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Development of prostaglandin D_2 receptor antagonist: discovery of highly potent antagonists

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Abstract—The process of discovery for highly potent prostaglandin D_2 (PGD_2) receptor antagonists is reported. A series of *N*-(*p*-alkoxy)benzoyl-2-methylindole-4-acetic acids were synthesized and identified as a new class of selective PGD_2 receptor antagonists. Most of them exhibited strong PGD_2 receptor antagonism in binding studies and the cAMP formation assay. The structure–activity relationships (SAR), including subtype selectivity of the synthesized compounds, are also discussed.

1. Introduction

Prostanoids consist of prostaglandins (PGs) and thromboxanes (TX) and are products of cyclooxygenase derived from arachidonic acid, which is the most abundant precursor fatty acid in mammals (including humans). Once prostanoids are produced by specific processes, they act on the tissues where they are synthesized via specific G-protein-coupled receptors. Each of these receptors has been cloned, expressed, and characterized.

Coleman et al. proposed the existence of receptors specific for TX, PGI, PGE, PGF, and PGD that were named the TP, IP, EP, FP, and DP receptors, respectively.[1] They further classified the EP receptor into four subtypes (EP_1, EP_2, EP_3, and EP_4), all of which respond to PGE_2 in different ways.

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.[2] 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 the same with regard to DP receptor antagonists and only a few antagonists such as S-5751[3] are under going clinical trials for the treatment of allergic rhinitis.

In the preceding paper,[4] we reported on the identification of Indomethacin analog **2** as a new chemical lead for a class of DP receptor antagonists. Optimization of **2** was continued to identify a superior mDP receptor antagonist. As a result, we found that structural transformation of **2** to **3a**, which includes transformation of *N*-benzoyl 2-methyl-5-methoxyindole-3-acetic acid to *N*-benzoyl-2-methylindole-4-acetic acid, led to a marked increase of binding affinity. Here we report on the discovery of more optimized mDP receptor antagonists **3c**, **3o–r**, and **3t–v** starting from our chemical lead **3a** (Fig. 1).

2. Chemistry

The synthesis of **4–7** is outlined in Scheme 1. A key intermediate **12** was prepared from 2-methyl-4-hydroxyindole **11** by the usual procedure.[5] Palladium catalyzed insertion of a carbon monoxide into **12** in the presence of methanol provided **13**,[6] alkaline hydrolysis of which,

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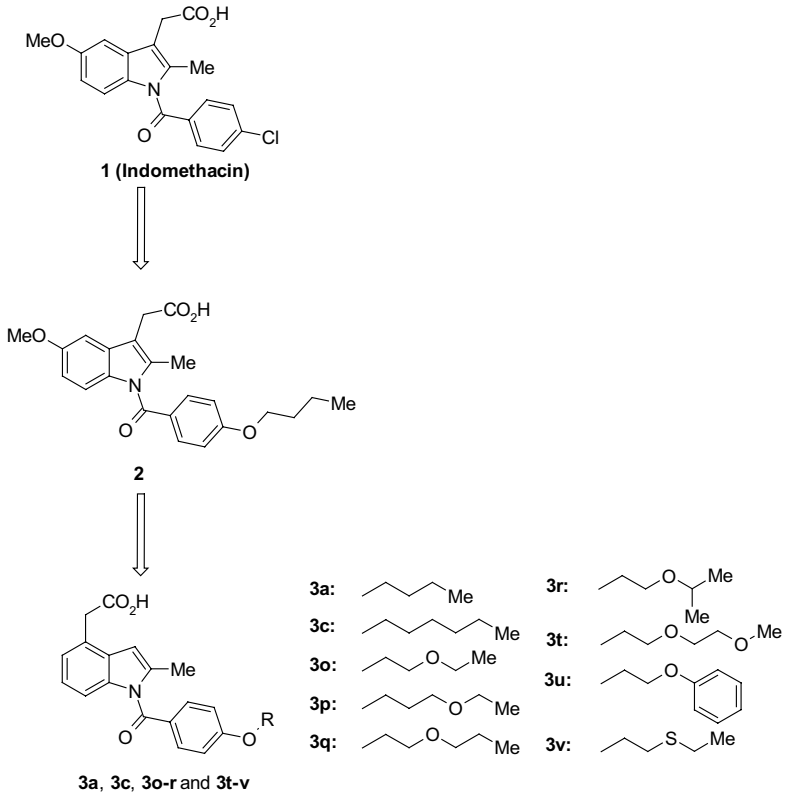
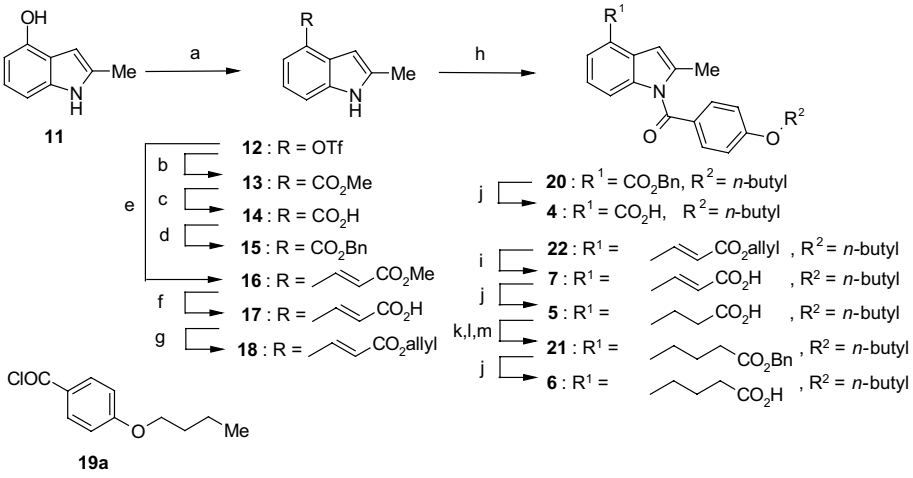


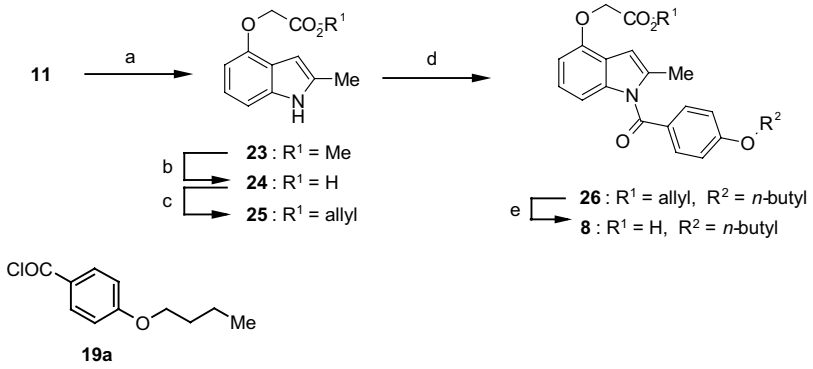
Figure 1. Discovery process of **3a**, **3c**, **3o–r**, and **3t–v**.



Scheme 1. Synthesis of **4**–**6** and **7a**–**g**. Reagents: (a) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78 °C; (b) $Pd(PPh_3)_4$, CO, TEA, MeOH, DMF; (c) NaOH, MeOH, 1,4-dioxane; (d) benzyl bromide, K_2CO_3, DMF; (e) $Pd(PPh_3)_2Cl_2$, methyl acrylate, DIPEA, DMF; (f) NaOH, MeOH, 1,4-dioxane; (g) allyl bromide, K_2CO_3, DMF; (h) NaH, **19a**, DMF (Method A), NaOH (powdered), **19a**, TBACl, CH_2Cl_2 (Method B); (i) morpholine, $Pd(PPh_3)_4$, THF; (j) Pd–C, H_2, MeOH, EtOAc; (k) $(COCl)_2$, DMF, toluene; (l) $TMSCHN_2$, THF, CH_3CN; (m) 2,4,6-collidine, benzyl alcohol.

followed by benzylation, led to the benzyl ester **15**. Palladium catalyzed coupling of **12** with methyl acrylate afforded **16**, alkaline hydrolysis of which, followed by allylation, gave **18**. *N*-Acylation of **15** with **19a** resulted in **20**, catalytic hydrogenation of which produced **4**. *N*-Acylation of **18** with **19a** led to **22**, palladium catalyzed deprotection of which produced the corresponding α,β-unsaturated acid **7**. Catalytic hydrogenation of **7** afforded **5**, which was converted to **21** by the conventional C1 unit homologation procedure.[7] Deprotection of **21** by catalytic hydrogenation gave **6**.

The synthesis of various *N*-benzoyl-2-methylindole-4-oxy acetic acids **8** is outlined in Scheme 2. *O*-Alkylation of 2-methyl-4-hydroxyindole **11** with a methyl bromoacetate afforded **23**. Alkaline hydrolysis of **23**, followed by



Scheme 2. Synthesis of **8**. Reagents: (a) methyl bromoacetate, K_2CO_3, DMF; (b) NaOH, MeOH, THF; (c) allyl bromide, K_2CO_3, DMF; (d) NaH, **19a**, DMF; (e) morpholine, $Pd(Ph_3)_4$, THF.

allylation with allyl bromide, resulted in **25**. *N*-Acylation of the ester **25** with benzoyl chloride **19a** gave **26**, deprotection of which produced **8**.

Compound **9** was prepared from **27** as described in Scheme 3. Starting with **27**, successive demethylation with pyridinium hydrochloride, *O*-alkylation with benzyl bromoacetate, and *N*-acylation with **19a** afforded **28**, deprotection of which produced **9**.

Synthesis of **3a**–**v** is outlined in Scheme 4. Protection of the NH moiety of **12** with a *tert*-butoxy carbonyl produced **29**, palladium catalyzed coupling reaction of which with trimethylsilyl acetylene gave **30**.[8] Hydroboration of **30**, followed by oxidation with alkaline hydrogen peroxide and deprotection, afforded **31**. Esterification of **31**with allyl bromide and benzyl bromide provided their corresponding esters **32** and **33**, respectively. *N*-Acylation of **32** and **33** with **19a**–**v** resulted in **34c**,**e**–**j**,**l**–**n**, **p**–**v** and **34a**,**b**,**d**,**k**,**o**, respectively, deprotection of which produced **3a**–**v**.

Compound **10** was prepared from **3v** by the oxidation using mCPBA as described in Scheme 5.

3. Results and discussion

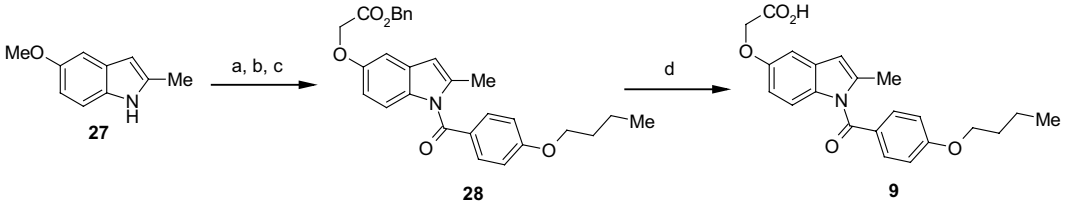
The test compounds listed in Tables 1–3 were evaluated for inhibition of the specific binding of a radiolabeled ligand, [^{3}H]PGD_2, to membrane fractions prepared from cells stably expressing each of the prostanoid receptors and for inhibition of cAMP formation evoked by PGD_2 in CHO cells in the presence of 0.1% BSA.[9] Test compounds were also evaluated for binding to all the mouse subtypes of PGE_2 (mEP1–4) and to the human PGI_2 receptor (hIP).

