Bioorganic & Medicinal Chemistry Letters 23 (2013) 3349–3353

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

2-(1*H*-Pyrazol-4-yl)acetic acids as CRTh2 antagonists

Miriam Andrés, Mónica Bravo, Maria Antonia Buil, Marta Calbet, Jordi Castro, Teresa Domènech, Peter Eichhorn, Manel Ferrer, Elena Gómez, Martin D. Lehner, Imma Moreno, Richard S. Roberts *, Sara Sevilla

Almirall R&D Centre, Laureano Miró, 408-410, 08980 Sant Feliu de Llobregat, Barcelona, Spain

ARTICLE INFO

Article history:
Received 14 February 2013
Revised 19 March 2013
Accepted 22 March 2013
Available online 2 April 2013

Keywords:
CRTh2
Prostaglandin D2
Antagonist
Pyrazole
Plasma protein binding

ABSTRACT

High throughput screening identified the pyrazole-4-acetic acid substructure as CRTh2 receptor antagonists. Optimisation of the compounds uncovered a tight SAR but also identified some low nanomolar inhibitors.

Prostaglandin D_2 (PGD_2) is derived from the metabolism of arachidonic acid by cyclooxygenases and downstream PGD_2 synthases. In the immune system, PGD_2 is mainly produced by mast cells[1] but also although at lower levels, by macrophages and Th2 lymphocytes.[2] PGD_2 binds to three different receptors, the thromboxane type prostanoid receptor (TP),[3] the PGD_2 receptor (DP, also known as DP1)[4] and the chemoattractant receptor homologous molecule expressed on Th2 lymphocytes (CRTh2, also known as DP2).[5] CRTh2 is a G_i coupling GPCR, signaling through reduction of intracellular cyclic adenosine monophosphate (cAMP) and calcium mobilization and it is involved in the chemotaxis of Th2 lymphocytes, eosinophils and basophils.[6] CRTh2 also inhibits the apoptosis of Th2 lymphocytes[7] and stimulates the production of IL4, IL5, and IL13,[8] cytokines involved in important biological responses such as eosinophil recruitment and survival, mucus secretion, airway hyper-responsiveness and immunoglobulin E (IgE) production among others. Therefore, molecules that antagonize the pro-inflammatory PGD_2 effects mediated by CRTh2 on key cell types associated with allergic inflammation (basophils, eosinophils and Th2 lymphocytes) should have a potential benefit in related pathologies.

Several recent reviews have highlighted the progress and most advanced series of CRTh2 antagonists.[9,10] Many of the chemical series owe their origins to the observations that the indoleacetic acid indomethacin **1** inhibited the binding of PGD_2 to CRTh2,[11] and that the thromboxane inhibitor, the indolepropionic acid Ramatroban (Baynas®) **2** was also a fairly potent antagonist of CRTh2 (Fig. 1).[12]

While robust structural information on the receptor remains elusive, a general preference for an arylacetic acid pharmacophore has emerged. Furthermore, these structures may be conceptually broken into three areas (Fig. 2):[9b]

(1) The acetic acid 'head' group. The chain length affects binding affinity, with acetic acid chains generally best. Alpha-substitution tends to lose potency and common bioisosteres of carboxylic acids (for example tetrazoles or acyl sulfonamides) are not well tolerated.
(2) The 'core' ring. This may be monocyclic or bicyclic, and with the exception of a few cases where activity is lost, no clear preference has emerged in this position.
(3) The 'tail' group. This position seems to be the most open in terms of structure–activity relationship (SAR) and a great number of variations in chain length, directionality and polarity have been published.

We sought to take advantage of known SAR around the indoleacetic acid pharmacophore but applying a conceptual ring expansion of the indole core, revealing a substituted pyrazole (Fig. 2). Two possible ring-openings were considered: this letter addresses our work on pyrazole-4-acetic acids **3**, while the work on pyrazole-1-acetic acids **4** is described elsewhere.[13]

* Corresponding author. Tel.: +34 93 291 7540; fax: +34 93 291 3420.
E-mail address: rick.roberts@almirall.com (R.S. Roberts).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.03.093

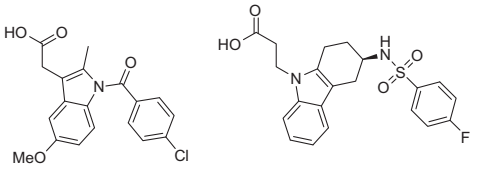


Figure 1. Chemical structures of Indomethacin **1** and Ramatroban **2**.

