
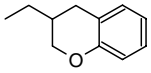
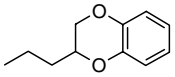
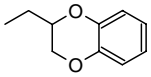
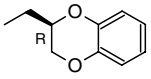
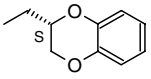


Compd	R	Binding K_i (μM)							IC_{50} (μM)[b]
		mEP1	mEP2	mEP3	mEP4	hIP	mDP	hDP	hDP
4	H	0.40	1.3	2.2	1.9	0.14	0.0018	0.087	0.15
5a	*o*-OMe	2.4	0.42	4.1	1.9	0.099	0.0039	0.10	0.25
5b	*m*-OMe	>10	0.55	>10	2.9	NT[a]	0.045	0.190	0.015
5c	*p*-OMe	0.69	0.68	>10	2.6	0.95	0.0064	0.14	0.40
5d	*o*-Me	0.65	0.23	2.3	1.8	0.050	0.026	0.11	0.032
5e	*m*-Me	1.4	0.79	>10	1.8	0.083	0.013	0.13	0.016
5f	*p*-Me	1.1	1.7	>10	3.9	0.29	0.025	0.30	0.28
5g	*o*-F	0.27	0.24	1.4	1.9	0.026	0.0024	0.046	0.15
5h	*m*-F	1.2	0.73	2.0	1.6	NT[a]	0.019	0.20	0.37
5i	*p*-F	0.20	0.71	2.8	3.5	0.29	0.0093	0.13	0.47

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

Table 3. Activity profiles of benzofuran, benzopyran, and benzodioxane derivatives



Compd	R	Binding K_i (μM)							IC_{50} (μM)[b]
		mEP1	mEP2	mEP3	mEP4	hIP	mDP	hDP	hDP
1		5.6	0.54	>10	>10	>10	0.017	0.29	0.25
2a		7.8	0.45	0.98	>10	0.27	0.022	0.029	0.036
2b		3.1	0.78	1.4	2.9	0.33	0.068	0.042	0.013
2c		0.78	0.25	0.75	>10	0.33	0.0094	0.039	0.056
2d		3.8	0.092	>10	3.1	0.16	0.061	0.052	0.030
2e		>10	0.28	>10	3.5	NT[a]	0.18	0.39	NT[a]
2f		7.0	2.9	>10	>10	NT[a]	0.18	NT[a]	0.24
2g		>10	0.24	>10	>10	0.27	0.012	0.032	0.024
2h		2.0	0.064	>10	>10	0.22	0.016	0.018	0.031
2i		2.3	1.3	>10	1.3	1.6	0.12	0.13	0.27

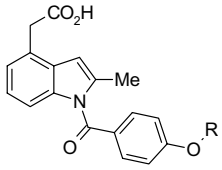
[a] NT: not tested.
[b] IC_{50} (μM): hDP receptor antagonist activity.

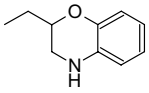
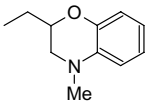
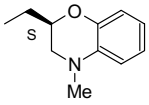
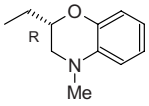
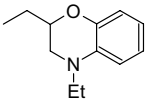
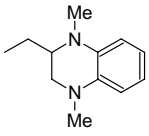
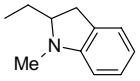
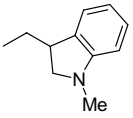
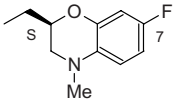
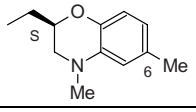
antagonist activity. Subtype selectivity of **2a–c** was reduced relative to that of **4**, mainly because of the increased mEP3 and hIP receptor affinity.

Benzopyran derivative **2d** showed 3.5-fold weaker mDP receptor affinity, and 5.5-fold stronger hDP receptor affinity relative to **1**, while it showed nearly 8-fold higher hDP receptor antagonist activity. Another dihydrobenzopyran **2e** showed weaker affinity for the mDP and hDP receptors compared with **2d**. Introduction of another phenolic oxygen at the fused dihydropyran of **2d** afforded **2g**, which showed nearly 5-fold stronger mDP receptor affinity and 2-fold stronger hDP receptor affinity. As illustrated in the transformation from **2d** to **2g**, introduction of another oxygen was effective for improving subtype selectivity and maintaining strong hDP antagonist activity. Replacement of the benzodioxane-2-yl methyl with a benzodioxane-2-yl ethyl moiety afforded **2f**, which showed nearly 10-fold weaker mDP receptor affinity and 10-fold lower hDP receptor antagonist activity.

Synthesis and biological evaluation of the optically active forms **2h** and **2i** was also conducted. The *R*-isomer **2h** demonstrated nearly equal mDP receptor affinity, nearly 2-fold stronger hDP receptor affinity, and nearly equal hDP receptor antagonist activity relative to its racemic form **2g**, while the *S*-isomer **2i** showed more than 10-fold weaker affinity for the mDP and hDP receptors and nearly 10-fold lower hDP receptor antagonist activity. The subtype selectivity of the dihydrobenzofuran analogs **2a–c** and the benzodioxane analog **2g** was lower than that of chemical lead **1**, while the subtype selectivity of the optically active *R*-isomer **2h** was higher than that of the *S*-isomer **2i**.

Table 4. Activity profiles of benzomorpholine, benzopiperazine, and indoline derivatives



Compd	R	Binding K_i (μM)							IC_{50} (μM)[b]
		mEP1	mEP2	mEP3	mEP4	hIP	mDP	hDP	hDP
2j		2.0	0.45	>10	2.8	NT[a]	0.16	0.087	0.021
2k		>10	0.11	>10	4.7	0.13	0.027	0.0038	0.0039
2l		6.2	0.10	1.7	>10	0.037	0.016	0.0035	0.0012
2m		>10	0.41	>10	>10	NT[a]	0.23	0.17	0.019
2n		>10	1.7	>10	3.3	0.94	0.024	0.018	0.013
2o		>10	2.7	>10	>10	NT[a]	0.19	0.21	0.022
2p		8.1	3.9	>10	>10	NT[a]	0.22	0.22	0.16
2q		>10	0.74	1.4	>10	0.19	0.30	0.066	0.011
2r		>10	2.1	1.7	>10	0.26	0.023	0.0053	0.00081
2s		>10	2.4	>10	>10	1.2	0.021	0.018	0.0021

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

Further optimization of **2a**–**b** and **2g** was successfully carried out by introducing a nitrogen instead of an oxygen at the same position, as illustrated in Table 4. Replacing the 4-oxygen of the benzodioxane moiety of **2g** with NH led to **2j**, which showed reduced mDP and hDP receptor affinity, while **2j** showed equal hDP receptor antagonist activity relative to **2g**. *N*-Methylation of the nitrogen of the benzomorpholine of **2j** gave **2k**, with improved mDP and hDP receptor affinity as well as a marked increase of hDP receptor antagonist activity. *N*-Ethylation of the benzomorpholine of **2j** afforded **2n**, which showed increased mDP and hDP receptor affinity as well as nearly 2-fold higher hDP receptor antagonist activity, although the increase of antagonist activity was not as great as that of the *N*-methylated compound. Replacement of the oxygen of the benzomorpholine moiety of **2k** with a *N*-methyl moiety afforded **2o**, which demonstrated a marked decrease

of mDP and hDP receptor affinity as well as 5-fold lower antagonist activity. Accordingly, synthesis and biological evaluation of the optically active forms of the most potent analog **2k** was conducted.

The 2*S*-isomer **2l**, which showed an increase of receptor affinity and antagonist activity compared with the racemate **2k**, demonstrated markedly higher DP receptor affinity and antagonist activity than the 2*R*-isomer **2m**. Replacement of the oxygen atom of the benzofuran derivative **2a** with an *N*-methyl moiety afforded **2p**, which showed nearly 10-fold weaker affinity for mDP and hDP receptors and nearly 4-fold lower hDP antagonist activity. The regioisomer **2q** showed nearly 14-fold higher hDP antagonist activity than **2p**, while it showed nearly equal affinity and nearly 3-fold stronger affinity for the mDP and hDP receptors, respectively.

Introduction of a 7-fluoro moiety and a 6-methyl moiety at the *N*-methylbenzomorpholine of the more active enantiomer **2l** afforded **2r** and **2s**, respectively, with nearly equal mDP receptor affinity. Compound **2r** exhibited nearly 1.5-fold lower hDP receptor affinity relative to **2l**, while **2s** showed 5-fold weaker hDP receptor affinity. With regard to hDP receptor antagonist activity, that of **2r** was 1.5-fold higher relative to **2l**, while that of **2s** was nearly 1.8-fold lower. As a result, **2r** exhibited the most potent hDP antagonist activity among the test compounds. Thus, **2r** and **2s** were identified as more optimized subtype selective hDP antagonist because of the increased affinity of **2l** for the hIP receptor.