During optimization of our new chemical lead **2**, it was found that **3a** (in which the acetic acid residue is attached to position 4) showed a marked increase of mDP receptor affinity and antagonist activity (Fig. 1).[4]

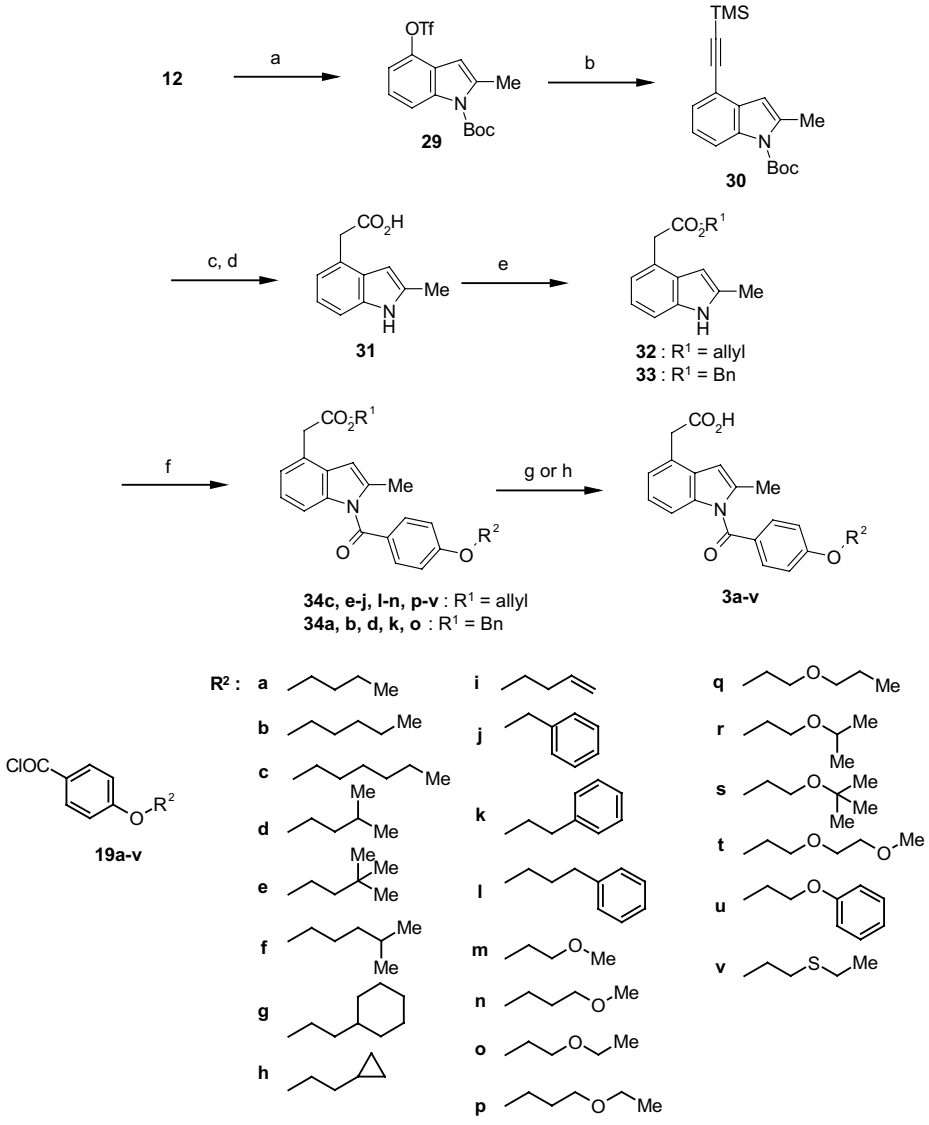
As described in Figure 2, the new chemical lead **3a** was thought to have a structural analogy to PGs regarding the acidic part, which corresponds to α-chain, and the lipophilic part, which corresponds to ω-chain. Based on many historical evidences, chemical modification of the α- and ω-chains of PGs has been known to be crucial for the receptor affinity and/or subtype selectivity. In such a reason, optimization of these acidic part and the lipophilic part of **3a** was considered to be one of the promising approaches for the increase of the receptor affinity and subtype selectivity.

First, focus was placed on the optimization of 4-acetic acid moiety. As described in Table 1, replacement of the acetic acid residue of **3a** with a carboxylic acid residue afforded **4** with 3-fold lower affinity for the mDP receptor and 4-fold less potent antagonist activity. The subtype selectivity of **4** was lower than that of **3a**, mainly because of a nearly 100-fold increase of its affinity for the mEP3 receptor.

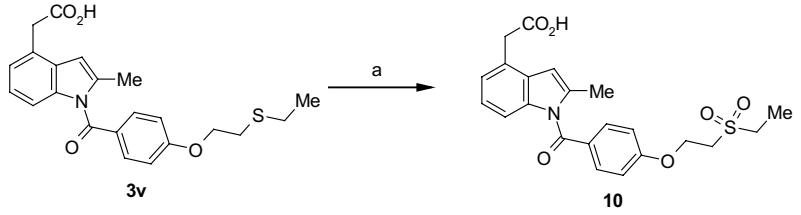
Replacement of the acetic acid residue of **3a** with a propionic acid residue and butanoic acid residue afforded **5**



Scheme 3. Synthesis of **9**. Reagents: (a) HCl–pyridine; (b) benzyl bromoacetate, K_2CO_3, DMF; (c) NaOH (powdered), **19a**, TBACl, CH_2Cl_2; (d) H_2, Pd–C, MeOH, EtOAc.



Scheme 4. Synthesis of **3a**–**v**. Reagents: (a) Boc_2O, DMAP (cat.), CH_3CN; (b) $PdCl_2(PPh_3)_2$, trimethylsilylacetylene, CuI, TBAI, TEA, DMF, 70 °C (c) (1) dicyclohexylborane, (2) NaOHaq, H_2O_2, THF; (d) NaOH aq, MeOH, 1,4-dioxane; (e) allyl bromide or benzyl bromide, K_2CO_3, DMF; (f) NaOH (powdered), **19a**–**v**, TBACl, CH_2Cl_2; (g) morpholine, $Pd(PPh_3)_4$, THF; (h) H_2, Pd–C, *i*-PrOH, EtOAc.

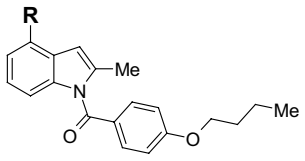


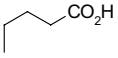
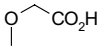
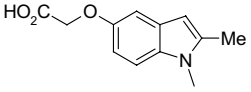
Scheme 5. Synthesis of **10**. Reagents: (a) mCPBA, CH_2Cl_2.

and **6**, respectively, which showed lower affinity for the mDP receptor and less potent antagonist activity, while **5** and **6** showed lower subtype selectivity.

Introduction of a 2,3-*trans* double bond into the propionic acid residue of **5** gave **7** with nearly 3-fold lower affinity to mDP receptor and more than 2-fold weaker antagonist activity, while the subtype selectivity of **7** was lower relative to that of **5** mainly because of a nearly 10-fold increase of mEP2 receptor affinity. Replacement of the acetic acid residue of **3a** with a glycolic acid residue afforded **8** with nearly 4-fold lower affinity for the mDP receptor and a more than 2-fold decrease of antagonist activity, while **8** showed much lower subtype selec-

Table 1. Effect of the 4-acidic moieties on the activity profiles



Compd	R	Binding K_i (μM)					IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	mDP	mDP
4		>10	5.1	0.029	>10	0.033	1.2
3a		>10	2.0	3.3	>10	0.010	0.30
5		>10	3.5	>10	3.0	0.013	0.43
6		>10	0.046	>10	>10	0.033	0.57
7		>10	0.32	>10	>10	0.032	1.1
8		>10	4.3	0.50	2.6	0.047	0.69
9		>10	>10	>10	>10	1.2	NT[a]

[a] NT: not tested.

tivity mainly because of its increased affinity for the mEP3 and mEP4 receptors. Transfer of the glycolic acid residue of **8** from position-4 to position-5 led to **9**, which showed a marked decrease of mDP receptor affinity. Accordingly, the optimization of *N*-benzoyl-2-methylindole-4-acetic acid was pursued.

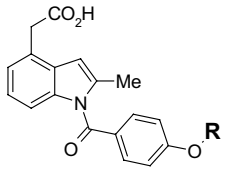
Our second focus was placed on the optimization of the *p*-alkoxy moiety of **3a** (Tables 2 and 3). As described in Table 2, replacement of the *n*-butoxy moiety of **3a** with longer alkoxy moieties, such as *n*-pentyloxy and *n*-hexyloxy, gave **3b** and **3c**, which had slightly higher and more than 10-fold higher antagonist activity relative to **3a**, respectively, but subtype selectivity was not improved compared with **3a**. Replacement of the *p*-butoxy moiety of **3a** with branched *p*-alkoxy moieties provided **3d–f**. Compound **3d** (*p*-isoamyloxy moiety) demonstrated slightly higher affinity for the mDP receptor and stronger antagonist activity relative to **3a**, while its subtype selectivity was not improved.

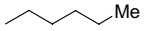
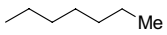
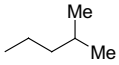
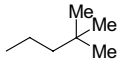
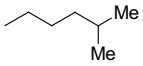
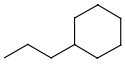
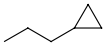
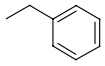
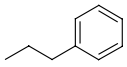
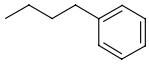
Two other branched alkoxy derivatives, **3e–f**, showed slightly lowert and 2-fold higher antagonist activity, respectively, while their mDP receptor affinity was more than 10-fold lower than that of **3a**. Introduction of *p*-(2-cycloalkyl)ethoxy moieties as the alkoxy moiety created **3g** and **3h**. Compound **3g**, with a *p*-(2-cyclohexyl)ethoxy benzoyl moiety, showed slightly stronger antagonist activity relative to **3a**, while its mDP receptor affinity was nearly 20-fold lower. Compound **3h**, with a *p*-(2-cyclopropyl)ethoxy benzoyl moiety, showed stronger antagonist activity relative to **3a**, while its mDP receptor affinity was slightly lower. The subtype selectivity of these two compounds was estimated to be inferior to **3a**. Replacement of the butoxy moiety of **3a** with a 3-butenyloxy moiety provided **3i**, which showed 4.6-fold lower mDP affinity.

Replacement of the *p*-butoxy moiety of **3a** with various phenylalkoxy moieties, such as *p*-phenylmethoxy, *p*-phenylethoxy and *p*-phenylpropyloxy moieties, resulted in **3j–l**, respectively. Among these compounds, **3j** and **3l** showed lower mDP receptor affinity while **3k** showed nearly 5-fold higher mDP receptor affinity and more than 2-fold stronger antagonist activity. The subtype selectivity of these three compounds **3j–l** seemed to be lower than that of **3a**. Thus, **3c** was found to show the highest antagonist activity, although its subtype selectivity was not satisfactory.