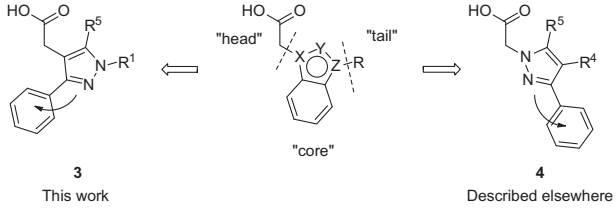
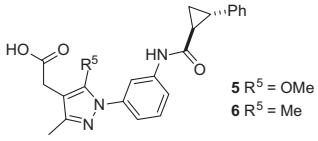


Figure 2. Conceptual opening of the indole core to reveal the pyrazoleacetic acids.

A high throughput screening (HTS) campaign of our compound collection[14] revealed compound **5** as a micromolar hit. The methoxypyrazole motif appeared a potential metabolic weak-spot of the molecule so we directly synthesized and characterized dimethyl analogue **6**. Encouragingly, this compound was more active than the original hit **5** in the blockade of radio-labelled [^{35}S]-GTPγS binding (Table 1).[15] Functional activity was confirmed through the eosinophil shape change assay (ESC) in human whole blood (hWB),[16] showing a modest 10-fold drop in activity, a shift undoubtedly due to binding to plasma proteins. The physicochemical properties were acceptable at this stage for an oral compound so we proceeded to profile the pharmacokinetics in rat. Compound **6** showed low clearance, and good exposure after intravenous dosing. The oral bioavailability was low, but as this was only a hit compound, we were encouraged to continue.

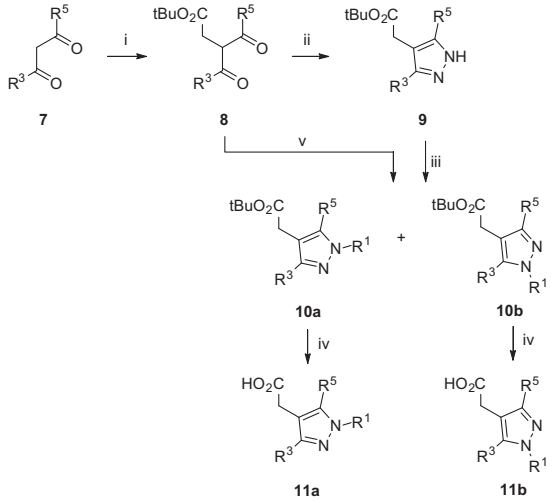
The general synthetic route is outlined in Scheme 1. The appropriate diketones **7** were alkylated with *tert*-butyl bromoacetate and cyclised with hydrazine to give the pyrazoles **9**. Functionalisation of the pyrazole NH (alkylation, arylation or sulfonylation) followed by acidic deprotection led to the desired pyrazoles **11**. Alternatively, the diketones **8** could be cyclised directly with the appropriate hydrazine to give **10**, followed by acid deprotection.

Table 1
Pyrazole HTS hit **5** and dialkyl analogue **6**



Property	5	6
GTPγS binding IC_{50} (nM)	1600	230
ESC hWB IC_{50} (nM)		2100
Caco-2 AB/BA ($P_{app} \times 10^{-6}$ cm/s)		3/6
Solubility (mg/ml) pH 1/7.4		0.5/0.8
p*Ka*[a]/log$D_{7.4}$[b]		4.2/0.9
iv Pharmacokinetics[c]		
$AUC_{0-24\ h}$ (ng*h/ml)		3364
Clearance (ml/min/kg)		2.5
$t_{½}$ (h)		1.3
V_{ss} (l/kg)		0.14
F_{oral}[d]		12%

[a] Potentiometric.
[b] Shake-flask.
[c] Rat iv dose 0.5 mg/kg.
[d] Rat oral dose 1 mg/kg.



Scheme 1. Representative syntheses of final compounds. Reagents and conditions: (i) NaH, THF, 0 °C; *tert*-butyl bromoacetate, rt; (ii) $NH_2NH_2{\cdot}H_2O$, AcOH, rt; (iii) alkyl halide, K_2CO_3, DMF, rt or NaH, THF, 0 °C; sulfonyl chloride, rt or aryl boronic acid, $Cu(OAc)_2$, O_2, pyridine, CH_2Cl_2, rt; (iv) TFA, $CHCl_3$, rt; (v) R^1NHNH_2, AcOH, rt.

