Bioorganic & Medicinal Chemistry Letters 21 (2011) 1036–1040

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Sodium [2′-[(cyclopropanecarbonyl-ethyl-amino)-methyl]-4′-(6-ethoxy-pyridin-3-yl)-6-methoxy-biphenyl-3-yl]-acetate (AM432): A potent, selective prostaglandin D_2 receptor antagonist

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ARTICLE INFO

Article history:
Received 11 November 2010
Revised 30 November 2010
Accepted 2 December 2010
Available online 9 December 2010

Keywords:
Prostaglandin D_2
CRTH2
Antagonist
DP_2 receptor

ABSTRACT

Compound **21** (AM432) was identified as a potent and selective antagonist of the DP_2 receptor (CRTH2). Modification of a bi-aryl core identified a series of tri-aryl antagonists of which compound **21** proved a viable clinical candidate. AM432 shows excellent potency in a human whole blood eosinophil shape change assay with prolonged incubation, a comparatively long off-rate from the DP_2 receptor, excellent pharmacokinetics in dog and in vivo activity in two mouse models of inflammatory disease after oral dosing.

Arachidonic acid (AA), once liberated from the phospholipid membrane wall via the action of phospholipase A2 ($sPLA_2$), serves as a precursor for a variety of pro-inflammatory enzymes in the human body, notably the cyclooxygenase enzymes (COX-1/COX-2) and 5-lipoxygenase (5-LO) enzyme. The action of COX-1/2 on AA generates the prostaglandin PGH_2 which in turn is further metabolized to a variety of signaling molecules, each with their own specific set of receptors (Fig. 1). Prostaglandin D_2 (PGD_2) is the major product released from activated mast cells and exerts its biological actions through selective binding to the G-protein coupled receptors DP_1 and DP_2 (CRTH2).[1] The DP_2 receptor is expressed on basophils, eosinophils and Th2 cells and activation of DP_2 by PGD_2 promotes the release of a variety of Th2 cytokines, eliciting a continued immune response.[2] The DP_2 receptor has been implicated in a number of inflammatory conditions such as asthma, allergic rhinitis and, more recently, chronic obstructive pulmonary disease (COPD). As such, DP_2 poses an interesting target for pharmacological intervention in these disorders.[3]

We have recently reported the discovery of several biphenyl acetic acid based DP_2 antagonists that demonstrated efficacy in a murine model of allergic rhinitis, compound **1**[4] and AM156[5] (**2**) (Fig. 2). As part of our efforts to develop potent, selective DP_2 antagonists suitable for clinical development, further SAR of the biphenyl acetic acid series was undertaken culminating in the discovery of AM432 (**21**) as a potential candidate for clinical development.

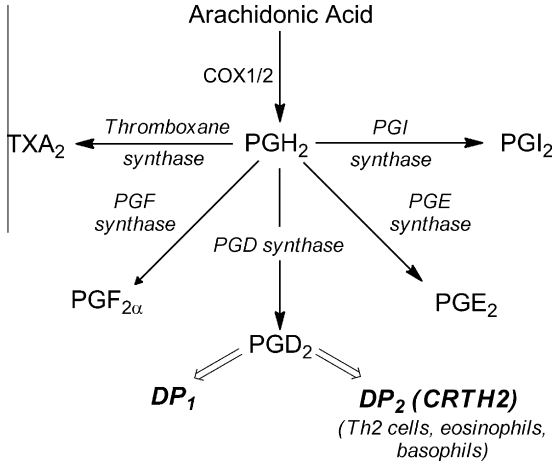

Figure 1. Arachidonic acid processing through the prostaglandin pathway.

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doi:10.1016/j.bmcl.2010.12.016

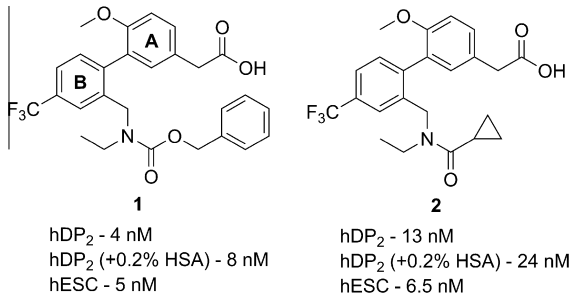
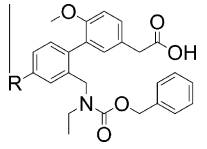


