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Exploration of SAR features by modifications of thiazoleacetic acids as CRTH2 antagonists

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ABSTRACT

The SAR features have been further explored for (2-benzhydryl-4-phenyl-thiazol-5-yl)acetic acids as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) antagonists. The introduction of a nitrogen or a methyl substituent in the benzhydrylic position offer two alternative drugable scaffolds attractive for unsymmetrically substituted derivatives. An imidazole analogue lacks activity due to formation of a favored coplanar intramolecular hydrogen bond. The pyrimidine derivative **18** represents a potent and selective compound that will be subject to continued investigations.

Prostaglandin D2 (PGD_2) and one of its receptors CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) have been implicated in the pathogenesis of various inflammatory conditions.[1] The CRTH2 receptor is expressed on eosinophils, basophils and Th2 cells, mediating their chemotaxis in response to PGD_2 and several other arachidonate metabolites.[1–3] The Th2 cells act as central orchestrators of allergic asthma, driving IgE response and eosinophilia. Hence, CRTH2 induces the production of proinflammatory cytokines in Th2 cells,[1,4] enhances the release of histamine from basophils[1,5] mediates the respiratory burst and degranulation of eosinophils,[1,6] and inhibits apoptosis of human Th2 cells induced by cytokine deprivation.[7] Accordingly, CRTH2 antagonists are being under development for the treatment of asthma, COPD and allergic disease.[8–10]

We have earlier described the hit-to-lead process of hits obtained from screening an in silico derived library that successfully delivered some novel chemotypes of CRTH2 antagonists (Fig. 1).[11–14] Initially a pyrazole series **1** was developed[12] and in more recent publications[13,14] we described how thiazole hits were optimized into compounds with western lipophilic moieties (**2**) and with eastern benzydrylic motifs (**3**,**4**). In this Letter we describe the efforts of making compounds of the latter type with lower lipophilicity and further exploration of the structure–activity relationships.

The introduction of different substituents in the phenyl rings in the benzyhydryl series leads to a chiral centre. We envisaged one

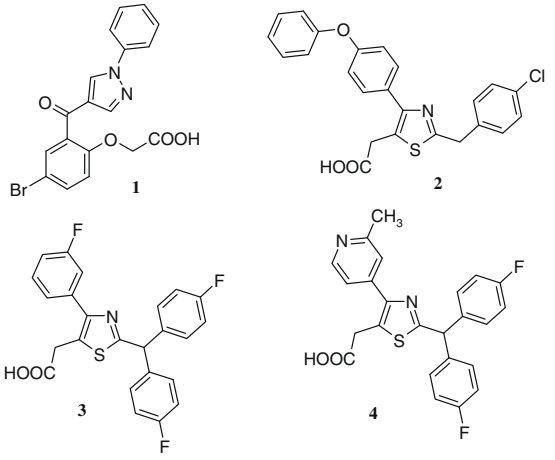

Figure 1. Representative chemotypes identified after hit-to-lead process of in silico screening hits. CRTH2 receptor binding IC_{50}: **1** (4 nM),[12] **2** (3.7 nM),[13] **3** (1.4 nM)[14] and **4** (3.1 nM).[14]

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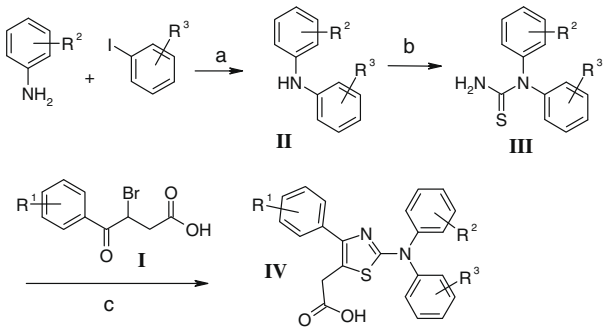
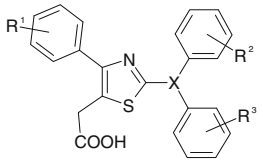


Scheme 1. Reagents and conditions: (a) $(DPPF)PdCl_2.CH_2Cl_2$, DPPF, sodium *t*-butoxide, THF, 100 °C or $Pd(OAc)_2$, X-Phos, Cs_2CO_3, toluene, *t*-BuOH, microwave reactor, 150 °C, 5 min; 80–95%; (b) (i) FmocNCS, CH_2Cl_2; (ii) piperidine, MeOH; 40–60%; (c) MeCN or DMF, microwave reactor 100–120 °C; 10–20%.

way to circumvent chirality, and simultaneously add another acceptor site, was replacement of the benzydryl carbon with nitrogen. Diarylamines **II**, required for the synthesis of the target molecules **IV**, were prepared by palladium assisted cross-coupling. Subsequent conversion of **II** into the corresponding thioureas **III**, followed by microwave assisted condensation with 3-bromo-4-oxo-butyric acid derivatives **I** led to the desired 2-aminothiazoles **IV** (Scheme 1).

