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Discovery of new chemical leads for prostaglandin D_2 receptor antagonists

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Abstract—A series of Indomethacin analogs were synthesized and biologically evaluated. Among the compounds tested, *N*-(*p*-butoxy)benzoyl-2-methylindole-4-acetic acid **2** was discovered as a new chemical lead for a prostaglandin D_2 (PGD_2) receptor antagonist. Structure–activity relationship data are also presented.

1. Introduction

Coleman and co-workers proposed the existence of specific receptors for thromboxane (TX), PGI, PGE, PGF, and PGD namely the TP, IP, EP, FP, and DP receptors, respectively.[1] A number of specific ligands for these receptors have already been described in the literature.[2] Among them, the DP receptor is the newest and the least characterized.[3] PGD_2 is the major prostanoid released from mast cells after challenging with IgE[4] and it has also been shown to affect the sleep cycle[5] and body temperature.[6] The discovery of a DP selective receptor antagonist[7] seems to offer significant advantages, for investigating the role of this receptor in the various pathologies described above. However, the role of the DP receptor remains unclear because of the lack of potent and subtype-selective ligands. Here we report on the discovery of a new chemical lead **2** for DP receptor antagonists that were obtained from the chemical modification of Indomethacin analogs **1a–c** (Fig. 1).

2. Chemistry

Synthesis of the compounds listed in Tables 1–4 is outlined in Schemes 1–3. Compounds **1a–c** and **9a–b** were prepared as described in Scheme 1. *p*-Substituted phenylhydrazines **11** were converted to the corresponding hydrazones followed by *N*-acylation of the remaining

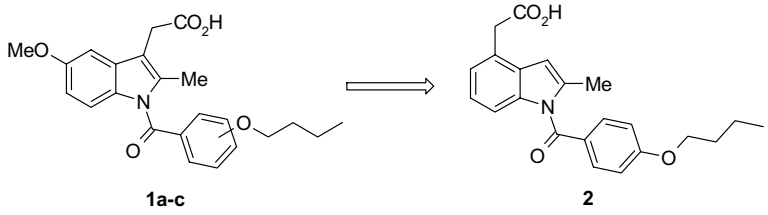

Figure 1. Discovery of a new chemical lead for a DP receptor antagonist.

Keywords: Prostaglandin; DP receptor; Antagonist.
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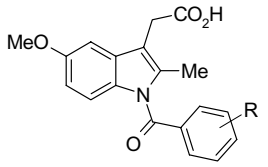
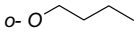
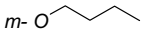
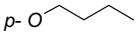
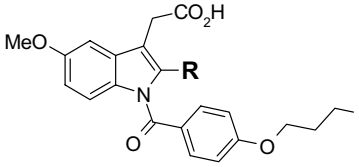
Table 1. Activity profile of Indomethacin and *N*-benzoyl-2-methyl-5-methoxyindole-3-acetic acids **2a–c**



Compd	R	Binding K_i (μM)					IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	mDP	mDP
	Indomethacin	>10	>10	>10	>10	>10	>10
1a	*o*- 	6.5	>10	>10	>10	>10	NT[a]
1b	*m*- 	>10	1.7	>10	>10	0.41	2.5
1c	*p*- 	>10	4.7	>10	>10	0.27	1.6

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.[9] with some modifications.[8] With regard to the mDP 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 BSA (bovine serum albumin) (0.1%) evoked by PGD_2 in mDPreceptor expressing CHO cells.
[a] NT: not tested.

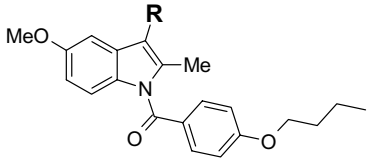
Table 2. Effect of a 2-substituent on the activity profiles

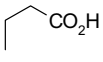
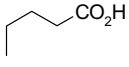
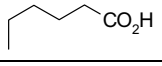


Compd	R	Binding K_i (μM)					IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	mDP	mDP
3	H	>10	8.9	>10	>10	2.2	NT[a]
1c	Me	>10	4.7	>10	>10	0.27	1.6
4	Et	3.8	2.9	>10	>10	1.5	NT[a]

[a] NT: not tested.