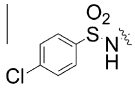
Figure 2. Biphenylacetic acid DP_2 inhibitors previously reported by our laboratories. hDP_2, human DP_2 membrane binding assay[6]; HSA, human serum albumin; hESC, human whole blood eosinophil shape change assay.[7]

Compound **1** was discovered through internal screening efforts and structural optimization, primarily focusing on ring 'A' and the moieties attached to the benzylic amine on ring 'B'.[4] We sought to optimize potency, off-target activities and pharmacokinetic parameters of this scaffold through modification of the 'B' ring and the substituents attached. All compounds were screened in a DP_2 membrane binding assay in the absence and presence of 0.2% human serum albumin (HSA) to evaluate intrinsic potency at the receptor and ascertain the degree of protein shift associated with these compounds.[6] Compounds of interest were further profiled in a human whole blood eosinophil shape change assay (hESC) whereby activation of the DP_2 receptor by PGD_2 results in eosinophil degranulation and changes in cell morphology which can be assayed using flow cell cytometry.[7]

Initial replacement of the tri-fluoromethyl moiety of compound **1** with a variety of groups (Table 1) indicated a wide tolerance for diverse functionalities. These compounds were prepared as previously disclosed using the appropriately functionalized 'B' ring.[4] All the compounds tested showed good intrinsic potency in the DP_2 membrane binding assay. However, in the presence of 0.2% HSA varying degrees of protein shift were observed, the more polar functions (amide **3**, sulfonamide **4** and carboxylic acid – **8**) showing the greatest loss of activity in the presence of serum, presumably due to greater protein binding for these compounds.

Table 1
In vitro hDP_2 membrane binding IC_{50}'s for compounds **3**–**8**[a]



Compound	R	hDP_2 binding (nM)	
		−HSA	+0.2% HSA
3		7	60
4		3	90
5	F	1	9
6		5	7
7		12	24
8		8	254

[a] hDP_2 binding values are the average of three experiments.

Compound **1** possesses a carbamate group, a potential structural alert with regards to cross-reactivity through serine proteases.[8] Furthermore, significant quantities of the secondary amine **9** (from cleavage of the carbamate) were also detected after oral dosing in rat, indicating the potential for complications in development of a carbamate bearing inhibitor (see Fig. 3).

Concurrent work leading to the discovery of compound **2** (AM156) identified the cyclopropyl amide as a replacement for the benzyl carbamate of **1** which was well tolerated and metabolically stable.[5] Further SAR therefore focused on modifications with the cyclopropyl amide in place of the benzyl carbamate moiety employing relatively non-polar aromatic substituents on the 'B' ring with the aim of minimizing the observed protein shift (Table 2).

Synthesis of these compounds is outlined in Scheme 1. Commercially available 5-bromo-2-iodobenzonitrile was reduced to the aldehyde **10** using standard reduction conditions (DIBAl-H) in good yield. Reductive amination using sodium cyanoborohydride in the presence of ethylamine followed by acylation with Boc anhydride afforded the *t*-butylcarbamate **11**. Cross coupling of the previously disclosed boronate **12**[4] with the more reactive aryl iodide under Suzuki[9] conditions furnished the biphenyl acetic ester **13** which could be then further transformed to the boronate **14**

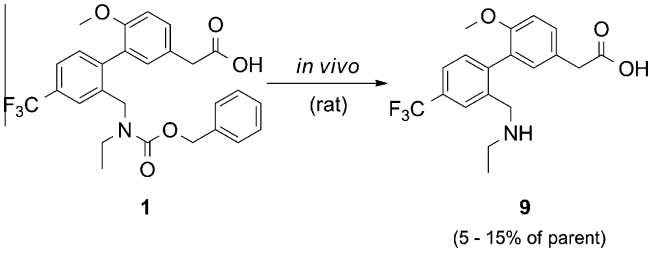
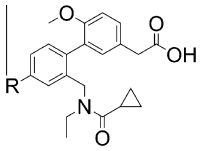
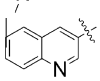
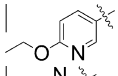
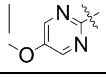


