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Identification of prostaglandin D_2 receptor antagonists based on a tetrahydropyridoindole scaffold

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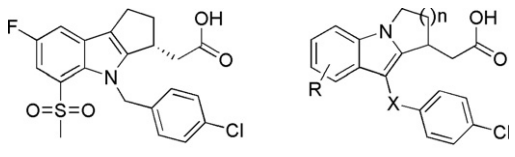
Abstract—A new series of indole-based antagonists of the PGD_2 receptor subtype 1 (DP1 receptor) was identified and the progress of the structure–activity relationship study to the identification of potent and selective antagonists is presented. Selective DP1 antagonists with high potency and selectivity were prepared. Of particular interest is the DP1 antagonist **26** with a K_i value of 1 nM for the DP1 receptor and an IC_{50} value of 4.6 nM in a DP1 functional assay for the inhibition of the PGD_2 induced cAMP production in platelet rich plasma (PRP).

Selective antagonists of the prostaglandin D_2 receptor subtype 1 (DP1) are being considered for use in the treatment of symptoms associated with niacin-induced flushing[1,2] and also for the treatment of allergic airway responses.[3–6] Niacin (nicotinic acid, vitamin B_3) has shown efficacy in reducing cardiovascular events in patients with dyslipidemia. However, symptoms associated with niacin-induced vasodilatation (e.g., flushing) have limited its use. The flushing response is mediated in large part by PGD_2 and recently the DP1 receptor antagonist laropiprant (MK-0524) has proven to be effective in suppressing both subjective and objective manifestations of niacin-induced vasodilatation.[1,2] In addition, it has been demonstrated that intranasal instillation of PGD_2 results in upper airway obstruction with 10-fold greater potency than histamine.[7]

The role of the PGD_2 receptor (DP1 receptor) in the regulation of mucous secretion and the efficacy of DP1 receptor blockade in allergic disease models prompted us to identify a suitable DP1 receptor antagonist for clinical studies to block the inflammatory effects of PGD_2.[8–12]

Recently, we reported the identification of the potent and selective DP1 antagonist MK-0524 (**1**) (Fig. 1).[13] While MK-0524 clinical trials were underway, part of our efforts were focused on finding additional potential development candidates. One of our main criteria was to identify a DP1 antagonist structurally distinct from our first clinical candidate MK-0524.

Towards this end, we elected to preserve the 6-5-5 or 6-5-6 tricyclic scaffold which was a common motif to our more potent DP1 antagonists. Thus, one of the modification considered (Fig. 1) was to invert the indole



1 **2**

Figure 1. Structure of MK-0524 (**1**) and of proposed novel indole scaffold (**2**).

Keywords: DP1 antagonist; Prostanoid; PGD2 antagonist; PGD2 receptor; Tetrahydropyridoindole; Indole.
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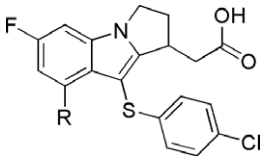
central core to form a new structure (**2**) in which the MK-0524's indole nitrogen atom and the carbon on 3-position of indole were exchanged. Based on the similarities between both indole structures, we expected some features of the structure–activity relationship (SAR) to be common to both series. In this letter, we describe the SAR of this new series of DP1 receptor antagonists based on the above proposal.

