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Discovery of orally active prostaglandin D_2 receptor antagonists

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Abstract—A series of *N*-(*p*-alkoxy)benzoyl-2-methylindole-4-acetic acids were synthesized and evaluated for prostaglandin D_2 (DP) receptor affinity and antagonist activity. Some of them exhibited strong receptor binding and were potent in the cAMP formation assays. These antagonists also suppressed allergic inflammatory responses such as the PGD_2-induced increase of microvascular permeability. Structure–activity relationship (SAR) data are presented.

1. Introduction

Prostaglandin D_2 (PGD_2) is considered to play an important role in various allergic diseases such as allergic rhinitis,[1] atopic asthma,[2] allergic conjunctivitis,[3] and atopic dermatitis.[4] However, there have been very few reports about the efficacy of PGD_2 receptor (DP) antagonists in animal models of allergy or patients with allergic diseases.[5] A DP receptor selective antagonist was considered to possess potential therapeutic value for various allergic disorders.

In the preceding paper,[6] we reported on the discovery of a new chemical lead **1** for DP receptor selective antagonists starting from the chemical modification of indomethacin analogs. Further optimization of the new chemical lead **1** was carried out. Modification of the chemical lead **1** was concentrated on the terminal alkyl moiety, which was still not optimized. The *p*-phenyloxyethyloxy derivative **2d** showed the most potent mDP receptor antagonist activity among the compounds listed in Table 1. However, the human (h)DP receptor antagonist activity of **2d** was found to be relatively lower than the mDP receptor antagonist activity as described in Table 1. Further optimization of **2d** was continued to increase its hDP receptor antagonist activity.

Here we report on the identification of an orally active hDP receptor antagonist **3i**, which exhibited efficacy in animal models, starting from chemical modification of **2d** (Fig. 1).

2. Chemistry

A series of *N*-benzoyl-2-methylindole-4-acetic acids **1**, **2a–d**, and **3a–i** listed in Tables 1 and 2 were synthesized as outlined in Scheme 1. Efficient synthetic method of 2-methylindole-4-acetic acid has been unknown. In the preceding paper,[6] we reported on the synthesis of 2-methylindole-4-acetic acid using palladium-catalyzed carbon monoxide insertion reaction starting from 2-methyl-4-hydroxyindole **4a**. Here we report on the more efficient synthesis consisting of palladium-catalyzed C2 homologation followed by the hydroboration.

Replacement of the trifluoromethane sulfonate function of **4c** with trimethylsilylacetylene moiety was successfully carried out to afford **5** by the palladium catalyzed reaction in the presence of cuprous iodide.[7] Hydroboration of **5** followed by an alkaline deprotection of indole nitrogen and the appropriate esterification afforded **6a** and **6b**, which were converted to **1**, **2a–d**, and **3a–i** by the following sequential procedures: (1) *N*-acylation with *p*-acetyloxybenzoyl chloride; (2) aminolysis with pyrollidine; (3) Mitsunobu reaction with an appropriate alcohol; (4) deprotection. The alcohol **11** for **3i** were prepared from **7** as outlined in Scheme 2. *N*-Tosylation

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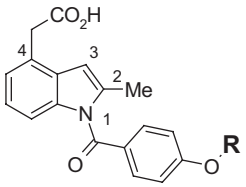
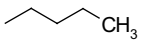
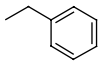
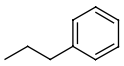
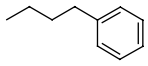
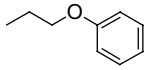
Table 1. Effect of the *p*-alkoxy substituent of the *N*-benzoyl moiety on the activity profiles



Compound	R	Binding K_i (μM)					IC_{50} (μM)	
		mEP1	mEP2	mEP3	mEP4	mDP	mDP	hDP
1		>10	2.0	3.3	>10	0.010	0.30	NT[a]
2a		1.2	0.33	1.2	>10	0.068	1.3	NT[a]
2b		0.40	1.3	2.2	1.9	0.0018	0.12	NT[a]
2c		7.7	NT[a]	3.3	2.1	0.17	NT[a]	NT[a]
2d		5.6	0.54	>10	>10	0.017	0.053	0.25

