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Identification of an indole series of prostaglandin D_2 receptor antagonists

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Abstract—A novel indole series of PGD_2 receptor (DP receptor) antagonists is presented. Optimization of this series led to the identification of potent and selective DP receptor antagonists. In particular, antagonists **35** and **36** were identified with K_i values of 2.6 and 1.8 nM, respectively. These two antagonists are also potent in a DP functional assay where they inhibit the PGD_2 induced cAMP production in platelet rich plasma with IC_{50} values of 7.9 and 8.6 nM, respectively. The structure–activity relationships of this indole series of DP receptor antagonists will also be discussed.

Seasonal allergic rhinitis (SAR) is a multisymptom disease characterized by nasal congestion, itching, and rhinorrhea. The nasal congestion in SAR results from tissue edema and vasodilatation in the nasal mucosa. A number of treatments are currently available for SAR but they are either ineffective at treating all the symptoms of this disease or suffer from unwanted side-effects. For example, antihistamines are effective at controlling the rhinorrhea associated with SAR but are not effective at controlling the congestive response. The symptoms of SAR are induced by a number of mediators such as histamine, cysteinyl leukotrienes, and PGD_2. It has been demonstrated in humans that intranasal instillation of prostaglandin D_2 results in upper airway obstruction with 10-fold greater potency than histamine.[1] PGD_2 is the major cyclooxygenase derived metabolite produced by degranulation of mast cells[2,3] and is present in the nasal washings of atopic individuals following allergen challenge. The potential role of PGD_2 in allergic rhinitis prompted us to initiate a program aimed at identifying PGD_2 receptor[4] (DP receptor) antagonists to block the inflammatory effects of PGD_2.[5–9] To this end, we wish to disclose herein the identification of an indole series of potent and selective PGD_2 receptor antagonists.

The search for PGD_2 receptor antagonists was initiated through the screening of the Merck sample collection. From this effort, a number of hits were identified. One of these hits was the difluoroindole **1** (Fig. 1). This compound displayed good binding affinity[10] (11 nM) for the DP receptor although this compound was actually more potent on the thromboxane A_2 (TP) receptor (1.7 nM).

Compound **1** displayed moderate to good selectivity versus the remaining six prostanoid receptors (Fig. 1).

At this point, medicinal chemistry was initiated in an effort to improve the DP potency and selectivity of this indole series. At the outset, with the structure–activity information available to us from the screening effort,

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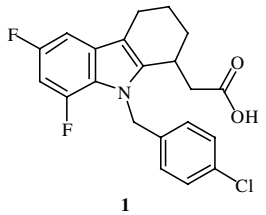


1

Ki (nM)

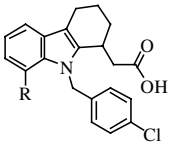
DP	TP	EP1	EP2	EP3	EP4	FP	IP
11 ± 5.1	1.7 ± 1.6	>7000	>4600	>300	>2900	>20000	>2000

Figure 1. Prostanoid binding profile of lead difluoroindole **1**.

we decided to focus our initial attention on modifying the benzoid substituents of the indole core. Table 1 summarizes the results from modification of the indole 7-position. The unsubstituted indole **2** possess good DP activity (K_i = 36 nM) but is 15-fold more potent on the TP receptor (K_i = 2.4 nM). Introduction of an isopropyl group (**3**) not only increased the DP activity but also inverted the selectivity in favour of the DP receptor.

A bromine or aldehyde substituent as in **4** or **5** leads to potent, non-selective, antagonists. The dimethylamide analog **7** is somewhat less active on DP but displays good selectivity versus TP (100-fold). The corresponding phenyl amide **8** is less potent on DP and is also non-selective. Interestingly, while the methyl sulfide analog **9** leads to a potent, non-selective antagonist, the corresponding sulfoxide **10** is significantly more selective versus the TP receptor (60-fold). Other sulfoxide analogs (**11**–**14**) did not result in improved DP activity or selectivity relative to the methylsulfoxide. While the sulfone analog **15** exhibited similar DP potency as the corresponding methylsulfoxide analog, it was significantly less selective versus the TP receptor.