Pharmacological evaluation of the antagonist activity of the optimized compounds was carried out as follows. Some of the selected compounds were evaluated for inhibition of the increase of vascular permeability in the guinea pig conjunctiva after oral administration.

As shown in Table 5, when chemical lead **4** was given orally (po), it caused 46% inhibition and 51% inhibition at a dose of 1 and 3 mg/kg, respectively. The optically active isomer **2r** had significantly greater potency than **4**, and achieved 60% inhibition at 0.3 mg/kg, po. The optically active *N*-methyl benzomorpholine analog **2s** was equipotent to **2r**, but exhibited more potency than **4**.

These compounds were also evaluated for their ability to inhibit the increase of vascular permeability caused by OVA (1%, 20 μL) in the guinea pig conjunctiva.

As shown in Table 6, when compounds **2r** and **2s** were given orally, they caused 21% inhibition and 40% inhibition at 10 mg/kg, respectively, while a positive control (Pyrilamine) caused 68% inhibition when a dose of 1 mg/kg was given intravenously.

Table 5. Inhibitory effects of **4**, **2r**, and **2s** on PGD_2-induced vascular permeability in guinea pig conjunctiva (n = 8). ** 0.01 versus Control with Dunnett's test

Compd	Dose (mg/kg, po)	% inhibition
4	1	46
	3	51
2r	0.3	60**
2s	0.3	61**

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.

Table 6. Inhibitory effects of **2r** and **2s** on OVA-induced vascular permeability in guinea pig conjunctiva (n = 7) *0.1 versus Control with Dunnett's test

Compd	Dose (mg/kg, po)	% inhibition
2r	10	21
2s	10	40*
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.

Based on these findings, the newly discovered DP antagonists such as **2r** and **2s** were expected to have therapeutic potential for allergic diseases.

The results of pharmacokinetic (PK) study of compound **2s** was shown in Table 7 as a representive example. When **2s** was administered orally to male rats at a dose of 10 mg/kg under fasting condition. Compound **2s** had a good pharmacokinetic profile in rats (Table 7). Administration of this compound to rats (1 mg/kg, iv; 10 mg/kg, po; $n = 3$) led to detectable plasma levels, with a $T_{1/2}$ of 9.2 h (iv) and 7.8 h (po). The volume of distribution (Vss) was calculated to be 1408 mL/kg, indicating distribution to the tissues. Systemic clearance (CL) was 2.10 mL/min/kg. The peak plasma level (C_{max}) was 4371 μg/mL at 0.7 h, and the oral bioavailability was 48% at a dose of 10 mg/kg. The AUC was 8.69 μg h/mL (iv) and 42.1 μg h/mL (po).

4. Summary

Further optimization of the new chemical leads **1** and **4** was successfully achieved. Modification of these chemical leads was concentrated on their terminal phenyl moiety, which was still not optimized. The *N*-methyl benzomorpholine derivative **2k** showed the most potent hDP receptor antagonist activity among the racemic compounds tested. Optically active **2r** and **2s** were identified as more optimum hDP receptor selective

Table 7. Pharmacokinetic data for **2s** in rats

	Dose (mg/kg)	AUC_{inf} (μg h/mL)	T_{max} (h)	C_{max} (μg/mL)	CL_{tot} (mL/min/kg)	$T_{1/2}$ (h)	Vss (mL/kg)	BA (%)
iv	1	8.69			2.1	9.2	1408	
po	10	42.1	0.7	4371		7.8		48

antagonists relative to **2l** because both showed weaker hIP receptor affinity. Some of the selected compounds were evaluated in animal models, and compound **2s** exhibited good biological and PK profiles.

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 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. 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), acetic acid (AcOH), triethylamine (TEA) and diisopropylethylamine (DIPEA).

5.2. General procedure for the preparation of *N*-(*p*-(2-arylethyloxy)benzoyl)-2-methyl-indole-4-acetic acids (9a–i)

5.2.1. Allyl (2-methyl-1-{4-[2-(2-methylphenyl)ethoxy]-benzoyl}-1*H*-indol-4-yl)acetate (9d). To a stirred mixture of **6** (100 mg, 0.436 mmol) and NaOH (powdered, 87.2 mg, 2.18 mmol) in CH_2Cl_2 (5 mL) were added TBACl (6 mg, 0.02 mmol) and *p*-((2-(*o*-methylphenyl)ethyloxy))benzoyl chloride (180 mg, 0.655 mmol) at room temperature. After stirring for 30 min at room temperature, the reaction mixture was quenched with 2 M HCl aq and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over Na_2SO_4 and evaporated to afford the residue, which was purified by column chromatography on silica gel to yield **9d** (107 mg, 53%); TLC R_f = 0.38 (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, J = 8.8 Hz, 2H), 7.22–7.17 (m, 4H), 7.02–6.92 (m, 5H), 6.50 (s, 1H), 6.01–5.81 (m, 1H), 5.31–5.18 (m, 2H), 4.61 (dt, J = 5.6, 1.4 Hz, 2H), 4.23 (t, J = 7.2 Hz, 2H), 3.86 (s, 2H), 3.16 (t, J = 7.2 Hz, 2H), 2.44 (s, 3H), 2.40 (s, 3H); MS (APCI, Pos, 20V.) m/z 468 $(M+H)^+$.

5.2.2. Allyl (1-{4-[2-(2-methoxyphenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (9a). 65% yield; TLCC R_f = 0.35 (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 7.2 Hz, 2H), 7.04–6.90 (m, 7H), 6.50 (s, 1H), 6.02–5.80 (m, 1H), 5.32–5.17 (m, 2H), 4.63–4.60 (m, 2H), 4.24 (t, J = 7.4 Hz, 2H), 3.85 (s, 2H), 3.84 (s, 3H), 3.12 (t, J 7.4 Hz, 2H), 2.45 (s, 3H).

5.2.3. Allyl (1-{4-[2-(3-Methoxyphenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (9b). 70% yield; TLC R_f = 0.37 (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, J = 8.8 Hz, 2H), 7.44–7.36 (m, 1H), 7.27–7.20 (m, 2H), 7.06–6.90 (m, 6H), 6.50 (s, 1H), 6.02–5.79 (m, 1H), 5.32–5.18 (m, 2H), 4.63–4.60 (m, 2H), 4.24 (t, J = 7.0 Hz, 2H), 3.88 (s, 5H), 3.15 (t, J = 7.0 Hz, 2H), 2.45 (s, 3H).

5.2.4. Allyl (2-methyl-1-{4-[2-(3-methylphenyl)ethoxy]-benzoyl}-1*H*-indol-4-yl)acetate (9e). 81% yield; TLC R_f = 0.40 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J = 9.0 Hz, 2H), 7.22–6.92 (m, 9H), 6.50 (s, 1H), 6.01–5.81 (m, 1H), 5.32–5.18 (m, 2H), 4.61 (dt, J = 5.4, 1.2 Hz, 2H), 4.25 (t, J = 7.0 Hz, 2H), 3.86 (s, 2H), 3.10 (t, J = 7.0 Hz, 2H), 2.44 (d, J = 1.0 Hz, 3H), 2.40 (s, 3H); **MS** (APCI, Pos, 20V.) m/z 468 $(M+H)^+$.

5.2.5. Allyl (2-methyl-1-{4-[2-(4-methylphenyl)ethoxy]-benzoyl}-1*H*-indol-4-yl)acetate (9f). 34% yield; TLC R_f = 0.25 (*n*-hexane/EtOAc, 4/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J = 8.6 Hz, 2H), 7.18–7.16 (m, 4H), 7.02–6.92 (m, 5H), 6.50 (s, 1H), 6.01–5.81 (m, 1H), 5.32–5.18 (m, 2H), 4.61 (dt, J = 5.6, 1.4 Hz, 2H), 4.23 (t, J = 7.2 Hz, 2H), 3.86 (s, 2H), 3.10 (t, J = 7.2 Hz, 2H), 2.44 (d, J = 0.8 Hz, 3H), 2.34 (s, 3H); MS (APCI, Pos, 20V.) m/z 468 $(M+H)^+$.

5.2.6. Allyl (1-{4-[2-(2-fluorophenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (9g). 87% yield; TLC R_f = 0.47 (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.68 (d, J = 8.8 Hz, 2H), 7.40–7.10 (m, 4H), 7.06–6.89 (m, 5H), 6.49 (s, 1H), 6.01–5.80 (m, 1H), 5.32–5.18 (m, 2H), 4.63–4.60 (m, 2H), 4.25 (t, J 6.9 Hz, 2H), 3.86 (s, 2H), 3.18 (t, J = 6.9 Hz, 2H), 2.42 (s, 3H).