For asymmetrical pyrazoles ($R^3 \neq R^5$), the step to incorporate the R^1 group inevitably led to the formation of both regioisomers **10a** and **10b**.[17] According to the conceptual ring opening of Fig. 2, the desired isomer generally required R^3 = aryl and R^5 = methyl.[18] In this case, functionalisation of pyrazoles **9** gave the desired regioisomer **10a** as the major product, typically in a ratio of ~90:10. Direct cyclisation of diketones **8** with aryl hydrazines (R^1 = aryl) also gave regioisomer **10a** as the major products. Only in the case of direct cyclisation with alkyl hydrazines (R^1 = alkyl) did the selectivity change, giving the undesired compounds **10b** as the major isomer. The separation of the regioisomers **10a** and **10b** required careful chromatography in each case and the stereochemistry of the products was confirmed by nuclear Overhauser effect (NOE) studies in many cases.

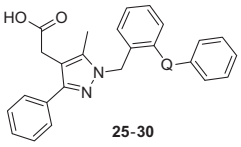
From the general synthetic route, we first synthesized a directed set of analogues of general formula **12**, keeping the synthesis simple by using a symmetrical pyrazole ($R^3 = R^5$ = Me). The SAR of a set of 30 compounds in the GTPγS binding assay turned out to be quite tight: only 4 (13%) compounds showed moderate activity of <1 μM, with a further 6 (20%) compounds between 1 and 5 μM.

A recent letter by Pfizer of a directed library of pyrazole-4-acetic acids also highlighted a general absence of activity for this substructure.[19] However, this was in contrast to some very potent pyrazoles of similar general structure exemplified by Boehringer Ingelheim.[20]

When we investigated further, we saw that there was a clear separation of the tail SAR between the pyrazole series and reported indole-based compounds (Fig. 4). However, in the case of the ortho-sulphonyl benzyl tails (**23**–**25**) the activity was maintained in the pyrazole derivatives and we observed very good potencies.

Compound **25** was used as the starting point for further SAR investigations around the tail, and we found a clear preference for the sulfone linker (Table 2). The next most active linker after the SO_2 group was the methylene **27**. It is interesting to consider that in these linked bisaryl systems, the conformational preference for both sulfone and methylene is similar, with the two aromatic rings positioned orthogonal to each other.[22] Carbonyl and oxygen linkers are markedly different, favouring conformations in which

Table 2
SAR of the sulfone variations



25-30

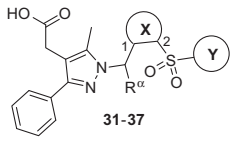
Compd	Q	GTPγS IC_{50} (nM)
25	SO_2	7
26	CH_2SO_2	490
27	CH_2	100
28	C=O	1400
29	bond	5000
30	O	2000

both aryl rings try to achieve the maximum planarity with respect to the linker group. In accordance, these linkers **28** and **30** respectively lost orders of magnitude of potency with respect to **25**. Hence we reasoned that the position of the terminal aromatic group was important for binding and was generally maintained in subsequent molecules.

With the tail linker group fixed as sulfone, we carried out further SAR around the other positions of the tail (Table 3). Replacement of the terminal phenyl group with a cycloalkyl group (sulphonamides **32** and **33**) lost an order of magnitude of potency. More radical changes such as a strongly basic group at the terminus (**34**) or a small terminal group (**35**) lost even more potency. We also saw that alpha-substitution adjacent to the pyrazole core (**36**) showed a dramatic drop in potency. Here the loss was attributed to one (or both) of the following reasons: steric clash of the alpha-methyl group with the pyrazole-5-methyl disrupted the relative conformation between the pyrazole and ring X. Alternatively, the proximity of the alpha-methyl to the sulfone linker was sufficient to disturb the relative conformation between the X and Y rings, a sensitive part of the molecule as we had already observed. Finally replacement of the proximal phenyl ring with a pyrrolidine (**37**) lost all activity. Clearly in this series, the SAR was very sensitive to conformational changes of the tail group, with both the rings X and Y preferably aromatic and requiring the correct relative orientation between both themselves and the pyrazole core.

