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Use of libraries to access new chemical space: Applications to CRTH2

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ABSTRACT

The generation of novel CRTH2 ligands in heavily congested chemical space, by de novo design of libraries is disclosed. Novel (1719) compounds across seven libraries were synthesised. More than 100 of these compounds showed binding potency <3 μM against CRTH2, with the most potent being 247 nM. These libraries produced novel series and demonstrated that this approach is a viable one.

PGD_2 is an arachadonic acid-derived prostaglandin[1] produced in large quantities when asthmatic lung tissues are challenged by allergens. PGD_2 contracts the airway tissue as well as stimulating an inflammatory response.[2]

PGD_2 was also found to be the ligand for a second receptor, DP2 (originally known as CRTH2). Chemoattractant receptor homologous molecule expressed on TH_2 lymphocytes (CRTH2) are activated by PGD_2 and a range of responses such as chemotaxis and mediator release are triggered. These findings have resulted in considerable interest in the development of CRTH2 antagonists as treatments for asthma and allergic rhinitis.

The structures of some known CRTH2 antagonists[3] and examples of generic structures that have been patented are shown in Figure 1.

The non-selective cyclooxygenase (COX) inhibitor indomethacin **5**, is also a potent CRTH2 agonist,[4] whereas the thomboxane receptor antagonist Ramatroban **6** is a CRTH2 antagonist.[5]

As part of Pfizer's effort towards discovering a novel CRTH2 antagonist, an analysis of the CRTH2 ligand patent literature (>60 patents) revealed the common motif/pharmacophore to be an aryl group attached to a carboxylic acid.[6]

In-silico analysis of the Pfizer screening compound collection was carried out to identify the number and structures of the carboxylic acids it contained. On comparison of these structures with the known chemical space for prostanoid ligands, it was apparent that novel acids sharing some related chemical features were under-represented and consequently finding novel chemical space

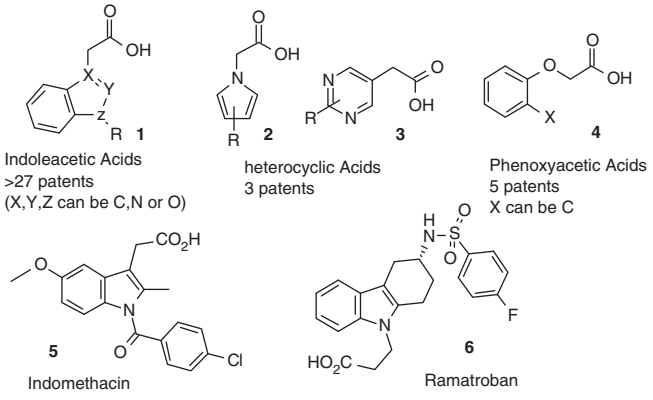

Figure 1. Examples of CRTH2 ligands.

with activity for CRTH2 from the compound file or commercial compound collections would be difficult.

Libraries were therefore designed with the goal of delineating new chemical space for these prostanoid targets. In addition, screening a set of specifically designed library compounds against prostanoid targets would likely give an improved hit rate as compared to a less targeted, very resource intensive HTS.

Ulven et al.[7] used a pharmacophore model based on the genetic similarity of CRTH2 to the AT1 and AT2 receptors for in-silico screening to identify leads from commercial compound collections, and Frimurer et al. generated a pharmacophore model of the core ligand-binding site generated using the known crystal structure of bovine Rhodopsin combined with data on mutagenesis and

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SAR from known ligands for 7TM receptor proteins (GPCRs) to the same end. Both these approaches successfully identified leads from commercial libraries which led to development candidates but were very resource intensive and in a heavily patented arena may not have delivered novel chemical leads.

Our strategy was to first explore some new synthetic routes to add to our synthetic protocols and then to design libraries according to the following criteria:

- The final compounds to be carboxylic acids; a key feature of most prostanoid ligands.
- Inclusion of key H-bond acceptor and lipophilic motifs and aromatic scaffold.
- Compounds to be out of the scope of current patents of known CRTH2 antagonists.
- Good 3D topology comparison between new and known ligands.
- Molecular weight <450.
- Majority of compounds with $c\log D_{7.4}$[8] between −0.5 and 1.5, so as to maximise the probability of having selective, safe orally absorbed acids.
- Libraries should differ in terms of chemistry (e.g., with different functionalities to maximise diversity) but still be amenable to rapid prosecution.

Based on these criteria, the following core templates were selected and libraries designed around them (Fig. 2).

The virtual library compounds were overlayed with a set of known CRTH2 ligands. Of these Laropripant, a known CRTH2 antagonist was found to have the fewest conformations (Fig. 3), and this compound was overlayed with each template and used to help filter down the number of library templates and final library sizes.

In all the libraries, the carboxylic acids were protected as *tert*-butyl esters so that final stage deprotection could be performed using a strong acid.

Phenoxyacetic acids are a commonly reported pharmacophore for CRTH2 ligands[9] and this structural feature was conserved in the majority of the library templates.