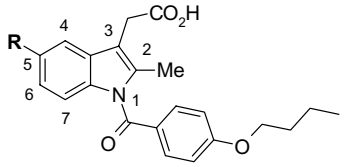
Table 3. Effect of a 3-substituent on the activity profiles



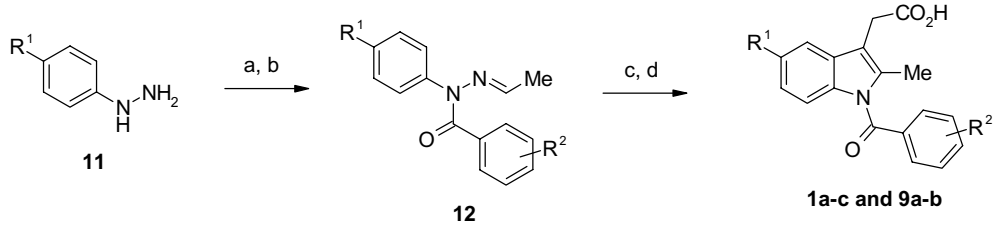
Compd	R	Binding K_i (μM)					IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	mDP	mDP
5		>10	>10	>10	>10	0.94	4.0
1c		>10	4.7	>10	>10	0.27	1.6
6		>10	5.7	>10	3.0	0.28	1.5
7		>10	2.7	2.4	1.5	0.19	0.91
8		>10	5.2	>10	4.2	0.40	4.5

nitrogen to afford **12**, which was cyclized using levulinic acid to form the *N*-benzoyl indoles **1a–c** and **9a–b**.[8] Compounds **3** and **10** were prepared as described in Scheme 2. Demethylation of the 5-methoxy group of **13** gave **14**, *o*-benzylation of which provided **15**. *N*-Benzoylation of **15** gave **16**, catalytic hydrogenation of which resulted in **10**. Compound **3** was prepared from **17** according to the same procedures described for the preparation of **10** from **13**. Compounds **4–8** were prepared from **18** according to the procedures described in

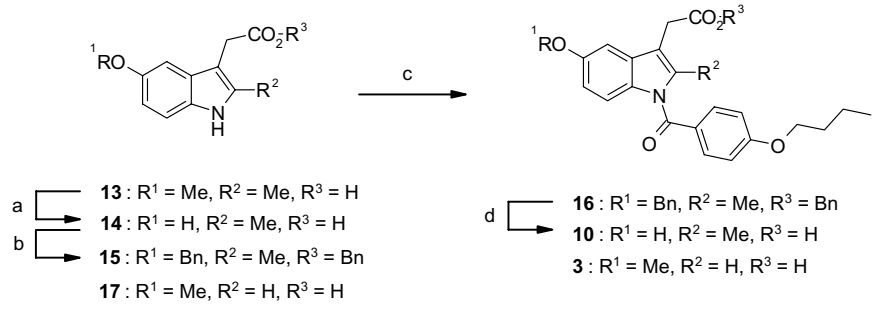
Table 4. Effect of a 5-substituent on the activity profiles