Previous SAR studies from the MK-0524 (**1**) series demonstrated the advantage of fluorine substitution at the 5-position of the indole ring which corresponds to the 6-position of our new indole series.[13] The presence of an acetic acid side chain was also found to be essential for activity. To initiate SAR work in this new series, we kept the same substitution pattern and initially focused our attention on identifying an optimal substituent for the 4-position of the indole ring. The template used for this SAR was a dihydropyrroloindole scaffold substituted with a 4-chlorophenylthioether at the 3-position of the indole, chosen for its ease of synthesis. In general, compounds were screened for affinity against all nine human prostanoid receptors and were synthesized as racemates. However, our attention was mainly directed at the thromboxane A2 receptor (TP) which was the most significant 'off-target' activity for the compounds in this series. In addition, antagonism of compounds was characterized by a DP1 CRE-SEAP assay.[14] As shown in Table 1, the unsubstituted compound **3** maintains a modest binding affinity[15] for the DP1 receptor (K_i = 132 nM) and is actually 8-fold more potent on the TP receptor (K_i = 17 nM). Substitution of the 4-position with a bromide atom (**4**) resulted in a gain of potency on the DP1 receptor and led to a modestly DP1 selective antagonist. Introduction of more polar groups such as methyl ketone and secondary alcohols (compounds **5**–**7**) resulted in potent compounds with improved selectivity profiles. However, compounds **5**–**7** were only modestly potent in a DP1 functional assay (DP1 PRP) in inhibiting the PGD_2 induced production of cAMP in platelet rich plasma (PRP)[16] with IC_{50} values of 101, 56 and 509 nM, respectively. Gratifyingly, potency in this DP1 PRP assay was improved by the introduction of a methoxyether (**8**) or a methylthioether (**9**) with IC_{50} values of 6.7 and 4.1 nM, respectively. Therefore, the DP1 selectivity profile of **8** was far superior to **9** (180-fold vs 17-fold selectivity). Interestingly, replacement of the methoxyether by a methylsulfone (**11**) resulted in an antagonist as potent and selective as **8**. The bulkier ethylsulfone **10** was 3-fold less potent than **11** in the DP1-PRP functional assay. Further SAR with alkyl chains and/or carbocycles as for compounds **12**–**14** resulted in less potent and/or less selective derivatives. Metabolic profiles of **8** and **11** were evaluated (data not shown) and revealed higher rate of metabolism in human microsomes and hepatocytes for **8** compared to **11**. The overall profile of **11** being superior, the methylsulfone group was identified as optimal at the 4-position position of the indole.

Table 1. SAR of the indole 4-position



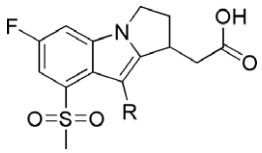
Compound	R	DP1[a] K_i (nM)	TP[a] K_i (nM)	DP1 PRP[b] IC_{50} (nM)
3	H	132	17	—
4	Br	7.7	17	—
5	COMe	3.6	674	101
6	CH(OH)Me	2.5	273	56
7	CH(OH)*i*-Pr	7.9	484	509
8	CH(OMe)Et	1.1	200	6.7
9	CH(SMe)Me	1.6	27.5	4.1
10	SO_2Et	2.3	250	34
11	SO_2Me	1.3	226	10.4
12	*i*-Pr	2.4	46	8.6
13	$CH(Et)_2$	8.1	363	21.6
14	Cyclopropyl	5.7	136	—

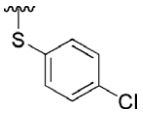
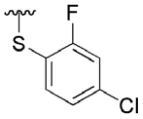
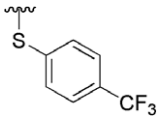
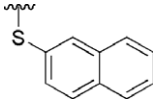
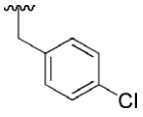
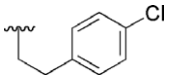
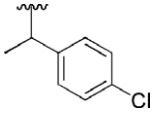
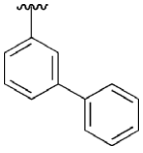
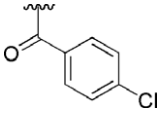
[a] Radioligand competition binding assays using recombinant human prostaglandin D_2 (DP1) and recombinant human thromboxane receptor (TP).

[b] Inhibition of the accumulation of cAMP in platelet rich plasma (PRP) challenged with PGD_2.