With a tight SAR emerging, we profiled compound **25** to evaluate the series (Table 4).[23] The first detail that emerged was the almost complete loss of potency in the human whole blood ESC assay. This was followed up with plasma protein binding (PPB)

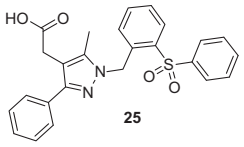
Table 3
SAR of ortho-sulphone tails



31-37

Compd	R^{α}	X	Y	GTPγS IC_{50} (nM)
25	H	Ph	Ph	7
31	H	5-OMe-Ph	4-F-Ph	36
32	H	Ph	4-Morpholinyl	89
33	H	Ph	1-Piperidinyl	66
34	H	4-F-Ph	1-Piperazinlyl	5000
35	H	Ph	NMe_2	850
36	Me	Ph	Ph	2900
37	H		Ph	Inactive

Table 4
Profile of pyrazole **25**



25

Property	**25**
GTPγS binding IC_{50} (nM)	7
ESC hWB IC_{50} (nM)	2400
PPB%[a] (rat/human)	98.8/>99.9
Caco-2 AB/BA ($P_{app} \times 10^{-6}$ cm/s)	13/17
Solubility (mg/ml) pH 1, 7.4	0.003/0.95
p*Ka*[b]/log$D_{7.4}$[c]	4.2/0.7
iv Pharmacokinetics[d]	
$AUC_{0-24\ h}$ (ng*h/ml)	1955
Clearance (ml/min/kg)	8.5
$t_{½}$ (h)	4.8
V_{ss} (l/kg)	1.6
F_{oral}%[e]	73

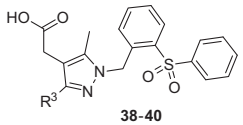
[a] Plasma protein binding, rapid equilibrium dialysis.
[b] Potentiometric.
[c] Shake-flask.
[d] Rat iv dose 1 mg/kg.
[e] Rat oral dose 10 mg/kg.

measurements: in human plasma, the true extent of binding was hard to measure and even diluting the plasma to 10% concentration, the extent of binding as measured by equilibrium dialysis was around 99.9%. Protein binding of this order was seen as an issue. While the compound is bound to plasma proteins, it is essentially shielded from the various clearance mechanisms of the body, and an attractive overall PK profile may result. However, the bound compound is also impeded from interacting with the target receptor: large circulating levels of an inactive compound (or very long-lived but very very low free concentrations) lead to little.[24] In terms of physical chemical properties, **25** showed properties expected for a lipophilic carboxylic acid. Permeability and, due to the presence of the carboxylate at physiological pH, solubility were good. At low pH, re-protonation to the carboxylic acid gave a lipophilic, neutral molecule and so solubility dropped away. Both the PPB and PK profile in rat were acceptable and revealed a well absorbed, low clearance compound with a long terminal half-life.

With the narrow SAR of the tail favouring the ortho sulfone group, we examined the remaining position open to substitution at the pyrazole 3-position, in an attempt to at least moderate the excessive human PPB (Table 5). Lipophilic groups in this position maintained huge shifts in potency in the absence or presence of plasma proteins. Only the polar pyridyl substitution (**39**) was capable of maintaining high potency in the presence of albumin. In this case we used a substitute assay of running the GTPγS binding in the presence of 1% human serum albumin (HSA). This assay allowed a much higher throughput than the ESC assay and we always observed a good correlation between the two (data not shown).

At this point, through the tail SAR we had found an optimum substitution pattern in the ortho-sulfonylbenzyl tail. This in turn had led to an optimisation of the substituents of the pyrazole core, resulting in potent inhibitor **39**. However, it was evident that these substitution patterns of both tail and core were quite specific and that we were left with little further room for manoeuvre. We could however see parallels with a series of thiazole-4-acetic acid compounds reported by 7TM,[25] in which the equivalent R^3 group of the core was also 4-pyridyl. The tail groups of this series were dibenzyl derivatives, so we decided to pursue this line of investigation.