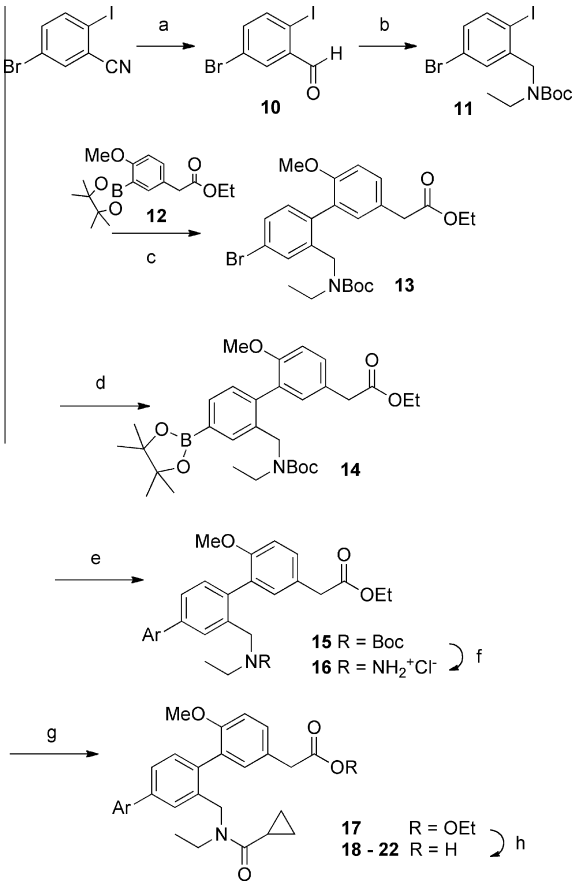
Figure 3. Benzyl carbamate cleavage of **1** to afford amine metabolite **9** after oral dosing in rat.

Table 2
In vitro hDP_2 membrane binding and human whole blood eosinophil shape change (hESC) IC_{50}'s for compounds **18**–**22**[a]



Compound	R	hDP_2 binding (nM)		hESC (nM)
		−HSA	+0.2% HSA	
18		27	37	4
19		7	52	12
20		9	60	23
21		6	31	5
22		27	107	18

[a] hDP_2 binding values are the average of three experiments; hESC IC_{50}'s are the average of two experiments from separate donors. For compound **21** hDP_2 binding values are the average of five experiments and the hESC IC_{50} is the average of eight experiments from separate donors.



Scheme 1. (a) DIBAl-H, THF, −78 °C to rt (88%); (b) (i) $Na(CN)BH_3$, AcOH, $EtNH_2$, MeOH, rt (81%); (ii) Boc_2O, CH_2Cl_2, rt (99%); (c) boronate **12**, $Pd(PPh_3)_4$ (cat.), K_2CO_3, DME, H_2O, 80 °C (66%); (d) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (cat.), KOAc, bis(pinacolato)diboron, dioxane, 85 °C (88%); (e) aryl iodide/bromide, $Pd(PPh_3)_4$ (cat.), K_2CO_3, DME, H_2O, 80 °C; (f) 4 N HCl in dioxane, CH_2Cl_2, rt (99%); (g) (i) cyclopropane carbonyl chloride, Hünigs base, CH_2Cl_2, rt (99%); (h) $LiOH{\cdot}H_2O$, THF, H_2O, rt (quant).

under standard conditions ($Pd(dppf)Cl_2$, dioxane, KOAc, bis(pinacolato)diboron). A variety of heterocycles could then be cross coupled with the boronate **14** under Suzuki conditions to yield the tri-aryl intermediates **15**. Removal of the Boc group under standard conditions (4 N HCl in dioxane) afforded the amine hydrochloride **16**. Preparation of the desired cyclopropyl amides through reaction of the secondary amine with cyclopropane carbonyl chloride and Hünigs base afforded the esters **17**. The desired acids (**18**–**22**) were obtained through hydrolysis of the ethyl ester under standard reaction conditions ($LiOH{\cdot}H_2O$, THF, H_2O).

These 'tri-aryl' antagonists proved intrinsically potent in the DP_2 binding assay, with the quinoline **19**, fluoro-pyridine **20** and ethoxy-pyridine **21** showing the greatest intrinsic binding affinity. All of these compounds showed a modest shift in the presence of +0.2% HSA but more notable was the hESC assay results for both the pyrazole **18** and ethoxy-pyridine **21**. The apparent increase in potency between the protein shifted binding assay and hESC assay is presumably due to the difference in the expression levels of DP_2 receptors in these assays. Pyrazole **18** had very poor exposure in rat PK (data not shown), however, the ethoxy-pyridine **21** upon dosing in rat had acceptable pharmacokinetic parameters (Fig. 4). Furthermore, po (2.5 mg/kg) and iv (2 mg/kg) dosing in dog showed extremely good PK properties (C_{max} = 30 μM, $t½$ = 6 h, Cl = 0.5 mL/min/kg, AUC = 87 h μg/mL).