The compounds were characterized with respect to binding affinity and functional antagonistic activity using a bioluminescence resonance energy transfer (BRET) assay in HEK385-7 cells (Table 1).[12, 13, 15] Superimposition of carbon and nitrogen analogues reveals a slightly different orientation of the eastern phenyl rings (Fig. 2A). However, the compounds **5**, **6** and **7**, lacking eastern substituents, display activities that are comparable to the carbon analogues described before having binding affinities of 14, 9 (compound **14**) and 3 nM, respectively.[14] Introduction of *para* fluoro or methoxy substituents in the eastern rings (**8** and **9**) lowers the

Table 1
Binding affinity and functional antagonism on hCRTH2 of 4-(*p*-chlorophenyl)thiazoleacetic acids



No.	X	R^1	R^2	R^3	IC_{50} Bind [a] (nM)	IC_{50} BRET [b] (μM)
5	N	4-Cl	H	H	18	0.072
6	N	4-F	H	H	15	0.098
7	N	3-F	H	H	4.4	0.079
8	N	4-F	4-F	4-F	23	0.24
9	N	3-F	4-OMe	4-OMe	15	0.26
10	N	4-F	$4\text{-}MeSO_2$	H	190	2.0[d]
11	N	4-F	4-CN	H	84	0.46[c]
12	N	4-F	3-py[e]	H	43	1.5[d]
13	CMe	4-F	H	H	7.9	0.085
14[f]	CH	4-F	H	H	8.7	0.077

[a] $[^3H]PGD_2$ equilibrium competition binding in HEK385-7 cells.
[b] Antagonistic activity as inhibition of β-arrestin translocation measured in a bioluminescence resonance energy transfer (BRET) assay in HEK385-7 cells. All compounds displayed efficacy above 80% unless noted. All values are single or mean of double determinations.
[c] Compound having 55–65% antagonistic efficacy.
[d] Compound having about 30% antagonistic efficacy.
[e] One phenyl ring replaced with 3-pyridyl system.
[f] Previously described.[14]

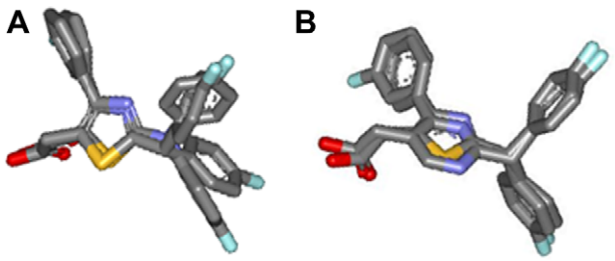


Figure 2. Superimpositions of A: thiazole **3** (X = CH) and the corresponding nitrogen analogue (X = N) and B: thiazole **3** and the corresponding pyrimidine **18**.

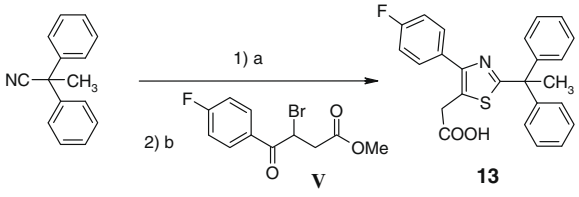
functional activity somewhat. Such reduction in activity caused by the introduction of eastern *para* substituents was not seen in the benzhydryl series,[14] which might be attributed to the different positioning of the *para* substituents imposed by the different hybridization stage for carbon and nitrogen as reflected in the superimpositions (cf. Fig. 2A). The more polar monosubstituted derivatives, that is, methylsulfone **10**, nitrile **11** and 3-pyridyl **12**, display a further reduction in activity.

We also explored the introduction of a methyl group in the benzhydrylic position to see if activity was retained to potentially be able to block an epimerisation of chiral compounds. We assessed this possibility by making the simple compound **13** by conversion of the commercial 2,2-diphenylpropionitrile to thioamide and condensation with the *p*-fluorophenyl butyric acid derivative **V** as shown in Scheme 2. This compound possessed a binding affinity of 8 nM and a full functional antagonistic activity comparable to the unmethylated compound **14**.[14] Thus, the methyl group can be assumed to be without interaction with the receptor binding site.