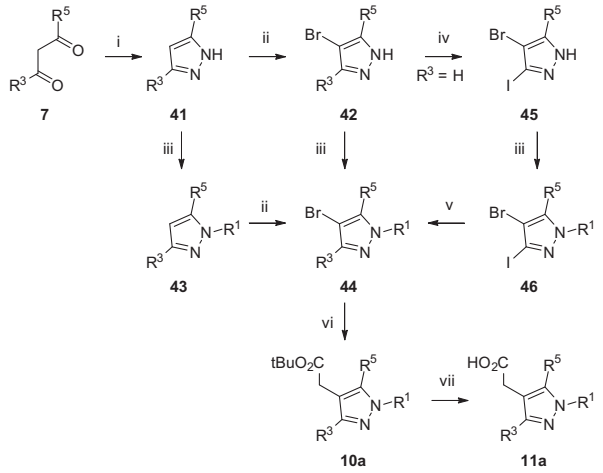
Table 5
SAR around the pyrazole R^3 group



38-40

Compd	R^3	GTPγS IC_{50} (nM)	ESC hWB IC_{50} (nM)
24	Me	81	1700
25	Ph	7	2400
38	4-Cl–Ph	24	8100
39	4-Pyr	4	66[a]
40	OH	160	1000

[a] Measured by surrogate assay: GTPγS binding in presence of 1% human serum albumin (HSA).



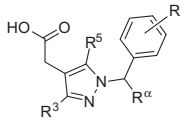
Scheme 2. Representative syntheses of final compounds. Reagents and conditions (i) $NH_2NH_2{\cdot}H_2O$, EtOH, rt; (ii) NBS, DMF, 55 °C; (iii) NaH, DMF, rt; alkyl halide; (iv) NIS, DMF, 115 °C; (v) aryl boronic acid, $PdCl_2$(dppf), dioxane, 80 °C; (vi) $Pd(dba)_2$, Q-Phos, $tBuO_2CCH_2ZnBr$, THF, microwave irradiation, 100 °C; (vii) HCl, dioxane, rt.

For some of these compounds, alternative synthetic routes were useful (Scheme 2). If the correct diketone **7** was available, this was cyclised with hydrazine (**41**) and then alkylated and brominated (in either order) to give the bromopyrazole **44**. Alternatively, to allow introduction of the R^3 group at a later stage of the synthesis the iodobromopyrazoles **45** were synthesised. Alkylation (**45**→**46**) proceeded with very high regioselectivity in this case. The R^3 group was then introduced via a Suzuki reaction. With the purified regioisomer of the bromopyrazole **44** in hand, the acetic acid head group was introduced via a palladium-catalysed Negishi reaction under microwave conditions. Finally, acid deprotection was carried out with HCl in dioxane, as when the R^1 tail group was benzhydryl, standard TFA treatments also cleaved the tails.

In terms of this new SAR, the 4-pyridyl group at the pyrazole 3-position was preferable (Table 6). Again, we found that small changes to the first compound of the series lost activity. For example, extending the hydrogen-bond acceptor capacity of the pyridyl group to a pyridone (**51**) lost all potency. In the end, the benzhydryl tail motif was unable to reach the same levels of potency as seen with the sulfone tails.

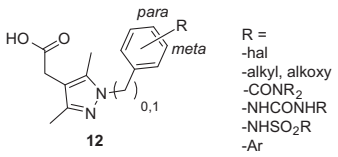
A selection of the compounds throughout the series, namely **24**, **25**, **27**, **30**, **32**, **40** and **47** were also tested for their selectivity against both the DP1 receptor and the thromboxane A2 receptor. All compounds were selective, showing <40% inhibition at 10 μM in all cases.

Table 6
SAR around branched tail groups



47-53

Compd	R^3	R^5	R^α	R	GTPγS IC_{50} (nM)
47	4-pyr	Me	4-F–Ph	4-F	46
48	4-pyr	Me	H	4-F	1900
49	4-pyr	H	4-F–Ph	4-F	290
50	2-F-4-pyr	Me	4-F–Ph	4-F	310
51	4-OH-3-pyr	Me	4-F–Ph	4-F	Inactive
52	Ph	Me	4-F–Ph	4-F	1100
53	Ph	Me	4-F–Ph	4-SO_2Me	5000



12

Figure 3. Directed set of phenyl and benzyl-substituted pyrazole analogues.

