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Potent and highly selective DP1 antagonists with 2,3,4,9-tetrahydro-1*H*-carbazole as pharmacophore ☆

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ABSTRACT

We discovered that the introduction of a methyl group to the benzylic position of the *N*-benzyl group in lead compound **1a** has a dramatic effect on improving the binding selectivity of this ligand for the prostanoid receptors DP1 (receptor for prostaglandin D_2) as compared to TP (receptor for thromboxane A_2). Based on this discovery, we have synthesized a series of potent and highly selective DP1 antagonists. Among them, compound **1h** was identified as a highly selective DP1 antagonist with excellent overall properties. It has a K_i of 0.43 nM to DP1 in binding assay and an IC_{50} of 2.5 nM in the DP1 functional assay. Its selectivity for DP1 over TP (the most potent receptor after DP1) exceeds 750-fold based on both binding and functional assays. These properties make **1h** a very potent and highly selective DP1 receptor antagonist suitable for investigating the biological functions of DP1 in normal physiology and models of disease

It is found that, in response to allergic stimulus, the production of prostaglandin D_2 (PGD_2) along with a variety of other lipid mediators such as histamine, some leukotrienes (cysLTs), and thromboxane A_2 (TxA_2) in mast cell are increased.[1] This leads to the therapeutic potential of using the receptors of PGD_2, namely, DP1 and $CRTH_2$/DP2 as targets for mediating various allergic disorders. Such potential has been supported by a series of discoveries that PGD_2 is involved in various allergic disorders and appears to be an important mediator of such disorders like allergic asthma,[2] atopic dermatitis,[3] allergic rhinitis[4] and allergic conjunctivitis.[5] The clinical utility of DP1 and $CRTH_2$/DP2 ligands in allergic inflammation are currently under investigation.[6] Recently, DP1 antagonists have found application for treatment of the side-effects of niacin-induced flushing. Thus, the combination of a DP1 antagonist, Laropiprant (Fig. 1), with niacin has the therapeutic potential to reduce flushing without lessening the beneficial effects of niacin on treating dyslipidemias.

Continuing the on-going research efforts in developing a potent and selective DP1 antagonist with an indole scaffold, we explored a 2,3,4,9-tetrahydro-1*H*-carbazole scaffold in stead of the 1,2,3,4-tetrahydrocyclopenta[*b*]indole scaffold used in our previous studies.[7,8] The first compound we synthesized in this new series is

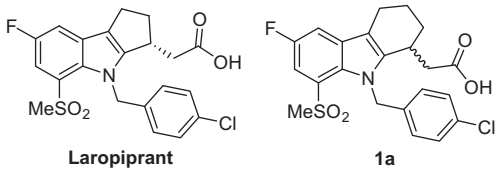

Figure 1.

1a. As a racemate, **1a** has a K_i of 0.89 nM and 2.51 nM to DP1 and TP, respectively. We hoped that the modification of the *N*-benzyl substituent could provide compounds with similar potency but with much improved selectivity for DP1 over TP. Previous SAR studies in the 1,2,3,4-tetrahydrocyclopenta[*b*]indole series showed that the following *N*-benzyl modifications, including (1) the replacement of the phenyl with other aromatic rings or heteroaromatic rings, and (2) the introduction of various substituents to various positions of the phenyl ring, could not help achieve the goal.[9] This prompted us to examine the possibilities of introducing alkyl substituents to the benzylic position. We hoped that, by doing so, the orientation of the benzyl substituent could be positioned to discriminate the ligand structural requirement between DP1 and TP, and thus led to the improvement of DP1 potency and selectivity over TP. In this communication, a detailed account of this study is provided.

Our initial synthetic pathway to benzylic alkylated analogs was outlined in Scheme 1.[10] By following a standard procedure of