Due to the structural similarity of the ligands in the arachidonic acid pathway, the potential for antagonist cross-reactivity between the various AA metabolite receptors needs to be evaluated. We therefore counter-screened **21** against a subset of these targets to ascertain its selectivity (Table 3). Gratifyingly, **21** (AM432) showed no cross-reactivity against the TP or IP receptors, COX-1 or COX-2 enzymes and minimal activity at the DP_1 receptor. Furthermore, there was no apparent agonist or antagonist activity against the three PPAR isoforms at concentrations up to 250 μM. Compound **21** also showed no significant inhibitory action against the human CYP450 isoforms tested at concentrations up to 50 μM and no time dependent inhibition of CYP3A4. Compound **21** was also devoid of any significant activity at the human ether-a-go-go receptor (hERG) in vitro at a concentration of 10 μM.[10]

It can be advantageous, when developing antagonists of G-protein coupled receptors, such as DP_2, to identify compounds with long off-rates from the receptor. Antagonists with longer off-rates may display greater clinical efficacy through prolonged receptor blockade and this potentially mitigates unforeseen poor pharmacokinetic parameters in human. Utilizing the DP_2 radioligand binding assay and a tritiated form of **21** with varying incubation times, we determined that **21** was fully bound to the DP_2 receptor after 30 min incubation at 37 °C and had an appreciable off-rate half-life of approximately 90 min compared to 11 min for PGD_2 (Fig. 5). Furthermore, utilizing the human whole blood eosinophil

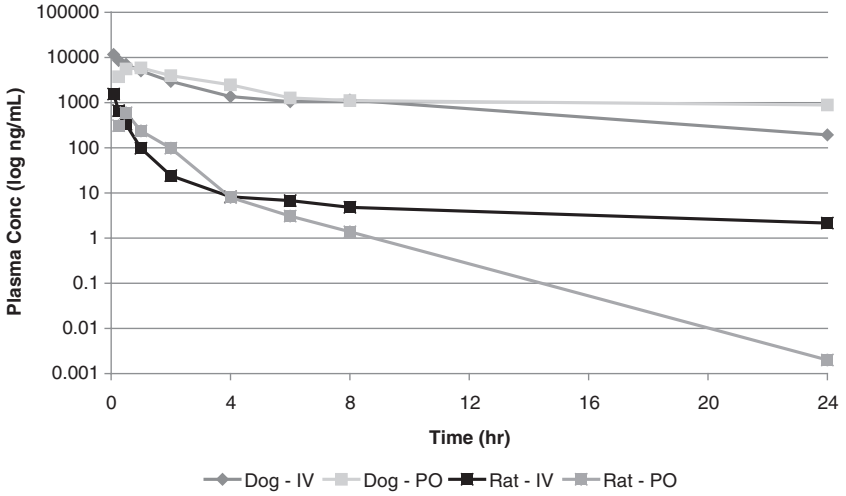


Figure 4. Rat and dog pharmacokinetics for **21**, dose normalized to 1 mg/kg.

Table 3
Counter-screen and CYP450 data for **21** (AM432)[a]

Assay target	IC_{50}
DP_1 receptor	26.7 μM
TP receptor	>100 μM
IP receptor	>100 μM
COX-1	>100 μM
COX-2	>100 μM
PPAR α, β and γ	>250 μM
CYP3A4	>50 μM
CYP2C9	>50 μM
CYP2C19	>50 μM
CYP2D6	>50 μM
CYP1A2	>50 μM
CYP3A4 TDI	0.002
hERG	11% @ 10 μM

[a] hDP_1 binding performed using ^{3}H-PGD_2 and human platelet membranes. hTP binding performed using human platelet membranes and ^{3}H-SQ-29548. hIP binding performed using hIP/293 membranes and ^{3}H-iloprost.

shape change assay (hESC) with a longer incubation time (4 h), **21** showed a 2.6-fold increase in potency with an IC_{50} of 1.7 nM ($n = 4$). This may be attributable to either the necessity of a longer incubation in human blood to fully equilibrate with the DP_2 receptor from other blood proteins or the compound's apparent long off-rate from the receptor or both.