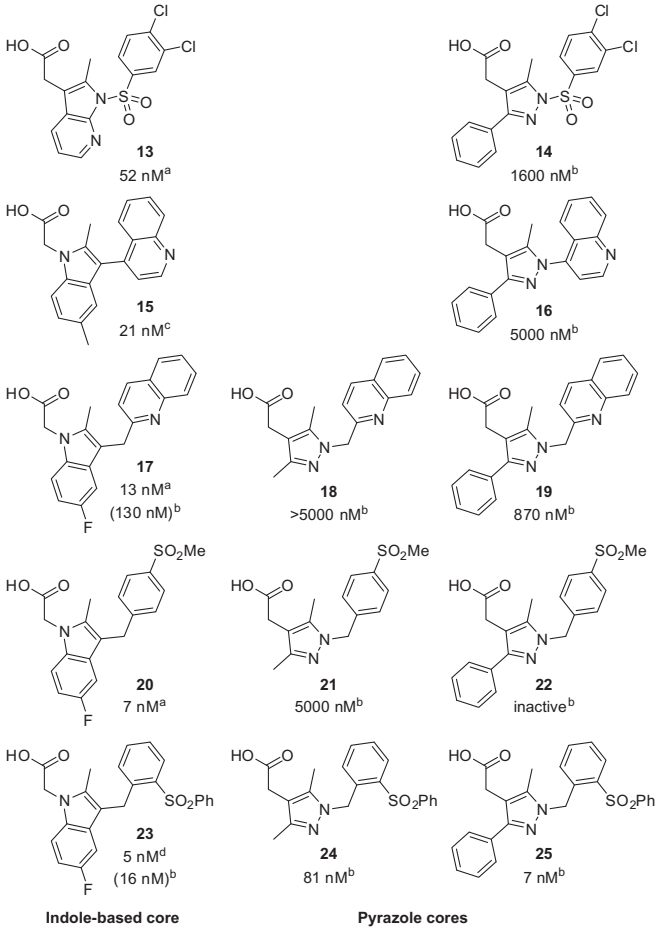


Figure 4. Comparative potency data (nM) between pyrazole and indole-based cores. [a]K_i data taken from Ref. 9b and references therein. [b]Internally measured GTPγS IC_{50} data. [c]IC_{50} data taken from Ref. 21. [d]ESC IC_{50} data taken from Ref. 9a and references therein.

We also characterised the dissociation kinetics of some of these compounds, following some reports of the potential for slow off-rate compounds from the CRTh2 receptor.[26] Briefly, membranes were incubated for 1 h with the test compound at $10 \times IC_{50}$. We then initiated compound dissociation by adding a huge excess of PGD_2 (100 μM and 0.1 nM ^{35}S-GTPγS) so that once the test compound dissociated, re-binding was prevented by mass-action law. We could then perform the standard GTPγS assay at intervals event to follow the loss of binding activity over time. Using this assay, benzyl-substituted compounds **24** and **25** showed rapid dissociation half-lives of 2 and 12 min respectively. Benzhydryl analogue **47** was somewhat slower with a half-life of around 30 min.

In conclusion, HTS identified the pyrazole-4-acetic acid substructure as a micromolar hit. The SAR of the scaffold was investigated, showing significant differences in some case to the SAR of reported indole acetic acid CRTh2 antagonists. SAR was able to identify **25** as a very potent inhibitor in vitro. The extremely high PPB of this series was a mitigating factor for the further development of these compounds. Pyridyl analogue **39** was seen as an attractive alternative. The SAR uncovered around the pyrazoles was of interest for other CRTh2 antagonist series which will be reported in due course.

The structures of the set of pyrazoles **12** of Figure 3 are contained in the Supplementary data supplied with this manuscript.

Acknowledgments

The authors thank the following for their valuable assistance in the experimental procedures: Laura Estrella, Manel de Luca, José Luís Gómez, Encarna Jiménez, Francisco Jiménez, Juan Navarro, Isabel Pagan and Sílvia Petit.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.093.