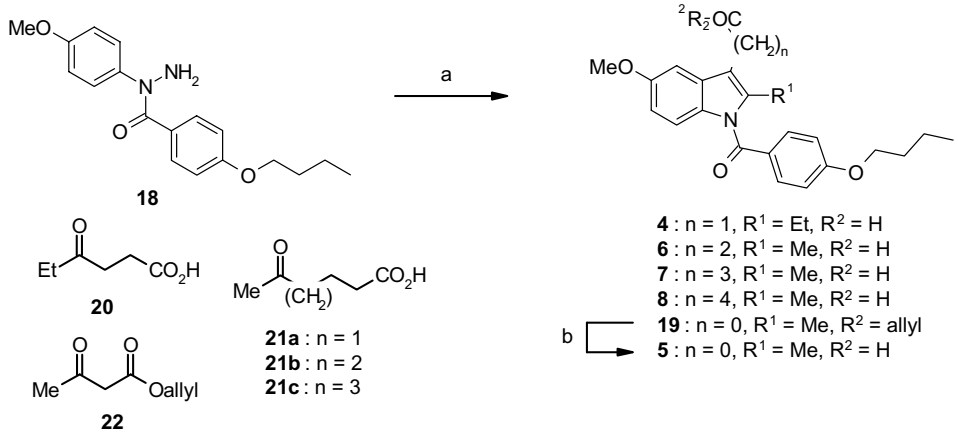
Compd	R	Binding K_i (μM)					IC_{50} (μM)
		mEP1	mEP2	mEP3	mEP4	mDP	mDP
1c	OMe	>10	4.7	>10	>10	0.27	1.6
9a	H	5.2	2.1	>10	>10	0.11	3.9
9b	Me	5.0	1.7	>10	2.2	0.15	2.9
10	OH	>10	3.3	>10	>10	1.9	>10



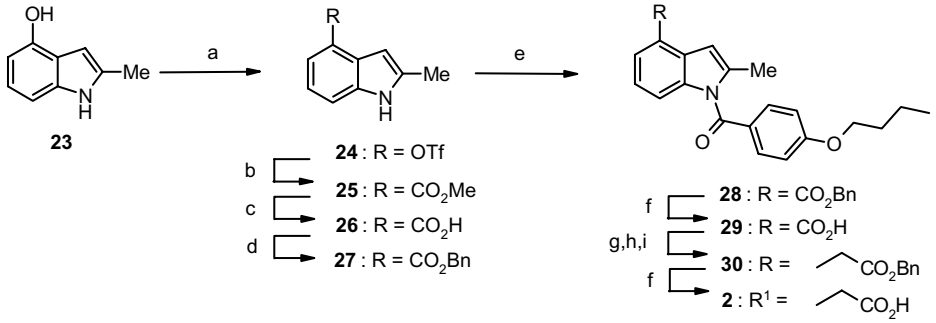
Scheme 1. Synthesis of compounds **1a**–**c** and **9a**–**b**. Reagents: (a) CH_3CHO, toluene; (b) ArCOCl, pyridine, CH_2Cl_2; (c) 4 N HCl, 1,4-dioxane; (d) levulinic acid, AcOH.



Scheme 2. Synthesis of **3** and **10**. Reagents: (a) HCl–pyridine, 180 °C; (b) benzyl bromide, K_2CO_3, DMF; (c) NaH, *p*-^{n}butoxybenzoyl chloride, DMF; (d) H_2, Pd–C, *i*-PrOH, EtOAc.



Scheme 3. Synthesis of **4**–**8**. Reagents: (a) **20** or **21a**–**c** or **22**, AcOH; (b) morpholine, $Pd(PPh_3)_4$, THF.



Scheme 4. Synthesis of **2**. 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) NaH, *p*-*n*butoxybenzoyl chloride, DMF; (f) Pd–C, H_2, MeOH, EtOAc; (g) $(COCl)_2$, DMF, toluene; (h) $TMSCHN_2$, THF, CH_3CN; (i) 2,4,6-collidine, benzyl alcohol.

Scheme 3, using an appropriate ketoester or keto carboxylic acid selected from **20**–**22** instead of levulinic acid. Compound **2** was prepared from 4-hydroxy-2-methylindole **23** in ten steps as briefly described in Scheme 4.