Table 1. DP and TP binding affinities of 7-substituted indoles

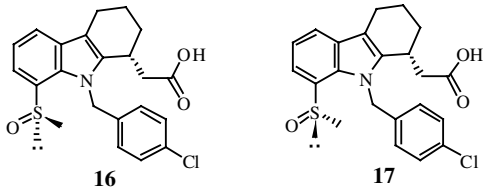


Compound	*R*	K_i[a] (nM)	
		DP	TP
2	H	36 (±11)	2.4 (±1.2)
3	*i*-Pr	1.7 (±0.6)	9.1 (±1.7)
4	Br	2.6 (±0.9)	2.2 (±0.8)
5	CHO	6.3 (±4.8)	6.6 (±5.8)
6	CH_2OH	16 (±5.3)	7.8 (±3.3)
7	$CONMe_2$	28 (±2.9)[b]	3700 (±1000)
8	CONHPh	110 (±74)	88 (±48)
9	SMe	1.0 (±0.0)	2.6 (±1.2)
10	SOMe	2.8 (±0.4)	172 (±24)
11	SOEt	4.0 (±0.4)	340 (±170)
12	SO-*i*-Pr	23 (±12)	1800 (±1200)
13	SOPh	29 (±7.8)	96 (±49)
14	SOBn	3.9 (±1.5)	140 (±45)
15	SO_2Me	1.9 (±0.2)	5.1 (±1.7)

[a] Values are means of at least three experiments unless otherwise stated.
[b] n = 2.

At this point sulfoxide **10**, which is a mixture of four stereoisomers, was resolved into its individual isomers. The DP and TP binding affinities of the two more potent isomers (**16** and **17**) are presented in Figure 2. These two isomers were found to have *R* configuration at the acetic acid side chain as determined by X-ray crystallography of sulfoxide **17** (Fig. 3). The corresponding *S* stereoisomers were 50- to 100-fold less active on the DP receptor. The stereochemistry at sulfur has a minimal effect on the activity or selectivity of these antagonists as illustrated in Figure 2.

Concurrent with the SAR studies at the 7-position of the indole, other benzoid positions were explored. The effect of modifying the right-hand ring was also investigated. Figure 4 below highlights two initial analogs that were prepared in this regard. Both of these methylsulfone analogs were found to be modestly selective for the DP receptor, though less potent than the corresponding 7-substituted sulfoxide analog **17**. Since both **18** and **19** displayed similar binding profiles, it was decided to focus the initial structure–activity studies on the cyclopentyl series (**19**). Table 2 highlights some of these results. Replacement of the methylsulfone with a sulfonamide group resulted in somewhat diminished activity. Interestingly, introduction of a bromine atom at the 7-position, as present in antagonist **21**, resulted in an approximate 270-fold gain in potency. Initial



16

DP Ki = 1.7 ± 0.7 nM
TP Ki = 130 ± 48 nM

17

DP Ki = 1.8 ± 0.7 nM
TP Ki = 47 ± 8.4 nM

Figure 2. Prostanoid binding profile of sulfoxides **16** and **17**.

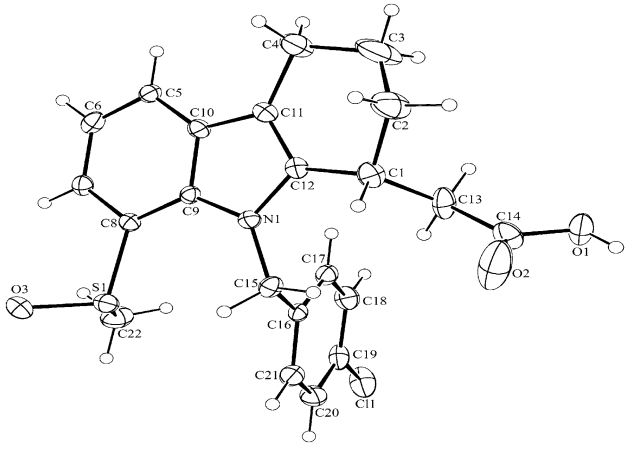


Figure 3. ORTEP representation of **17**. Non-hydrogen atoms are represented by ellipsoids corresponding to 20% probability. Hydrogen atoms have been drawn at arbitrary size.