The effect of the substituent at the 3-position of the indole was also studied and is presented in Table 2. Modification of the substitution pattern on the 4-chlorophenylthioether such as for compound **15** did not improve the potency profile of the series. Antagonists, for which the chloride atom was replaced, were found to be less active as observed with **16** which is 3-fold less active than **11** in the DP1 PRP functional assay. The replacement of the phenyl ring with a naphthyl ring (**17**) had the same effect. The benzyl analog **18** exhibited a better affinity for the DP1 receptor but the selectivity over the TP receptor was significantly reduced (23-fold selectivity only). The addition of an additional methylene to **18** to increase the distance between the phenyl and the indole ring (**19**) diminished the potency by 38-fold. A similar loss of activity was also observed for the α-methyl benzyl analog **20**. Surprisingly, the replacement of the phenylthioether with a 3-biphenyl group was well tolerated (**21**). However, this biphenyl analogs were not as potent as **11**. Of particular interest, the benzoyl analog **22** was somewhat less potent on DP1 than the corresponding phenylthioether but displayed an excellent selectivity versus TP (1000-fold). The SAR around the 3-position of indole did not result in significant gain in potency and the thioether analogs appeared as the most attractive for further studies. Nonetheless, due to the high selectivity profile of benzoyl **22**, we decided to evaluate the metabolic profile of both of these DP1 antagonists. Unfortunately for compound **11** and close analogs, high levels of covalent protein labeling were identified as a major issue with these antagonists. Indeed, all compounds tested from the thioether series exhibited in vitro covalent protein binding levels higher than 50 pmol equiv/mg of protein, which is considered as the upper limit for covalent binding modification.[17] Since high propensity of a compound for covalent protein labeling is potentially associated with liabilities in vivo such as organ toxicity and unwanted immune

Table 2. SAR of the indole 3-position

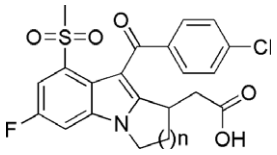


Compound	R	DP1 K_i (nM)	TP K_i (nM)	DP1 PRP IC_{50} (nM)
11		1.3	226	10.4
15		3.5	198	32.1
16		1.8	286	29.2
17		1.2	583	40
18		0.5	11.7	4.7
19		18.9	219	—
20		14.7	3190	—
21		1.7	310	31.6
22		6.9	6972	—

response,[17] we decided to focus the SAR studies around the benzoyl series which had acceptable levels of covalent protein labeling (<50 pmol equiv/mg of protein).

We next examined the importance of the size of the carbocycle fused to the indole. The expansion of the carbocycle from a cyclopentyl (**22**) to a cyclohexyl (**23**) improved the potency of the DP1 antagonist by 2-fold in the binding assay as shown in Table 3. Moreover, **23** was found to be highly potent in the DP1 PRP assay (4.6 nM) and also exhibited a satisfactory selectivity versus TP (>100-fold). Further expansion of the carbocycle to a cycloheptyl ring (**24**) had a minimal effect on the

Table 3. SAR for the size of the carbocycle ring fused to the indole ring



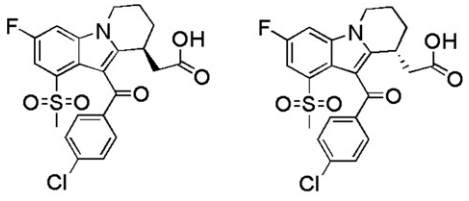
Compound	*n*	DP1 K_i (nM)	TP K_i (nM)	DP1 PRP IC_{50} (nM)
22	1	6.9	6972	—
23	2	3	513	4.6
24	3	1.4	675	5.2

activity and resulted in a compound more shifted than the cyclohexyl analog **23** in the PRP functional assay. At this point, **23** was identified as the optimal DP1 antagonist based on its excellent in vitro profile and its good pharmacokinetics profile in rats (100% bioavailability, 4.4 h half-life). Since indole **23** was a mixture of enantiomers, it was resolved by chiral HPLC separation into its individual isomers, namely **25** and **26**. As illustrated in Table 4, the orientation of the acetic acid side chain was important to maintain the activity on the DP1 receptor, **26** being 36-fold more potent than **25** in the DP1 binding assay. Not surprisingly, the absolute stereochemistry of the acetic acid side chain of **26** was found to be the same as in **1**.[18] The antagonist **26** exhibits a similar activity on the DP receptor to **1**,[13] and is at least 100-fold selective for the DP receptor over all the other prostanoids receptors.

