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Isosteric ramatroban analogs: selective and potent CRTH-2 antagonists

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Abstract—The chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH-2), also found on eosinophils and basophils, is a prostaglandin D_2 receptor involved in the recruitment of these cell types during an inflammatory response. In this report, we describe the synthesis and optimization of a ramatroban isostere that is a selective and potent antagonist of CRTH-2 which may be useful in the treatment of certain diseases.

Asthma is a chronic inflammatory disease of the airways. It is characterized by bronchial hyperresponsiveness, chronic pulmonary eosinophilia, and increased lung mucus production. The inflammation associated with asthma is characterized by an infiltration of many cell types, including but not limited to mast cells, eosinophils, T-lymphocytes, monocytes, and neutrophils.[1] These cells, along with their mediators, form a complex cascade of interactions, which ultimately result in inflammation of the airways.[2] The preferred treatment of asthma includes the use of anti-inflammatory agents (such as corticosteroids or leukotriene inhibitors) and bronchodilators (β2 adrenoceptor agonists). The increasing prevalence of asthma and the variable response to these agents indicate the need for novel treatments involving different mechanisms and approaches.

Leukotrienes, prostaglandins, and thromboxanes are a series of arachidonic acid metabolites known to exert a variety of physiological functions that are mediated through their respective receptors.[3–5] Prostaglandin D_2 (PGD_2) is one of the major inflammatory molecules released by activated mast cells and is greatly increased in the bronchiolar lavage fluid of asthmatics.[6] PGD_2 signals through two G-protein coupled receptors (GPCRs) from distinct GPCR subfamilies: PGD_2 receptor (DP), a prostanoid receptor[7] and chemoattractant receptor-homologous molecule expressed on T_H2 cells[8,9] (CRTH-2), a receptor that is most closely related to receptors for 'classical' chemoattractants, such as N-formyl peptide (FMLP), C3a, and C5a.[10] CRTH-2 is expressed in several tissues but has been most carefully studied on T_H2 cells, eosinophils, and basophils where it appears to mediate the chemoattractant effect of PGD_2 on each of these cell types.[8] In addition, PGD_2 can induce eosinophil degranulation via CRTH-2 stimulation.[11] Taken together, these findings suggest an involvement of PGD_2 and CRTH-2 in asthma and perhaps other inflammatory diseases.

Ramatroban (BAY u3405, **1**) has been marketed in Japan for allergic rhinitis as a selective thromboxane-type prostanoid receptor (TP) antagonist (Fig. 1). However, recently ramatroban was also identified as a CRTH-2 antagonist.[12] As an entry into SAR studies based on diverse leads generated from an internal HTS campaign, this finding prompted us to explore the hCRTH-2 pharmacophore by simply reversing the ramatroban backbone (Fig. 1, **2**).

Keywords: CRTH-2; Prostaglandin D_2; Ramatroban; Asthma.
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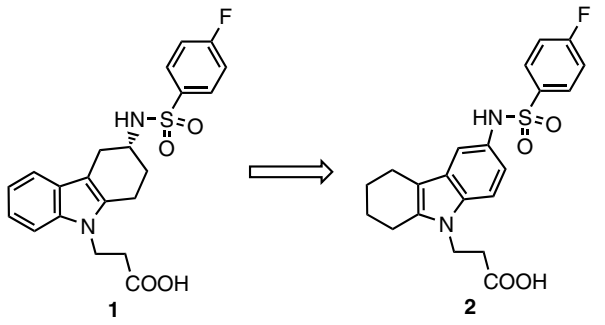


Figure 1. Isostere of ramatroban—'reverse scaffold'.

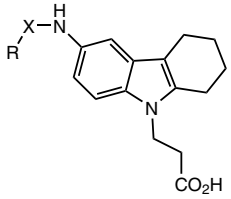
The synthesis of compound **2** began with tetrahydrocarbazole **3** (Scheme 1).[13] Michael addition to *t*-butyl acrylate and reduction gave aniline **4**. Sulfonylation and deprotection gave the requisite sulfonamides **2** and **5a**–**q** (Table 1). Treatment of aniline **4** with phenylisocyanate, benzoyl chloride, and benzaldehyde/$NaCNBH_3$ gave compounds **6**, **7**, and **8**, respectively.

Comparison of the activities of compounds **2**, **5a**–**q**, and **6**–**9** in a hCRTH-2 binding assay, using membranes from HEK293 cells stably expressing recombinant hCRTH-2, illustrates the necessity for aryl sulfonamides that are substituted in the *para*-position (Table 1). Removal of the sulfonamide resulted in loss of activity as shown for urea, **6**, amide, **7**, and amine, **9**, while benzyl amine, **8** showed a significant drop in activity. Interestingly, none of the substitutions attempted improved upon the concept analog, compound **2**, which incorporated a 4-fluorophenyl sulfonamide moiety.