5.2.7. Allyl (1-{4-[2-(3-fluorophenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (9h). 80% yield; TLC R_f = 0.45 (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J = 8.8 Hz, 2H), 7.38–7.20 (m, 2H), 7.09–6.85 (m, 7H), 6.49 (s, 1H), 6.01–5.81 (m, 1H), 5.33–5.16 (m, 2H), 4.63–4.60 (m, 2H), 4.25 (t, J = 6.6 Hz, 2H), 3.86 (s, 2H), 3.13 (t, J = 6.6 Hz, 2H), 2.42 (s, 3H).

5.2.8. Allyl (1-{4-[2-(4-fluorophenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (9i). 90% yield; TLC R_f = 0.50 (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.72–7.63 (m, 2H), 7.23–7.17 (m, 2H), 7.06–6.82 (m, 7H), 6.49 (s, 1H), 6.02–5.80 (m, 1H), 5.35–5.17 (m, 2H), 4.63–4.59 (m, 2H), 4.21 (t, J = 7.0 Hz, 2H), 3.88 (s, 2H), 3.08 (t, J = 7.0 Hz, 2H), 2.42 (s, 3H).

5.2.9. Benzyl (1-{4-[2-(4-methoxyphenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (9c). 28% yield; TLC $R_f = 0.68$ (*n*-hexane/EtOAc, 5/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.73–7.65 (m, 2H), 7.40–7.16 (m, 7H), 7.06–6.83 (m, 7H), 6.45 (s, 1H), 5.15 (s, 2H), 4.20 (t, $J = 7.0$ Hz, 2H), 3.86 (s, 2H), 3.80 (s, 3H), 3.08 (t, $J = 7.0$ Hz, 2H), 2.42 (s, 3H).

5.3. General procedure for the preparation of *N*-(*p*-(2-arylethyloxy)benzoyl)-2-methyl-indole-4-acetic acids (5a, b, d–i)

5.3.1. (2-Methyl-1-{4-[2-(2-methylphenyl)ethoxy]benzoyl}-1*H*-indol-4-yl)acetic acid (5d). To a stirred solution of **9d** (107 mg, 0.229 mmol) in THF (5 mL) was added $Pd(PPh_3)_4$ (26 mg, 0.022 mmol) at room temperature under argon atmosphere. After stirring for 15 min, morpholine (0.10 mL, 1.2 mmol) was added. After 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 a residue, which was purified by column chromatography on silica gel to yield **5d** (45 mg, 48%); TLC $R_f = 0.50$ ($CHCl_3/CH_3OH$, 9/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, $J = 9.0$ Hz, 2H), 7.23–6.91 (m, 9H), 6.48 (s, 1H), 4.23 (t, $J = 7.2$ Hz, 2H), 3.86 (s, 2H), 3.16 (t, $J = 7.2$ Hz, 2H), 2.44 (s, 3H), 2.39 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 426 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{25}NO_4$: 427.1784; found: 427.1765; IR (KBr) 3419, 2926, 1684, 1605, 1574, 1510, 1434, 1370, 1300, 1256, 1221, 1169, 1019, 912, 778, 760, 642, 615 cm^{-1}.

5.3.2. (1-{4-[2-(2-Methoxyphenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (5a). 42% yield; TLC $R_f = 0.45$ ($CHCl_3/CH_3OH$, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, $J = 8.8$ Hz, 2H), 7.22 (d, $J = 7.2$ Hz, 2H), 7.04–6.93 (m, 7H), 6.47 (s, 1H), 4.24 (t, $J = 7.4$ Hz, 2H), 3.85 (s, 2H), 3.85 (s, 3H), 3.14 (t, $J = 7.4$ Hz, 2H), 2.43 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 442 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{25}NO_5$: 443.1733; found:443.1732; IR (KBr) 2926, 1684, 1604, 1435, 1299, 1255, 756 cm^{-1}.

5.3.3. (1-{4-[2-(3-Methoxyphenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (5b). 50% yield; TLC $R_f = 0.35$ (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.68 (m, 2H), 7.44–7.36 (m, 1H), 7.28–7.22 (m, 2H), 7.06–6.88 (m, 6H), 6.48 (s, 1H), 4.24 (t, $J = 7.0$ Hz, 2H), 3.86 (s, 5H), 3.15 (t, $J = 7.0$ Hz, 2H), 2.44 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 442 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{25}NO_5$: 443.1733; found: 443.1734; IR (neat) 2936, 1682, 1602, 1494, 1433, 1369, 1253, 1168, 1019, 910, 731, 642, 520 cm^{-1}.

5.3.4. (2-Methyl-1-{4-[2-(3-methylphenyl)ethoxy]benzoyl}-1*H*-indol-4-yl)acetic acid (5e). 42% yield; TLC $R_f = 0.50$ (CH_3Cl/MeOH, 9/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, $J = 8.8$ Hz, 2H), 7.19–6.92 (m, 9H), 6.48 (s, 1H), 4.24 (t, $J = 7.2$ Hz, 2H), 3.86 (s, 2H), 3.10 (t, $J = 7.2$ Hz, 2H), 2.44 (s, 3H), 2.36 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 426 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{25}NO_4$: 427.1784; found: 427.1774; IR (KBr) 2924, 1683, 1604, 1574, 1510, 1434, 1370, 1299, 1256, 1221, 1169, 1025, 912, 779, 760, 701, 642, 542 cm^{-1}.

5.3.5. (2-Methyl-1-{4-[2-(4-methylphenyl)ethoxy]benzoyl}-1*H*-indol-4-yl)acetic acid (5f). 34% yield; TLC $R_f = 0.50$ (CH_3Cl/MeOH, 9/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, $J = 8.8$ Hz, 2H), 7.18–6.92 (m, 9H), 6.49 (s, 1H), 4.23 (t, $J = 6.6$ Hz, 2H), 3.87 (s, 2H), 3.10 (t, $J = 6.6$ Hz, 2H), 2.44 (s, 3H), 2.34 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 426 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{25}NO_4$: 427.1784; found: 427.1783; IR (KBr) 3419, 2923, 1696, 1680, 1606, 1511, 1435, 1371, 1318, 1255, 1222, 1172, 1021, 912, 811, 761, 639, 613, 554 cm^{-1}.

5.3.6. (1-{4-[2-(2-Fluorophenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (5g). 85% yield; TLC $R_f = 0.45$ (CH_3Cl/MeOH, 9/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.68 (d, $J = 8.8$ Hz, 2H), 7.40–7.08 (m, 4H), 7.05–6.90 (m, 5H), 6.47 (s, 1H), 4.26 (t, $J = 6.8$ Hz, 2H), 3.84 (s, 2H), 3.18 (t, $J = 6.8$ Hz, 2H), 2.43 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 430 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{26}H_{22}FNO_4$: 431.1533; found: 431.1539; IR (KBr) 2927, 1685, 1605, 1494, 1434, 1301, 1256, 1170 cm^{-1}.

5.3.7. (1-{4-[2-(3-Fluorophenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (5h). 86% yield; TLC $R_f = 0.45$ (CH_3Cl/MeOH, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, $J = 8.8$ Hz, 2H), 7.35–7.20 (m, 2H), 7.09–6.85 (m, 7H), 6.47 (s, 1H), 4.25 (t, $J = 6.6$ Hz, 2H), 3.84 (s, 2H), 3.12 (t, $J = 6.6$ Hz, 2H), 2.42 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 430 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{26}H_{22}FNO_4$: 431.1533; found: 431.1539; IR (KBr) 2928, 1686, 1605, 1510, 1434, 1256, 1170, 1142, 1027 cm^{-1}.

5.3.8. (1-{4-[2-(4-Fluorophenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (5i). 76% yield; TLC $R_f = 0.45$ (CH_3Cl/MeOH, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.72–7.65 (m, 2H), 7.23–7.17 (m, 2H), 7.06–6.84 (m, 7H), 6.47 (s, 1H), 4.21 (t, $J = 7.0$ Hz, 2H), 3.85 (s, 2H), 3.80 (s, 3H), 3.08 (t, $J = 7.0$ Hz, 2H), 2.43 (s, 3H); MS (APCI, Neg, 20V.) *m*/*z* 430 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{26}H_{22}FNO_4$: 431.1533; found: 431.1532; IR (neat) 2926, 1684, 1605, 1510, 1434, 1370, 1300, 1256, 1222, 1170, 760 cm^{-1}.

5.4. (1-{4-[2-(4-Methoxyphenyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (5c)

To a stirred solution of the compound **9c** (110 mg, 206 mmol) in EtOAc (1 mL) and MeOH (2 mL) was added 10 wt% Pd–C (10 mg) at room temperature. The resulting suspension was 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 **5c** (25.2 mg, 28%); TLC $R_f = 0.50$ ($CHCl_3/CH_3OH$, 10/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.72–7.65 (m, 2H), 7.23–7.17 (m, 2H), 7.06–6.84 (m, 7H), 6.47 (s, 1H), 4.21 (t, $J = 7.0$ Hz, 2H), 3.85 (s, 2H), 3.80 (s, 3H), 3.08 (t, $J = 7.0$ Hz, 2H), 2.43 (s, 3H); MS (APCI,

Neg 20V) *m/z* 442 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{25}NO_5$: 443.1733; found: 443.1714; IR (neat) 2931, 1708, 1683, 1604, 1513, 1434, 1370, 1301, 1250, 1169, 1025, 912, 829, 759 cm^{-1}.