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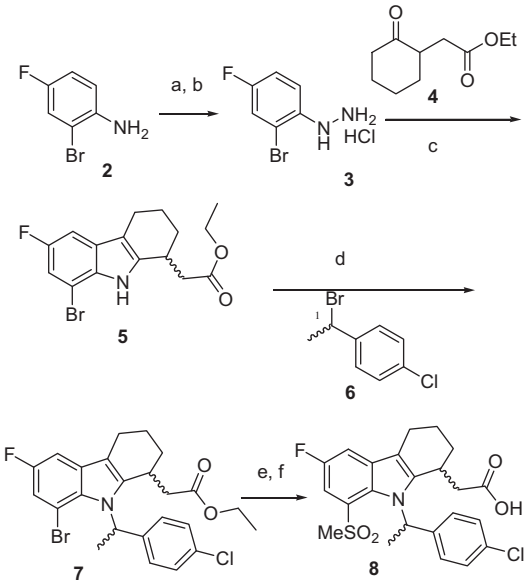
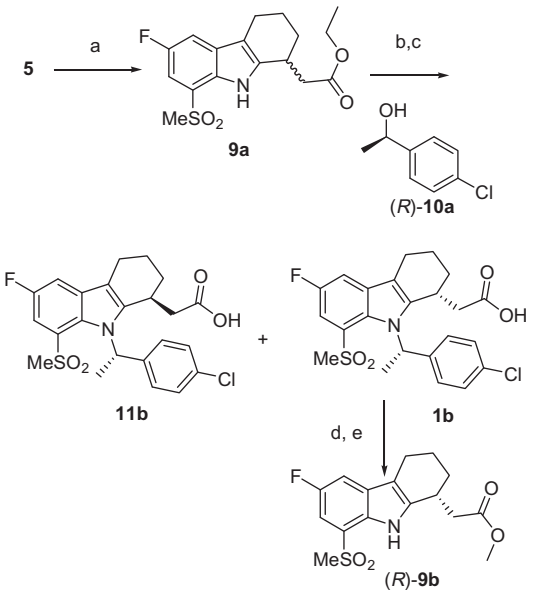


Scheme 1. Reagents and conditions: (a) $NaNO_2$, HCl, −4 °C; (b) $SnCl_2$, HCl, 0–5 °C; quantitative for two-steps; (c) Compound **4**, HOAC, reflux, 32%; (d) **6** (4 equiv), Cs_2CO_3 (8 equiv), MeCN, reflux, 5 h, 99%; (e) $MeSO_2Na$ (5 equiv), CuI (5 equiv), NMP/150 °C, 8 h, 73%; (f) NaOH (5 equiv), MeOH, THF, rt, 3 h, quantitative.



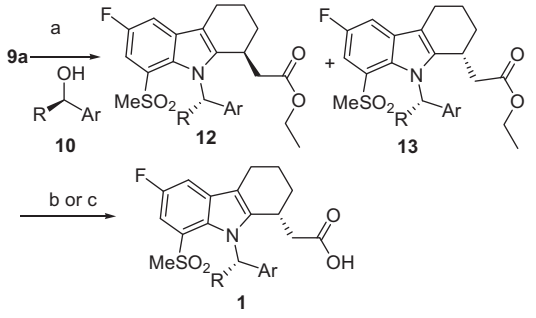
Scheme 2. Reagents and conditions: (a) $MeSO_2Na$ (5 equiv), CuI (5 equiv), NMP/150 °C, 8 h, 63%; (b) $tBuO_2CN{=}NCO_2tBu$, Ph_3P, (*R*)-**10a**, rt, quantative; (c) KOH (5 equiv), MeOH, THF, reflux, 3 h, quantitative; (d) H_2,Pd/C, MeOH, rt, 16 h; (e) CH_2N_2, THF, 95% yield for two-steps.

hydrazine preparation with **2** as starting material, compound **3** was obtained in quantitative yield. The Fisher indole reaction of **3** with ketoester **4** in refluxed acetic acid led to the formation of bromoindole **5**. Bromoindole **5** was alkylated at the indole nitrogen with 4 equivalents of benzyl bromide **6** in the presence of 8 equivalents of Cs_2CO_3 as a base in reflux acetonitrile. The alkylated product **7** was treated with 5 equiv of $MeSO_2Na$ and 5 equiv of CuI in NMP at 150 °C, followed by the hydrolysis of ester functional to afford the desired acid **8** as a mixture of four stereoisomers. The two pairs of diastereomers could be easily separated by flash chromatography on silica gel. In DP1 binding assay, the slow-eluting pair exhibited much higher potency with a K_i of 1.3 nM, as compared to the fast-eluting pair with a K_i of 12 nM. We were gratified to find that the more potent pair has a K_i of 219 nM to TP in binding assay; this represents a much improved selectivity for DP1 over TP compared to **1a**.