Insertion of a hetero atom, such as oxygen or sulfur, into the *p*-alkoxy substituent of the *N*-benzoyl moiety produced the compounds listed in Table 3 with better mDP receptor antagonist activity and subtype selectivity. Replacement of the *p*-butoxy moiety of **3a** with a methoxyethoxy moiety afforded **3m**, which showed more than 17-fold lower affinity for the mDP receptor. Methoxypropyloxy derivative **3n** showed 3-fold stronger antagonist activity and 3-fold lower affinity for the mDP receptor. Replacement of the *p*-butoxy moiety of **3a** with a ethoxyethoxy moiety gave **3o**, which showed more potent antagonist activity and much improved subtype selectivity, while **3o** showed 2-fold lower mDP receptor affinity relative to **3a**. Ethoxypropyloxy derivative **3p** demonstrated nearly 3-fold stronger antagonist activity and nearly 5-fold higher mDP receptor affinity than **3o**. The *n*-propyloxyethoxy derivative **3q** showed more potent antagonist activity and slightly higher

Table 2. Effect of the *p*-alkoxy moieties on the activity profiles



Compd	R	Binding K_i (μM)						IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	hIP	mDP	mDP
3b		3.1	0.80	2.8	3.1	1.9	0.0043	0.25
3c		5.3	1.0	>10	3.1	0.74	0.0085	0.020
3d		3.0	0.92	>10	2.3	>10	0.0092	0.20
3e		>10	2.8	>10	>10	NT[a]	0.34	0.46
3f		>10	2.6	>10	>10	NT[a]	0.19	0.14
3g		4.3	2.9	>10	4.0	NT[a]	0.22	0.20
3h		4.3	1.3	>10	4.9	0.85	0.016	0.080
3i		2.7	1.8	>10	>10	>10	0.046	NT[a]
3j		1.2	0.33	1.2	>10	2.5	0.068	1.3
3k		0.40	1.3	2.2	1.9	0.14	0.0018	0.12
3l		7.7	NT[a]	3.3	2.1	NT[a]	0.17	NT[a]

[a] NT: not tested.

mDP receptor affinity than **3o**, but its subtype selectivity was lower because of increased affinity for mEP1, mEP2, and mEP3 relative to **3o**. The *i*-propyloxyethoxy derivative **3r** demonstrated 1.5-fold higher antagonist activity than **3o**, while its subtype selectivity was lower mainly because of its increased affinity for the mEP1 receptor relative to **3o**. The *tert*-butoxyethoxy derivative **3s** unexpectedly showed lower antagonist activity compared with its high affinity for the mDP receptor. Methoxyethoxyethoxy derivative **3t** showed slightly stronger antagonist activity and nearly 2-fold higher affinity for the mDP receptor than **3q**. The subtype selectivity of compounds **3s** and **3t** was excellent. The unexpectedly weaker antagonist activity of **3s** relative to its high mDP receptor affinity was considered to be attributable to relatively stronger protein binding because of its more lipophilic *tert*-butoxy moiety. Phenoxyethoxy derivative **3u** showed slightly stronger antagonist activity and higher mDP receptor affinity relative to **3o**, while subtype selectivity was lower because of increased affinity for the mEP1 and mEP2 receptors. The subtype selectivity of compounds **3n** and **3p** was lower than that of **3o**, mainly because of increased mEP2 receptor affinity. Replacement of the ether oxygen of **3o** with a sulfur afforded **3v**, which showed slightly more potent antagonist activity and nearly 2-fold higher mDP receptor affinity relative to **3o**, while its subtype selectivity was much lower because of increased affinity for the mEP1, mEP2, and mEP3 receptor. Oxidation of the sulfur atom of **3v** to a sulfone moiety provided **10**, which showed markedly lower affinity for the mDP receptor than **3v**.

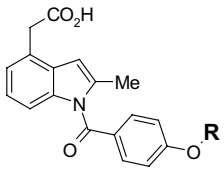
As a result, the optimal subtype-selective mDP antagonists were **3o** and **3t**. The subtype selectivity of **3s** was excellent, although its antagonist activity was reduced compared with its relatively high affinity for the mDP receptor because of its predicted high level of protein binding.

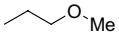
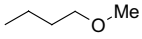
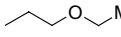
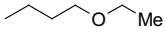
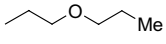
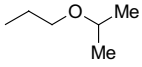
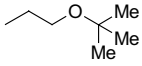
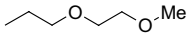
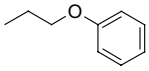
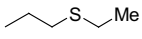
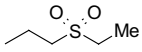
4. Conclusion

In summary, structural optimization of **3a** was continued to identify a more potent mDP receptor antagonist. Among the compounds tested, **3c**, **3o**–**r**, and **3t**–**v** demonstrated higher mDP receptor affinity and antagonist activity relative to **3a**. Compound **3c** also showed affinity for the hIP receptor at 0.74 μM (K_i), while **3o**–**r** and **3t**–**v** did not show hIP receptor affinity at 10 μM.

The acetic acid residue at position-4 of the indole moiety of **3a** was optimal among the residues investigated, as

Table 3. Effect of the hetero atom-containing *p*-alkoxy moieties on the activity profiles



Compd	R	Binding K_i (μM)						IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	hIP	mDP	mDP
3m		>10	>10	NT[a]	>10	NT[a]	0.17	NT[a]
3n		>10	0.42	>10	>10	>10	0.031	0.10
3o		>10	>10	>10	>10	>10	0.020	0.074
3p		>10	6.6	>10	>10	>10	0.0043	0.023
3q		4.7	0.57	2.0	>10	>10	0.014	0.027
3r		5.3	>10	>10	>10	>10	0.075	0.048
3s		>10	>10	>10	>10	>10	0.067	1.0
3t		>10	>10	>10	>10	>10	0.0078	0.063
3u		5.6	0.54	>10	>10	>10	0.017	0.053
3v		8.8	2.7	3.6	>10	>10	0.012	0.066
10		>10	>10	>10	>10	NT[a]	1.2	NT[a]

[a] NT: not tested.

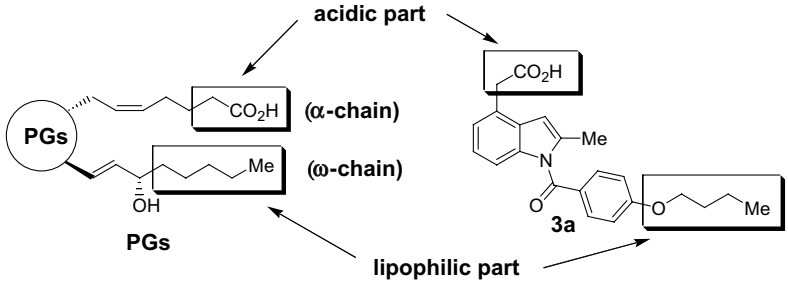


Figure 2. Structural analogy of PGs and **3a**.

illustrated in Table 1. Position-4 was also found to be better than position-5 for substitution by an acetic acid residue, as illustrated by the increased mDP affinity of **8** relative to that of **9**. Further optimization and in vivo evaluation of the optimum compound will be reported in due course.

5. Experimental

5.1. General directions

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{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. 2-Methyl-1*H*-indol-4-yl trifluoromethanesulfonate (12)

To a stirred solution of 2-methyl-1*H*-indol-4-ol (**11**) (10.0 g, 67.9 mmol) in CH_2Cl_2 (100 mL) were added lutidine (10.3 mL, 88.3 mmol) and Tf_2O (13.72 mL, 81.54 mmol) at 0 °C. After stirring for 1 h, 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 evaporated to afford a residue, which was used for the next reaction without further purification; **TLC** R_f = 0.57 (*n*-hexane/EtOAc, 7/3).

5.3. Methyl 2-methyl-1*H*-indole-4-carboxylate (13)

To a stirred solution of **12** (6.34 g, 22.7 mmol) in MeOH (33 mL) and DMF (200 mL) were added TEA (6.3 mL, 45.29 mmol) and $Pd(PPh_3)_4$ (2.6 g, 2.26 mmol) at room temperature. After stirring over night at 60 °C under an atmosphere of carbon monoxide, the reaction mixture was filtered through a pad of Celite to remove the catalyst. The filtrate was poured into water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, and dried over Na_2SO_4 and evaporated to afford a residue, which was purified by column chromatography on silica gel to yield **13** (100% yield); **TLC** R_f = 0.18 (toluene); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 8.14–8.00 (br s, 1H), 7.85 (m, 1H), 7.47 (m, 1H), 7.14 (t, J = 8.1 Hz, 1H), 6.87 (m, 1H), 3.97 (s, 3H), 2.50 (s, 3H).

5.4. 2-Methyl-1*H*-indole-4-carboxylic acid (14)

To a stirred solution of methyl ester **13** (4.30 g, 22.7 mmol) in MeOH (10 mL) and 1,4-dioxane (10 mL) was added 5 M NaOH aq (10 mL). After stirring for 16 h at 60 °C, the reaction mixture was acidified 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 residue, which was purified by column chromatography on silica gel to yield **14** (1.6 g, 40%); **TLC** R_f = 0.48 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (300 MHz, CD_3OD) δ 8.14–8.04 (br, 1H), 7.93 (dd, J = 8.1,0.9 Hz, 1H), 7.52 (m, 1H), 7.18 (dd, J = 8.1, 0.9 Hz, 1H), 6.94 (m, 1H), 3.71 (s, 3H).

5.5. Benzyl 2-methyl-1*H*-indole-4-carboxylate (15)

To a stirred solution of **14** (690 mg, 3.94 mmol) in DMF (10 mL) were added K_2CO_3 (815 mg, 5.91 mmol) and benzyl bromide (0.7 mL, 5.91 mmol) at room temperature. After stirring for 2 h at 80 °C, the reaction mixture was poured into ice-water and extracted with EtOAc (×3). The combined organic layers were washed with water, brine, dried over Na_2SO_4. The solvent was removed by evaporation and the residue was purified by column chromatography on silica gel to afford **15** (610 mg, 58%). **TLC** R_f = 0.44 (*n*-hexane/EtOAc, 8/3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.05 (br s, 1H), 7.91 (d, J = 7.2 Hz, 1H), 7.54–7.24 (m, 7H), 6.88 (m, 1H), 5.44 (s, 2H), 2.48 (s, 3H).

5.6. Methyl (2*E*)-3-(2-methyl-1*H*-indol-4-yl)acrylate (16)

To a stirred solution of **12** (3.16 g, 11.3 mmol) in DMF (200 mL) were added methyl acrylate (2.24 mL, 24.9 mmol), DIPEA (5.9 mL, 33.9 mmol) and $Pd(PPh_3)_2Cl_2$ (238 mg, 0.339 mmol) at room temperature. After stirring for 16 h at 95 °C, the reaction mixture was poured into water and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, and dried over Na_2SO_4 and evaporated to afford a residue, which was purified by column chromatography on silica gel to yield **16** (950 mg, 39% yield); **TLC** R_f = 0.50 (*n*-hexane/EtOAc, 8/2); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.97 (d, J = 16.2 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 6.82 (s, 1H), 6.54 (d, J = 16.2 Hz, 1H), 3.84 (s, 3H), 2.61 (s, 3H).

5.7. (2*E*)-3-(2-Methyl-1*H*-indol-4-yl)acrylic acid (17)

To a stirred solution of methyl ester **16** (950 mg, 4.41 mmol) in MeOH (10 mL) and 1,4-dioxane (10 mL) was added 5 M NaOH aq (5 mL). After stirring for 16 h at room temperature, the reaction mixture was acidified 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 **17** (700 mg, 85% yield), which was used for the next reaction without further purification; **TLC** R_f = 0.54 ($CHCl_3$/MeOH, 9/1).

5.8. Allyl (2*E*)-3-(2-methyl-1*H*-indol-4-yl)acrylate (18)

To a stirred solution of **17** (300 mg, 1.60 mmol) in DMF (5 mL) were added K_2CO_3 (332 mg, 2.40 mmol) and allyl bromide (0.21 mL, 2.40 mmol) at room temperature. After stirring for 2 h at 80 °C, the reaction mixture was poured into ice-water and extracted with EtOAc (×3). The combined organic layers were washed with water, brine, and dried over Na_2SO_4. The solvent was removed by evaporation and the residue was purified by column chromatography on silica gel to afford **18** (240 mg, 62%). **TLC** R_f = 0.43 (*n*-hexane/EtOAc, 8/2); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.10–8.00 (m, 2H), 7.35–7.30 (m, 2H), 7.12 (t, J = 7.8 Hz, 1H), 6.61 (d, J = 16.2 Hz, 1H), 6.54 (br s, 1H), 6.10–5.95 (m, 1H), 5.95–5.85 (m, 1H), 5.80–5.75 (m, 1H), 4.80–4.70 (m, 2H), 2.50 (s, 3H).