Given the favorable in vitro profile of **21** and suitable pharmacokinetics, it was further profiled in several animal models of inflammatory disease. PGD_2 and its receptor DP_2 have been associated with both allergic rhinitis and asthma in human.[11] The former can be modeled in mice through sensitization of the animals with ovalbumin (OVA) and subsequent nasal challenge.[12] Therefore, female BALB/c mice were sensitized by intraperitoneal administration of 10 μg OVA complexed with Imject Alum on days 0 and 7. On days 21–25, mice were challenged intranasally with 200 μg OVA or 20 μL saline and were orally dosed with either 0.5% methylcellulose vehicle or compound **21** (10 mg/kg). The number of sneezes and nasal rubs were counted during an 8 min period immediately following OVA challenge on days 21, 23 and 25. OVA challenge increased the number of sneezes and nasal rubs and this was significantly reduced by **21** (Fig. 6). These data provide further support for the beneficial effects of DP2 antagonists in murine models of allergic rhinitis.[13]

We recently reported on the pharmacological blockade of the DP_2 receptor inhibiting cigarette smoke induced inflammation in the mouse lung.[14] In this model female BALB/c mice were exposed to the smoke of seven cigarettes on days 0–2. Mice were orally dosed with compounds **21** (10 or 50 mg/kg) or 0.5% methylcellulose vehicle on days −1 through 2. On day 3, mice were euthanized by isoflurane inhalation and bronchoalveolar lavage and differential cell counts were performed as described previously.[14] Cigarette smoke exposure produced an influx of neutrophils, lymphocytes and macrophages into the airways and this was significantly reduced by 50 mg/kg of **21** (Fig. 7). At 10 mg/kg, **21** significantly reduced neutrophils and caused a non-significant trend toward a reduction in lymphocytes and macrophages. These data are consistent with previously published reports describing efficacy of novel DP_2 antagonists in murine cigarette smoke exposure models.[15]

In conclusion, we have identified a potent and selective DP_2 antagonist, compound **21** (AM432), with a 4 h hESC potency of 1.7 nM, acceptable pharmacokinetics in two species and efficacy in both a murine model of allergic rhinitis and a cigarette smoke induced inflammatory model of COPD.

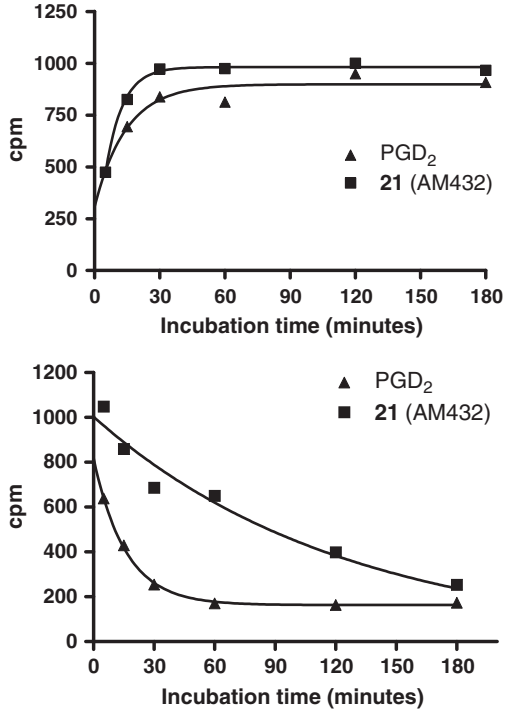


Figure 5. On- and off-rate analysis of PGD_2 and compound **21** (AM432) at 37 °C. Off- and on-rate determinations were performed using the hDP_2 membrane binding assay with varying incubation times at 37 °C. (Top) Both PGD_2 and **21** (AM432) are fully bound after 30 min. (Bottom) PGD_2 $t½ = 11$ min, **21** (AM432) $t½ = 86$ min.