The general synthetic route used to prepare compounds of the dihydropyrroloindole, the tetrahydropyridoindole and the tetrahydroazepinoindole series is outlined in Scheme 1. The indole ring formation involved the thermolysis of azidocinnamate[19] **28** which affords the methyl indole-2-carboxylate **29**. Indole **29** is then alkylated with a halo alkyl alkanoate to afford a 5, 6 or 7 membered ring carbocycle fused to the indole[20] and the resulting ketoester is decarboxylated under acidic conditions to afford ketone **30**. Introduction of the acetic acid side chain is then accomplished by a Reformatsky type addition on ketone **30** followed by deoxygenation[21] of hydroxyl ester **31**. The reaction proceeds using in situ generated TMSI to provide the important intermediate **32** which was used as a common building block for the thioester series as well as for the benzoyl series of DP1 antagonist. Thus, the sulfenylation[22] of **32** allowed the preparation of thioester **33** while Friedel–Crafts acylation permitted the preparation of the benzoyl analog **34**. The presence of a bromide atom on 4-position of indoles **33** and **34** allowed the introduction of a variety of substituents on the indole ring such as methylsulfone, introduced by Ullmann type coupling[23] to afford the desired DP1 antagonists **35** and **36** after ester hydrolysis.[24]

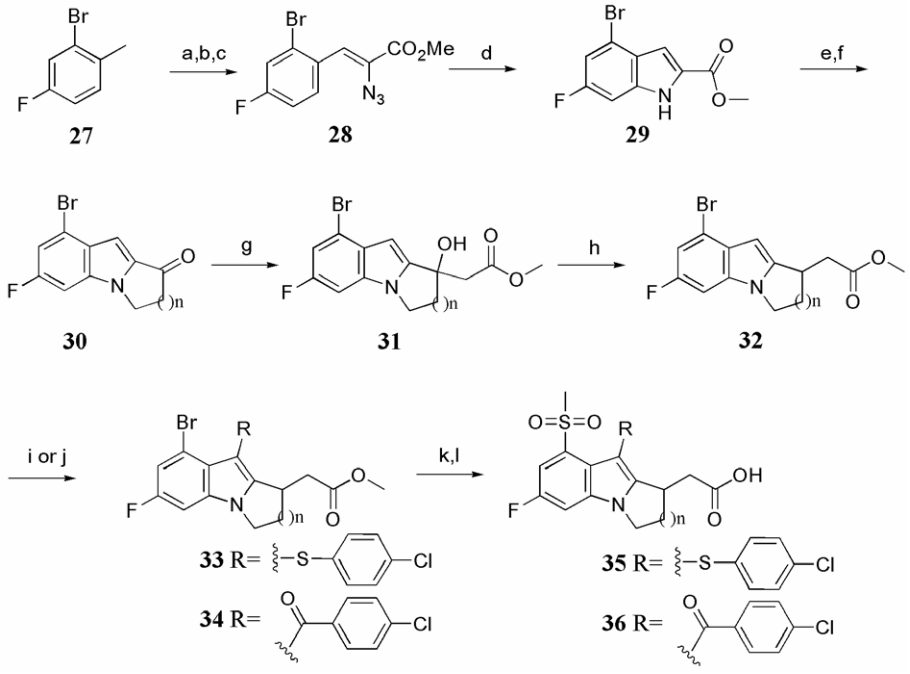
In conclusion, a new series of potent and selective DP1 receptor antagonists was identified by modification of the tetrahydrocarbazole core of MK-0524 (**1**). Tetrahydropyridoindole **26** displayed the best overall profile among the compounds studied and fulfills the criteria of