References and notes

1. Lewis, R. A.; Soter, N. A.; Diamond, K. F.; Oates, J. A.; Roberts II, L. J. *J. Immunol.* **1982**, *129*, 1627.
2. (a) Urade, Y.; Ujihara, M.; Horiguchi, Y.; Ikai, K.; Hayaishi, O. *J. Immunol.* **1989**, *143*, 2982; (b) Tanaka, K.; Ogawa, K.; Sugamura, K.; Nakamura, M.; Takano, S.; Nagata, K. *J. Immunol.* **2000**, *164*, 2277.
3. (a) Coleman, R. A.; Sheldrick, R. L. G. *Br. J. Pharmacol.* **1989**, *96*, 688; (b) Francis, H. P.; Greenham, S. J.; Patel, U. P.; Thompson, A. M.; Gardiner, P. J. *Br. J. Pharmacol.* **1991**, *104*, 596.
4. Boie, Y.; Saywer, N.; Slipetz, D. M.; Metters, K. M.; Abramovitz, M. *J. Biol. Chem.* **1995**, *270*, 18910.
5. (a) Nagata, K.; Tanaka, K.; Ogawa, K.; Kemmotsu, K.; Imai, T.; Yoshie, O.; Abe, H.; Tada, K.; Nakamura, M.; Sugamura, K.; Takano, S. *J. Immunol.* **1999**, *162*, 1278; (b) Powell, W. S. *Prostaglandins Leukot. Essent. Fatty Acids* **2003**, *69*, 179.
6. (a) Hirai, H.; Tanaka, K.; Yoshie, O.; Ogawa, K.; Kenmotsu, K.; Takamori, Y.; Ichimasa, M.; Sugamura, K.; Nakamura, M.; Takano, S.; Nagata, K. *J. Exp. Med.* **2001**, *193*, 255; (b) Shichijo, M.; Sugimoto, H.; Nagao, K.; Inbe, H.; Encinas, J. A.; Takeshita, K.; Bacon, K. B.; Gantner, F. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 518.
7. Xue, L.; Barrow, A.; Pettipher, R. *J. Immunol.* **2009**, *182*, 7580.
8. Xue, L.; Gyles, S. L.; Wettey, F. R.; Gazi, L.; Townsend, E.; Hunter, M. G.; Pettipher, R. *J. Immunol.* **2005**, *175*, 6531.
9. (a) Chen, J. J.; Budelsky, A. L. *Prog. Med. Chem.* **2011**, *50*, 49; (b) Pettipher, R.; Whittaker, M. *J. Med. Chem.* **2012**, *55*, 2915.
10. (a) Medina, J. C.; Liu, J. *Annu. Rep. Med. Chem.* **2006**, *41*, 221; (b) Ulven, T.; Kostenis, E. *Exp. Opin. Ther. Patents* **2010**, *20*, 1505; (c) Norman, P. *Exp. Opin. Invest. Drugs* **2010**, *19*, 947; (d) Burgess, L. *Annu. Rep. Med. Chem.* **2011**, *46*, 119.
11. Hirai, H.; Tanaka, K.; Takano, S.; Ichimasa, M.; Nakamura, M.; Nagata, K. *J. Immunol.* **2002**, *168*, 981.
12. Sugimoto, H.; Shichijo, M.; Iino, T.; Manabe, Y.; Watanabe, A.; Shimazaki, M.; Gantner, F.; Bacon, K. B. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 347.
13. Andrés, M.; Bravo, M.; Buil, M. A.; Calbet, M.; Castillo, M.; Castro, M.; Eichhorn, P.; Ferrer, M.; Lehner, M. D.; Moreno, I.; Roberts, R. S.; Sevilla, S. *Eur. J. Med. Chem.* **2013**, manuscript in preparation.
14. Approximately 100 K compounds were screened by homogeneous cAMP assay in 384-well format using the cAMP dynamic 2 jumbo kit (Ref. 62AM4PEJ, CisBio) using HTRF energy transfer technology. A hit rate of 0.45% was observed.
15. 4-8 μg of membranes obtained from CHO.K1 cells stably over-expressing CRTh2 were pre-incubated with the compound to be tested for 1 h, followed by incubation with 50 nM PGD2 and 0.1 nM guanosine [gamma-thio]triphosphate ([^{35}S]-GTPγS) in incubation buffer (20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, 10 μM GDP, 10 μg/ml saponine and either 0.2% BSA or 1% HSA as appropriate) for 2 h at rt. The reaction was terminated by filtration using GF/C plates pre-treated with 20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl and 0.1% BSA, washing 6 times with buffer (20 mM NaH_2PO_4, 20 mM Na_2HPO_4). The plates were dried and scintillation buffer Optiphase was added. The radioactivity retained in the filter was counted using a Microbeta liquid scintillation counter. Compound IC_{50}s were determined using Excel XL-fit for calculations. Antagonism was observed by the reduction of retained radioactivity from control. Agonism could be measured using a similar assay set-up but without the 1 h pre-incubation and without the addition of PGD_2.
