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Novel selective thiazoleacetic acids as CRTH2 antagonists developed from in silico derived hits. Part 1

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ABSTRACT

Structure–activity relationships of three related series of 4-phenylthiazol-5-ylacetic acids, derived from two hits emanating from a focused library obtained by in silico screening, have been explored as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) antagonists. Several compounds with double digit nanomolar binding affinity and full antagonistic efficacy for human CRTH2 receptor were obtained in all subclasses. The most potent compound was [2-(4-chloro-benzyl)-4-(4-phenoxy-phenyl)-thiazol-5-yl]acetic acid having an binding affinity of 3.7 nM and functional antagonistic effect of 66 nM in a BRET and 12 nM in a cAMP assay with no functional activity for the other PGD2 DP receptor (27 μM in cAMP).

Prostaglandin D_2 (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-type T lymphocytes, and mediates their chemotaxis in response to PGD_2.[1,2] In addition to PGD_2, a number of other arachidonate metabolites activate the CRTH2 receptor, including 13,14-dihydro-15-keto-PGD_2, PGJ_2, $\Delta^{12}PGJ_2$, 15-deoxy-PGJ_2 and 11-dehydro-TXB_2.[1,3] The Th2 cells are known 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] and mediates the respiratory burst and degranulation of eosinophils.[1,6] Recently, CRTH2 has also been implicated in mediating an inhibitory effect of PGD_2 on the apoptosis of human Th2 cells induced by cytokine deprivation.[7] Accordingly, CRTH2 antagonists are being developed for the treatment of asthma and allergic disease.[8,9] Some compounds have also advanced to clinical trials directed towards asthma, allergic rhinoconjunctivitis and chronic obstructive pulmonary disease.[10]

We have earlier designed small target-specific libraries using a physicogenetic approach to identify binding pocket-related 7TM receptors associated with ligand information.[11] As one example we have described the generation of a pharmacophore for the CRTH2 receptor that was derived with input from the binding pocket-related AT1 and AT2 receptors and associated ligands.[11,12] The pharmacophore, containing a negatively charged site and three hydrophobic regions, was used to extract about 600 compounds from approximately 1 million publically available compounds from vendors. In vitro binding of this library showed 10% of the compounds to have IC_{50} values less than 10 μM. Some identified representative compounds (**1–2**) are shown in Figure 1.[11,12] The phenoxyacetic acid derivative **2** provided input to the design of potent and selective agents of the pyrazole-4-carbonyl type **3** displaying oral activity in allergic in vivo models.[12]

In this Letter we describe another set of compounds, that is, the thiazoleacetic acids **4a** and **4b** shown in Figure 2 derived from the same in silico screening campaign, and the optimization towards more potent and selective compounds. Initially we expanded the mining of public compound sources for additional compounds with related structures to enrich the SAR. Using simple search

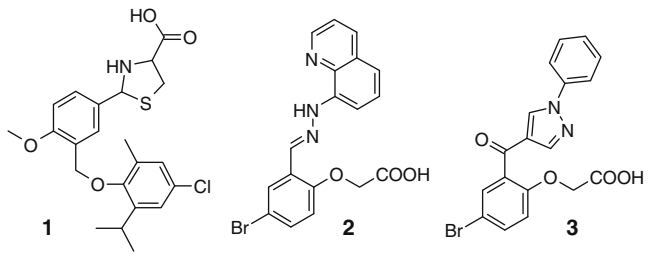

Figure 1. Some representative chemotypes identified after in silico screening, that is, **1** (IC_{50}1.9 μM)[11] and **2** (IC_{50} 0.044 μM).[12] The pyrazole **3** was developed as an orally active potent antagonist with IC_{50} 4 nM.[12]

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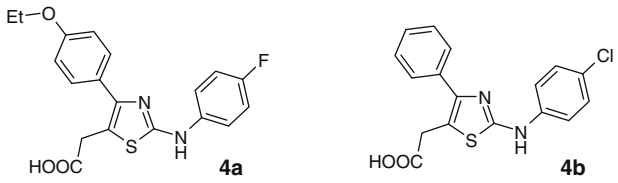


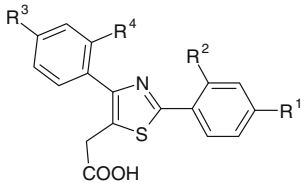
Figure 2. Thiazoleacetic acid **4a** (IC_{50} 3.0 μM) and **4b** (IC_{50} 0.35 μM) identified in the initial in silico screening.

queries based on the hits **5a** and **5b** and the previous pharmacophore led us to a set of thiazoleacetic acids **5a–5g** with both phenyl rings directly attached to the thiazole core (Table 1). The available compounds did not display a great variation in potency, but one may conclude that substituents are allowed in *para* (R^1/R^3) as well as *ortho* (R^2/R^4) positions of both phenyl rings. Potency benefits from having a larger halogen substituent in either *para* position R^1 or R^3 of the two phenyl rings.