5.9. Benzyl 1-(4-butoxybenzoyl)-2-methyl-1*H*-indole-4-carboxylate (20)

To a stirred solution of **15** (690 mg, 2.60 mmol) in DMF (8 mL) was added sodium hydride (60% oil dispersion,

114 mg, 2.86 mmol) in several portions at 0 °C under argon atmosphere, and the resulting suspension was stirred for 30 min at 0 °C. After the addition of *p*-(*n*-butoxy) benzoyl chloride (0.54 mL, 2.86 mmol), the reaction mixture was stirred for 12 h at 0 °C, quenched with water and extracted with EtOAc (×3). 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 **20** (1.02 g, 89%); **TLC** R_f = 0.61 (*n*-hexane/EtOAc, 8/2); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.10–6.90 (m, 13H), 5.45 (s, 2H), 4.05 (t, J = 6.3 Hz, 2H), 2.44 (s, 3H), 1.86–1.74 (m, 2H), 1.60–1.45 (m, 2H), 0.99 (t, J = 7.5 Hz, 3H).

5.10. Allyl (2*E*)-3-[1-(4-butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]acrylate (22)

98% yield; **TLC** R_f = 0.59 (*n*-hexane/EtOAc, 8/2); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.10–8.00 (m, 1H), 7.70 (d, J = 11.7 Hz, 2H), 7.39 (d, J = 7.2 Hz, 1H), 7.10–6.90 (m, 4H), 6.72 (s, 1H), 6.58 (d, J = 15.9 Hz, 1H), 6.10–5.95 (m, 1H), 5.95–5.85 (m, 1H), 5.82–5.75 (m, 1H), 4.80–4.70 (m, 2H), 4.10–4.00 (m, 2H), 2.47 (s, 3H), 1.90–1.70 (m, 2H), 1.70–1.40 (m, 2H), 1.10–0.95 (m, 3H).

5.11. (2*E*)-3-[1-(4-Butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]acrylic acid (7)

To a stirred solution of **22** (337 mg, 1 mmol) in THF (7 mL) was added $Pd(PPh_3)_4$ (115 mg, 0.10 mmol) at room temperature under argon atmosphere. After stirring for 15 min, morpholine (0.45 mL, 5.0 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 recrystallization from EtOAc to yield **7** (50 mg, 85%); **TLC** R_f = 0.53 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.16 (d, J = 16.2 Hz, 1H), 7.70 (d, J = 9.0 Hz, 2H), 7.43 (br d, J = 7.2 Hz, 1H), 7.15–7.03 (m, 2H), 6.96 (d, J = 9.0 Hz, 2H), 6.74 (br s, 1H), 6.59 (d, J = 16.2 Hz, 1H), 4.06 (t, J = 6.3 Hz, 2H), 2.48 (s, 3H), 1.88–1.76 (m, 2H), 1.60–1.46 (m, 2H), 1.00 (t, J = 7.2 Hz, 3H); **MS** (APCI, Neg, 20V.) *m*/*z* 376 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for C_{23} $H_{23}NO_4$: 377.1627; found: 377.1642; **IR** (neat) 2959, 1684, 1626, 1604, 1509, 1427, 1371, 1294, 1256, 1168, 1066, 950, 912, 779, 760, 616 cm^{-1}.

5.12. 1-(4-Butoxybenzoyl)-2-methyl-1*H*-indole-4-carboxylic acid (4)

To a stirred solution of the compound **20** (1.02 g, 2.31 mmol) in MeOH (10 mL) and EtOAc (5 mL) was added 10% Pd–C (100 mg) at room temperature, and the resulting mixture was stirred for 30 min at room temperature under hydrogen atmosphere. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to give a residue, which was purified by column chromatography on silica gel to yield **4** (433 mg, 53%); **TLC** R_f = 0.59 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.99 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 9.0 Hz, 2H), 7.36 (d, J = 8.1 Hz, 1H), 7.21 (br s, 1H), 7.13 (t, J = 8.1 Hz, 1H), 6.97 (d, J = 9.0 Hz, 2H), 4.06 (t, J = 6.6 Hz, 2H), 2.48 (s, 3H), 1.88–1.76 (m, 2H), 1.60–1.46 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H); **MS** (FAB, Pos.) *m*/*z* 352 $(M+H)^+$; **HRMS** (EI, Pos.) calcd for C_{21} $H_{21}NO_4$: 351.1471; found: 351.1493; **IR** (neat) 2960, 1682, 1604, 1511, 1481, 1432, 1371, 1257, 1171, 912, 804, 757 cm^{-1}.

5.13. 3-[1-(4-Butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]propanoic acid (5)

8% yield; **TLC** R_f = 0.58 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.70 (d, J = 9.3 Hz, 2H), 7.00–6.86 (m, 5H), 6.48 (s, 1H), 4.05 (t, J = 6.6 Hz, 2H), 3.75–3.65 (br, 1H), 3.19 (t, J = 8.4 Hz, 2H), 2.79 (t, J = 8.4 Hz, 2H), 2.45 (s, 3H), 1.87–1.72 (m, 2H), 1.60–1.40 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H); **MS** (APCI, Neg, 20V.) *m*/*z* 378 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{25}NO_4$: 379.1784; found: 379.1790; **IR** (neat) 2960, 2873, 1682, 1605, 1573, 1511, 1432, 1369, 1300, 1256, 1226, 1169, 1115, 1068, 1025, 912, 849, 777, 759, 617 cm^{-1}.

5.14. Benzyl 4-[1-(4-butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]butanoate (21)

To a stirred solution of **5** (1.77 g, 4.67 mmol) in toluene (20 mL) were added oxalyl chloride (0.64 mL, 7.4 mmol) at room temperature. After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the corresponding acid chloride, which was used for the next reaction without further purification. To a stirred solution of the acid chloride described above in THF (4 mL) and CH_3CN (4 mL) was added (trimethylsilyl)diazomethane (2 mol/L in ether, 4.67 mL, 9.34 mmol) at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was concentrated in vacuo to afford a residue which was dissolved with benzyl alcohol (4 mL) and 2,4,6-collidine (4 mL) at room temperature. After stirring for 30 min at 180 °C, the reaction mixture was concentrated in vacuo to afford a residue, which was directly purified by column chromatography on silica gel to yield **21** (460 mg, 20%); **TLC** R_f = 0.51 (*n*-hexane/EtOAc, 8/2); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.67 (d, J = 9.0 Hz, 2H), 7.40–7.20 (m, 5H), 7.03–6.85 (m, 5H), 6.47 (s, 1H), 5.10 (s, 2H), 4.03 (t, J = 6.6 Hz, 2H), 2.90 (t, J = 7.2 Hz, 2H), 2.50–2.32 (m, 5H), 2.13–2.00 (m, 2H), 2.00–1.40 (m, 4H), 1.00 (t, J = 7.5 Hz, 3H).

5.15. 4-[1-(4-Butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]butanoic acid (6)

To a stirred solution of the compound **21** (460 mg, 0.95 mmol) in EtOAc (5 mL) and MeOH (5 mL) was added 10 wt% Pd–C (45 mg) at room temperature. The resulting suspension was stirred for 1 h at room temperature under hydrogen atmosphere. Insoluble substance was removed by filtration. The filtrate was concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel to yield **6** (170 mg, 45%); **TLC** R_f = 0.50 ($CHCl_3$/CH_3OH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.71 (d, J = 9.0 Hz, 2H), 7.00–6.86 (m, 5H), 6.48 (s, 1H), 4.05 (t, J = 6.6 Hz, 2H), 2.90 (t,

J=7.2 Hz, 2H), 2.48–2.38 (m, 5H), 2.14–2.00 (m, 2H), 2.00–1.40 (m, 5H), 1.00 (t, J=7.5 Hz, 3H); **MS** (APCI, Neg 20V) m/z 392 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{24}H_{27}NO_4$: 393.1940; found: 393.1949; **IR** (neat) 3351, 2959, 1682, 1605, 1573, 1510, 1484, 1432, 1369, 1301, 1257, 1227, 1169, 912, 779, 618 cm^{-1}.

5.16. Methyl [(2-methyl-1*H*-indol-4-yl)oxy]acetate (23)

To a stirred solution of 2-methyl-4-hydroxy indole **11** (5 g, 33.9 mmol) in DMF (50 mL) were added K_2CO_3 (11.7 g, 84.9 mmol) and methyl bromoacetate (3.54 mL, 37.4 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 2 h. The resulting mixture was quenched with ice water. The resulting precipitates were collected by filtration to yield **23** (5.4 g, 73%) as a gray powder; **TLC** R_f=0.75 (n-hexane/EtOAc, 7/3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.00–7.84 (br, 1H), 7.04–6.94 (m, 2H), 6.45–5.36 (m, 2H), 4.77 (s, 2H), 3.80 (s, 3H), 2.43(s, 3H).

5.17. [(2-Methyl-1*H*-indol-4-yl)oxy]acetic acid (24)

To a stirred solution of a methyl ester **23** (5.4 g, 24.6 mmol) in MeOH (18 mL) and 1,4-dioxane (36 mL) was added 5 N NaOH aq (15 mL). After stirring for 1 h at room temperature, the reaction mixture was acidified with 2 M HCl aq, and the resulting precipitates were collected by filtration to afford **24** (3.5 g, 69%) as a white powder; **TLC** R_f=0.20 ($CHCl_3$/CH_3OH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 10.89 (s, 1H), 6.90–6.80 (m, 2H), 6.29 (dd, J=1.5,6.9 Hz, 1H), 6.11 (s, 1H), 4.68 (s, 2H), 2.33 (s, 3H).

5.18. Allyl [(2-methyl-1*H*-indol-4-yl)oxy]acetate (25)

To a stirred solution of **24** (2 g, 9.8 mmol) in DMF (20 mL) were added K_2CO_3 (2.02 g, 14.6 mmol) and allyl bromide (1.27 mL, 14.6 mmol) at room temperature. After stirring for 2 h at 80 °C, the reaction mixture was poured into ice water and extracted with EtOAc (×3). The combined organic layers were washed with water, brine, and dried over Na_2SO_4. The solvent was removed by evaporation and the residue was purified by column chromatography on silica gel to afford **25** (1.88 g, 79%) as a white solid. **TLC** R_f=0.50 (n-hexane/EtOAc, 7/3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.03–6.94 (m, 2H), 6.45–6.37 (m, 2H), 6.00–5.85 (m, 1H), 5.34 (dq, J=5.7,1.5 Hz, 2H), 5.25 (dq, J=10.2,1.5 Hz, 1H), 4.79 (s, 2H), 4.71 (dt, J=5.7,1.5 Hz, 2H), 2.43 (s, 3H).