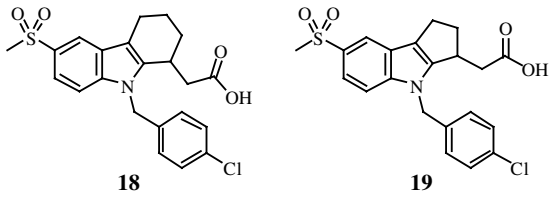


Figure 4. Prostanoid binding profile of sulfones **18** and **19**.

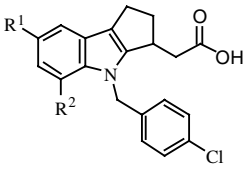
pharmacokinetic studies in rats with sulfonamide **21** revealed that this compound was extensively demethylated. Re-introduction of the more metabolically stable methylsulfone group, as in **22**, improved both the DP potency and TP selectivity. Compound **22** is thus a potent (K_i = 1.7 nM) DP antagonist with a high level of TP selectivity (120-fold). At this point, having achieved desirable level of DP potency we turned our attention on exploring additional bromine replacements with the aim of maintaining DP activity while improving the TP selectivity. To this end, replacement of the bromine with a vinyl, cyclopropyl, cyano or thiophene unit provided potent compounds though with comparable levels of selectivity. Gratifyingly, the level of selectivity could be further improved with the introduction of a methyl ketone (**27**) or a secondary alcohol (**28**) substituent at the 7-position. These data illustrate how, within this indole series, both the DP activity and TP selectivity can be effectively modulated with the proper choice of substituents.

We next examined potential replacements for the *para*-chloro benzylic group. Table 3 presents some of the analogs prepared in this series. Replacement of the chlorine atom with the larger *tert*-butyl substituent, as in **29**, resulted in a modest loss of activity. Introduction of a methylsulfonyl unit to generate the bis-sulfone **30** resulted in an approximate 1000-fold loss of DP activity. The *meta*-chloro analog **31** or the *meta*-trifluoromethoxy analog **32** provided equipotent antagonists relative to the chloro analog **22**. Interestingly, several heterocycles were found to be good 4-chlorobenzyl replacements leading to antagonists such as the 2,3-dichloro-5-thieno[3,2-*b*]pyridine **33** and the chloroquinoline **34**.

While a number of benzyl replacements were identified, none proved to further enhance the TP selectivity relative to the corresponding 4-chlorobenzyl analog **22**. Thus, from the above studies, antagonists **27** and **28** were found to display the optimal in vitro profiles. As before, since antagonists **27** and **28** are mixtures of isomers, it was deemed advantageous at this point to synthesize and evaluate the prostanoid binding profile of the individual isomers. Table 4 summarizes the prostanoid binding profile of the most active isomers, namely **35** and **36**.[11] Both **35** and **36** are potent DP receptor antagonists with high levels of selectivity versus the remaining seven prostanoid receptors as measured by the receptor binding affinities. In addition, antagonists **35** and **36** display excellent potencies in a DP functional assay where they inhibit the PGD_2 induced production of cAMP in platelet rich plasma[12] (PRP) with IC_{50} values of 7.9 and 8.6 nM, respectively. Also, the TP selectivities of **35** and **36** were maintained in a TP functional assay[13] which measures a compound's ability to inhibit the U46619-induced platelet aggregation in platelet rich plasma (see Table 5).

The preparation of the tetrahydrocarbazole compounds **2**–**12** presented in Table 1 involved a Fischer-indole reaction as the key step in their synthesis (Scheme 1).[14,15] Thus, arylhydrazines (**37**) are treated with ethyl 2-oxocyclohexanecarboxylate (**38**) in acetic acid to furnish the corresponding indoles (**39**) in moderate to good yields. Standard benzylation and ester hydrolysis provided the target compounds (**41**).