Using membrane fractions of CHO cells expressing the prostanoid receptors, the mouse (m) EP receptor or (m) DP receptor, K_i values were determined by competitive binding assay, which was performed according to the method of Kiriyama et al. with some modifications.[7] With regard to the mDP and human (h) DP receptor antagonist activity, IC_{50} values were determined based on the effects of the test compounds on the increase in intracellular cAMP formation in the presence of bovine serum albumin (BSA) (0.1%) evoked by PGD_2 in mDP or hDP receptor expressing CHO cells.

[a] NT: not tested.

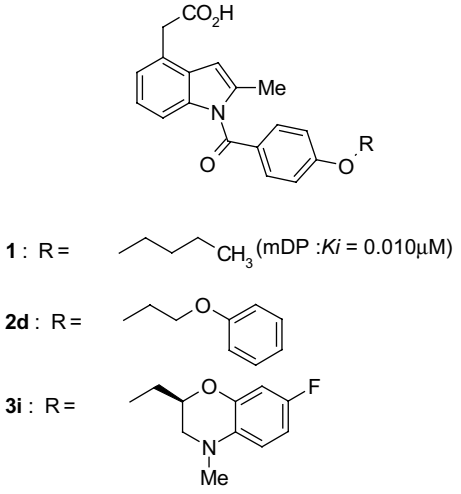


Figure 1. Discovery of new DP receptor antagonists.

of **7** with tosyl chloride gave **8**. *N*-Alkylation of **8** with (*S*)-(−)-glycidyl triphenylmethyl ether provided **9**. Cyclization reaction of **9**, followed by removal of the *N*-tosyl moiety, produced **10**. *N*-Methylation of **10** followed by acidic deprotection produced **11**.

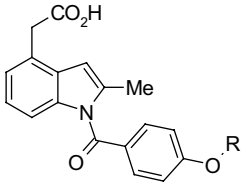
3. Results and discussion

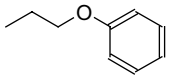
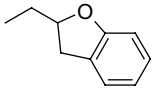
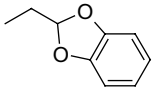
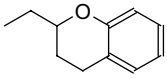
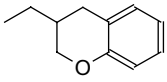
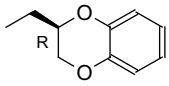
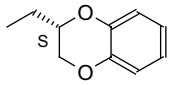
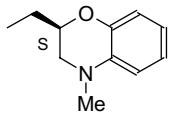
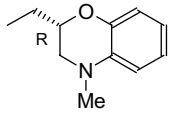
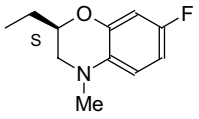
The compounds listed in Tables 1 and 2 were evaluated for inhibition of the specific binding of a radioligand, [^{3}H]PGD_2, to membrane fractions prepared from cells stably expressing each prostanoid receptor and for inhibition of cAMP formation evoked by PGD_2 in CHO cells[8] in the presence of BSA (0.1%). Test compounds were also evaluated for binding to all subtypes of the mouse PGE_2 receptor (mEP1, mEP2, mEP3, and mEP4).[9]

In the preceding paper,[6] we reported on the discovery of **1** as a new chemical lead for DP receptor antagonists (Fig. 1). We then conducted further optimization of *N*-benzoyl-2-methylindole-4-acetic acid as a subtype-selective DP receptor antagonist.