5.19. Allyl {[1-(4-butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]oxy}acetate (26)

To a stirred solution of **25** (900 mg, 3.67 mmol) in DMF (10 mL) was added sodium hydride (60% oil dispersion, 147 mg, 3.67 mmol) in several portions at 0 °C under argon atmosphere, and the resulting suspension was stirred for 30 min at 0 °C. After the addition of p-(n-butyloxy) benzoyl chloride (0.70 mL, 3.67 mmol), the reaction mixture was stirred for 12 h at 0 °C, quenched with water and extracted with EtOAc. 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 **26** (800 mg, 52%); **TLC** R_f=0.63 (n-hexane/EtOAc, 7/3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.69 (d, J=8.7 Hz, 2H), 7.00–6.85 (m, 3H), 6.68 (d, J=8.4 Hz, 1H), 6.67 (s, 1H), 6.47 (d, J=7.2 Hz, 1H), 6.00–5.87 (m, 1H), 5.40–5.30 (m, 1H), 5.30–5.24 (m, 1H), 4.78 (s, 2H), 4.75–4.68 (m, 2H), 4.05 (t, J=6.3 Hz, 2H), 2.42 (s, 3H), 1.87–1.75 (m, 2H), 1.60–1.45 (m, 2H), 1.00 (t, J=7.5 Hz, 3H).

5.20. {[1-(4-Butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]oxy}acetic acid (8)

To a stirred solution of **26** (800 mg, 1.90 mmol) in THF (100 mL) was added $Pd(PPh_3)_4$ (220 mg, 0.19 mmol) at room temperature under argon atmosphere. After stirring for 15 min, morpholine (0.83 mL, 9.54 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 **8** (570 mg, 79%) as a yellow powder; **TLC** R_f=0.38 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.70 (d, J=9.0 Hz, 2H), 6.98–6.89 (m, 3H), 6.71 (d, J=8.4 Hz, 1H), 6.60–6.57 (m, 1H), 6.51 (d, J=8.4 Hz, 1H), 4.84 (s, 2H), 4.05 (t, J=6.6 Hz, 2H), 2.43 (s, 3H), 1.87–1.75 (m, 2H), 1.59–1.44 (m, 2H), 1.00 (t, J=7.5 Hz, 3H); **MS** (APCI, Neg, 20V.) m/z 380 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{22}H_{23}NO_5$: 381.1576; found: 381.1577; **IR** (neat) 2959, 2873, 1734, 1684, 1604, 1571, 1509, 1494, 1438, 1384, 1325, 1295, 1258, 1226, 1170, 1107, 1068, 1029, 952, 912, 850, 768, 731, 668, 620 cm^{-1}.

5.21. Benzyl {[1-(4-butoxybenzoyl)-2-methyl-1*H*-indol-5-yl]oxy}acetate (28)

A mixture of **27** (2.5 g, 15.5 mmol) and pyridinium hydrochloride (25 g) was heated for 2 h at 190 °C. After cooling to room temperature, the reaction mixture was poured into water and extracted with EtOAc (×3). The combined organic layers were washed with 1 M HCl aq, water, brine, dried over Na_2SO_4 and evaporated to give a residue, which was used for the next reaction without further purification; **TLC** R_f=0.33 (n-hexane/EtOAc, 7/3).

To a stirred solution of 2-methyl-5-hydroxy indole described above (15.5 mmol) in DMF (25 mL) were added K_2CO_3 (5.35 g, 38.77 mmol) and benzyl bromoacetate (2.7 mL, 17.06 mmol) at room temperature. After stirring for 30 min at 80 °C, 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 to give a residue, which was purified by column chromatography on silica gel to yield an O-alkylated product (1.17 g, 26%); **TLC** R_f=0.51 (n-hexane/EtOAc, 7/3).

To a stirred solution of the above-described product (300 mg, 1.02 mmol) and NaOH (powdered, 203 mg, 5.08 mmol) in CH_2Cl_2 (4 mL) were added TBACl

(28 mg, 0.102 mmol) and *p*-(*n*-butyloxy) benzoyl chloride (0.29 mL, 1.53 mmol) at room temperature. After stirring for 30 min at room temperature, the reaction mixture was purified by column chromatography on silica gel to yield **28** (220 mg, 46%); **TLC** R_f = 0.59 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.68 (d, J = 9.0 Hz, 2H), 7.40–7.30 (m, 5H), 6.97–6.88 (m, 4H), 6.68 (dd, J = 9.0,2.4 Hz, 1H), 6.34–6.28 (m, 1H), 5.24 (s, 2H), 4.68 (s, 2H), 4.05 (t, J = 6.6 Hz, 2H), 2.42 (s, 3H), 1.88–1.76 (m, 2H), 1.66–1.46 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H).

5.22. {[1-(4-Butoxybenzoyl)-2-methyl-1*H*-indol-5-yl]oxy}acetic acid (9)

To a stirred solution of **28** (220 mg, 0.47 mmol) in MeOH (3 mL) and EtOAc (3 mL) was added 10 wt% Pd–C (20 mg) at room temperature, and the resulting suspension was stirred for 20 min at room temperature under hydrogen atmosphere. The resulting mixture was filtered and the filtrate was concentrated in vacuo to give a residue, which was recrystallized from EtOAc/*n*-hexane to yield **9** (80 mg, 46%) as pale pink powder; **TLC** R_f = 0.40 ($CHCl_3$/CH_3OH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.68 (d, J = 8.7 Hz, 2H), 7.00–6.92 (m, 4H), 6.70 (dd, J = 8.7,2.1 Hz, 1H), 6.36 (br s, 1H), 4.68 (s, 2H), 4.05 (t, J = 6.6 Hz, 2H), 2.42 (s, 3H), 2.00–1.40 (m, 5H), 1.00 (t, J = 7.5 Hz, 3H); **MS** (APCI, Neg. 20V) *m/z* 380 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for C_{22} $H_{23}NO_5$: 381.1576; found: 381.1556; **IR** (KBr) 3510, 2952, 1748, 1682, 1608, 1512, 1477, 1432, 1372, 1315, 1254, 1195, 1177, 1150, 1111, 1075, 913, 846, 793, 761, 652 cm^{-1}.

5.23. *tert*-Butyl 2-methyl-4-{[(trifluoromethyl)sulfonyl]oxy}-1*H*-indole-1-carboxylate (29)

To a stirred solution of **12** (380 g, 1.36 mol) in CH_3CN (750 mL) were added a solution of Boc_2O (326 g, 1.50 mol) in CH_3CN (750 mL) and *N*,*N*′-dimethylaminopyridine (1.66 g, 13.6 mmol) at room temperature. After stirring for 1 h at room temperature, the resulting mixture was concentrated in vacuo. The residue was dissolved with *n*-hexane/EtOAc and washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to give a residue, which was used for the next reaction without further purification; **TLC** R_f = 0.72 (*n*-hexane/EtOAc, 10/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.14 (m, 1H), 7.23 (dd, J = 8.4, 8.1 Hz, 1H), 7.12 (d, J = 8.1 Hz, 1H), 6.44 (m, 1H), 2.62 (d, J = 0.9 Hz, 3H), 1.69 (s, 9H).

5.24. *tert*-Butyl 2-methyl-4-[(trimethylsilyl)ethynyl]-1*H*-indole-1-carboxylate (30)

To a stirred solution of **29** (516 g, 1.36 mol) in DMF (1000 mL) were added CuI (2.59 g, 13.6 mmol), TEA (284 mL, 2.04 mmol), TBAI (5.02 g, 13.6 mmol), $Pd(PPh_3)Cl_2$ (9.55 g, 13.6 mmol) and trimethylsilylacetylene (160 g, 163 mol) at room temperature. After stirring for 7 h at 70 °C, the reaction mixture was poured into saturated NH_4Cl aq and extracted with EtOAc (×2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to give a residue, which was purified by column chromatography on silica gel to yield **30** (446 g, 100%); **TLC** R_f = 0.80 (*n*-hexane/EtOAc, 20/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.06 (m, 1H), 7.30 (dd, J = 7.5,0.9 Hz, 1H), 7.13 (dd, J = 8.1,7.5 Hz, 1H), 6.49 (m, 1H), 2.62 (d, J = 0.9 Hz, 3H), 1.68 (s, 9H), 0.29 (s, 9H).

5.25. (2-Methyl-1*H*-indol-4-yl)acetic acid (31)

To a stirred solution of borane-tetrahydrofuran complex (1.0 mol/L in THF, 666 mL, 666 mmol) in THF (350 mL) was added cyclohexene (135 mL, 1332 mmol) at 15 °C. After stirring for 1 h at same temperature, a solution of **30** (117 g, 333 mmol) in THF (200 mL) was added dropwise. After stirring for 3 h at room temperature, 2 M NaOH aq (1000 mL, 2 mol) was added dropwise at 0 °C, and then 30% aqueous hydrogen peroxide (300 mL) was added at 50 °C. The reaction mixture was concentrated in vacuo to remove THF. The resulting aqueous layer was washed with *n*-hexane, and acidified by the addition of conc HCl to pH 4 and extracted with EtOAc (×3). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to give a residue, which was used for the next reaction without further purification; **TLC** R_f = 0.53 (*n*-hexane/EtOAc, 1/1).

To a stirred solution of the above-described intermediate in MeOH (500 mL) and 1,4-dioxane (500 mL) was added 5 M NaOH aq (1000 mL). After stirring for 14 h at 70 °C, the reaction mixture was acidified with conc HCl and extracted with EtOAc (×3). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo to give a residue, which was recrystallized from EtOAc/*n*-hexane to yield **31** (142 g, 56%); **TLC** R_f = 0.29 (*n*-hexane/EtOAc=1/1); (300 MHz, $CDCl_3$/CD_3OD) δ 9.55 (br s, 1H), 7.20 (d, J = 7.8 Hz, 1H), 7.02 (dd, J = 7.8,7.8 Hz, 1H), 6.93 (d, J = 7.8 Hz, 1H), 6.23 (s, 1H), 3.83 (s, 2H), 2.41 (s, 3H).

5.26. Allyl (2-methyl-1*H*-indol-4-yl)acetate (32)

To a stirred solution of **31** (53 mg, 280 mmol) in DMF (500 mL) were added K_2CO_3 (59 g, 427 mmol) and allyl bromide (31 mL, 358 mmol) at room temperature. After stirring for 1 h at 50 °C, the reaction mixture was poured into ice-water containing EtOAc (×3). 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 **32** (43.5 g, 68%); **TLC** R_f = 0.50 (*n*-hexane/EtOAc, 2/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.92 (br s, 1H), 7.19 (d, J = 7.0 Hz, 1H), 7.06 (t, J = 7.0 Hz, 1H), 6.96 (dd, J = 7.0,1.2 Hz, 1H), 6.27 (m, 1H), 5.90 (ddt, J = 17.2, 10.4, 5.4 Hz, 1H), 5.25 (d, J = 17.2 Hz, 1H), 5.18 (d, J = 10.4 Hz, 1H), 4.59 (m, 2H), 3.88 (s, 2H), 2.43 (s, 3H).

5.27. Benzyl (2-methyl-1*H*-indol-4-yl)acetate (33)

To a stirred solution of compound **31** (650 mg, 3.44 mmol) in DMF (7 mL) were added K_2CO_3 (1.02 g, 7.38 mmol) and benzyl bromide (0.49 mL, 4.12 mmol)

at room temperature under argon atmosphere, and stirring was continued for 4 h. The reaction mixture was quenched with 1 M HCl aq and extracted two times 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 **33** (600 mg, 62%); **TLC** R_f = 0.60 (*n*-hexane/EtOAc, 3/1); (300 MHz, $CDCl_3/CD_3OD$) δ 7.93 (br s, 1H), 7.30 (m, 5H), 7.15 (d, J = 7.8 Hz, 1H), 7.04 (dd, J = 7.8,7.8 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 6.22 (s, 1H), 5.12 (s, 2H), 3.89 (s, 2H), 2.37 (s, 3H).