5.5. Benzyl {1-[4-(acetyloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (10)

To a stirred solution of **7** (15 g, 53.7 mmol) and NaOH (powdered, 10.74 g, 269 mmol) in CH_2Cl_2 (400 mL) were added TBACl (746 mg, 2.69 mmol) and *p*-acetoxy benzoyl chloride (20.4 g, 102 mmol) at room temperature. After stirring for 30 min at room temperature, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel to yield **10** (12.2 g, 51%); TLC $R_f = 0.34$ (*n*-hexane/EtOAc, 7/3); ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 8.7$ Hz, 2H), 7.40–7.20 (m, 7H), 7.08–6.92 (m, 3H), 6.47 (s, 1H), 5.15 (s, 2H), 3.88 (s, 2H), 2.40 (s, 3H), 2.35 (s, 3H).

5.6. {1-[4-(Acetyloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (11)

To a stirred solution of **10** (12.2 g, 27.6 mmol) in EtOAc (250 mL) was added $Pd(OH)_2$ (1.2 g) at room temperature. The resulting suspension was stirred for 2 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 recrystallization from EtOAc to yield **11** (7.39 g, 80%); TLC $R_f = 0.52$ ($CHCl_3/CH_3OH$, 9/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, $J = 8.7$ Hz, 2H), 7.24 (d, $J = 8.7$ Hz, 2H), 7.10–6.94 (m, 3H), 6.51 (s, 1H), 3.87 (s, 2H), 2.43 (s, 3H), 2.35 (s, 3H).

5.7. Polymer supported 2-chlorotriphenylmethyl{1-[4-(acetyloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (12)

To an agitated solution of **11** (7.77 g, 23.2 mmol) and DIPEA (16.0 mL, 92.7 mmol) in CH_2Cl_2 (200 mL) were added polymer supported 2-chlorotriphenylmethyl chloride (1.2 mmol/g, 100–200 mesh, 1% DVB) (19.3 g, 23.2 mmol) at room temperature. After agitating for 2.5 h at room temperature, the reaction mixture was filtered to collect a insoluble substance, which was washed with CH_2Cl_2 (×5).

To a suspension of this insoluble substance in CH_2Cl_2 (150 mL) were added a solution of AcOH (13.4 mL, 232 mmol), DIPEA (160 mL, 927 mmol). After agitating for 1 h at room temperature, the reaction mixture was filtered, washed with CH_2Cl_2 (×5) to afford the **12** (26.1 g, 100%); IR (KBr) 3569, 3026, 2923, 1736, 1686, 1601, 1492, 1432, 1367, 1304, 1191, 1042, 908, 826, 740, 697, 457 cm^{-1}.

5.8. Polymer supported 2-chlorotriphenylmethyl [1-(4-hydroxybenzoyl)-2-methyl-1*H*-indol-4-yl]acetate (13)

To an agitated mixture of **12** (26.1 g, 23.2 mmol) in CH_2Cl_2 (240 mL) was added piperidine (12.5 mL, 127 mmol) at room temperature. After agitating for 30 min at room temperature, the reaction mixture was filtered, washed with CH_2Cl_2 (×5), dried in vacuo to afford **13** (24.7 g, 100%); IR (KBr) 3410, 3059, 3025, 2923, 2849, 1738, 1684, 1604, 1492, 1432, 1367, 1285, 1219, 1163, 1040, 981, 957, 909, 826, 754, 735, 698, 634, 607, 589, 461 cm^{-1}.

5.9. General procedure for the preparation of *N*-(*p*-(alkyloxy)benzoyl)-2-methylindole-4-acetic acids (2a–q)

5.9.1. {1-[4-(2,3-Dihydro-1-benzofuran-2-ylmethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (2a). To an agitated mixture of **13** (500 mg, 0.470 mmol) in CH_2Cl_2 (5 mL) were added a solution of **15a** (423 mg, 2.82 mmol) in CH_2Cl_2 (1 mL), Ph_3P (740 mg, 2.82 mmol) and DEAD (40% in toluene) (1.29 mL, 2.82 mmol) at room temperature. After agitating for 2 h at room temperature, the reaction mixture was filtered to collect insoluble substance, which was washed with CH_2Cl_2 and dried in vacuo to afford **14a**.

To a stirred suspension of **14a** described above in CH_2Cl_2 (2 mL) and CF_3CH_2OH (0.6 mL) was added acetic acid (0.3 mL) at room temperature. After agitating for 2 h at room temperature, 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 **2a** (91.4 mg, 44%); TLC $R_f = 0.34$ ($CHCl_3/CH_3OH$, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.73–7.69 (m, 2H), 7.23–7.12 (m, 2H), 7.06–6.82 (m, 7H), 6.49 (d, $J = 1.2$ Hz, 1H), 5.20 (m, 1H), 4.29 (dd, $J = 9.9$, 6.3 Hz, 1H), 4.20 (dd, $J = 9.9$, 4.2 Hz, 1H), 3.86 (s, 2H), 3.42 (dd, $J = 15.9$, 9.6 Hz, 1H), 3.17 (dd, $J = 15.9$, 8.4 Hz, 1H), 2.44 (d, $J = 1.2$ Hz, 3H); MS (EI, Pos) *m/z* 441 (M^+); HRMS (EI, Pos.) calcd for $C_{27}H_{23}NO_5$: 441.1576; found: 441.1573; IR (KBr) 2925, 1684, 1604, 1509, 1481, 1434, 1369, 1257, 1169, 1041, 911, 753 cm^{-1}.

5.9.2. {1-[4-(2,3-Dihydro-1-benzofuran-3-ylmethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (2b). 21% yield; TLC $R_f = 0.50$ (CH_3Cl/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.70 (m, 2H), 7.31 (d, $J = 7.4$ Hz, 1H), 7.21 (m, 1H), 7.10–6.82 (m, 7H), 6.49 (s, 1H), 4.73 (t, $J = 9.6$ Hz, 1H), 4.55 (dd, $J = 9.6$, 4.6 Hz, 1H), 4.29–3.88 (m, 3H), 3.86 (s, 2H), 2.44 (s, 3H); MS (FAB, Pos.) *m/z* 442 $(M+H)^+$; HRMS (EI, Pos.) calcd for $C_{27}H_{23}NO_5$: 441.1576; found: 441.1566; IR (neat) 3348, 1708, 1682, 1603, 1574, 1509, 1483, 1461, 1434, 1369, 1300, 1254, 1221, 1169, 1097, 1021, 960, 911, 837, 753, 614 cm^{-1}.

5.9.3. {1-[4-(1,3-Benzodioxol-2-ylmethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (2c). 43% yield; TLC $R_f = 0.33$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.74–7.69 (m, 2H), 7.06–6.85 (m, 9H), 6.50–6.47 (m, 2H), 4.35 (d, $J = 4.2$ Hz, 2H), 3.85 (s, 2H), 2.43 (d, $J = 0.9$ Hz, 3H); MS (FAB, Pos.) *m/z* 444 $(M+H)^+$; HRMS (EI, Pos.) calcd for $C_{26}H_{21}NO_6$: 443.1369; found: 443.1360; IR (KBr) 2925, 1685, 1604, 1509, 1484, 1434, 1231, 1043, 911, 743, 612 cm^{-1}.

5.9.4. {1-[4-(3,4-Dihydro-2*H*-chromen-2-ylmethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (2d). 48% yield; TLC $R_f = 0.49$ (CH_3Cl/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.74–7.67 (m, 2H), 7.06–6.87 (m, 9H), 6.49 (s, 1H), 4.45 (m, 1H), 4.38–4.15 (m, 2H), 3.86 (s, 2H), 3.00–2.80 (m, 2H), 2.44 (s, 3H), 2.30–1.90 (m, 2H); MS (APCI, Neg. 20V) *m/z* 454 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{25}NO_5$: 455.1733; found: 455.1716; IR (KBr) 2926, 1684, 1604, 1575, 1509, 1434, 1369, 1301, 1257, 1170, 1039 cm^{-1}.

5.9.5. {1-[4-(3,4-Dihydro-2*H*-chromen-3-ylmethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (2e). 41% yield; TLC $R_f = 0.34$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.72–7.43 (m, 5H), 7.15–6.82 (m, 6H), 6.49 (s, 1H), 4.36 (m, 1H), 4.17 (m, 1H), 4.08 (d, $J = 6.9$ Hz, 2H), 3.87 (s, 2H), 3.04 (dd, $J = 16.5$, 6.0 Hz, 1H), 2.78 (dd, $J = 16.5$, 7.2 Hz, 1H), 2.63 (m, 1H), 2.44 (d, $J = 1.2$ Hz, 3H); **MS** (APCI, Neg. 20V) *m/z* 454 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{25}NO_5$: 455.1733; found: 455.1740; IR (KBr) 2924, 1683, 1604, 1509, 1490, 1435, 1369, 1255, 1169, 911, 837, 757, 723, 694, 614, 542 cm^{-1}.