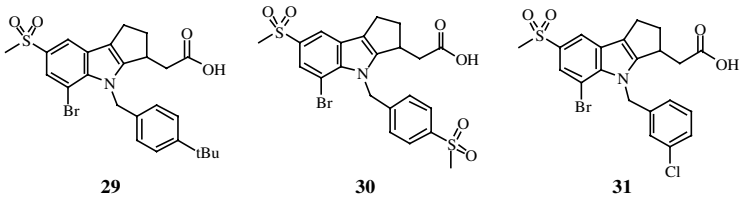
Table 2. Modification of the indole 5- and 7-positions



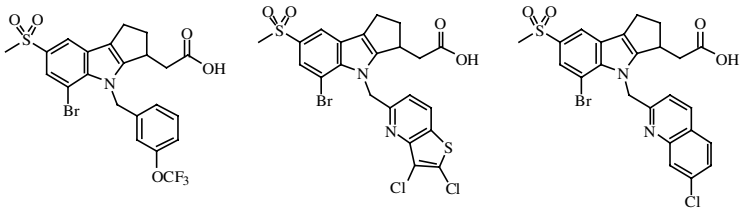
Compound	R^1	R^2	K_i[a] (nM)	
			DP	TP
19	SO_2Me	H	300 (±95)	2100 (±750)
20	SO_2NMe_2	H	1100 (±850)	2300 (±1300)
21	SO_2NMe_2	Br	4.1 (±0.7)[b]	100 (±9.1)
22	SO_2Me	Br	1.7 (±0.2)	200 (±40)
23	SO_2Me	$CH{=}CH_2$	3.6 (±2.0)	520 (±210)
24	SO_2Me	Cy	2.8 (±1.4)	330 (±210)
25	SO_2Me	CN	9.7 (±0.8)	1100 (±240)
26	SO_2Me	2-Thiophene	4.5 (±0.7)	730 (±300)
27	SO_2Me	COMe	9.5 (±3.0)	3600 (±2300)
28	SO_2Me	CH(OH)Me	8.5 (±2.7)	16,000 (±16,000)

[a] Values are means of at least three experiments unless otherwise stated.
[b] n = 2.

Table 3. Modification of the indole benzylic substituents



29 **30** **31**

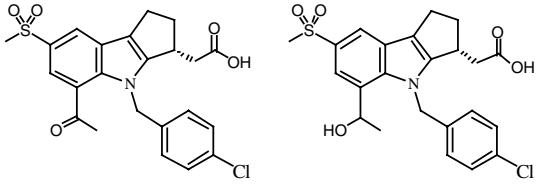


32 **33** **34**

Compound	K_i[a] (nM)	
	DP	TP
29	7.5 (±1.0)	820 (±120)
30	1900 (±720)	2700 (±1100)
31	3.5 (±0.6)	760 (±390)
32	4.1 (±1.1)	260 (±130)
33	4.4 (±2.3)	220 (±73)
34	1.0 (±0.4)	180 (±43)

[a] Values are means of at least three experiments.

Table 4. Prostanoid receptor binding profiles of **35** and **36**



35 **36**

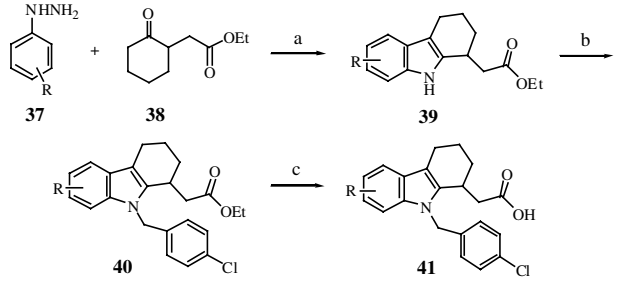
Compound	K_i[a] (nM)							
	DP	TP	EPI	EP2	EP3	EP4	FP	IP
35	2.6 ± 0.7	1200 ± 470	>20,000	230	>20,000	>20,000	>20,000	>20,000
36	1.8 ± 0.7	7100 ± 820	>20,000	390	>20,000	>2900	>20,000	>20,000

[a] Values are means of at least three experiments.