16. Whole blood obtained from consenting donors was mixed with heparin and kept in rotation until use. For compound treatment, 90 μl of blood was mixed with 10 μl of test compound solution and incubated for 10 min at room temperature followed by addition of 10 μl 500 nM PGD_2 for exactly 4 min at 37 °C. The reaction was stopped by placing the tubes on ice and adding of 0.25 ml of ice cold 1:5 diluted CellFix (BD Biosciences). Red blood cells were lysed in two steps by adding 2 ml of lysis solution (150 mM ammonium chloride, 10 mM potassium hydrogencarbonate), incubating for 20 and 10 min respectively and centrifuging at 300*g* for 5 min at 6 °C. The pellet was re-suspended in 0.2 ml fixative solution and immediately analysed on a FACSCalibur (BD Biosciences). Eosinophils were gated on the basis of autofluorescence in the FL2 channels and shape change of 600 cells was assayed by forward scatter and side scatter analysis. Compound IC_{50}s were determined using Excel XL fit for calculations.
17. Elguero, J. In *Comprehensive Heterocyclic Chemistry II*; Katritzky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Pergamon Press, Oxford, 1996; Vol. 3, p 1.
18. Compounds of formula **11b** were found to be weakly active if at all (data not shown).
19. Masood, M. A.; Gardner, M.; Dack, K.; Winpenny, D.; Lunn, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3682.
20. (a) Oost, T.; Anderskewitz, R.; Hamprecht, D. W.; Hoenke, C.; Martyres, D.; Rist, W.; Seither, P. PCT Int. Appl. 2011, WO2011092140.; (b) Anderskewitz, R.; Martyres, D.; Oost, T.; Rist, W.; Seither, P. PCT Int. Appl. 2012, WO2012101043.; (c) Martyres, D.; Anderskewitz, R.; Hoenke, C.; Kriegl, J.; Oost, T.; Rist, W.; Seither, P. PCT Int. Appl. 2012, WO2012130633.
21. Birkinshaw, T. N.; Teague, S. J.; Beech, C.; Bonnert, R. V.; Hill, S.; Patel, A.; Reakes, S.; Sanganee, H.; Dougall, I. G.; Phillips, T. T.; Salter, S.; Schmidt, J.; Arrowsmith, E. C.; Carrillo, J. J.; Bell, F. M.; Paine, S. W.; Weaver, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4287.
22. Brameld, K. A.; Kuhn, B.; Reuter, D. C.; Stahl, M. *J. Chem. Inf. Model.* **2008**, *48*, 1.
23. *Representative data for* ***25***: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.83 (s, 3H), 3.47 (s, 2H), 5.53 (s, 2H), 6.54 (d, J = 7.03 Hz, 1H), 7.29–7.36 (m, 1H), 7.40 (t, J = 7.42 Hz, 2H), 7.53 (d, J = 7.42 Hz, 2H), 7.60–7.71 (m, 4H), 7.72–7.80 (m, 1H), 8.00 (d, J = 7.42 Hz, 2H), 8.21 (dd, J = 5.47, 3.52 Hz, 1H), 12.36 (s, 1H). HPLC/MS (15 min) retention time 8.18 min. LRMS: m/z 447 (M+1).
24. (a) Trainor, G. L. *Exp. Opin. Drug Disc.* **2007**, *2*, 51; (b) Smith, D. A.; Di, L.; Kerns, E. H. *Nat. Rev. Drug Disc.* **2010**, *9*, 919; (c) Liu, X.; Chen, C.; Hop, C. E. C. A. *Curr. Top. Med. Chem.* **2011**, *11*, 450.
25. Grimstrup, M.; Rist, Ø.; Receveur, J.-M.; Frimurer, T. M.; Ulven, T.; Mathiesen, J. M.; Kostenis, E.; Högberg, T. *Bioog. Med. Chem. Lett.* **2009**, *20*, 1181.
26. (a) Mathiesen, J. M.; Christpoulos, A.; Ulven, T.; Royer, J. F.; Campillo, M.; Heinemann, A.; Pardo, L.; Kostenis, E. *Mol. Pharmacol.* **2006**, *69*, 1441; (b) Gervais, F. G.; Sawyerm, N.; Stocco, R.; Hamel, M.; Krawczyk, C.; Sillaots, S.; Denis, D.; Wong, E.; Wang, Z.; Gallant, M.; Abraham, W. M.; Slipetz, D.; Crackower, M. A.; O'Neill, G. P. *Mol. Pharmacol.* **2011**, *79*, 69; (c) Stock, N.; Volkots, D.; Stebbins, K.; Broadhead, A.; Stearns, B.; Roppe, J.; Parr, T.; Baccei, C.; Bain, G.; Chapman, C.; Correa, L.; Darlington, J.; King, C.; Lee, C.; Lorrain, D. S.; Prodanovich, P.; Santini, A.; Evans, J. F.; Hutchinson, J. H.; Prasit, P. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1036.