5.9.6. (1-{4-[2-(2,3-Dihydro-1,4-benzodioxin-2-yl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (2f). 12% yield; TLC $R_f = 0.44$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, $J = 8.7$ Hz, 2H), 7.10–6.80 (m, 9H), 6.49 (s, 1H), 4.50–4.40 (m, 1H), 4.38–4.17 (m, 3H), 4.01 (dd, $J = 11.4$, 7.2 Hz, 1H), 3.86 (s, 2H), 2.44 (s, 3H), 2.17 (q, $J = 6.0$ Hz, 2H); MS (APCI, Neg. 20V) *m/z* 470 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{25}NO_6$: 471.1682; found: 471.1669; IR (neat) 2926, 1709, 1683, 1604, 1509, 1494, 1434, 1369, 1303, 1253, 1221, 1169, 1044, 910, 837, 749, 641 cm^{-1}.

5.9.7. {1-[4-(2,3-Dihydro-1,4-benzodioxin-2-ylmethoxy)-benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (2g). 35% yield; TLC $R_f = 0.34$ (CH_3Cl/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.71 (d, $J = 8.8$ Hz, 2H), 7.09–6.82 (m, 9H), 6.48 (s, 1H), 4.61 (m, 2H), 4.42 (dd, $J = 11.8$, 2.6 Hz, 1H), 4.38–4.18 (m, 3H), 3.84 (s, 2H), 2.43 (s, 3H); MS (APCI, Neg. 20V) *m/z* 456 $(M-H)^-$; HRMS (MALDI-TOF, Pos.) calcd for $C_{27}H_{23}NO_6+H^+$: 458.1604; found: 458.1064; IR (KBr) 3046, 2925, 1734, 1708, 1684, 1603, 1574, 1509, 1494, 1433, 1369, 1302, 1246, 1220, 1170, 1044, 958, 911, 842, 780, 751, 637, 614 cm^{-1}.

5.9.8. (1-{4-[(2*R*)-2,3-Dihydro-1,4-benzodioxin-2-ylmethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (2h). 38% yield; 98.6% ee; TLC $R_f = 0.34$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.75–7.70 (m, 2H), 7.07–6.86 (m, 9H), 6.49 (m, 1H), 4.61 (m, 1H), 4.42 (dd, $J = 11.4$, 2.1 Hz, 1H), 4.33 (dd, $J = 10.2$, 5.1 Hz, 1H), 4.26 (dd, $J = 11.4$, 6.3 Hz, 1H), 4.25 (dd, $J = 10.2$, 6.0 Hz, 1H), 3.87 (s, 2H), 2.44 (d, $J = 1.2$ Hz, 3H); MS (APCI, Neg. 20V) *m/z* 456 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{23}NO_6$: 457.1525; found: 457.1523; IR (KBr) 2927, 1685, 1604, 1494, 1434, 1369, 1248, 1170, 1044, 910, 751, 542 cm^{-1}.

5.9.9. (1-{4-[(2*S*)-2,3-Dihydro-1,4-benzodioxin-2-ylmethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (2i). 24% yield; 98.6% ee; TLC $R_f = 0.30$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.75–7.70 (m, 2H), 7.07–6.86 (m, 9H), 6.49 (m, 1H), 4.61 (m, 1H), 4.42 (dd, $J = 11.4$, 2.1 Hz, 1H), 4.33 (dd, $J = 10.2$, 5.1 Hz, 1H), 4.26 (dd, $J = 11.4$, 6.3 Hz, 1H), 4.25 (dd, $J = 10.2$, 6.0 Hz, 1H), 3.87 (s, 2H), 2.44 (d, $J = 1.2$ Hz, 3H); MS (FAB, Pos.) *m/z* 458 $(M+H)^+$; HRMS (EI, Pos.) calcd for $C_{27}H_{23}NO_6$: 457.1525; found: 457.1540; IR (KBr) 2925, 1685, 1604, 1495, 1434, 1369, 1248, 1171, 1045, 911, 841, 752, 615 cm^{-1}.

5.9.10. {1-[4-(3,4-Dihydro-2*H*-1,4-benzoxazin-2-ylmethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (2j). 28% yield; TLC $R_f = 0.25$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.76–7.40 (m, 3H), 7.10–6.89 (m, 4H), 6.89–6.60 (m, 4H), 6.49 (s, 1H), 4.60 (m, 1H), 4.31 (dd, $J = 9.8$, 5.0 Hz, 1H), 4.24 (dd, $J = 9.8$, 6.2 Hz, 1H), 3.86 (s, 2H), 3.58 (dd, $J = 11.8$, 3.0 Hz, 1H), 3.42 (dd, $J = 11.8$, 6.6 Hz, 1H), 2.44 (s, 3H); MS (APCI, Neg. 20V) *m/z* 455 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{27}H_{24}N_2O_5$: 456.1685; found: 456.1687; IR (neat) 3384, 2924, 1681, 1603, 1574, 1502, 1433, 1369, 1301, 1256, 1215, 1169, 1120, 1038, 958, 910, 836, 779, 729, 695, 639, 616, 541 cm^{-1}.

5.9.11. (2-Methyl-1-{4-[(4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)methoxy]benzoyl}-1*H*-indol-4-yl)acetic acid (2k). 28% yield; TLC $R_f = 0.26$ (CH_3Cl/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.78–7.40 (m, 3H), 7.10–6.78 (m, 6H), 6.73 (d, $J = 8.0$ Hz, 2H), 6.49 (s, 1H), 4.68 (m, 1H), 4.31 (dd, $J = 10.0$, 5.2 Hz, 1H), 4.20 (dd, $J = 10.0$, 6.4 Hz, 1H), 3.86 (s, 2H), 3.41 (dd, $J = 11.6$, 2.8 Hz, 1H), 3.27 (dd, $J = 11.6$, 6.6 Hz, 1H), 2.92 (s, 3H), 2.44 (s, 3H); MS (APCI, Neg. 20V) *m/z* 469 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{26}N_2O_5$: 470.1842; found: 470.1836; IR (neat) 2924, 2359, 1683, 1604, 1505, 1434, 1368, 1299, 1254, 1221, 1170, 1041, 959, 911, 837, 727, 542 cm^{-1}.

5.9.12. [2-Methyl-1-(4-{[(2*S*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)-1*H*-indol-4-yl]acetic acid (2l). 30% yield; TLC $R_f = 0.26$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, $J = 9.0$ Hz, 2H), 7.20–6.78 (m, 7H), 6.78–6.64 (m, 2H), 6.49 (s, 1H), 4.68 (m, 1H), 4.30 (dd, $J = 9.9$, 5.4 Hz, 1H), 4.21 (dd, $J = 9.9$, 6.0 Hz, 1H), 3.86 (s, 2H), 3.40 (dd, $J = 11.4$, 2.7 Hz, 1H), 3.27 (dd, $J = 11.4$, 6.6 Hz, 1H), 2.92 (s, 3H), 2.44 (s, 3H); MS (APCI, Neg. 20V) *m/z* 469 $(M-H)^-$; HRMS (MALDI-TOF, Pos.) calcd for $C_{28}H_{26}N_2O_5+H^+$: 471.1920; found: 470.1914; IR (KBr) 2923, 1699, 1604, 1506, 1434, 1368, 1240, 1172, 1043, 962, 844, 745 cm^{-1}.

5.9.13. [2-Methyl-1-(4-{[(2*R*)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)-1*H*-indol-4-yl]acetic acid (2m). 48% yield; TLC $R_f = 0.26$ (CH_3Cl/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.72 (d, $J = 8.8$ Hz, 2H), 7.12–6.79 (m, 7H), 6.72 (d, $J = 7.4$ Hz, 2H), 6.49 (s, 1H), 4.68 (m, 1H), 4.31 (dd, $J = 9.6$, 5.2 Hz, 1H), 4.20 (dd, $J = 9.6$, 6.2 Hz, 1H), 3.87 (s, 2H), 3.40 (dd, $J = 11.6$, 2.8 Hz, 1H), 3.28 (dd, $J = 11.6$, 6.2 Hz, 1H),

2.92 (s, 3H), 2.45 (s, 3H); MS (APCI, Neg. 20V) *m/z* 469 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{26}N_2O_5$: 470.1842; found: 470.1846; IR (neat) 2925, 1708, 1682, 1604, 1504, 1433, 1368, 1299, 1255, 1220, 1170, 1136, 1041, 958, 910, 836, 735, 616 cm^{-1}.