3. Results and discussion

As shown in Tables 1–5, the test compounds were biologically evaluated for inhibition of the specific binding of a radioligand, $[^3H]PGD_2$, to membrane fractions prepared from cells stably expressing each prostanoid receptor and for inhibition of cAMP formation evoked by PGD_2 in CHO cells[9] in the presence of BSA (bovine serum albumin) (0.1%). Test compounds were also evaluated for binding to all subtypes of the mouse PGE_2 receptor (mEP1, mEP2, mEP3, and mEP4).[2]

In the course of screening of our library focused on lipid mediators, Indomethacin analog **1c** was found to show moderate affinity for the mouse DP (mDP) receptor, while Indomethacin did not show any affinity at 10 μM (Table 1). Indomethacin is a well-known orally active anti-inflammatory drug that acts by inhibition of cyclooxygenase in the arachidonic acid cascade. Thus, Indomethacin analog **1c** was considered to be an excellent chemical lead for an orally active DP receptor antagonist. Based on the above information, structural optimization was initiated with chemical modification of **1c**. *o*-Isomer **1a** and *m*-isomer **1b** were synthesized and evaluated as described in Table 1. *m*-Isomer **1b** demonstrated slightly lower receptor affinity and antagonist activity relative to *p*-isomer **1c**, while *o*-isomer **1a** did not show any mDP receptor affinity at 10 μM. Compounds **1a** and **1b**–**c** also showed weak mEP1 receptor affinity and mEP2 receptor affinity, respectively. As a result, *p*-isomer **1c**, which showed the strongest mDP receptor affinity and antagonist activity among the three isomers, was reconfirmed as a chemical lead for further optimization.

As shown in Table 2, the effect of the 2-substituent of the *N*-(*p*-butoxy)benzoyl-5-methoxyindole-3-acetic acid skeleton on receptor affinity and mDP receptor antagonist activity was evaluated.

Removal of the methyl moiety of **1c** led to **3**, with nearly 10-fold lower mDP receptor affinity. Replacement of the methyl moiety of **1c** with an ethyl moiety provided **4**, which showed nearly 5.6-fold lower mDP receptor affinity. Compound **4** also demonstrated weak affinity for the mEP1 receptor in addition to the mEP2 and mDP receptors, while **3** showed similar subtype selectivity to **1c**. As a result, the 2-substituent was optimized as a methyl group. As shown in Table 3, the effect of the 3-substituent on the activity profile was investigated. Removal of the methylene moiety from the acetic acid moiety of **1c** resulted in **5**, with nearly 3-fold lower mDP receptor affinity, 2.5-fold less potent antagonist activity, and similar subtype selectivity. One carbon homologation of **1c** gave **6**, which showed retention of mDP receptor affinity and antagonist activity.

Compound **6** also demonstrated weak affinity for the mEP2 and mEP4 receptors. Two carbon homologation of **1c** afforded **7**, which had slightly increased mDP receptor affinity and a slightly increased antagonist activity, while it showed decreased subtype selectivity because of an increase in mEP2, mEP3, and mEP4 receptor affinity. Three carbon homologation of **1c** gave **8**, with nearly 1.5-fold lower mDP receptor affinity and 2.8-fold less potent antagonist activity. It also showed less subtype selectivity because of increased mEP4 receptor affinity. As a result, **1c** was considered to be the best chemical lead based on its high mDP receptor affinity and the greatest subtype selectivity among the compounds listed in Table 3.

Table 5. Effect of transformation of 2-methylindole-3-acetic acid to 2-methylindole-4-aceic acid on the activity profiles

Compd	Binding K_i (μM)					IC_{50} (μM)
	mEP1	mEP2	mEP3	mEP4	mDP	mDP
1c	>10	4.7	>10	>10	0.27	1.6
2	>10	2.0	3.3	>10	0.010	0.30