As shown in Table 1, the effect of the *p*-alkoxy substituent of the *N*-benzoyl moiety of 2-methylindole-4-acetic acid was investigated. Replacement of the *p*-butyloxy moiety of **1** with benzyloxy, phenylethyloxy, and phenylpropyloxy moieties provided **2a**–**c**, respectively. *N*-(*p*-Benzyloxy)benzoyl analog **2a** demonstrated nearly 7-fold lower mDP receptor binding affinity, while the *N*-(*p*-phenylethyloxy)benzoyl and *N*-(*p*-phenylpropyloxy)benzoyl analogs **2b**–**c** demonstrated nearly 5-fold stronger and 17-fold weaker mDP receptor binding affinity, respectively. Regarding mDP receptor antagonist activity, **2a** showed nearly 4-fold lower potency relative to **1**, while **2b** showed nearly 2-fold greater potency. Replacement of the *p*-butyloxy moiety of **1** with a *p*-phenyloxyethyloxy moiety, which was designed for further optimization of the chain length, expected formation of another hydrogen bond and others, provided **2d** with 2-fold weaker mDP receptor binding affinity and nearly 6-fold more potent mDP receptor antagonist activity.

Table 2. Further optimization of the *p*-alkoxy substituent of the *N*-benzoyl moiety

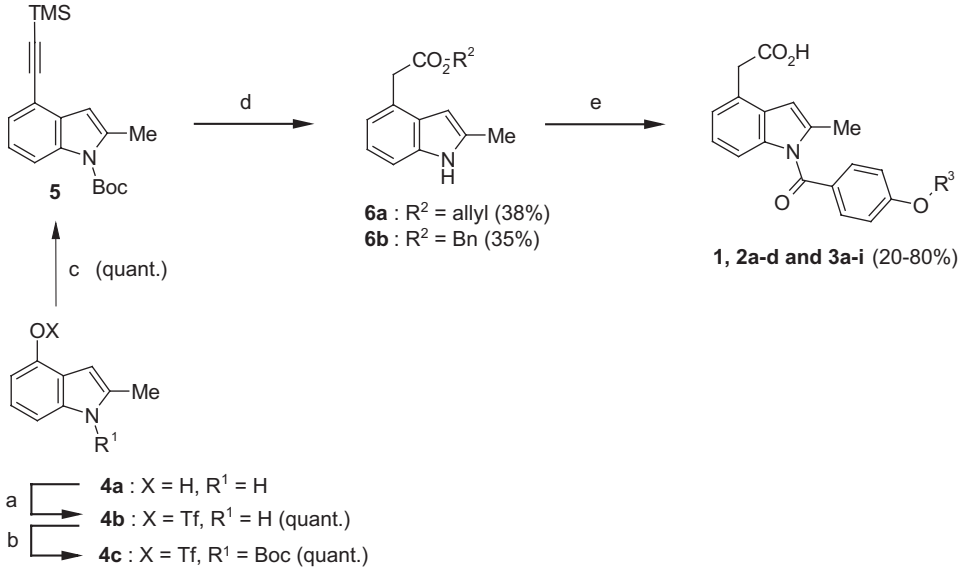


Compound	R	Binding K_i (μM)						IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	mDP	hDP	hDP
2d		5.6	0.54	>10	>10	0.017	0.29	0.25
3a		7.8	0.45	0.98	>10	0.022	0.029	0.036
3b		0.78	0.25	0.75	>10	0.0094	0.039	0.056
3c		3.8	0.092	>10	3.1	0.061	0.052	0.030
3d		>10	0.28	>10	3.5	0.18	0.39	NT[a]
3e		2.0	0.064	>10	>10	0.016	0.018	0.031
3f		2.3	1.3	>10	1.3	0.12	0.13	0.27
3g		6.2	0.10	1.7	>10	0.016	0.0035	0.0012
3h		>10	0.41	>10	>10	0.23	0.17	0.019
3i		>10	2.1	1.7	>10	0.023	0.0053	0.00081