5.28. General procedure for the preparation of *N*-benzoyl-2-methyl-indole-4-acetic acids (34c, e–j, l–n, p–v)

5.28.1. Allyl {1-[4-(2-cyclopropylethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34h). To a stirred solution of **32** (230 mg, 1.0 mmol) and NaOH (powdered, 200 mg, 5.0 mmol) in CH_2Cl_2 (5 mL) were added TBACl (28 mg, 0.102 mmol) and *p*-(2-cyclopropylethoxy) benzoyl chloride (450 mg, 2 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 **34h** (380 mg, 91%); **TLC** R_f = 0.78 (*n*-hexane/EtOAc, 1/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.73–7.69 (m, 2H), 7.06–6.93 (m, 5H), 6.51 (s, 1H), 6.02–5.62 (m, 1H), 5.32–5.19 (m, 2H), 4.63–4.60 (m, 2H), 4.16–4.09 (m, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.73 (q, J = 7.0 Hz, 2H), 0.98–0.78 (m, 1H), 0.58–0.49 (m, 2H), 0.20–0.13 (m, 2H).

5.28.2. Allyl {1-[4-(hexyloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34c). 90% yield; **TLC** R_f = 0.36 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.72–7.68 (m, 2H), 7.08–6.85 (m, 5H), 6.50 (s, 1H), 6.02–5.60 (m, 1H), 5.33–5.18 (m, 2H), 4.63–4.58 (m, 2H), 4.04 (t, J = 6.8 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.91–1.82 (m, 2H), 1.62–1.33 (m, 6H), 1.00–0.90 (m, 3H).

5.28.3. Allyl {1-[4-(3,3-dimethylbutoxy)benzoyl]-2-methyl 2*H*}-1*H*-indol-4-yl}acetate (34e). 92% yield; **TLC** R_f = 0.36 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.69 (d, J = 8.7 Hz, 2H), 7.08–6.85 (m, 5H), 6.48 (s, 1H), 6.03–5.62 (m, 1H), 5.32–5.18 (m, 2H), 4.64–4.58 (m, 2H), 4.09 (t, J = 7.0 Hz, 2H), 3.83 (s, 2H), 2.43 (s, 3H), 1.75 (t, J = 7.0 Hz, 2H), 1.02 (s, 9H).

5.28.4. Allyl (2-methyl-1-{4-[(4-methylpentyl)oxy]benzoyl}-1*H*-indol-4-yl)acetate (34f). 83% yield; **TLC** R_f = 0.35 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.72 (d, J = 8.8 Hz, 2H), 7.08–6.88 (m, 5H), 6.49 (s, 1H), 6.04–5.60 (m, 1H), 5.33–5.19 (m, 2H), 4.65–4.60 (m, 2H), 4.03 (t, J = 6.8 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.95–1.50 (m, 3H), 1.45–1.10 (m, 2H), 0.95 (d, J = 6.8 Hz, 6H).

5.28.5. Allyl {1-[4-(2-cyclohexylethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34g). 80% yield; **TLC** R_f = 0.41 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 8.8 Hz, 2H), 7.10–6.90 (m, 5H), 6.49 (s, 1H), 6.02–5.60 (m, 1H), 5.32–5.19 (m, 2H), 4.65–4.60 (m, 2H), 4.07 (t, J = 6.8 Hz, 2H), 3.85 (s, 2H), 2.45 (s, 3H), 1.85–0.90 (m, 13H).

5.28.6. Allyl {1-[4-(but-3-en-1-yloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34i). 89% yield; **TLC** R_f = 0.60 (*n*-hexane/EtOAc, 1/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.72–7.68 (m, 2H), 7.08–6.90 (m, 5H), 6.49 (s, 1H), 6.03–5.60 (m, 2H), 5.32–5.10 (m, 4H), 4.63–4.59 (m, 2H), 4.10 (t, J = 6.6 Hz, 2H), 3.86 (s, 2H), 2.70–2.53 (s, 2H), 2.45 (s, 3H).

5.28.7. Allyl {1-[4-(benzyloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34j). 78% yield; **TLC** R_f = 0.32 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70–7.50 (m, 2H), 7.50–7.35 (m, 5H), 7.06–6.90 (m, 5H), 6.45 (s, 1H), 6.02–5.60 (m, 1H), 5.32–5.10 (m, 4H), 4.63–4.60 (m, 2H), 3.85 (s, 2H), 2.42 (s, 3H).

5.28.8. Allyl {2-methyl-1-[4-(3-phenylpropoxy)benzoyl]-1*H*-indol-4-yl}acetate (34l). 85% yield; **TLC** R_f = 0.67 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 9.0 Hz, 2H), 7.35–7.16 (m, 5H), 7.08–6.85 (m, 5H), 6.51 (s, 1H), 6.02–5.62 (m, 1H), 5.32–5.20 (m, 2H), 4.63–4.59 (m, 2H), 4.05 (t, J = 6.5 Hz, 2H), 3.87 (s, 2H), 2.84 (t, J = 6.5 Hz, 2H), 2.45 (s, 3H), 2.20–2.09 (m, 2H).

5.28.9. Allyl {1-[4-(2-methoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34m). 64% yield; **TLC** R_f = 0.20 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 8.8 Hz, 2H), 7.10–6.90 (m, 5H), 6.50 (s, 1H), 6.05–5.60 (m, 1H), 5.35–5.20 (m, 2H), 4.63–4.60 (m, 2H), 4.20 (t, J = 5.0 Hz, 2H), 3.85 (s, 2H), 3.80 (t, J = 5.0 Hz, 2H), 3.46 (s, 3H), 2.45 (s, 3H).

5.28.10. Allyl {1-[4-(3-methoxypropoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34n). 63% yield; **TLC** R_f = 0.25 (*n*-hexane/EtOAc, 2/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.72 (d, J = 8.7 Hz, 2H), 7.09–6.90 (m, 5H), 6.50 (s, 1H), 6.02–5.60 (m, 1H), 5.35–5.20 (m, 2H), 4.65–4.60 (m, 2H), 4.15 (t, J = 6.7 Hz, 2H), 3.87 (s, 2H), 3.58 (t, J = 6.0 Hz, 2H), 3.37 (s, 3H), 2.43 (s, 3H), 2.20–2.05 (m, 2H).

5.28.11. Allyl {1-[4-(3-ethoxypropoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34p). 83% yield; **TLC** R_f = 0.33 (*n*-hexane/EtOAc, 2/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.71 (d, J = 9.0 Hz, 2H), 7.10–6.85 (m, 5H), 6.52 (s, 1H), 6.05–5.60 (m, 1H), 5.37–5.18 (m, 2H), 4.65–4.60 (m, 2H), 4.17 (t, J = 6.5 Hz, 2H), 3.85 (s, 2H), 3.62 (t, J = 6.5 Hz, 2H), 3.50 (q, J = 6.8 Hz, 2H), 2.45 (s, 3H), 2.08 (m, 2H), 1.21 (t, J = 6.8 Hz, 3H).

5.28.12. Allyl {2-methyl-1-[4-(2-propoxyethoxy)benzoyl]-1*H*-indol-4-yl}acetate (34q). 62% yield; **TLC** R_f = 0.39 (*n*-hexane/EtOAc, 2/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72 (d, J = 8.9 Hz, 2H), 7.05–6.90 (m, 5H), 6.49 (s, 1H), 6.02–5.60 (m, 1H), 5.32–5.20 (m, 2H), 4.65–4.58 (m, 2H), 4.23–4.20 (m, 2H), 3.86 (s, 2H), 3.86–3.80 (m, 2H), 3.50 (t, J = 7.0 Hz, 2H), 2.44 (s, 3H), 1.65 (m, 2H), 0.95 (t, J = 7.5 Hz, 3H).

5.28.13. Allyl {1-[4-(2-isopropoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34r). 100% yield; **TLC** R_f=0.65 (*n*-hexane/EtOAc, 1/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.72–7.68 (m, 2H), 7.08–6.80 (m, 5H), 6.50 (s, 1H), 6.02–5.62 (m, 1H), 5.32–5.19 (m, 2H), 4.63–4.59 (m, 2H), 4.22–4.14 (m, 2H), 3.86 (s, 2H), 3.86–3.65 (m, 3H), 2.44 (s, 3H), 1.21 (d, J=6.0 Hz, 6H).

5.28.14. Allyl {1-[4-(2-*tert*-butoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34s). 80% yield; **TLC** R_f=0.68 (*n*-hexane/EtOAc, 1/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J=9.0 Hz, 2H), 7.04–6.93 (m, 5H), 6.50 (s, 1H), 6.01–5.82 (m, 1H), 5.32–5.19 (m, 2H), 4.61 (dt, J=5.8,1.6 Hz, 2H), 4.17 (t, J=5.2 Hz, 2H), 3.86 (s, 2H), 3.76 (t, J=5.2 Hz, 2H), 2.45 (s, 3H), 1.25 (s, 9H); **MS** (APCI, Pos, 20V.) *m*/*z* 450 $(M+H)^+$.

5.28.15. Allyl (1-{4-[2-(2-methoxyethoxy)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (34t). 97% yield; **TLC** R_f=0.49 (*n*-hexane/EtOAc, 1/2); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.71–7.68 (m, 2H), 7.10–6.80 (m, 5H), 6.50 (s, 1H), 5.98–5.70 (m, 1H), 5.62–5.40 (m, 2H), 4.63–4.60 (m, 2H), 4.25–4.11 (m, 2H), 3.92–3.89 (m, 2H), 3.86 (s, 2H), 3.78–3.73 (m, 2H), 3.60–3.58 (m, 2H), 3.40 (s, 3H), 2.45 (s, 3H).

5.28.16. Allyl {2-methyl-1-[4-(2-phenoxyethoxy)benzoyl]-1*H*-indol-4-yl}acetate (34u). 97% yield; **TLC** R_f=0.50 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72 (d, J=9.0 Hz, 2H), 7.32 (t, J=7.7 Hz, 2H), 7.10–6.90 (m, 8H), 6.50 (s, 1H), 6.00–5.70 (m, 1H), 5.40–5.20 (m, 2H), 4.63–4.60 (m, 2H), 4.45–4.30 (m, 4H), 3.86 (s, 2H), 2.45 (s, 3H).

5.28.17. Allyl (1-{4-[2-(ethylthio)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetate (34v). 85% yield; **TLC** R_f=0.46 (*n*-hexane/EtOAc, 7/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.71 (d, J=9.0 Hz, 2H), 7.05–6.90 (m, 5H), 6.50 (s, 1H), 6.00–5.75 (m, 1H), 5.35–5.19 (m, 2H), 4.63–4.58 (m, 2H), 4.23 (t, J=7.0 Hz, 2H), 3.86 (s, 2H), 2.95 (t, J=7.0 Hz, 2H), 2.67 (q, J=7.4 Hz, 2H), 2.45 (s, 3H), 1.30 (t, J=7.4 Hz, 3H).

5.29. General procedure for the preparation of *N*-benzoyl-2-methylindole-4-acetic acids (34a, b, d, k, o)

5.29.1. Benzyl [1-(4-butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]acetate (34a). To a stirred solution of **33** (285 mg, 1.02 mmol) and NaOH (powdered, 203 mg, 5.08 mmol) in CH_2Cl_2 (4 mL) were added TBACl (28 mg, 0.102 mmol) and *p*-(*n*-butyloxy) benzoyl chloride (0.29 mL, 1.53 mmol) at room temperature. After stirring for 30 min at room temperature, the reaction mixture was purified by column chromatography on silica gel to yield **3b** (395 mg, 85%); **TLC** R_f=0.56 (*n*-hexane/EtOAc, 4/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.70 (d, J=9.0 Hz, 2H), 7.40–7.25 (m, 5H), 7.08–6.88 (m, 5H), 6.45 (s, 1H), 5.15 (s, 2H), 4.05 (t, J=6.6 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.90–1.74 (m, 2H), 1.60–1.45 (m, 2H), 1.00 (t, J=7.5 Hz, 3H).