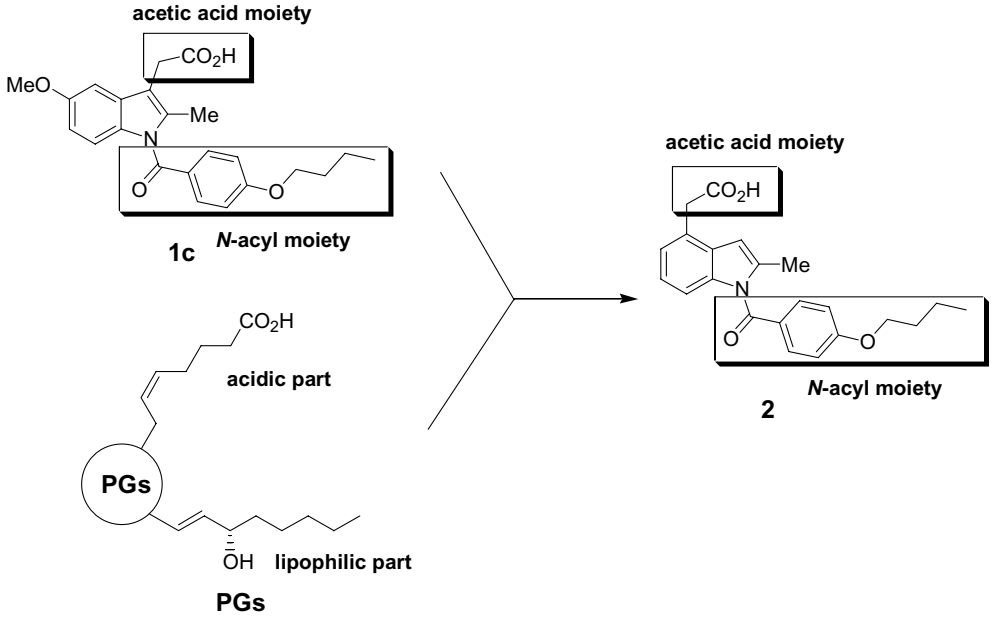


Figure 2. Molecular design of 2-methyl-4-indole acetic acid **2**.

The effect of a 5-substituent on the activity profile was investigated as described in Table 4. Replacement of the 5-methoxy group of **1c** with a hydrogen afforded **9a** with nearly 2.5-fold higher mDP receptor affinity, although it showed 2.4-fold less potent antagonist activity.

Replacement of the 5-methoxy group of **1c** with a methyl group provided **9b**, with nearly 1.9-fold higher mDP receptor affinity although it showed 1.8-fold less potent antagonist activity. Compound **9b** showed lower subtype selectivity relative to **1c** because of increased mEP4 receptor affinity. Demethylation of the 5-methoxy group of **1c** provided the corresponding hydroxy analog **10**, with nearly 7-fold weaker mDP receptor affinity and no antagonist activity at 10 μM. Compound **10** also showed very weak mEP2 receptor affinity. Among the compounds listed in Table 4, **1c** again showed the most promising activity profile with respect to both the antagonist activity and subtype selectivity. On the basis of the SAR described in Tables 1–4, further optimization of *N*-acyl-2-methylindole-3-acetic acid analogs seemed to be difficult. A breakthrough for further chemical modification of this template was strongly needed.

Accordingly to our consideration, these new Indomethacin analogs described above could be thought to possess structural similarity to PGs as described in Figure 2. Thus more optimized arrangement of the acidic part (acetic acid moiety) and the lipophilic part (*N*-acyl moiety) was strongly suggested for further increase of the activity. Based on this analysis, transfer of the 3-acetic acid moiety of **1c** to the other position was carried out while the lipophilic *N*-acyl moiety was fixed at the original position for the synthetic reason.

A breakthrough was obtained by structural transformation of the above-described indole-3-acetic acid template to an indole-4-acetic acid template such as **2**. As shown in Table 5, *N*-(*p*-butoxy)benzoyl-2-methylidole-4-acetic acid **2** was synthesized and evaluated. Compound **2** demonstrated 27-fold more potent mDP receptor affinity and more than 5-fold stronger mDP antagonist activity relative to **1c**.

In summary, a series of Indomethacin analogs were synthesized and evaluated as a new chemical lead for mDP receptor antagonists. Their subtype selectivity was also evaluated. As a result, *N*-(*p*-butoxy)benzoyl-2-methylindole-4-acetic acid **2** was discovered as a new chemical lead for DP receptor antagonists. Full details (including more detailed chemistry) will be reported in due course.

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