Having identified the optimal sulfonamide substitution, we next investigated various carboxylic acid modifications. Propionic acid derivatives were generally synthesized via a Michael addition to tetrahydrocarbazole **3** (Scheme 2). The use of stoichiometric base gave incomplete conversion to the Michael product. However, use of a catalytic amount (10 mol%) of NaHMDS was found to be effective at promoting this transformation. In the case of ethylpropiolate, TBAF was found to be a far more effective base than NaHMDS.

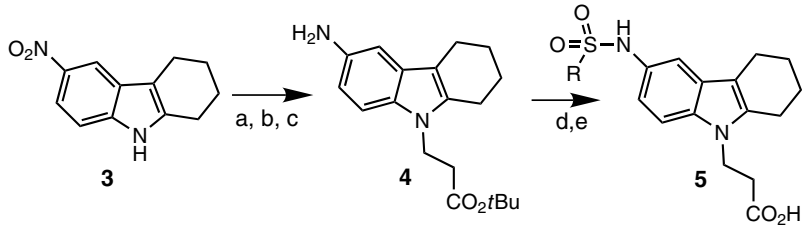
Lengthening and shortening of the carboxylic acid spacer to give **18**–**22** was accomplished through alkylation with the appropriate bromoalkyl ester (Scheme 3). The carboxylic acid was also attached through an aromatic linker via a recently reported modification of the Ullmann coupling[14] to give **23**–**25**, as shown in Scheme 4. The bromobenzoic ester was sufficiently reactive for the synthesis of **23** and **24**; however, in the case of the *ortho* derivative, **25**, the iodobenzoic ester was necessary.

Table 1. Structures and activities of sulfonamide analogs of **2** at the hCRTH-2 receptor

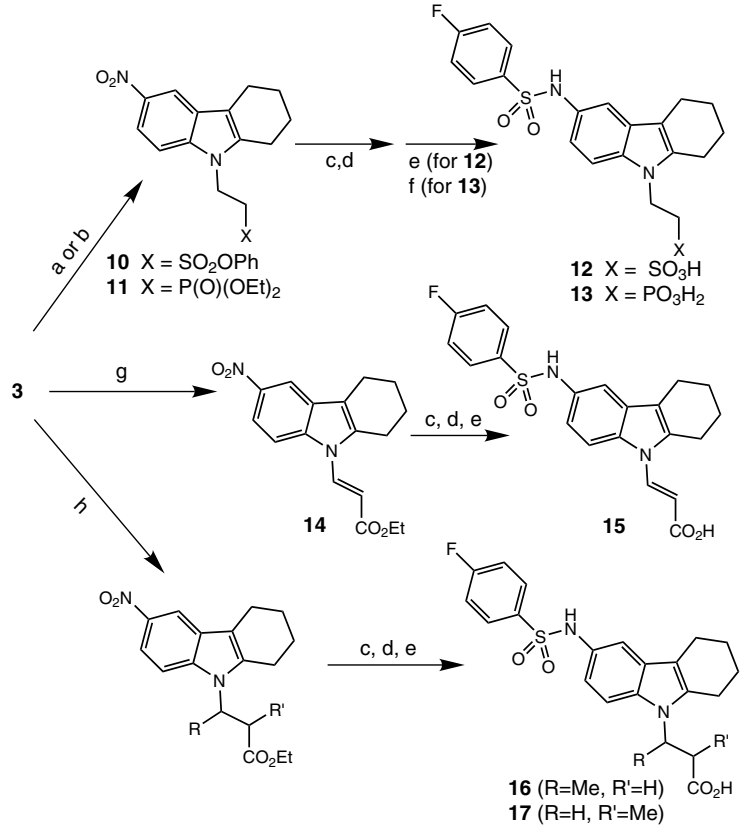


Compd	X	R	K_i (μM)
2	SO_2	4-F-phenyl	0.25
5a	SO_2	3-F-phenyl	1.3
5b	SO_2	2-F-phenyl	0.97
5c	SO_2	Phenyl	1.2
5d	SO_2	4-Me-phenyl	3.2
5e	SO_2	3-Me-phenyl	4.2
5f	SO_2	2-Me-phenyl	>50
5g	SO_2	2-Thienyl	7.6
5h	SO_2	4-OH-phenyl	7.4
5i	SO_2	2-Naphthyl	7.4
5j	SO_2	4-OPh-phenyl	7.5
5k	SO_2	4-OMe-phenyl	2.1
5l	SO_2	4-Cl-phenyl	3
5m	SO_2	4-Ph-phenyl	3.3
5n	SO_2	4-F, 3-Cl-phenyl	1.7
5o	SO_2	2,4-Di-F-phenyl	3.6
5p	SO_2	3,4-Di-F-phenyl	4.2
5q	SO_2	*n*-Propyl	>50
6	C(O)NH	Phenyl	>50
7	C(O)	Phenyl	>50
8	CH_2	Phenyl	17
9	—	H	>50
1, Ramatroban		N/A	0.29