5.9.14. (1-{4-[(4-Ethyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)methoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (2n). 35% yield; TLC $R_f = 0.28$ (CH_3Cl/MeOH, 10/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.80–7.57 (m, 2H), 7.10–6.79 (m, 7H), 6.79–6.56 (m, 2H), 6.49 (s, 1H), 4.68–4.50 (m, 1H), 4.31 (dd, $J = 9.6$, 5.2 Hz, 1H), 4.21 (dd, $J = 9.6$, 6.2 Hz, 1H), 3.86 (s, 2H), 3.57–3.20 (m, 4H), 2.44 (s, 3H), 1.17 (t, $J = 7.4$ Hz, 3H); MS (APCI, Neg. 20V) *m/z* 483 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{29}H_{28}N_2O_5$: 484.1998; found: 484.2000; IR (neat) 2970, 2925, 1708, 1684, 1604, 1576, 1503, 1434, 1368, 1300, 1255, 1219, 1169, 1043, 958, 910, 836, 779, 741, 614, 542 cm^{-1}.

5.9.15. (1-{4-[(1,4-Dimethyl-1,2,3,4-tetrahydroquinoxalin-2-yl)methoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (2o). 10% yield; TLC $R_f = 0.48$ (CH_3Cl/MeOH, 9/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, $J = 9.0$ Hz, 2H), 7.08–6.92 (m, 5H), 6.80–6.66 (m, 2H), 6.62–6.48 (m, 3H), 4.27–4.07 (m, 2H), 3.87 (s, 2H), 3.83–3.73 (m, 1H), 3.36–3.20 (m, 2H), 3.05 (s, 3H), 2.88 (s, 3H), 2.45 (s, 3H); MS (APCI, Neg. 20V) *m/z* 482 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{29}H_{29}N_3O_4$: 483.2158; found: 483.2161; IR (neat) 2925, 1682, 1602, 1508, 1433, 1369, 1298, 1254, 1221, 1169, 1022, 911, 737 cm^{-1}.

5.9.16. (2-Methyl-1-{4-[(1-methyl-2,3-dihydro-1*H*-indol-2-yl)methoxy]benzoyl}-1*H*-indol-4-yl)acetic acid (2p). 45% yield; TLC $R_f = 0.49$ (CH_3Cl/MeOH, 9/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.76–7.66 (m, 2H), 7.20–6.46 (m, 10H), 5.00–2.80 (m, 5H), 3.87 (s, 2H), 2.94 and 2.91 (s × 2, total 3H), 2.45 (s, 3H); MS (APCI, Neg. 20V) *m/z* 453 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{26}N_2O_4$: 454.1893; found: 454.1893; IR (neat) 2925, 2360, 1708, 1683, 1603, 1573, 1507, 1433, 1368, 1300, 1254, 1221, 1168, 1036, 958, 910, 837, 779, 733, 668, 614 cm^{-1}.

5.9.17. (2-Methyl-1-{4-[(1-methyl-2,3-dihydro-1*H*-indol-3-yl)methoxy]benzoyl}-1*H*-indol-4-yl)acetic acid (2q). 43% yield; TLC $R_f = 0.47$ (CH_3Cl/MeOH, 9/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.74–7.68 (m, 3H), 7.22–7.10 (m, 2H), 7.08–6.90 (m, 4H), 6.72 (t, $J = 6.6$ Hz, 1H), 6.53 (d, $J = 8.1$ Hz, 1H), 6.49 (s, 1H), 4.24–4.06 (m, 2H), 3.87 (s, 2H), 3.78–3.70 (m, 1H), 3.49 (t, $J = 8.1$ Hz, 1H), 3.38 (dd, $J = 9.0$, 5.1 Hz, 1H), 2.79 (s, 3H), 2.45 (s, 3H); MS (APCI, Neg. 20V) *m/z* 453 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{26}N_2O_4$: 454.1893; found: 454.1896; IR (neat) 3376, 1682, 1604, 1493, 1434, 1369, 1300, 1254, 1168, 1022, 910, 727, 542 cm^{-1}.

5.9.18. [2-Methyl-1-(4-{[(2*S*)-7-fluoro-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)-1*H*-indol-4-yl]acetic acid (2r). 33% yield; TLC $R_f = 0.49$ (CH_3Cl/MeOH, 9/1); ^1H NMR (MHz, $CDCl_3$) δ 7.72 (d, $J = 8.7$ Hz, 2H), 7.08–6.85 (m, 5H), 6.60 (d, $J = 8.7$ Hz, 3H), 6.49 (s, 1H), 4.73–4.64 (m, 1H), 4.30 (dd, $J = 9.9$, 5.1 Hz, 1H), 4.21 (dd, $J = 9.9$, 6.3 Hz, 1H), 3.87 (s, 2H), 3.36 (dd, $J = 11.7$, 2.7 Hz, 1H), 3.22 (dd, $J = 11.7$, 6.6 Hz, 1H), 2.88 (s, 3H), 2.45 (s, 3H); MS (APCI, Neg. 20V) *m/z* 487 $(M-H)^-$; HRMS (EI, Pos.) calcd for $C_{28}H_{25}FN_2O_5$: 488.1748; found: 488.1741; IR (KBr) 3438, 2925, 1684, 1603, 1574, 1512, 1434, 1369, 1299, 1254, 1170, 1151, 1039, 984, 959, 911, 846, 780, 760, 703, 615 cm^{-1}.

5.9.19. [2-Methyl-1-(4-{[(2*S*)-4, 6-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methoxy}benzoyl)-1*H*-indol-4-yl]-acetic acid (2s). 32% yield; TLC $R_f = 0.37$ (CH_3Cl/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.74–7.69 (m, 2H), 7.06–6.82 (m, 5H), 6.73 (d, $J = 8.1$ Hz, 1H), 6.54–6.47 (m, 3H), 4.64 (m, 1H), 4.30 (dd, $J = 9.9$, 5.1 Hz, 1H), 4.20 (dd, $J = 9.9$, 6.3 Hz, 1H), 3.86 (s, 2H), 3.39 (dd, $J = 11.4$, 2.7 Hz, 1H), 3.26 (dd, $J = 11.4$, 6.6 Hz, 1H), 2.90 (s, 3H), 2.45 (d, $J = 0.9$ Hz, 3H), 2.28 (s, 3H); MS (EI, Pos.) *m/z* 484 (M^+); Anal. Calcd for $C_{29}H_{28}N_2O_5$: C, 71.88; H, 5.82; N, 5.78. Found: C, 71.95; H, 5.91; N, 5.85; IR (KBr) 3430, 2921, 1699, 1666, 1604, 1576, 1510, 1478, 1435, 1419, 1368, 1338, 1288, 1240, 1189, 1171, 1136, 1065, 1039, 1015, 963, 912, 845, 807, 777, 758, 744, 706, 677, 641, 607, 591, 554, 521, 461 cm^{-1}.

5.10. Methyl 1,3-benzodioxole-2-carboxylate (17)

To a stirred suspension of sodium hydride (60% oil dispersion, 8.0 g, 210 mmol) were added **16** (11.0 g, 100 mmol) in DMF (400 mL) and methyl dichloroacetate (52 mL, 500 mmol) at room temperature under argon atmosphere. After stirring for 3 h at 95 °C, the reaction mixture was quenched with 2 M HCl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, then 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 **17** (4.86 g, 27%); TLC $R_f = 0.43$ (*n*-hexane/EtOAc, 3/1; ^1H NMR (300 MHz, $CDCl_3$) δ 6.90–6.87 (m, 4H), 6.33 (s, 1H), 3.86 (s, 3H).

5.11. 1,3-Benzodioxol-2-ylmethanol (15c)

To a stirred suspension of lithium aluminium hydride (760 mg, 20.0 mmol) in THF (10 mL) was added a solution of **17** (3.0 g, 16.7 mmol) in THF (20 mL) at 0 °C. After stirring for 20 min at room temperature, the reaction mixture was diluted with ether (50 mL) and quenched with saturated aqueous sodium sulfate under cooling. The resulting mixture was stirred for additional 1 h at room temperature. Insoluble substances were removed by filtration and the filtrate was evaporated to give a crude product, which was purified by column chromatography on silica gel to yield **15c** (602 mg, 8% for 2 steps); TLC $R_f = 0.39$ (*n*-hexane/EtOAc, 2/1); ^1H NMR (300 MHz, $CDCl_3$) δ 6.83–6.81 (m, 4H), 6.18 (t, $J = 3.6$ Hz, 1H), 3.93 (dd, $J = 6.6$, 3.6 Hz, 2H), 1.88 (t, $J = 6.6$ Hz, 1H).

5.12. (4-Methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-methanol (15k)

To a stirred solution of **18** (4.47 g, 21.6 mmol) in acetone (50 mL) were added K_2CO_3 (5.96 g, 43.1 mmol) and methyl iodide (2.7 mL, 43.1 mmol) at room temperature under argon atmosphere, and stirring was continued for 16 h at 40 °C. The reaction mixture was diluted with EtOAc/*n*-hexane. The resulting precipitates were removed by filtration. The filtrate was concentrated in vacuo, to give a residue, which was used for the next reaction without further purification.