We decided to further explore this chemotype and synthesize a more diverse range of structures. The 2,5-diphenylthiazoles **5h–5s** were conveniently obtained by condensing the appropriate 3-bromo-4-oxo-butyric acid derivatives **II** with thioamides **IV** by microwave assisted heating in DMF. Standard Friedel–Crafts succinoylation afforded butyric acid **I** and subsequent treatment with bromine in diethyl ether gave the monobromo compound **II**. The thioamides **IV** were obtained by treating the nitriles **III** with hydrogen sulfide in a mixture of pyridine and triethylamine. The compounds **5h–5s** were characterized with respect to binding affinity (COS7 or HEK385-7 cells with comparable affinities observed) and functional antagonistic activity using a bioluminescence resonance energy transfer (BRET) assay (Table 2).[12–14]

The introduction of a *para* halogen substituent in the western (**5i** and **5j**) or eastern (**5n** and **5o**) phenyl ring gave compounds equipotent to the parent compound **5h**. Notably, introduction of a *para* methoxy substituent in the western ring (**5k**) led to a 10-fold drop in potency whereas the activity was retained by substituting the eastern phenyl ring (**5p**). A *para* ethyl substituent (**5l**) in the western ring gave a functionally more active compound but only with partial antagonistic activity. Better potency gains were obtained by increasing lipophilicity by introduction of a phenyl group in the western *para* position (**5m**) or phenoxy groups in the eastern *para* position (**5q–5s**). The influence of electron donating (**5r**) or attracting (**5s**) substituents only had marginal effects on the activity of the unsubstituted **5q**.

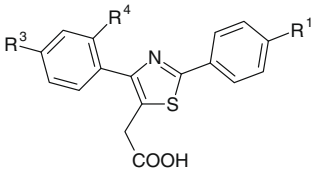
Table 1
Binding affinity on hCRTH2 of 2,4-diphenylthiazoleacetic acids extracted by ligand-based searches from publicly available compound collections



No.	R^1	R^2	R^3	R^4	IC_{50} Bind[a] (μM)
5a	H	OCH_3	Et	H	1.4
5b	H	OCH_3	Br	H	0.35
5c	OCH_3	H	OCH_3	H	4.1
5d	OCH_3	H	Br	H	0.39
5e	NMe_2	H	Br	H	0.46
5f	Cl	H	OCH_3	H	0.21
5g	Cl	H	CH_3	CH_3	0.99

[a] $[^3H]PGD_2$ equilibrium competition binding in COS7 cells.

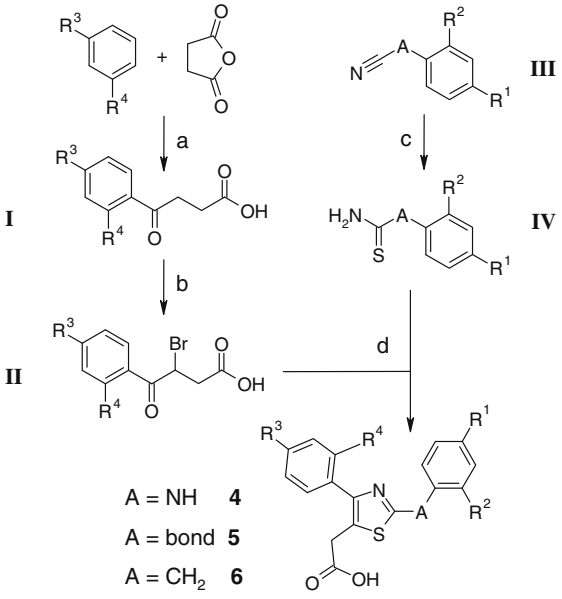
Table 2
Binding affinity and functional antagonism on hCRTH2 of 2,4-diphenylthiazoleacetic acids