5.29.2. Benzyl {2-methyl-1-[4-(pentyloxy)benzoyl]-1*H*-indol-4-yl}acetate (34b). 91% yield; **TLC** R_f=0.58 (*n*-hexane/EtOAc, 4/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72–7.65 (m, 2H), 7.39–7.28 (m, 5H), 7.05–6.88 (m, 5H), 6.45 (s, 1H), 5.15 (s, 2H), 4.04 (t, J=6.6 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.87–1.20 (m, 6H), 0.95 (t, J=6.9 Hz, 3H).

5.29.3. Benzyl {2-methyl-1-[4-(3-methylbutoxy)benzoyl]-1*H*-indol-4-yl}acetate (34d). 80% yield; **TLC** R_f=0.58 (*n*-hexane/EtOAc, 4/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.73–7.67 (m, 2H), 7.42–7.20 (m, 5H), 7.06–6.90 (m, 5H), 6.45 (s, 1H), 5.15 (s, 2H), 4.06 (t, J=6.6 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.85 (m, 1H), 1.72 (m, 2H), 0.99 (t, J=6.2 Hz, 6H).

5.29.4. Benzyl {2-methyl-1-[4-(2-phenylethoxy)benzoyl]-1*H*-indol-4-yl}acetate (34k). 100% yield; **TLC** R_f=0.64 (*n*-hexane/EtOAc, 8/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 8.07 (d, J=9.0 Hz, 1H), 7.69 (d, J=8.6 Hz, 2H), 7.40–7.24 (m, 9H), 7.10–6.90 (m, 5H), 6.45 (s, 1H), 5.15 (s, 2H), 4.26 (t, J=7.0 Hz, 2H), 3.88 (s, 2H), 3.15 (t, J=7.0 Hz, 2H), 2.42 (s, 3H).

5.29.5. Benzyl {1-[4-(2-ethoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetate (34o). 100% yield; **TLC** R_f=0.35 (*n*-hexane/EtOAc, 4/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J=9.0 Hz, 2H), 7.39–7.28 (m, 5H), 7.05–6.88 (m, 5H), 6.45 (s, 1H), 5.15 (s, 2H), 4.21 (t, J=4.8 Hz, 2H), 3.87 (s, 2H), 3.83 (t, J=4,8 Hz, 2H), 3.62 (q, J=6.6 Hz, 2H), 2.42 (s, 3H), 1.26 (t, J=6.6 Hz, 3H).

5.30. General procedure for the preparation of *N*-benzoyl-2-methylindole-4-acetic acids (3c, e–j, l–n)

5.30.1. {1-[4-(2-Cyclopropylethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3h). To a stirred solution of **34h** (380 mg, 0.91 mmol) in THF (5 mL) was added $Pd(PPh_3)_4$ (50 mg, 0.05 mmol) at room temperature under argon atmosphere. After stirring for 15 min, morpholine (0.40 mL, 4.55 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 **3h** (214 mg, 62%); **TLC** R_f=0.37 (EtOAc); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.73–7.69 (m, 2H), 7.08–6.94 (m, 5H), 6.49 (s, 1H), 4.12 (t, J=6.5 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.72 (q, J=6.5 Hz, 2H), 0.96–0.80 (m, 1H), 0.56–0.47 (m, 2H), 0.18–0.13 (m, 2H); **MS** (APCI, Neg, 20V.) *m*/*z* 376 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{23}NO_4$: 377.1627; found: 395.1811; **IR** (neat) 2944, 1703, 1604, 1510, 1436, 1372, 1302, 1214, 1173, 1010, 911, 757, 707, 638 cm^{-1}.

5.30.2. {1-[4-(Hexyloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3c). 45% yield; **TLC** R_f=0.44 (EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72–7.69 (m, 2H), 7.06–6.93 (m, 5H), 6.49 (s, 1H), 4.04 (t, J=6.5 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.9–1.8 (m, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.9–1.8 (m, 2H), 1.6–1.4 (m, 2H), 1.4–1.3 (m, 4H), 1.0–0.9 (m, 3H); **MS** (APCI, Neg, 20V.) *m*/*z* 392 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{24}H_{27}NO_4$: 393.1940; found: 393.1949; **IR** (KBr)

2953, 1678, 1606, 1511, 1436, 1374, 1322, 1260, 1211, 1172, 1020, 911, 839, 812, 775, 759, 706, 640 cm^{-1}.

5.30.3. {1-[4-(3,3-Dimethylbutoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3e). 68% yield; **TLC** R_f = 0.45 ($CHCl_3$/MeOH, 10/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.68 (d, J = 8.8 Hz, 2H), 7.08–6.86 (m, 5H), 6.47 (s, 1H), 4.09 (t, J = 7.2 Hz, 2H), 3.83 (s, 2H), 2.43 (s, 3H), 1.76 (t, J = 7.2 Hz, 2H), 1.00 (s, 9H); **MS** (APCI, Neg, 20V.) m/z 392 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{24}H_{27}NO_4$: 393.1940; found: 393.1934; **IR** (KBr) 2955, 1686, 1605, 1573, 1510, 1435, 1369, 1299, 1259, 1169 cm^{-1}.

5.30.4. (2-Methyl-1-{4-[(4-methylpentyl)oxy]benzoyl}-1*H*-indol-4-yl)acetic acid (3f). 84% yield; **TLC** R_f = 0.43 (*n*-hexane/EtOAc, 1/3); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 8.6 Hz, 2H), 7.07–6.88 (m, 5H), 6.48 (s, 1H), 4.03 (t, J = 6.8 Hz, 2H), 3.86 (s, 2H), 2.45 (s, 3H), 1.92–1.73 (m, 2H), 1.73–1.52 (m, 1H), 1.43–1.11 (m, 2H), 0.94 (d, J = 6.8 Hz, 6H); **MS** (FAB, Pos.) m/z 394 $(M+H)^+$; **HRMS** (EI, Pos.) calcd for $C_{24}H_{27}NO_4$: 393.1940; found: 393.1950; **IR** (KBr) 2962, 1704, 1683, 1607, 1577, 1510, 1471, 1436, 1373, 1317, 1260, 1214, 1170, 1114, 1047, 1006, 913, 861, 837, 812, 776, 759, 743, 708, 642, 621, 609 cm^{-1}.

5.30.5. {1-[4-(2-Cyclohexylethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3g). 65% yield; **TLC** R_f = 0.50 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 8.8 Hz, 2H), 7.06–6.91 (m, 5H), 6.48 (s, 1H), 4.08 (t, J = 6.8 Hz, 2H), 3.86 (s, 2H), 2.44 (s, 3H), 1.81–0.95 (m, 13H); **MS** (APCI, Neg, 20V.) m/z 418 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{26}H_{29}NO_4$: 419.2097; found: 419.2108; **IR** (KBr) 3436, 2923, 2851, 1684, 1605, 1574, 1510, 1435, 1370, 1300, 1257, 1222, 1169, 1043, 960, 911, 837, 760, 642 cm^{-1}.

5.30.6. {1-[4-(But-3-en-1-yloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3i). 43% yield; **TLC** R_f = 0.28 (EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72–7.69 (m, 2H), 7.08–6.94 (m, 5H), 6.49 (s, 1H), 5.98–5.85 (m, 1H), 5.23–5.13 (m, 2H), 4.10 (t, J = 6.5 Hz, 2H), 3.86 (s, 2H), 2.64–2.56 (m, 2H), 2.45 (s, 3H); **MS** (APCI, Neg, 20V.) m/z 362 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{22}H_{21}NO_4$: 363.1471; found: 363.1467; **IR** (KBr) 2928, 1705, 1675, 1603, 1438, 1373, 1300, 1258, 1214, 1174, 911, 757, 639 cm^{-1}.

5.30.7. {1-[4-Benzyloxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3j). 56% yield; **TLC** R_f = 0.55 ($CHCl_3$/MeOH, 10/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.65–7.33 (d, J = 8.8 Hz, 2H), 7.45–7.36 (m, 5H), 7.05–6.90 (m, 5H), 6.48 (s, 1H), 5.14 (s, 2H), 3.85 (s, 2H), 2.43 (d, J = 1.0 Hz, 3H); **MS** (APCI, Neg, 20V.) m/z 398 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{25}H_{21}NO_4$: 399.1471; found: 399.1494; **IR** (KBr) 3437, 1698, 1686, 1671, 1602, 1509, 1435, 1372, 1320, 1303 cm^{-1}.

5.30.8. {2-Methyl-1-[4-(3-phenylpropoxy)benzoyl]-1*H*-indol-4-yl}acetic acid (3l). 86% yield; **TLC** R_f = 0.41 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 9.3 Hz, 2H), 7.34–7.16 (m, 5H), 7.08–6.88 (m, 5H), 6.50 (s, 1H), 4.05 (t, J = 6.3 Hz, 2H), 3.88 (s, 2H), 2.84 (t, J = 6.3 Hz, 2H), 2.45 (s, 3H), 2.20–2.10 (m, 2H); **MS** (APCI, Neg, 20V.) m/z 426 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{27}H_{25}NO_4$: 427.1784; found: 427.1772; **IR** (neat) 3369, 2922, 1682, 1604, 1510, 1435, 1370, 1300, 1257, 1169, 1031, 912, 837, 743, 701, 590 cm^{-1}.

5.30.9. {1-[4-(2-Methoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3m). 67% yield; **TLC** R_f = 0.44 ($CHCl_3$/MeOH, 10/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 8.8 Hz, 2H), 7.03–6.93 (m, 5H), 6.48 (s, 1H), 4.21 (t, J = 5.0 Hz, 2H), 3.85 (s, 2H), 3.79 (t, J = 5.0 Hz, 2H), 3.47 (s, 3H), 2.44 (s, 3H); **MS** (APCI, Neg, 20V.) m/z 366 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{21}H_{21}NO_5$: 367.1420; found: 367.1425; **IR** (KBr) 2941, 1687, 1609, 1437, 1373, 1318, 1259, 1216, 1175, 1123 cm^{-1}.

5.30.10. {1-[4-(3-Methoxypropoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3n). 74% yield; **TLC** R_f = 0.12 (EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72–7.69 (m, 2H), 7.08–6.94 (m, 5H), 6.48 (s, 1H), 4.15 (t, J = 6.5 Hz, 2H), 3.86 (s, 2H), 3.58 (t, J = 6.0 Hz, 2H), 3.37 (s, 3H), 2.44 (s, 3H), 2.13–2.05 (m, 2H); **MS** (APCI, Neg, 20V.) m/z 380 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{22}H_{23}NO_5$: 381.1576; found: 381.1575; **IR** (KBr) 2931, 1677, 1608, 1435, 1337, 1253, 1174, 964, 852, 759, 622 cm^{-1}.

5.30.11. {1-[4-(3-Ethoxypropoxy)benzoyl]-2-methyl-1*H*-indol-4- yl}acetic acid (3p). 87% yield; **TLC** R_f = 0.22 (*n*-hexane/EtOAc, 1/2); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.70 (d, J = 9.0 Hz, 2H), 7.10–6.86 (m, 5H), 6.49 (s, 1H), 4.16 (t, J = 6.4 Hz, 2H), 3.86 (s, 2H), 3.62 (t, J = 6.4 Hz, 2H), 3.51 (q, J = 6.8 Hz, 2H), 2.45 (s, 3H), 2.09 (m, 2H), 1.21 (t, J = 6.8 Hz, 3H); **MS** (APCI, Neg, 20V.) m/z 394 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{25}NO_5$: 395.1733; found: 395.1762; **IR** (neat) 2974, 2873, 1684, 1604, 1573, 1510, 1434, 1370, 1300, 1258, 1221, 1169, 1116, 959, 911, 838, 761, 641, 617 cm^{-1}.