Table 5. DP and TP receptor functional activity of indoles **35** and **36**

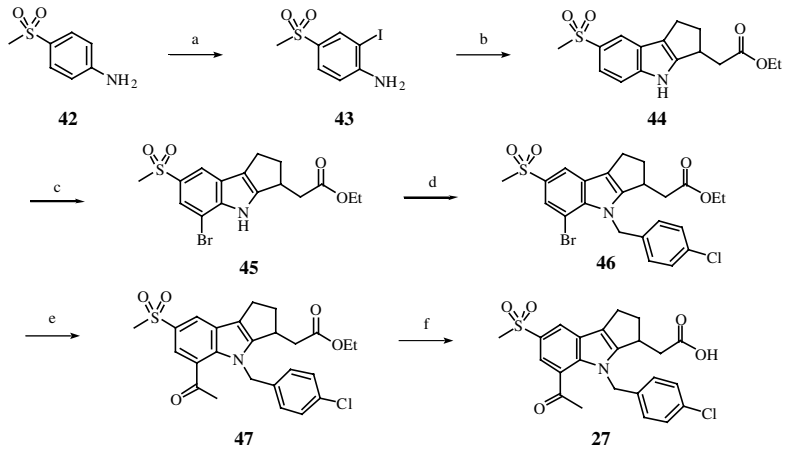
Compound	IC_{50}[a] (nM)		
	DP WP[b]	DP PRP[c]	TP PRP[d]
35	2.0 (±0.7)	7.9 (±2.8)	8400 (±4700)
36	2.0 (±1.4)	8.6 (±4.3)	43,000 (±16,000)

[a] Values are means of at least three experiments.
[b] DP WP assay, inhibition of the accumulation of cAMP in washed platelets challenged with PGD_2.
[c] DP PRP assay, inhibition of the accumulation of cAMP in platelet rich plasma challenged with PGD_2.
[d] TP PRP assay, the inhibition of U46619-induced platelet aggregation in platelet rich plasma.



Scheme 1. Synthesis of tetrahydrocarbazole series. Reagents and condition: (a) AcOH; (b) NaH, DMF, 0 °C, 4-$ClC_6H_4CH_2Br$; (c) NaOH, H_2O, MeOH, THF.

In the case of the tetrahydrocyclopenta[*b*]indole series, a direct Fischer-indole approach proved unsuccessful, giving rise to low yields and mixtures of products. To overcome this shortcoming, the indole core was constructed via a palladium mediated reaction[16] between an *ortho*-iodoaniline and a cyclopentanone as illustrated



Scheme 2. Synthesis of tetrahydrocyclopenta[*b*]indole series. Reagents and conditions: (a) I_2, EtOH, $AgSO_4$; (b) ethyl 2-oxocyclopentane-carboxylate, $Pd(OAc)_2$ DMF; (c) pyrHBr–Br_2, pyr, Zn, AcOH; (d) NaH, DMF, 0 °C; 4-$ClC_6H_4CH_2Br$; (e) 1—1-ethoxyvinylstannane, $Pd_2(dba)_3$, Ph_3As, DMF, 100 °C; 2—aq HCl; (f) NaOH, H_2O, MeOH, THF.

by the synthesis of **27** shown in Scheme 2. Thus, after iodination of aniline **42**, condensation with ethyl 2-oxocyclopentane-carboxylate followed by treatment with $Pd(OAc)_2$ in DMF yielded the desired indole **44** in good yields. Selective bromination at the 7-position was achieved through the actions of pyridinium tribromide to provide **45**.[17] Following standard benzylation, a Stille coupling of **46** with 1-(ethoxyvinyl) tributylstannane followed by acidic work-up generated ketone **47**.[18] Ester hydrolysis thus delivered the DP antagonist **27**.

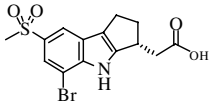
In summary, we have presented a number of potent and selective DP receptor antagonists. In particular, indoles **35** and **36** were shown to have the optimal potency and selectivity. These compounds demonstrate high levels of DP activity in the DP PRP functional assay and are amongst the most potent DP antagonist reported to date. In addition, the structure–activity profile highlighted herein illustrate how, within this indole class of compounds, one can successfully modulate both the DP potency and TP selectivity of these antagonists.

Acknowledgment

The authors thank Mr. Oliver Bouchard for his help in exploring with some of the chemistry described in Schemes 1 and 2.