Encouraged by these results, a more efficient synthetic method was developed to identify the most potent single diastereomer of mixture **8** responsible for the DP1 affinity and selectivity (Scheme 2).[10] Direct alkylation of compound **5** with chiral alcohol (*R*)-**10a** under Mitsunobu conditions failed to afford desired products. However, when compound **5** was transformed into methyl sulfone **9** and then alkylated with (*R*)-**10a** under Mitsunobu conditions, the desired alkylation products were obtained in quantitative yield. The increased reactivity of **9** is likely due to the enhanced acidity by the electron-withdrawing nature of the methyl sulfonyl substituent. The alkylated diastereomeric esters, which have an absolute (*S*)-configuration at the *N*-benzylic position, were hydrolyzed to their corresponding acids and separated by silica gel flash chromatography. Compound **1b**, the slow-eluting diastereomer exhibits a K_i of 0.75 nM and 184 nM toward DP1 and TP, respectively. The potency and selectivity of **1b** on DP1 indicates that it is the most potent single diastereomer among the mixture of four (compound **8**) obtained by the pathway outlined in Scheme 1. Thus a (*S*)-configuration at *N*-benzylic position was determined to be essential to DP1 potency and selectivity over TP. The absolute stereochemistry at the carbon center bearing the acetic acid group was established to be (*R*)-configuration by X-ray crystallography of **9b**, which is obtained by the debenzylation of **1b** under Pd-catalyzed hydrogenation conditions following by treatment with diazomethane. The confirmed stereochemistry is in consistence with same stereochemistry required for DP1 activity observed for the 1,2,3,4-tetrahydrocyclopenta[*b*]indole series. Interestingly, it was found later that the ester which leads to the desired slow-eluting diastereomer could be selectively hydrolyzed to acid **1b** by stirring with 0.6 equiv of LiOH (1 M solution) in a solvent mixture (THF/MeOH 3:1) at 0 °C, thus facilitating the isolation of **1b** in preparative scale.

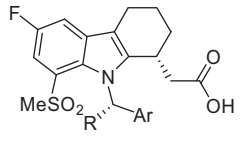
More analogs in the series were synthesized as outlined in Scheme 2[10] by using suitable chiral alcohols with absolute stereochemistry represented by **10**. Some of the alkylation products thus obtained, as a mixture of **12** and **13**, which contained chemically reactive functional groups on R or on Ar, were further exploited as starting point for preparation of other analogs. For example, when R = CH_2OH, it can be transformed into R = CH_2F or CHF_2; when Ar = 4-BrC_6H_4, it can be transformed into Ar = 4-NCC_6H_4 or 4-$MeSO_2C_6H_4$.

The newly synthesized compounds were evaluated in the DP1 and TP binding assays, and the results are summarized in Table 1. As discussed above, the introduction of a methyl group to the benzylic position of the *N*-benzyl group in the initial lead



Scheme 3. Reagents and conditions: (a) $tBuO_2CN{=}NCO_2tBu$, Ph_3P, **10**, rt; (b) KOH (5 equiv), MeOH, THF, reflux, 3 h, quantitative; (c) LiOH (0.6 equiv), MeOH, THF, 0 °C, 24 h.

Table 1
DP1 and TP receptor binding affinities



1

Entry	Compound			K_i (nM) [a]		
	No.	R	Ar	DP1	TP	DP1/TP (fold)
1	**1a**[b]	H	4-ClC_6H_4	0.89	2.51	<3
2	**1b**	Me	4-ClC_6H_4	0.75	184	245
3	**1c**	Me	3-F-4-ClC_6H_4	0.38	200	526
4	**1d**	Me	3,4-$Cl_2C_6H_4$	0.33	112	339
5	**1e**	Me	2-F-4-ClC_6H_4	0.41	270	658
6	**1f**	Me	C_6H_5	0.36	362	1005
7	**1g**	Me	3-ClC_6H_4	0.31	292	941
8	**1h**	Me	4-$CF_3C_6H_4$	0.43	338	786
9	**1i**	Me	4-FC_6H_4	0.33	100	303
10	**1j**	Me	4-BrC_6H_4	0.38	106	278
11	**1k**	Me	4-CNC_6H_4	0.51	93	182
12	**1l**	Me	4-$MeSO_2C_6H_4$	16.5	1481	89
13	**1m**	CFH_2	4-ClC_6H_4	0.36	130	361
14	**1n**	CF_2H	4-ClC_6H_4	0.39	138	353

[a] Average of three or more assays.
[b] Racemate.

compound **1a** led to compound **1b** with a dramatic improvement of the selectivity for DP1 over TP. In the TP binding assay, a 184-nM K_i for **1b** as compared to a 2.5-nM one for **1a** was observed; this represents an over 80-fold improvement of selectivity for DP1 over TP by comparing to **1a**.