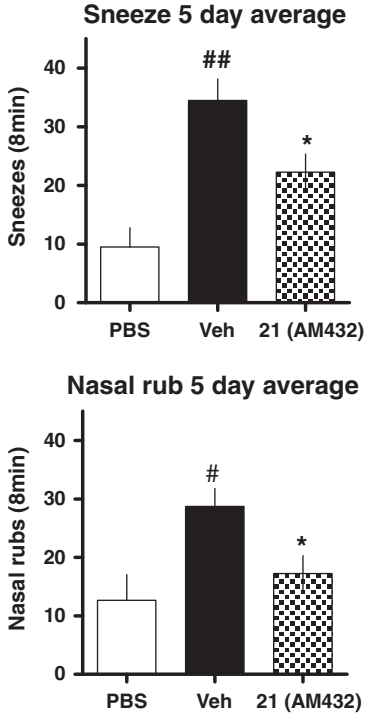


Figure 6. Mouse allergic rhinitis data for **21** (AM432) (10 mg/kg) versus PBS treated unsensitized mice and vehicle treated sensitized mice. Bars represent ±SEM of $n = 7$ mice per group. (Top) Number of sneezes, 5-day average; $^{\#\#}P$ <0.01 versus PBS, $^{*}P$ <0.05 versus vehicle; Tukey's post hoc following ANOVA: (bottom) nasal rub counts, 5-day average; $^{\#}P$ <0.05 versus PBS, $^{*}P$ <0.05 versus vehicle; Tukey's post hoc following ANOVA.

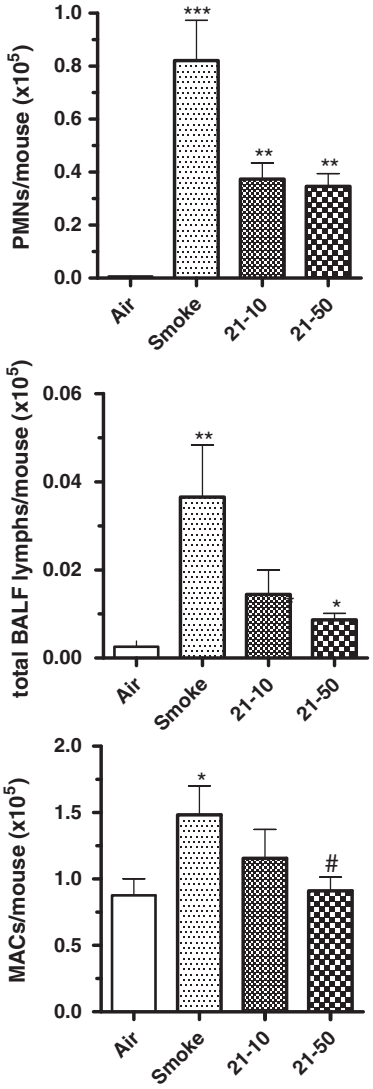


Figure 7. Broncoalveolar lavage (BAL) fluid cell count from mice after cigarette smoke exposure after 10 and 50 mg/kg po dosing of compound **21** (AM432). (Top) Neutrophil count; $^{**}P$ <0.01 versus smoke Tukey's post hoc following ANOVA, $^{***}P$ <0.001 versus air, Tukey's post hoc following ANOVA; (middle) lymphocyte count; $^{*}P$ <0.05 versus smoke, Tukey's post hoc following ANOVA, $^{**}P$ <0.01 versus air Tukey's post hoc following ANOVA; (bottom) macrophage count; $^{*}P$ <0.05 versus air, one-tailed *t* test, $^{\#}P$ <0.05 versus smoke, one-tailed *t* test.

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6. The DP_2 radioligand binding assay was performed on membranes from 293 cells stably expressing human DP_2. To measure binding, [^{3}H]-PGD_2 was incubated together with 293(hDP_2) membranes in the presence of increasing concentrations of compounds. Membranes were harvested and washed using a Brandel Harvester and the amount of [^{3}H]-PGD_2 that remained bound to the cells was measured on a TopCount. The concentration of compounds required to achieve a 50% inhibition of [^{3}H]-PGD_2 binding (the IC_{50}) was determined. The binding assay was carried out both in the absence and presence of 0.2% human serum albumin (HSA) to evaluate the protein shift associated with the compounds.
7. Human blood was drawn into EDTA tubes and used within 2 h of draw. One hundred microliter aliquots of fresh blood were incubated for 15 min at 37 °C plus or minus test compound in 50% DMSO/water. PGD_2 (50 nM final concentration) or vehicle was added from a 1 μM stock in PBS and incubations were continued for 5 min at 37 °C. The reactions were placed on ice and 250 μL of ice-cold 1:4 diluted Cytofix (BD Biosciences) in PBS immediately added. The reactions were transferred to FACS tubes and lysed with ammonium chloride lysing solution at room temperature for 15 min. Tubes were centrifuged and the cells washed once with 3 mL cold PBS before re-suspension in 200 μL of ice-cold 1:4 diluted Cytofix in PBS. Eosinophil shape change was analyzed on a FACS calibur by analyzing forward light scatter of the autofluorescent cells.
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