In the pyrazole series exemplified by compound **1** the carboxyl function is connected with a two atom linker to the phenyl ring so we wanted to explore this possibility also in the thiazole series by making **15** and **16** (Table 2). The thiazolepropionate **15** was made by reaction of 4-bromo-5-(4-fluorophenyl)-5-oxopentanoic acid and diphenylthioacetamide analogous to the synthesis of the acetate (cf. Scheme 2).

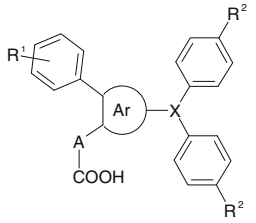
The oxymethylene-linked compound **16** was made in a modest yield according to Scheme 3 by alkylation with bromoacetate of the hydroxythiazole **VI** followed by hydrolysis. The intermediate **VI** was conveniently obtained from the thioamide **VII**, which was made by treatment or the corresponding amide with Lawesson's reagent. Both compounds **15** and **16** having the acidic side chain extended with one atom showed a considerable drop in potency and functional activity compared to **3**.

To increase the polarity in the core nucleus the thiazole was replaced with an imidazole ring according to the synthesis in Scheme 4. The nitrile was converted to 2,2-diphenylacetamidine that was reacted with the *p*-fluorophenyl butyrate ester **V** to produce the imidazoleacetic acid ester that was hydrolyzed to give **17** in a poor yield. The imidazole analogue is virtually devoid of CRTH2 affinity and lacks functional activity. This observation is likely to be ex-



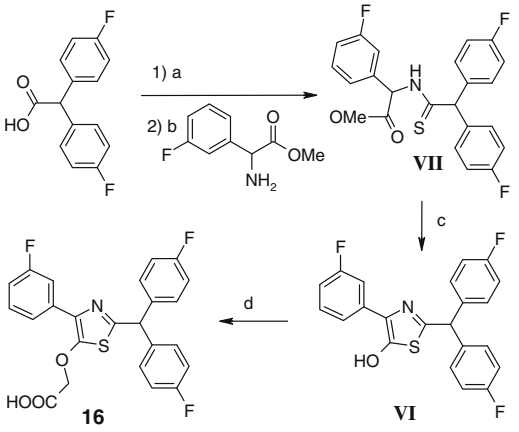
Scheme 2. Reagents and conditions: (a) H_2S in pyridine/Et_3N, rt, 17%; (b) (i) DMF, microwave reactor, 100 °C, 10 min; (ii) aq LiOH, THF; 63%.

Table 2
Binding affinity and functional antagonism on hCRTH2 of modified benzhydryl substituted heterocyclic acids



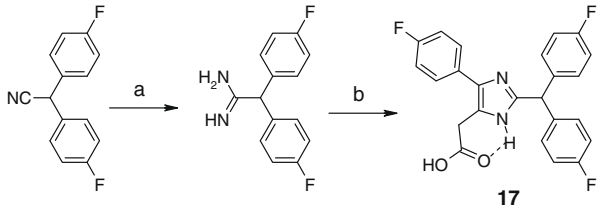
No.	Ar	X	A	R^1	R^2	IC_{50} Bind[a] (nM)	IC_{50} BRET[b] (μM)
3[c]		CH	CH_2	3-F	F	1.4	0.018
15		CH	CH_2CH_2	3-F	F	75	1.0
16		CH	OCH_2	3-F	F	83	0.20
17		CH	CH_2	4-F	F	9140	>100
18		CH	CH_2	3-F	F	1.9	0.012
19		CH	CH_2CH_2	3-F	F	2070	>100
20		N	CH_2	3-F	F	1260	>100
21		N	CH_2	3-F	OMe	2790	>100
22[c]		CH	CH_2	4-F	OMe	3.9	0.031

[a] and [b] as in Table 1.
[c] Previously described.[14]



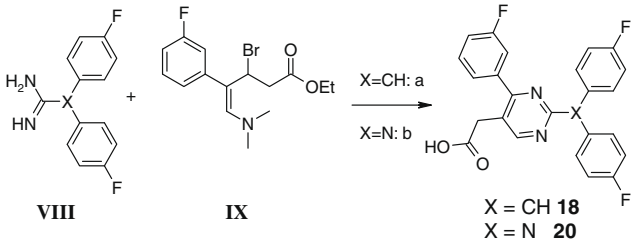
Scheme 3. Reagents and conditions: (a) isobutyl chloroformate, *N*-methylmorpholine; (b) (i) microwave reactor, 100 °C, 30 min; (ii) Lawesson's reagent, toluene, 80 °C; (c) (i) aq LiOH, THF; (ii) TFA, 80 °C; 13% overall yield from bis-(4-fluorophenyl)acetic acid; (d) (i) ethyl bromoacetate, K_2CO_3, acetone; (ii) aq LiOH, THF; 17%.

plained by the formation of a favored coplanar intramolecular hydrogen bond, indicated in Scheme 4, making the receptor bound acetic acid conformation energetically inaccessible (vide infra).