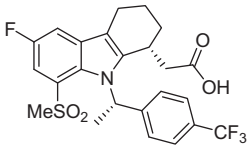
Having identified **1b** as a more selective lead, we tried to further improve its DP1 potency and selectivity over TP by introducing other substituents to the phenyl ring of its *N*-benzyl group. Introducing a 3-fluoro (compound **1c**) improves the DP1 affinity by two-fold while maintains the TP affinity, thus improves the selectivity for DP1 over TP to more than 520-fold (entry 3, Table 1). In comparison, the introduction of a 3-chloro, which leads to **1d**, increases potencies on both DP1 and TP and slightly improves the selectivity to over 330-fold (entry 4, Table 1). The introduction of a 2-fluoro to **1b** produces a similar effect on potencies and selectivity (entry 5, **1e**, Table 1) as of a 3-fluoro and improves the selectivity to more than 600-fold.

Further improvement of the selectivity for DP1 over TP can be achieved by removing the 4-chloro substituent from the phenyl ring of *N*-benzyl group in **1b** or by moving it to the 3-position. When the 4-chloro group was removed, compound **1f** was obtained. It is among the most potent DP1 antagonists discovered in this series and is the only compound that has a 1000-fold selectivity for DP1 over TP in binding assays (entry 6, Table 1). When the 4-chloro substituent in **1b** was moved to the corresponding 3-position, DP1 antagonist (**1g**) with a two-fold higher potency was obtained. It exhibits about 940-fold selectivity for DP1 over TP.

Our further SAR exploration was focused on replacing the 4-chloro substituent in **16b** with other substituents such as 4-CF_3 (entry 8, **1h**, Table 1), 4-F (entry 9, **1i**, Table 1), 4-Br (entry 10, **1j**, Table 1), and 4-NC (entry 11, **1k**, Table 1). Compared with **1b**, these replacements all help in increasing the DP1 potency (entries 8–11, Table 1) by up to slightly more than two-fold; however, only the replacement with 4-CF_3 has an effect on improving the selectivity to above 780-fold in binding assays. In contrast, the use of $MeSO_2$ as a replacement of the 4-chloro led to much less potent DP1 receptor antagonist **1l** (entry 12, Table 1).

The replacement of the methyl group substituted at the benzylic position of the *N*-benzyl group in **1b** (R = Me) with fluorine contained methyl group such as CF_2H (entry 13, **1m**, Table 1) or CFH_2 (entry 14, **1n**, Table 1) was also attempted. When compared to **1b**, both compounds **1m** and **1n** are more potent DP1 antagonists with slight improvement of selectivity for DP1 over TP.

Table 2
Binding affinities of other receptors with **1h**



1h

Compound	K_i (nM) [a]					
	EP_1	EP_2	EP_3	EP_4	FP	IP
1h	4590	2270	830	6610	4320	15,510

[a] Average of three or more assays.

Among all the selective and potent antagonists we discovered above, compound **1h** was selected for further evaluations due to its excellent overall properties. The binding affinities of **1h** to other prostanoid receptors are summarized in Table 2. Compound **1h** has a more than 1900-fold selectivity for DP1 over EP_3 and even much higher selectivities over other receptors. In cell based assays in human platelet rich plasma, compound **1h** inhibits the PGD_2-induced accumulation of cAMP with an IC_{50} of 2.5 nM and platelet aggregation induced with the thromboxane A_2 mimetic U46619 (TP-PRP) with a mean IC_{50} of 19.4 μM (5.77–38.8 μM, n = 16). This represents a 7760-fold selectivity for DP1 over TP. Besides the binding profiles, another main factor that effects compound selection is the stabilities of these DP1 antagonists under acidic conditions. It was found that when treated with acid at low pH, the substituted benzyl groups of most of the selective antagonists presented in Table 1 could be cleaved. For example, only 79% of **1b** could be recovered after 2 h incubation with 0.01 N of HCl at 37 °C. In comparison, under same conditions compound **1h** was found to be stable (99.6% of **1h** remained after 6 h).

To conclude, we discovered that the introduction of a methyl group to the benzylic position of the *N*-benzyl group in lead compound **1a** has a dramatic effect on improving binding selectivity for DP1 over TP. Based on this discovery, we have synthesized a series of potent and highly selective DP1 antagonists. Among them, compound **1h** has a K_i of 0.43 nM to DP1 in binding assay and an IC_{50} of 2.5 nM in DP1 functional assay. Its selectivity for DP1 over TP (the most potent receptor after DP1) is over 780- and 7760-fold in binding and functional assays, respectively. The excellent overall properties of **1h** warrant it for further preclinical development.

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