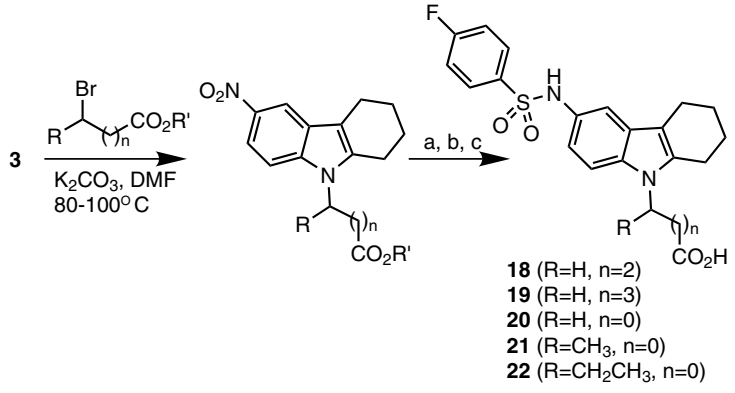
The ability of the carboxylic acid modified derivatives to inhibit the binding of PGD_2 to CRTH-2 is summarized in Table 2. Substituted propionic acids **16** and **17** resulted in complete loss of activity while acrylic acid analog (**15**) gave an ~75-fold decrease in potency indicating the narrow structural and conformational requirement for binding of the acid moiety. Attempts to change acid from carboxylic to sulfonic (**12**) or phosphonic (**13**) resulted in loss of potency. While the benzoic acid analogs (**23**–**25**) were also found to be less potent than the concept analog (**2**), the *para*-benzoic acid moiety was better tolerated. Next, we explored the effects of lengthening



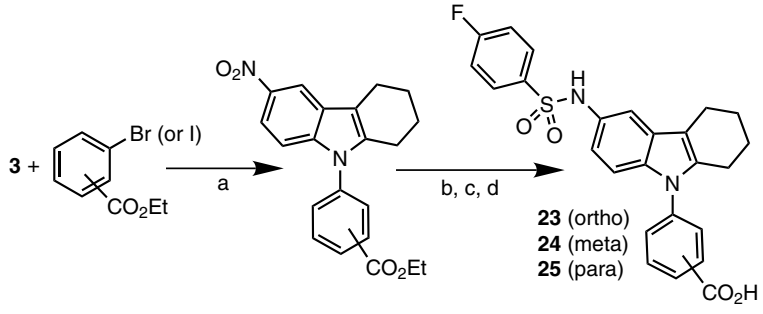
Scheme 1. Reagents and conditions: (a) NaH, (b) *t*-butyl acrylate, (c) H_2/Pd, (d) R–SO_2Cl, Et_3N, (e) TFA.



Scheme 2. Reagents and conditions: (a) Phenyl vinylsulfonate, NaHMDS (cat), DMF; (b) diethyl vinylphosphonate, NaHMDS (cat), DMF; (c) H_2/Pd; (d) 4-F–Ph–SO_2Cl, Et_3N; (e) NaOH, EtOH, heat; (f) TMS–Br, DCM; (g) ethyl propiolate, TBAF, THF; (h) RCH=CHR′CO_2Et, NaHMDS (cat), DMF, 60–70 °C.

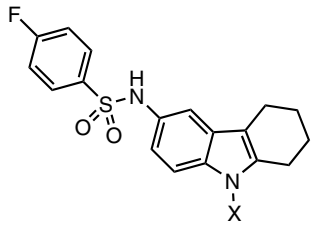


Scheme 3. Reagents and conditions: (a) H_2/Pd; (b) 4-F–Ph–SO_2Cl, Et_3N; (c) NaOH, EtOH, heat or TFA.



Scheme 4. Reagents and conditions: (a) CuI, NH(Me)CH_2CH_2NHMe, K_2CO_3, toluene, reflux; (b) H_2/Pd; (c) 4-F–Ph–SO_2Cl, Et_3N; (d) NaOH, EtOH, heat.