Scheme 4. Reagents and conditions: (a) NH_4Cl, Me_3Al, toluene, 80 °C; 78%; (b) (i) bromo ester **V**, $KHCO_3$, THF, 80 °C; (ii) aq LiOH, THF, 5%.

Thus, our interest of making a somewhat more polar core moiety turned to a central pyrimidine ring. Compared to the nitrogen analogues discussed above (Fig. 2A), superimpositions of thiazole **3** and pyrimidine **18** showed almost identical spatial arrangements including the eastern *para* substituents (Fig. 2B). This indicates that SAR generated for the thiazole series is to a large extent transferrable to the pyrimidine series. The pyrimidines were synthesized by reacting an amidine **VIII** with the bromo enamine **IX** as exemplified in Scheme 5 for **18** and **20**. The *N*,*N*-diphenylguanidines (**VIII**, X = N) required for synthesis of the 2-aminopyrimidines **20** and **21** were obtained by treatment of anilines **II** with cyanamide and trimethylsilyl chloride in acetonitrile during microwave conditions at 180 °C.



Scheme 5. Reagents and conditions: (a) (i) sodium *t*-pentoxide, *t*BuOH, microwave reactor, 135 °C, 1 h; (ii) aq LiOH, THF; 20% or (i) sodium *t*-butoxide, EtOH, 50 °C, overnight; (ii) aq LiOH, THF; 17%; (b) (i) EtOH, reflux, overnight; (ii) aq LiOH, THF; 19%.

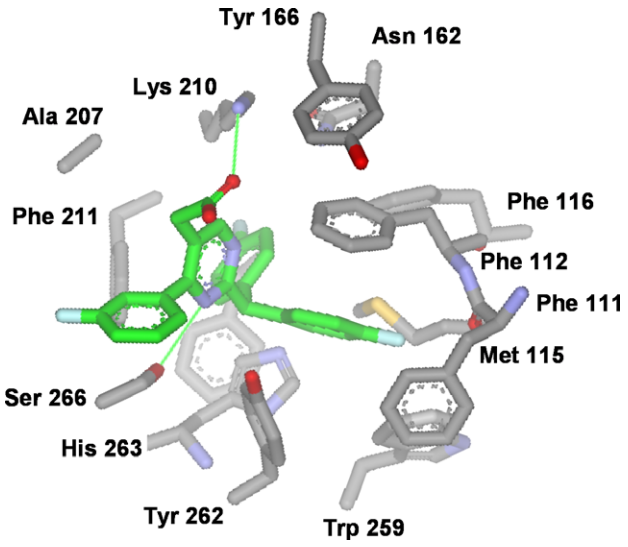


Figure 3. Docking of pyrimidine derivative **18** in the hCRTH2 receptor.

The pyrimidine **18** is a very potent CRTH2 receptor antagonist (Table 2) also showing appreciable activity in other species suitable for PD studies, that is rat 7.5 nM, mouse 3.4 nM, and 29 nM guinea pig, in contrast to the other PGD_2 receptor DP (human 8.5 μM, guinea pig >100 μM, mouse 5.9 μM), and thromboxane A_2 (31 μM). Molecular modelling of **18** in the CRTH2 receptor binding site shows a tight fit in the hydrophobic subpocket containing several aromatic residues, a hydrogen bond to one of the pyrimidine nitrogens with ^{266}Ser and a crucial interaction with ^{210}Lys to the carboxyl function that is out of the pyrimidine plane (Fig. 3). This latter side chain conformation is not achieved for the poorly binding imidazole **17** having the carboxyl group oriented in the imidazole ring plane by an intramolecular hydrogen bond. The chain elongated compound **19** shows a pronounced drop in potency also in line with poor adherence to proper interaction with the lysine residue. In comparison to the thiazole series, the eastern *para* substituted nitrogen analogues **20** and **21** display a much more drastic reduction in activity, that is, being 100-fold less active than the benzhydryl derivatives, which could be due to conformational or electronic reasons that we do not fully understand.

In conclusion, we have explored the SAR features further for the (2-benzhydryl-4-phenyl-thiazol-5-yl)acetic acids series and extended our understanding regarding the carboxyl side chain conformation and other interaction requirements for binding to the CRTH2 receptor. The introduction of a nitrogen or a methyl substituent in the benzhydrylic position offer two alternative drugable scaffolds that could be attractive for unsymmetrically substituted derivatives. The pyrimidine derivative **18** represents a potent and selective compound that will be subject to continued investigations.

Acknowledgements

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