References and notes

1. Doyle, W. J.; Boehm, S.; Skoner, D. P. *J. Allergy Clin. Immunol.* **1990**, *86*, 924.
2. Lewis, R. A.; Soter, N. A.; Diamond, P. T.; Austen, K. F.; Oates, J. A.; Roberts, L. J., II *J. Immunol.* **1982**, *129*, 1672.
3. Matsuoka, T.; Hirata, M.; Tanaka, H.; Takahashi, Y.; Murata, T.; Kabashima, K.; Sugimoto, Y.; Kobayashi, T.; Ushikubi, F.; Aze, Y.; Eguchi, N.; Urade, Y.; Yoshida, N.; Kimura, K.; Mizoguchi, A.; Honda, Y.; Nagai, H.; Narumiya, S. *Science* **2000**, *287*, 2013.
4. Boie, Y.; Sawyer, N.; Slipetz, D. M.; Metters, K. M.; Abramovitz, M. *J. Biol. Chem.* **1995**, *270*, 18910.
5. Giles, H.; Leff, P.; Bolofo, M. L.; Kelly, M. G.; Robertson, A. D. *Br. J. Pharmacol.* **1989**, *96*, 291.
6. Torisu, K.; Kobayashi, K.; Iwahashi, M.; Egashira, H.; Nakai, Y.; Okada, Y.; Nanbu, F.; Ohuchida, S.; Nakai, H.; Toda, M. *Eur. J. Med. Chem.* **2005**, *40*, 505.
7. Mitsumori, S.; Tsuri, T.; Honma, T.; Hiramatsu, Y.; Okada, T.; Hashizume, H.; Inagaki, M.; Arimura, A.; Yasui, K.; Asanuma, F.; Kishino, J.; Ohtani, M. *J. Med. Chem.* **2003**, *46*, 2436.
8. Mitsumori, S.; Tsuri, T.; Honma, T.; Hiramatsu, Y.; Okada, T.; Hashizume, H.; Kida, S.; Inagaki, M.; Arimura, A.; Yasui, K.; Asanuma, F.; Kishino, J.; Ohtani, M. *J. Med. Chem.* **2003**, *46*, 2446.
9. Tsuri, T.; Honma, T.; Hiramatsu, Y.; Okada, T.; Hashizume, H.; Mitsumori, S.; Inagaki, M.; Arimura, A.; Yasui, K.; Asanuma, F.; Kishino, J.; Ohtani, M. *J. Med. Chem.* **1997**, *40*, 3504.
10. Abramovitz, M.; Adam, M.; Boie, Y.; Carriere, M.-C.; Denis, D.; Godbout, C.; Lamontagne, S.; Rochette, C.; Sawyer, N.; Tremblay, N. M.; Belley, M.; Gallant, M.; Dufresne, C.; Gareau, Y.; Ruel, R.; Juteau, H.; Labelle, M.; Ouimet, N.; Metters, K. M. *Biochim. Biophys. Acta* **2000**, *1483*, 285.
11. The absolute stereochemistry of the acetic acid side chain present in **35** and **36** was established by X-ray analysis of the following acid which was obtained from ester hydrolysis and resolution of **45**:



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Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 297169 (**17**) and CCDC 297363 (**48**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [e-mail: deposit@ccdc.cam.ac.uk].
12. The DP 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 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).

13. TP functional assay was performed as follows: Test compounds were assessed for their ability to inhibit platelet aggregation in human PRP using aggregometry. Two hundred and fifty microliters of PRP was stirred in a cuvette at 37 °C for 3 min. Twenty-five microliters of the test compound diluted in buffer [0.9% (w/v) or 0.15 M NaCl containing 0.25% (w/v) BSA] or vehicle (buffer), as control was added to 250 μL stirring platelets for a 10 min preincubation period. Twenty microliters of agonist (1.36 μM U46619, final concentration) was then added to the stirring PRP mixture to induce aggregation and the incubation continued for five additional minutes. Maximum aggregation was determined at approximately 5 min. The maximum extent of aggregation achieved in the presence of U46619 was set to 100%.
14. All new compounds provided satisfactory spectral data along with mass spectral and/or elemental analysis.
15. Eberle, M. K.; Kahle, G. G.; Talati, S. M. *Tetrahedron* **1973**, *29*, 4045.
16. Chen, C.; Lieberman, D. R.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. *J. Org. Chem.* **1997**, *62*, 2676.
17. Lachance, N.; Chan, W. Y. *J. Heterocycl. Chem.* **2003**, *40*, 289.
18. Gan, T.; Van Ornum, S. G.; Cook, J. M. *Tetrahedron Lett.* **1997**, *38*, 8453.