Table 2. Inhibition constants of various carboxylic acid modifications at the hCRTH-2 receptor



Compd	X	K_i (μM)
12	$CH_2CH_2SO_3H$	2.4
13	$CH_2CH_2PO_3H_2$	>50
15	$CH{=}CH{-}CO_2H$	19
16	$CH(Me)CH_2CO_2H$	>50
17	$CH_2CH(Me)CO_2H$	>50
18	$(CH_2)_3CO_2H$	8.3
19	$(CH_2)_4CO_2H$	4.6
20	CH_2CO_2H	0.030
21	$CH(Me)CO_2H$	4.4
22	$CH(Et)CO_2H$	18
23	*ortho*-Ph–CO_2H	33
24	*meta*-Ph–CO_2H	27
25	*para*-Ph–CO_2H	6.4
2	$(CH_2)_2CO_2H$	0.25
1, Ramatroban	N/A	0.29

and shortening the methylene-spacer between the acid moiety and the indole. Increasing the chain length (compounds **18** and **19**) resulted in loss of potency while decreasing the chain length resulted in an ~10-fold improvement in potency (compound **20**). Finally, substituted acetic acids were explored. As in the case with substituted propionic acids, substituted acetic acids also showed a reduced potency, the magnitude of which was proportional to the size of the substituent (compounds **21** and **22**).

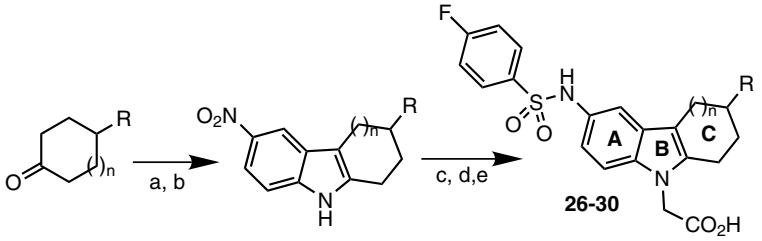
Next, we evaluated 'C-ring' modified analogs incorporating the acetic acid moiety determined previously to enhance potency. Various derivatives of tetrahydrocarbazole **3** were readily prepared via a Fischer indole synthesis with an appropriate cyclic ketone (Table 3). Standard transformations resulted in analogs **26**–**30**. The size of the 'C-ring' was found to have a profound effect on activity: the order of potency being 7-membered (**30**) ⩾ 6-membered (**20**) ≫ 5-membered (**29**). Lastly, the substitution effect on the 6-membered 'C-ring' series resulted in the finding that a methyl group in the 3-position was optimal as typified by compound **26**. A select group of the isosteric ramatroban analogs were tested for their ability to inhibit PGD_2-mediated receptor activation in a fluorescence assay that measures changes in intracellular calcium (Table 3). The IC_{50} values determined in this functional assay were very similar to the inhibition constants determined in the CRTH-2 binding assay.

Ramatroban (**1**) was initially developed as a thromboxane A_2 antagonist that was subsequently determined to also be an inhibitor of CRTH-2. Since the compounds reported herein were derived from ramatroban, select analogs were counter-screened against the human thromboxane receptor (hTP) using membranes from HEK293 cells stably expressing recombinant hTP to determine if any of them had dual activity. Interestingly, ramatroban (**1**) is ~16-fold selective for TP over CRTH-2 while the concept compound (**2**) is ~6-fold selective for CRTH-2 over TP. This effect is amplified upon examination of our lead compounds **20**, **26**, **27**, and **30**, which are all very selective, exhibiting >400-fold preference for CRTH-2 over TP (Table 4).

Table 4. Counter screening against hTP receptor

Compd	hTP % inhibition @ 50 μM	K_i (μM) hTP	K_i (μM) hCRTH-2
2	92	1.5	0.25
20	21	>20	0.030
26	30	>20	0.013
27	66	>20	0.050
30	28	>20	0.020
1, Ramatroban	98	0.018	0.29

Table 3. Synthesis and activity at the hCRTH-2 receptor of saturated ring variants



Compd	R	n	K_i (μM)	IC_{50} (functional) (μM)
26	CH_3	1	0.013	0.0097
27	Phenyl	1	0.050	0.022
28	*t*-Butyl	1	0.15	0.12
29	H	0	0.20	0.13
30	H	2	0.020	0.022
1, Ramatroban		N/A	0.29	0.34

Reagents and conditions: (a) 4-Nitrophenylhydrazine, EtOH; (b) HOAc / HCl (aq), 120 °C; (c) $BrCH_2CO_2t$-Bu, K_2CO_3, DMF; (d) H_2/Pd; (e) 4-F–Ph–SO_2Cl, Et_3N; (f) TFA.

In conclusion, we have designed and synthesized a series of potent substituted indole acetic acids as CRTH-2 antagonists based on ramatroban as an initial foray into defining the CRTH-2 antagonist pharmacophore.[15] While ramatroban is moderately selective for TP over CRTH-2, we have shown the compounds described herein to be more than 400-fold selective for CRTH-2 over TP. Further studies have been undertaken to determine the potential clinical usefulness of these compounds in allergic diseases.

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