No.	R^1	R^3	R^4	IC_{50} Bind[a] (μM)	IC_{50} BRET[b] (μM)
5h	H	H	H	0.87	2.5
5i	H	F	H	0.86	3.5
5j	H	Cl	H	0.55	0.96
5k	H	OCH_3	H	7.0	5.0
5l	H	Et	H	1.3	0.35[c]
5m	H	Ph	H	0.038	0.63
5n	F	H	H	1.5	3.7
5o	Cl	H	H	0.99	1.6
5p	OCH_3	H	H	0.82	1.7
5q	OPh	H	H	0.16	0.66
5r	OPh	OCH_3	H	0.47	1.6
5s	OPh	Cl	H	0.093	1.3

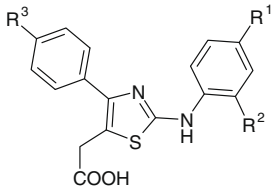
[a] $[^3H]PGD_2$ equilibrium competition binding (COS7 or HEK385-7).
[b] Antagonistic activity as inhibition of β-arrestin translocation measured in a bioluminescence resonance energy transfer (BRET) assay in HEK385-7 cells. All compounds displayed efficacy above 70% unless noted. All values are single or mean of double determinations.
[c] Compound having 50% antagonistic efficacy.

We expanded the SAR of the original 2-anilinothiazoleacetic acids hits **4a** and **4b** by making a diverse set of compounds **4c–4w** using the same chemical route outlined in Scheme 1 starting with thioureas **IV**. No significant activity differences by introducing halogen, trifluoromethyl or methyl substituents (**4d**, **4e**, **4l**, **4n, 4p**) into the unsubstituted compound **4c** were observed (Table 3). A drop in antagonistic potency was however noted for the isopropyl derivative **4o**. Introduction of a methoxy group in western or eastern *para* positions (**4f**, **4q**) or eastern *ortho* positions (**4h**, **4i**) led to comparable activities. A phenyl group in western *para* position (**4g**, **4j**, **4m**) did not improve potency. However, a more flexible phenoxy substituent gave rise to improved potency (**4k**, **4r**, **4s**) provided



Scheme 1. Reagents and conditions: (a) $AlCl_3$, <−10 °C with gradual warming to rt, over night; (b) 1.1 equiv Br_2 in Et_2O, rt, 4 h; (c) H_2S in pyridine/Et_3N 5:1, rt, 3 days; (d) equimolar amounts of II and IV, DMF, 100 °C, 10 min, microwave oven.

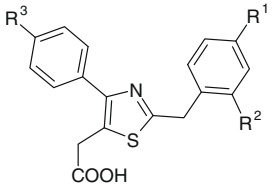
Table 3
Binding affinity and functional antagonism on hCRTH2 of 2-anilino-4-phenylthiazoleacetic acids

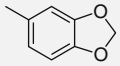


No.	R^1	R^2	R^3	IC_{50} Bind[a] (μM)	IC_{50} BRET[b] (μM)
4c	H	H	H	0.35	1.5
4d	H	H	F	0.65	1.3
4e	H	H	Cl	0.10	1.3
4f	H	H	OCH_3	1.9	2.4
4g	H	H	Ph	0.66	1.2
4h	H	OCH_3	F	1.5	2.1
4i	H	OCH_3	Cl	0.45	0.73
4j	H	OCH_3	Ph	0.31	1.9
4k	H	OCH_3	OPh	0.049	0.48
4l	Cl	H	F	0.96	1.9
4m	Cl	H	Ph	0.94	3.8[c]
4n	CF_3	H	Cl	0.70	1.1
4o	*i*Pr	H	Cl	1.2	16
4p	Me	H	Cl	0.75	0.56
4q	OCH_3	H	H	0.94	1.5
4r	H	H	OPh	0.084	0.35
4s	F	H	OPh	0.089	0.54
4t	H	F	OPh	0.21	>100
4u	H	Cl	OPh	0.13	>100
4v	2-Naphthyl		Br	0.22	0.37
4w	2-Naphthyl		OPh	0.094	0.26[c]

[a] and [b] as in Table 2.
[c] Compounds having 20–30% antagonistic efficacies.