To a suspension of lithium aluminium hydride (1.59 g, 42 mmol) in THF (20 mL) was added a solution of above-described ethyl ester (4.78 g, 21.6 mmol) in THF (100 mL) at room temperature. After stirring for 1 h at room temperature, the reaction mixture was diluted with ether and quenched with saturated aqueous sodium sulfate at room temperature. The resulting mixture was stirred for additional 1 h at room temperature. Insoluble substance was removed by filtration and the filtrate was evaporated to give a crude product, which was purified by column chromatography on silica gel to yield **15k** (2.80 g, 75%); TLC $R_f = 0.10$ (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (200 MHz, $CDCl_3$) 7.00–6.60 (m, 4H), 4.35 (m, 1H), 4.00–3.70 (m, 2H), 3.33–3.10 (m, 2H), 2.88 (s, 3H), 2.01 (m, 1H).

5.13. [(2*R*)-4-Methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methanol (15m)

To a stirred solution of **19** (3.60 g, 14.1 mmol) in acetone (50 mL) were added K_2CO_3 (5.90 g, 42.3 mmol) and methyl iodide (2.7 mL, 42.3 mmol) at room temperature under argon atmosphere, and stirring was continued for 3 h at 40 °C. The reaction mixture was diluted with EtOAc/*n*-hexane. The resulting precipitates were removed by filtration. The filtrate was concentrated in vacuo, to give a residue, which was used for the next reaction without further purification.

To a stirred solution of above-described benzyl ether in MeOH (30 mL) were added 10 wt% Pd–C (0.30 g) and 10% HCl–MeOH (10 mL) at room temperature. The resulting suspension was refluxed for 2 h 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 **15m** (0.590 g, 23%); TLC $R_f = 0.10$ (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.00–6.60 (m, 4H), 4.35 (m, 1H), 4.00–3.70 (m, 2H), 3.33–3.10 (m, 2H), 2.88 (s, 3H), 2.01 (m, 1H).

5.14. (4-Ethyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-methanol (15n)

To a stirred solution of **15j** (1.65 g, 10 mmol) in DMF (10 mL) were added K_2CO_3 (2.7 g, 20 mmol) and ethyl iodide (1.6 mL, 20 mmol) at room temperature under argon atmosphere, and stirring was continued for 16 h at 50 °C. The reaction mixture was diluted with EtOAc/*n*-hexane. The resulting precipitates were 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 **15n** (1.32 g, 68%); TLC $R_f = 0.30$ (*n*-hexane/EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.00–6.50 (m, 4H), 4.35 (m, 1H), 4.05–3.70 (m, 2H), 3.50–3.00 (m, 4H), 2.20–1.90 (m, 1H), 1.13 (t, $J = 7.5$ Hz, 3H).

5.15. 1,2,3,4-Tetrahydroquinoxalin-2-ylmethanol (2l)

To a stirred solution of **20** (500 mg, 2.47 mmol) in THF (3 mL) and MeOH (6 mL) were added sodium borohydride (196 mg, 5.19 mmol) portionwise at 0 °C. After stirring for 1 h, the reaction mixture was quenched with saturated aqueous ammonium chloride, 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 residue, which was purified by column chromatography on silica gel to yield **21** (180 mg, 44%); TLC $R_f = 0.24$ ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.66–6.48 (m, 4H), 3.73 (dd, $J = 10.5$, 4.8 Hz, 1H), 3.68–3.52 (m, 2H), 3.34 (dd, $J = 10.5$, 3.0 Hz, 1H), 3.24 (dd, $J = 10.5$, 6.0 Hz, 1H), 3.10–2.60 (br, 2H).

5.16. (1,4-Dimethyl-1,2,3,4-tetrahydroquinoxalin-2-yl)methanol (15o)

To a stirred solution of **21** (180 mg, 1.10 mmol) in acetone (10 mL) were added K_2CO_3 (455 mg, 3.29 mmol) and methyl iodide (0.40 mL, 6.58 mmol) at room temperature under argon atmosphere, and stirring was continued for 16 h at 40 °C. 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 a residue, which was purified by column chromatography on silica gel to yield **15o** (60 mg, 28%); TLC $R_f = 0.20$ (*n*-hexane/EtOAc, 7/3); ^{1} H NMR (200 MHz, $CDCl_3$) δ 6.85–6.73 (m, 1H), 6.72–6.53 (m, 3H), 3.86 (dd, $J = 10.8$, 6.0 Hz, 1H), 3.75 (dd, $J = 10.8$, 3.9 Hz, 1H), 3.46–3.36 (m, 1H), 3.27–3.18 (m, 1H), 3.16–3.07 (m, 1H), 3.00 (s, 3H), 2.88 (s, 3H).

5.17. Methyl 1-methylindoline-2-carboxylate (23)

To a stirred solution of **22** (2.0 g, 12.26 mmol) in acetone (20 mL) were added K_2CO_3 (6.78 g, 49.0 mmol) and methyl iodide (3.1 mL, 49.0 mmol) at room temperature under argon atmosphere. After stirring for 16 h at room temperature, the reaction mixture was diluted with EtOAc/*n*-hexane. The resulting precipitates were removed by filtration. The filtrate was concentrated in vacuo, to give a residue, which was purified by column chromatography on silica gel to yield **23** (1.70 g, 73%); TLC $R_f = 0.70$ (*n*-hexane/EtOAc, 7/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (t, $J = 7.5$ Hz, 1H), 7.05 (d, $J = 7.5$ Hz, 1H), 6.70 (t, $J = 7.5$ Hz, 1H), 6.51 (d, $J = 7.5$ Hz, 1H), 4.05 (t, $J = 9.6$ Hz, 1H), 3.80 (s, 3H), 3.34 (dd, $J = 15.6$, 9.6 Hz, 1H), 3.13 (dd, $J = 15.6$, 9.6 Hz, 1H), 2.84 (s, 3H).

5.18. (1-Methyl-2,3-dihydro-1*H*-indol-2-yl)methanol (15p)

To a stirred suspension of lithium aluminium hydride (675 mg, 17.8 mmol) in THF (20 mL) was added a solution of **23** (1.70 g, 8.89 mmol) in THF (10 mL) at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was diluted with ether and quenched with saturated aqueous sodium sulfate under cooling. The resulting mixture was stirred for additional 1 h at room temperature. Insoluble substance was removed by filtration and the filtrate was evaporated to give a crude product, which was purified by column chromatography on silica gel to yield **15p** (1.30 g, 90%); TLC $R_f = 0.30$ (*n*-hexane/EtOAc, 7/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–7.05 (m, 2H), 6.73 (t, $J = 7.5$ Hz, 1H), 6.55 (d, $J = 7.5$ Hz, 1H), 3.95 (dd, $J = 11.7$, 3.6 Hz, 1H), 3.74–3.60 (m, 1H), 3.56–3.44 (m, 1H), 3.16–2.96 (m, 2H), 2.77 (s, 3H), 1.94–1.76 (m, 1H).

5.19. (1-Methyl-2,3-dihydro-1*H*-indol-3-yl)methanol (15q)

To a stirred suspension of lithium aluminium hydride (276 mg, 7.20 mmol) in ether (7 mL) was added a solution of **23** (600 mg, 2.40 mmol) in ether (3 mL) at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was diluted with ether and quenched with saturated aqueous sodium sulfate under cooling. The resulting mixture was stirred for additional 1 h at room temperature. Insoluble substance was removed by filtration and the filtrate was evaporated to give a crude product, which was purified by column chromatography on silica gel to yield **15q** (350 mg, 89%); TLC $R_f = 0.23$ (*n*-hexane/EtOAc, 7/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–7.08 (m, 3H), 6.68 (dt, $J = 0.9$, 7.5 Hz, 1H), 6.49 (d, $J = 7.5$ Hz, 1H), 3.85–3.72 (m, 2H), 3.52–3.34 (m, 2H), 3.32–3.22 (m, 1H), 2.75 (s, 3H); MS (APCI, Pos, 20V.) *m/z* 164 $(M+H)^+$.

5.20. General procedure for the preparation of 26a and 26b

5.20.1. *N*-(2,4-Difluorophenyl)-4-methylbenzenesulfonamide (26a). To a stirred solution of **25a** (20.8 g, 160 mmol) and pyridine (15.6 mL, 190 mmol) in CH_2Cl_2 (160 mL) were added *p*-toluenesulfonyl chloride (32.2 g, 170 mmol) at room temperature. After stirring for 16 h at room temperature, the reaction mixture was quenched with 1 M HCl aq and extracted with CH_2Cl_2 (x2). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give **26a** (46.2 g, 100%); TLC $R_f = 0.46$ (CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65–7.50 (m, 2H), 7.35–7.15 (m, 3H), 6.92–6.80 (m, 1H), 6.78–6.65 (m, 1H), 6.52–6.42 (m, 1H), 2.39 (s, 3H); MS (APCI, Pos, 20V.) *m/z* 284 $(M+H)^+$.