5.30.12. {2-Methyl-1-[4-(2-propoxyethoxy)benzoyl]-1*H*-indol-4-yl}acetic acid (3q). 82% yield; **TLC** R_f = 0.20 (EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.71–7.69 (m, 2H), 7.04–6.92 (m, 5H), 6.48 (s, 1H), 4.23-4.19 (m, 2H), 3.86 (s, 2H), 3.86–3.81 (m, 2H), 3.51 (t, J = 7.0 Hz, 2H), 2.44 (s, 3H), 1.65 (tq, J = 7.0,7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H); **MS** (APCI, Neg, 20V.) m/z 394 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{25}NO_5$: 395.1733; found: 395.1740; **IR** (KBr) 2965, 1704, 1607, 1512, 1436, 1374, 1322, 1211, 1175, 1047, 916, 760, 621 cm^{-1}.

5.30.13. {1-[4-(2-Isopropoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3r). 50% yield; **TLC** R_f = 0.35 (EtOAc); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.72–7.67 (m, 2H), 7.04–6.90 (m, 5H), 6.48 (s, 1H), 4.22–4.17 (m, 2H), 3.86 (s, 2H), 3.84–3.80 (m, 2H), 3.78–3.62 (m, 1H), 2.44 (s, 3H), 1.21 (d, J = 6.0 Hz, 6H); **MS** (APCI, Neg, 20V.) m/z 394 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{25}NO_5$: 395.1733; found:

395.1725; **IR** (KBr) 2976, 1687, 1604, 1508, 1436, 1372, 1316, 1257, 1212, 1172, 1036, 912, 808, 757, 642 cm^{-1}.

5.30.14. {1-[4-(2-*tert*-Butoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3s). 58% yield; **TLC** R_f=0.50 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.69 (d, J=8.8 Hz, 2H), 7.04–6.94 (m, 5H), 6.48 (s, 1H), 4.17 (t, J=5.2 Hz, 2H), 3.86 (s, 2H), 3.76 (t, J=5.2 Hz, 2H), 2.44 (s, 3H), 1.25 (s, 9H); **MS** (APCI, Neg, 20V.) m/z 408 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{24}H_{27}NO_5$: 409.1889; found: 409.1891; **IR** (KBr) 2975, 2927, 1684, 1605, 1574, 1510, 1434, 1368, 1300, 1260, 1222, 1194, 1170, 1099, 961, 912, 779, 761, 641, 615 cm^{-1}.

5.30.15. (1-{4-[2-(2-Methoxyethoxy)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (3t). 75% yield; **TLC** R_f=0.20 (EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.71–7.68 (m, 2H), 7.06–6.91 (m, 5H), 6.48 (s, 1H), 4.24–4.21 (m, 2H), 3.92–3.89 (m, 2H), 3.85 (s, 2H), 3.76–3.73 (m, 2H), 3.61–3.58 (m, 2H), 3.40 (s, 3H), 2.44 (s, 3H); **MS** (APCI, Neg, 20V.) m/z 410 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{25}NO_6$: 411.1682; found: 411.1679; **IR** (KBr) 2935, 1681, 1608, 1511, 1373, 1319, 912, 759 cm^{-1}.

5.30.16. {2-Methyl-1-[4-(2-phenoxyethoxy)benzoyl]-1*H*-indol-4-yl}acetic acid (3u). 70% yield; **TLC** R_f=0.55 ($CHCl_3$/MeOH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72 (d, J=9.0 Hz, 2H), 7.32 (t, J=7.8 Hz, 2H), 7.08–6.92 (m, 8H), 6.49 (s, 1H), 4.46–4.34 (m, 4H), 3.87 (s, 2H), 2.45 (s, 3H); **MS** (APCI, Neg, 20V.) m/z 428 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{26}H_{23}NO_5$: 429.1576; found: 429.1567; **IR** (neat) 3369, 1684, 1602, 1497, 1477, 1434, 1370, 1301, 1244, 1170, 912, 756 cm^{-1}.

5.30.17. (1-{4-[2-(Ethylthio)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (3v). 75% yield; **TLC** R_f=0.50 ($CHCl_3$/MeOH, 10/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.71 (d, J=9.0 Hz, 2H), 7.05–6.93 (m, 5H), 6.48 (s, 1H), 4.22 (t, J=6.8 Hz, 2H), 3.86 (s, 2H), 2.95 (t, J=6.8 Hz, 2H), 2.67 (q, J=7.4 Hz, 2H), 2.44 (s, 3H), 1.31 (t, J=7.4 Hz, 3H); **MS** (APCI, Neg, 20V.) m/z 396 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{22}H_{23}NO_4S$: 397.1348; found: 397.1321; **IR** (KBr) 3405, 2928, 1702, 1603, 1509, 1436, 1372, 1320, 1256, 1213, 1174, 912 cm^{-1}.

5.31. General procedure for the preparation of *N*-benzoyl-2-methyl-indole-4-acetic acids (3a, b, k, d, o)

5.31.1. [1-(4-Butoxybenzoyl)-2-methyl-1*H*-indol-4-yl]acetic acid (3a). To a stirred solution of the compound **34a** (110 mg, 239 mmol) in EtOAc (4 mL) was added 10% Pd–C (15 mg) 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/*n*-hexane to yield **3a** (25 mg, 28%); **TLC** R_f=0.46 ($CHCl_3$/CH_3OH, 9/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.70 (d, J=9.0 Hz, 2H), 7.08–6.90 (m, 5H), 6.49 (s, 1H), 4.05 (t, J=6.6 Hz, 2H), 3.87 (s, 2H), 2.45 (s, 3H), 1.88–1.74 (m, 2H), 1.80–1.40 (br, 1H), 1.60–1.45 (m, 2H), 1.00 (t, J=7.5 Hz, 3H); **MS** (APCI, Neg 20V) m/z 364 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{22}H_{23}NO_4$: 365.1627; found: 365.1619; **IR** (neat) 3369, 2959, 1682, 1604, 1574, 1511, 1472, 1434, 1369, 1299, 1257, 1222, 1169, 1016, 912, 837, 779, 641 cm^{-1}.

5.31.2. {2-Methyl-1-[4-(pentyloxy)benzoyl]-1*H*-indol-4-yl}acetic acid (3b). 58% yield; **TLC** R_f=0.64 ($CHCl_3$/CH_3OH, 10/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.73–7.66 (m, 2H), 7.07–6.92 (m, 5H), 6.49 (s, 1H), 4.04 (t, J=6.6 Hz, 2H), 3.87 (s, 2H), 2.44 (s, 3H), 1.86–1.23 (m, 6H), 0.95 (t, J=6.9 Hz, 3H); **MS** (APCI, Neg, 20V.) m/z 378 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{25}NO_4$: 379.1784; found: 379.1800; **IR** (KBr) 2945, 1707, 1679, 1607, 1511, 1437, 1374, 1320, 1304, 1261, 1214, 1174, 1049, 1014, 913, 839, 808, 760, 743, 707, 642 cm^{-1}.

5.31.3. {2-Methyl-1-[4-(3-methylbutoxy)benzoyl]-1*H*-indol-4-yl}acetic acid (3d). 64% yield; **TLC** R_f=0.34 ($CHCl_3$/CH_3OH, 10/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.73–7.67 (m, 2H), 7.06–6.91 (m, 5H), 6.48 (s, 1H), 4.07 (t, J=6.6 Hz, 2H), 3.86 (s, 2H), 2.45 (s, 3H), 1.84 (m, 1H), 1.72 (m, 2H), 0.98 (d, J=6.4 Hz, 6H); **MS** (FAB Pos.) m/z 380 $(M+H)^+$; **HRMS** (EI, Pos.) calcd for $C_{23}H_{25}NO_4$: 379.1784; found: 379.1799; **IR** (KBr) 2958, 1704, 1680, 1606, 1438, 1374, 1320, 1304, 1259, 1216, 1174, 913, 760, 641 cm^{-1}.

5.31.4. {2-Methyl-1-[4-(2-phenylethoxy)benzoyl]-1*H*-indol-4-yl}acetic acid (3k). 58% yield; **TLC** R_f=0.60 ($CHCl_3$/MeOH, 10/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72–7.66 (m, 2H), 7.38–7.23 (m, 5H), 7.07–6.92 (m, 5H), 6.49 (s, 1H), 4.26 (t, J=6.9 Hz, 2H), 3.86 (s, 2H), 3.14 (t, J=6.9 Hz, 2H), 2.44 (s, 3H); **MS** (APCI, Neg, 20V.) m/z 412 $(M-H)^-$; **HRMS** (EI, Pos.) calcd for $C_{26}H_{23}NO_4$: 413.1627; found: 413.1637; **IR** (KBr) 1684, 1604, 1510, 1434, 1370, 1300, 1258, 1169, 911, 758, 701 cm^{-1}.

5.31.5. {1-[4-(2-Ethoxyethoxy)benzoyl]-2-methyl-1*H*-indol-4-yl}acetic acid (3o). 90% yield; **TLC** R_f=0.50 ($CHCl_3$/CH_3OH, 10/1); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.70 (d, J=8.7 Hz, 2H), 7.08–6.92 (m, 5H), 6.49 (s, 1H), 4.21 (t, J=4.8 Hz, 2H), 3.87–3.81 (m, 4H), 3.62 (q, J=7.5 Hz, 2H), 2.45 (s, 3H), 1.26 (t, J=7.5 Hz, 3H); **MS** (APCI, Pos, 20V.) m/z 382 $(M+H)^+$; **HRMS** (EI, Pos.) calcd for $C_{22}H_{23}NO_5$: 381.1576; found: 381.1590; **IR** (KBr) 2979, 1703, 1676, 1607, 1513, 1439, 1374, 1321, 1305, 1264, 1214, 1176, 1127, 1046, 913, 842, 806, 759, 743 cm^{-1}.

5.31.6. (1-{4-[2-(Ethylsulfonyl)ethoxy]benzoyl}-2-methyl-1*H*-indol-4-yl)acetic acid (10). To a stirred solution of **3v** (0.050 g, 0.126 mmol) in CH_2Cl_2 (5 mL) was added mCPBA (0.0870 g, 0.504 mmol) at room temperature. After stirring 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 a residue, which was purified by column chromatography

on silica gel to yield **10** (0.044 g, 81%); **TLC** R_f = 0.40 ($CHCl_3/CH_3OH$, 10/1); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.73 (d, J = 8.8 Hz, 2H), 7.07–6.90 (m, 5H), 6.49 (s, 1H), 4.52 (t, J = 5.6 Hz, 2H), 3.86 (s, 2H), 3.47 (t, J = 5.6 Hz, 2H), 3.20 (q, J = 7.4 Hz, 2H), 2.44 (s, 3H), 1.48 (t, J = 7.4 Hz, 3H); **MS** (APCI, Neg, 20V.) m/z 428 $(M-H)^-$; **IR** (KBr) 2927, 1681, 1606, 1435, 1317, 1256, 1172, 1127 cm^{-1}.

5.32. Prostanoid mEP, 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, mEP1, mEP2, mEP3α, mEP4, mDP, 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 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, 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 25 °C for 60 min except for mEP1 and mDP (20 min), IP (30 min). The incubation was terminated by filtration through Whatman GF/B filters. The filters were then 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, 50 mM Tris–HCl buffer containing 1 mM EDTA and 10 mM $MgCl_2$, pH 7.5, for IP-receptor), 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) 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 radiolabeled ligand; K_d: Dissociation constant of radiolabeled ligand towards the prostanoid receptors.

5.33. 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 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 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 0.1% 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%) 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 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 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.

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