Table 4
Binding affinity and functional antagonism on hCRTH2 of 2-benzyl-4-phenylthiazoleacetic acids



No.	R^1	R^2	R^3	IC_{50} Bind[a] (μM)	IC_{50} BRET[b] (μM)
6a	H	H	Cl	0.18	4.1
6b	Cl	H	H	0.088	0.58
6c	Cl	H	Cl	0.050	0.22
6d	Cl	H	OCH_3	0.36	0.85
6e	CH_3	H	H	0.25	1.8
6f	OCH_3	*m*-OCH_3	H	0.33	3.8
6g	H	H	F	0.14	0.18[c]
6h	H	Br	F	0.70	>100
6i	H	Ph	F	0.024	0.37
6j	H	Ph	Cl	0.041	0.51
6k	H	(*p*-CN)Ph	F	0.17	0.19[c]
6l	H	(*m*-CN)Ph	F	0.34	>100
6m	H	(*p*-OCH_3)Ph	F	0.072	0.27
6n	H	(*m*-OCH_3)Ph	F	0.040	0.39
6o	H	(*o*-OCH_3)Ph	F	0.17	>100
6p	H	(*m*-OCF_3)Ph	F	0.16	>100
6q	H		F	0.024	1.5
6r	H	OPh	Cl	0.16	>100
6s	Cl	H	OPh	0.0037	0.066
6t	2-Naphthyl		Cl	0.041	0.57

[a] and [b] as in Table 2.
[c] Compounds having about 30% antagonistic efficacies.

no halogen substituent was present in the *ortho* position (**4t**, **4u**) which led to loss in functional antagonistic activity. By annelating the eastern phenyl ring to a naphthyl substituent a high functional antagonistic potency was achieved with **4v,** whereas the very lipophilic **4w** only behaved as a weak partial antagonist.

Subsequently, a larger set of analogous compounds **6a–6t** having the anilinic nitrogen replaced with a methylene linker was synthesized according to Scheme 1 (Table 4). The mono and dichloro compounds **6a**–**6c** indicate that most potency enhancement is delivered by the eastern *para* chloro group. However, introduction of a western methoxy group reduces this enhancement (**6d** vs **6b** and **6c**). Methyl (**6e**) or methoxy (**6f**) in the eastern *para* position gives no improvement, which also is true for a fluoro substituent in the western ring (**6g**, **6h**). However, activity is gained when a phenyl group is introduced in the *ortho* position (**6i**, **6j**) (Table 4).

Additional functionalisations were investigated on this motif as illustrated with the biphenyl derivatives **6k–6q** carrying less lipophilic substituents to explore if this could be done with retained potency. The nitrile derivatives **6k** an **6l** had poor functional activity. The *para* and *meta* methoxy (**6m**, **6n**) and the methylenedioxy (**6q**) derivatives had potencies comparable to the parent **6i** whereas the *ortho* compound **6o** had inferior functional activity. A trifluoromethoxy group in *meta* position was also less active than the corresponding methoxy derivative (cf. **6p** and **6n**). The *ortho* biphenyl ether **6r** is also functionally inactive in contrast to the corresponding biphenyl compound **6j**. By bringing the additional phenyl system even closer (2-naphthyl **6t**) potency and functional activity is regained. Notably, a phenoxy group in the western ring (**6s**) gives rise to the most potent compound of these series exhibiting single digit binding affinity to the CRTH2 receptor (3.7 nM $n = 6$). It displays full functional antagonistic effect at 66 nM ($n = 2$) in BRET and 12 nM ($n = 6$) in a cAMP assay. Furthermore, it lacks functional activity for the other PGD_2 DP receptor (27 μM in cAMP).

In summary, we described the structure–activity relationships of a series of arylated thiazoleacetic acids derived from hits obtained after in silico screening and their progression to potent and selective compounds of potential use as anti-inflammatory agents.

Acknowledgements

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14. Radioligand binding assay was conducted with stably transfected COS7 or HEK385-7 cells, expressing human CRTH2 receptor, by competition binding using[3H]PGD_2. Total and nonspecific binding were determined in the absence and presence of 10 μM PGD_2. An improved functional Bioluminescence Resonance Energy Transfer (BRET2) assay was performed on CRTH2 transfected HEK385-7 cells. Antagonists were preincubated with the cell suspension using a shaking table for 5 min. PGD_2 was then added to each well to elicit about 75–80% of the maximal agonist efficacy and cells were further incubated for 5 min. After the incubation period, the 96-well microplate was placed in the Mithras LB 940 instrument and DeepBlueC coelenterazine was injected to one well at a time. Five seconds after the injection, the light output from the well was measured sequentially at 400 nm and 515 nm. The BRET signal was calculated by the ratio of the fluorescence emitted by GFP2-β-arr2 (515 nm) over the light emitted by the receptor-Rluc (400 nm).