5.20.2. *N*-(2-Fluoro-5-methylphenyl)-4-methylbenzenesulfonamide (26b). The title compound **26b** was prepared from **25b** according to the same procedure as described for the preparation of **26a** from **25a**: 88% yield; TLC $R_f = 0.50$ (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.63 (m, 2H), 7.39 (m, 1H), 7.24–7.20 (m, 2H), 6.86–6.81 (m, 2H), 6.63 (brs, 1H), 2.38 (s, 3H), 2.30 (s, 3H).

5.21. General procedure for the preparation of 27a and 27b

5.21.1. *N*-(2,4-Difluorophenyl)-*N*-[(2S)-3-triphenylmethyloxy-2-hydroxypropyl]-4-methylbenzenesulfonamide (27a). To a stirred solution of **26a** (46.2 g, 160 mmol) and (*S*)-(−)-glycidyl triphenylmethyl ether (55.7 g, 180 mmol) in 1,4-dioxane (90 mL) were added K_2CO_3 (2.21 g, 16 mmol) and benzyltriethylammonium chloride (3.64 g, 16 mmol) at room temperature under argon atmosphere, and stirring was continued for 5 h at 120 °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 give **27a** (115.6 g, 100%), which was used for the next reaction without further purification; TLC $R_f = 0.29$ (*n*-hexane/EtOAc, 3/1); MS (EI, Pos.) *m/z* 599 (M^+).

5.21.2. *N*-[(2*S*)-3-Trityloxy-2-hydroxypropyl]-*N*-(2-fluoro-5-methylphenyl)-4-methylbenzenesulfonamide (27b). The title compound **27b** was prepared from **26b** according to the same procedure as described for the preparation of **27a** from **26a**: 100% yield; TLC $R_f = 0.46$ (*n*-hexane/EtOAc, 2/1).

5.22. General procedure for the preparation of 28a and 28b

5.22.1. (2*S*)-2-[(Trityloxy)methyl]-7-fluoro-3,4-dihydro-2*H*-1,4-benzoxazine (28a). To a stirred solution of **27a** (109 g, 150 mmol) in THF (150 mL) was added ^{t}BuOK (20.2 g, 180 mmol) at room temperature under argon atmosphere, and then refluxed for 1 h. The reaction mixture was diluted with saturated NH_4Cl 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 used for the next reaction without further purification; TLC $R_f = 0.47$ (*n*-hexane/EtOAc, 3/1); MS (EI, Pos.) *m/z* 579 (M^+).

To a stirred solution of the above-described crude product in THF (800 mL) was added dropwise a solution of sodium napthalenide at −78 °C, which was prepared from Na (22.2 g, 0.96 mol) and naphthalene (74.4 g, 0.6 mol) in DME (600 mL) at room temperature. After stirring for 3 h at −78 °C, the reaction mixture was quenched with saturated NH_4Claq 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 **28a** (44.1 g, 69%) as a pale yellow oil; TLC $R_f = 0.46$ (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.18 (m, 15H), 6.58–6.42 (m, 3H), 4.38–4.28 (m, 1H), 3.48–3.39 (m, 2H), 3.28–3.18 (m, 2H); MS (EI, Pos.) *m/z* 425 (M^+).

5.22.2. (2*S*)-2-[(Trityloxy)methyl]-6-methyl-3,4-dihydro-2*H*-1,4-benzoxazine (28b). The title compound **28b** was prepared from **27b** according to the same procedure as described for the preparation of **28a** from **27a**: 94% yield; TLC $R_f = 0.36$ (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48–7.20 (m, 15H), 6.67 (d, $J = 8.1$ Hz, 1H), 6.45 (dd, $J = 8.1$, 1.8 Hz, 1H), 6.40 (d, $J = 1.8$ Hz, 1H), 4.29 (m, 1H), 3.49–3.42 (m, 2H), 3.27–3.20 (m, 2H), 2.19 (s, 3H).

5.23. General procedure for the preparation of 15c and 15s

5.23.1. [(2*S*)-7-Fluoro-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methanol (15c). To a stirred solution of **28a** (1.89 g, 4.4 mmol) in acetone (18 mL) were added K_2CO_3 (1.84 g, 13 mmol) and methyl iodide (0.83 mL, 13 mmol) at room temperature under argon atmosphere. After stirring for 9 h at 35 °C, the reaction mixture was quenched with saturated $NaHCO_3$ 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 used for the next reaction without further purification; TLC $R_f = 0.52$ (*n*-hexane/EtOAc, 3/1); MS (EI, Pos.) *m*/*z* 439 (M^+).

A solution of the above-described crude product in THF (5 mL), acetic acid (13 mL) and water (2 mL) was heated at 100 °C. After stirring for 40 min at 100 °C, the reaction mixture was concentrated in vacuo to give an oily residue, which was purified by column chromatography on silica gel to yield **15r** (0.368 mg, 42%) as a pale brown oil; TLC $R_f = 0.09$ (*n*-hexane/EtOAc, 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.62–6.55 (m, 3H), 4.40–4.31 (m, 1H), 3.92–3.78 (m, 2H), 3.22–3.08 (m, 2H), 2.84 (s, 3H), 2.10–1.98 (m, 1H); MS (APCI, Pos. 20V) *m*/*z* 439 (M^+).

5.23.2. [(2*S*)-4,6-Dimethyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]methanol (15s). The title compound **15s** was prepared from **28b** according to the same procedure as described for the preparation of **28a** from **15r**: 45% yield; TLC $R_f = 0.44$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.70 (d, $J = 7.8$ Hz, 1H), 6.50–6.46 (m, 2H), 4.30 (m, 1H), 3.90–3.75 (m, 2H), 3.23–3.13 (m, 2H), 2.87 (s, 3H), 2.26 (s, 3H), 2.03 (brs, 1H).

6. Biological assay method

6.1. Prostanoid mEP, mDP, hDP, and hIP receptor binding assay

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

Membranes from CHO cells expressing prostanoid receptors were incubated with radioligand (2.5 nM of [^{3}H]PGE_2 for mEP1-4 or 2.5 nM of [^{3}H]PGD_2 for mDP and hDP or 5.0 nM of [^{3}H]Iloprost for IP) and the test compounds at various concentrations in assay buffer (10 mM KH_2PO_4–KOH buffer containing 100 mM NaCl, pH 6.0 for mEP1-4, 25 mM HEPES-NaOH buffer containing 1 mM EDTA, 5 mM $MgCl_2$ and 10 mM $MnCl_2$, pH 7.4 for mDP and hDP, 50 mM Tris–HCl buffer containing 1 mM EDTA and 10 mM $MgCl_2$, pH 7.5, for IP-receptor). Incubation was carried out at room temperature for 60 min except for mEP1, mDP, and hDP (20 min), IP (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, 10 mM Tris–HCl buffer containing 100 mM NaCl and 0.01 w/v% BSA, pH 7.4 for mDP and hDP, 10 mM Tris–HCl buffer containing 100 mM NaCl, pH 7.5, for IP receptor), 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) or unlabeled PGD_2 (for mDP and hDP) 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 and hDP receptor antagonist activity

To confirm that test compounds antagonize the mDP and hDP receptors and to estimate potencies of antagonism for the mDP and hDP receptors, a functional assay was performed by measuring PGD_2-stimulated changes in intracellular second messenger cAMP (cyclic adenosine 3′, 5′-monophosphate) as an indicator of receptor function.

For the assessment of the antagonist activity of test compounds, a suspension of CHO cells expressing mDP or hDP receptor was seeded at a cell density of 1×10^5 cells/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 0.1or1% 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 containing 100 nM of PGD_2 for mDP or 10 nM of PGD_2 for hDP, or assay medium containing various concentrations of test compounds and 100 nM of PGD_2 for mDP or 10 nM of PGD_2 for hDP was added to each well zand 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%) and the

incubation mixture was frozen at −80 °C until the assay for cAMP.

The frozen incubation mixture was thawed, and the cells were detached with a cell scraper. After centrifugation of the reaction mixture, TCA was extracted by adding a mixture of 0.5 M tri-*n*-octylamine and chloroform (53/239, v/v) to the resultant supernatant, mixing and re-centrifugation. The cAMP level in the resultant aqueous layer (upper layer) was determined by radioimmunoassay using a cAMP assay kit (Amersham). 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 the IC_{50} values.

6.3. 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.

6.4. Inhibitory effects of 2r and 2s 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.

6.5. Single dose rat pharmacokinetic study of 2s

Single dose pharmacokinetics of **2s** was 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: predosing, 2, 5, 15 and 30 min, 1, 2, 4, 6 and 8 h; po: predosing, 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:

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

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

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