Table 4. DP1 and prostanoids binding affinities for enantiomers **25** and **26**



25 **26**

Compound	K_i (nM)									IC_{50} (nM) DP1 PRP
	DP1	CRTH2	TP	EP1	EP2	EP3	EP4	FP	IP	
25	36	—	7154	2174	>29,188	>21,360	>16,061	>24,826	>22,498	—
26	1	19,200	168	142	3404	5063	>16,500	21,057	>21,200	4.6



Scheme 1. General synthesis of compounds **11** and **22**–**24**. Reagents and conditions: (a) NBS, BzOOH, CCl_4, reflux; (b) *N*-methylmorpholine *N*-oxide, dioxane, 70 °C; (c) $N_3CH_2CO_2Me$, MeONa, MeOH, 70 °C; (d) xylene, reflux; (e) $n = 1$: methyl acrylate, KOtBu, THF, reflux; $n = 2$: i—$Br(CH_2)_3CO_2Et$, NaH, Bu_4NI, DMF, 0 °C; ii—KOtBu, THF, 0 °C; $n = 3$: i—$Br(CH_2)_4CO_2Et$, NaH, Bu_4NI, DMF, 0 °C; ii—KOtBu, THF, 0 °C; (f) HCl concd, EtOH, reflux; (g) $BrCH_2CO_2Me$, Zn–Cu couple, HMPA, THF; (h)TMSCl, NaI, CH_3CN, Et_2O; (i) bis(4-chlorophenyl)disulphide, SO_2Cl_2, 1,2-DCE, DMF; (j) 4-ClPhCOCl, $AlCl_3$, 1,2-DCE; (k) CuI, $NaSO_2Me$, DMSO, 100 °C; (l) LiOH, H_2O, MeOH, THF.

potency and selectivity deemed suitable for further development.

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16. The DP1 functional assays were performed on blood collected from normal human volunteers. Platelet rich plasma (PRP) was prepared by centrifugation at 150*g* for 15 min. A portion of the PRP fraction was used to prepare the washed platelet fraction by isolating the platelets by centrifugation (10 min at 800*g*) and resuspension of the platelet cell pellet in buffer (25 mM Hepes, pH 7.4, HBSS without Ca^{2+}, Mg^{2+}). The WP and PRP assays were conducted as follows: isobutylmethylxanthine (IBMX; 2 mM final concentration) was added to prevent degradation of cAMP. Samples (100 μL) of either human WP or PRP were then preincubated (10 min at 37 °C) with increasing concentrations of test compound in DMSO. Samples were then challenged with PGD_2 (300 nM final) added in DMSO and incubated for an additional 2 min at 37 °C. The reaction was then terminated by the addition of 200 μL of ethanol to disrupt the cells and extract the cAMP. The samples were mixed thoroughly and centrifuged at 1400*g* for 10 min at 4 °C. Supernatant aliquots (100 μL) were removed and the ethanol removed by evaporation. cAMP was measured by [^{125}I] cAMP scintillation proximity assay (SPA) (RPA556), Amersham).
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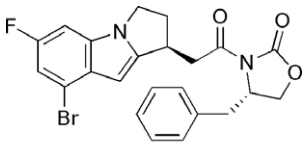
18. The absolute stereochemistry of the acetic acid side chain presents in **25** and **26** was established by X-ray analysis of benzyl oxazolidinone **37** obtained from the ester hydrolysis of **32**, followed by the resolution of the resulting acid by chiral HPLC separation and coupling reaction to afford **37** which was associated to the corresponding isomer of **32** leading to the less potent enantiomer of **35** and **36**.



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24. It was found that the resolution of the racemic corresponding acid of **32** allowed the enantioselective synthesis of the more potent isomer of **35** and **36** as well as the HPLC chiral resolution of **35** and **36**.