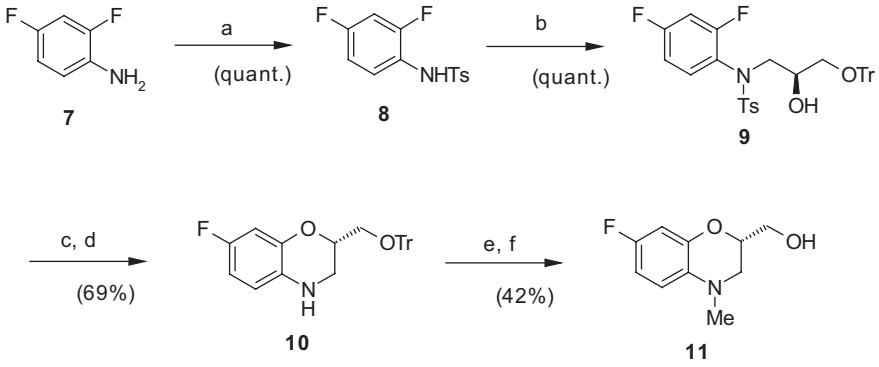
[a] NT: not tested.

The three compounds **2a**–**b** and **2d** also demonstrated weak to moderate affinity for other EP receptors. In particular, **2b** showed moderate affinity for all of the EP receptors despite its increased mDP receptor binding affinity and antagonist activity, while the *p*-phenyloxyethyloxy analog **2d** showed relatively higher subtype selectivity with more potent mDP receptor antagonist activity relative to **1**.

Compound **2d** was also evaluated for hDP[10] receptor antagonist activity. Unexpectedly, **2d** showed decreased hDP receptor antagonist activity despite its high mDP receptor binding affinity. Since our final goal was to identify a potent hDP receptor antagonist, we focused attention on improvement of the hDP receptor antagonist activity of **2d** (IC_{50} = 0.25 μM). Further optimization of **2d** was continued to increase its hDP receptor antagonist activity. A breakthrough was achieved by synthesis and evaluation of the series of compounds listed in Table 2. Replacement of the *p*-phenyloxyethyloxy moiety of **2d** with a dihydrobenzofuranyl-2-methyloxy moiety and a 1,3-dioxaindanyl-2-methyloxy moiety afforded **3a**–**b**, respectively, which showed increased hDP receptor binding affinity and antagonist activity relative to **2d** and nearly equivalent mDP receptor binding affinity. Replacement of the phenyloxyethyloxy moiety of **2d** with a benzopyranyl-2-methyloxy moiety or benzopyranyl-3-methyloxy moiety gave **3c**–**d**,



Scheme 1. Synthesis of *N*-benzoyl-2-methylindole-4-acetic acid analogs **1**, **2a**–**d**, and **3a**–**i**. Reagents and conditions: (a) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78 °C; (b) Boc_2O, DMAP (cat.), CH_3CN; (c) $PdCl_2(PPh_3)_2$, trimethylsilylacetylene, CuI, TBAI, TEA, DMF, 70 °C; (d) (1) dicyclohexylborane; (2) NaOH aq, H_2O_2, THF; (3) NaOH aq, MeOH, 1,4-dioxane; (4) allyl bromide or benzyl bromide, K_2CO_3, DMF; (e) (1) NaOH (powdered), *p*-acetyloxybenzoyl chloride, TBACl, CH_2Cl_2; (2) pyrollidine, CH_2Cl_2; (3) alcohol, DEAD, PPh_3; (4) morpholine, $Pd(PPh_3)_4$, THF or H_2, Pd–C, *i*-PrOH, EtOAc.



Scheme 2. Synthesis of **11**. Reagents and conditions: (a) TsCl, pyridine, CH_2Cl_2; (b) (*S*)-(−)-glycidyl triphenylmethyl ether, K_2CO_3, $BnEt_3NCl$, 1,4-dioxane; (c) ^tBuOK, THF; (d) Na, naphthalene, DME; (e) MeI, K_2CO_3, acetone; (f) AcOH, THF, H_2O.

respectively. Compound **3c** showed weaker mDP receptor binding affinity relative to **2d**, while it showed stronger hDP receptor binding affinity and antagonist activity. Its regioisomer **3d** showed nearly equivalent in hDP receptor binding affinity relative to **2d**. Benzo-1,4-dioxan- yl-2-methyloxy derivatives **3e**–**f** were also synthesized as optically active forms and evaluated. Compound **3e** (2*R*-form) showed nearly equal mDP receptor binding affinity relative to **2d**, while it showed stronger hDP receptor binding affinity and more potent hDP receptor antagonist activity. On the other hand, **3f** (2*S*-form) demonstrated nearly 10-fold weaker mDP receptor binding affinity and nearly equal hDP receptor antagonist activity relative to **2d**. The 4*N*-methylbenzomorpholinyl-2-methyloxy analogs **3g**–**i** were also synthesized as their optically active forms and evaluated.

Compound **3g** (2*S*-form) showed equal mDP receptor binding affinity relative to **2d**, while it showed a marked increase of hDP receptor binding affinity and antagonist activity.

The enantiomer **3h** (2*R*-form) was more than 10-fold weaker in the three assays, that is, mDP receptor binding affinity, hDP receptor binding affinity and hDP receptor antagonist activity. Introduction of a 7-fluoro moiety into the *N*-methylbenzomorpholin moiety of the more active enantiomer **3g** afforded **3i** with slightly less potent mDP receptor binding affinity and hDP receptor binding affinity although **3i** showed nearly 1.5-fold more potent hDP receptor antagonist activity. Regarding subtype selectivity, **3a**–**b** and **3g** showed moderate to weak affinity for other mEP receptors, such as mEP1, mEP2, and mEP3, while **3c** and **3f** showed moderate to weak affinity for the mEP1, mEP2, and mEP4 receptors. Compound **3d** showed moderate to weak affinity for the mEP2 and mEP4 receptors. The less potent enantiomer **3h** showed moderate mEP2 receptor affinity.

Table 3. Inhibitory effect of **3i** on PGD_2 (0.01%, 20 μL/eye) induced vascular permeability in guinea pig conjunctiva ($n = 8$)

Compound	Dose (mg/kg, po)	%Inhibition
3i	0.3	60**

** < 0.01 versus control with Dunnett's test. Inhibition of increase in conjunctival vascular permeability caused by topical application of PGD_2 (0.01%, 20 μL/eye) in guinea pig. **3i** was administered po 1 h before the challenge.

Compound **3i** exhibited better subtype selectivity with more optimized hDP receptor antagonist activity relative to **3g** mainly because of its decreased mEP2 receptor affinity with the retained potent antagonist activity.

Overall, the compounds listed in Table 2 demonstrated stronger hDP receptor binding affinity and their hDP receptor antagonist activity (IC_{50} value) was relatively close to their receptor affinity (K_i value). One of the reasons for the increased hDP receptor binding and antagonist activity of these analogs was estimated to be ascribed to the restricted conformation of the terminal phenyl moiety in addition to the interaction of these analogs with hDP receptor by the newly introduced heteroatoms such as ether oxygen and/or *N*-methyl functions.

These compounds were also evaluated for their TP receptor affinity, because PGD_2 has been known to be a TP agonist. All of the compounds listed in Tables 1 and 2 showed less than 1000-fold potent affinity to TP receptor.

To assess potential DP receptor antagonist activity in vivo, the inhibitory effect of **3i** on the PGD_2 (0.01%) induced increase of vascular permeability in guinea pig conjunctiva was evaluated ($n = 8$).[11] Compound **3i** showed 60% inhibition of the PGD_2-induced increase of vascular permeability at an oral dose of 0.3 mg/kg (Table 3).

In summary, a series of *N*-(*p*-alkoxy)benzoyl-2-methylindole-4-acetic acid analogs were synthesized and evaluated as a new class of DP receptor antagonists. Among the compounds tested, **3i** demonstrated the strongest hDP receptor antagonist activity and good subtype selectivity. In addition, **3i** showed in vivo DP receptor antagonist activity. Thus, a highly potent orally active DP receptor antagonist **3i** was discovered by starting from chemical modification of Indomethacin analogs. This could be a potential orally active drug. Full details (including more detailed chemistry) will be reported in due course.

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