Library 1: The first library attempted utilised the Suzuki cross coupling of aryl boronic acids with the heteroaryl bromide templates **15** and **16**, which were made by alkylation of bromoimidazole and bromopyrazole with *tert*-butyl bromoacetate. Alkylation was carried out in quantitative yield by stirring with cesium carbonate in dimethyformamide (DMF) at room temperature overnight. The Suzuki cross coupling reactions were carried out under optimised conditions using Buchwald's *ortho*-methoxy-Kitphos ligand[10] **17** and palladium acetate in toluene at 100 °C with potassium phosphate as the base (Scheme 1). Deprotection of the

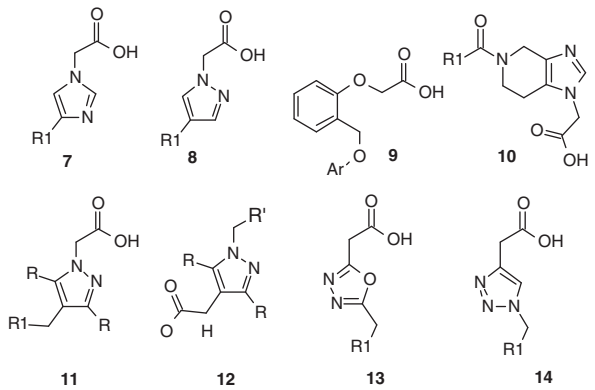


Figure 2. Library templates.

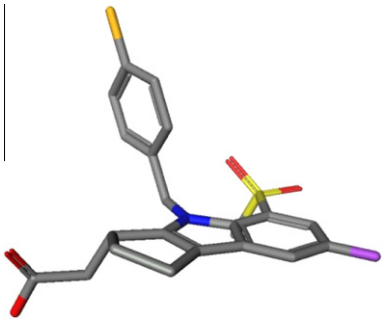
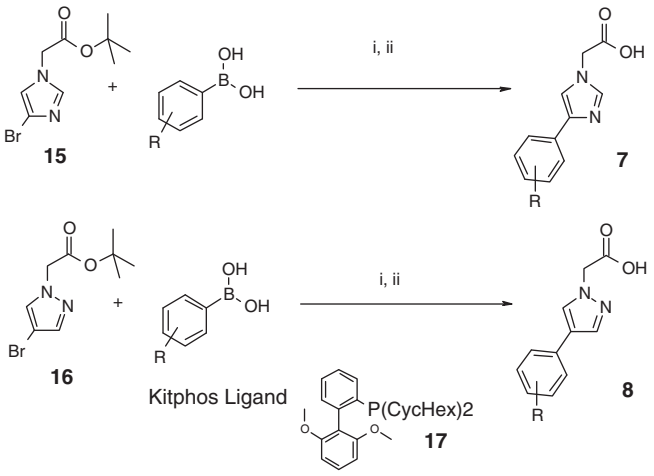


Figure 3. Minimized structure of Laropripant.



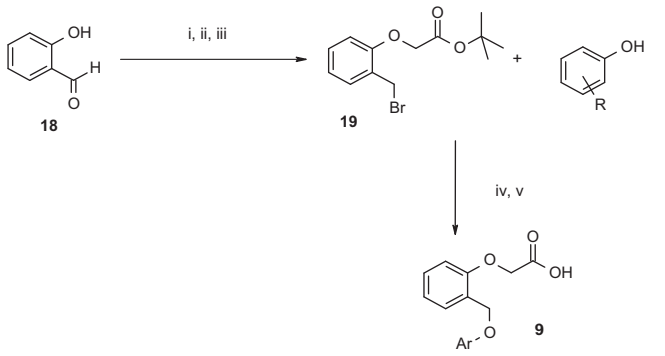
Scheme 1. Reagents and conditions: (i) $Pd(OAc)_2$ (5 mol %), Kitphos **17**, K_3PO_4, toluene, 100°C, 14 h; (ii) trifluoroacetic acid, dichloromethane, rt, 40 min.

tert-butyl ester was carried by treatment with trifluoroacetic acid at room temperature for 18 h to give the carboxylic acid products **7** and **8**. Although Novartis had previously investigated N-alkylated pyrroles as CRTH2 ligands;[12] the templates described in this communication have not been reported.

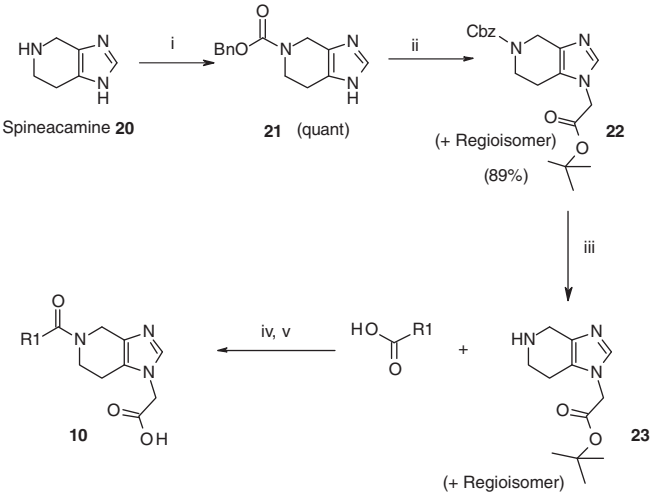
The library synthesis was carried out on 120 μmol scale and a combined success rate[11] of 60% was obtained with all compounds being isolated in >3 μmol yield and 85% or greater purity (as determined by UV and ELSD).

Library 2: This library series was structurally most similar to known CRTH2 ligands, but was still outside known CRTH2 chemical space. The library template was synthesised from commercially available salicaldehyde **18**. Alkylation of **18** with *tert*-butyl bromoacetate in DMF using potassium carbonate at rt for 3 h gave the alkylated aldehyde in quantitative yield. Reduction of the aldehyde with sodium borohydride in ethanol and then treatment with carbon tetrabromide and triphenylphosphine gave the bromomethyl compound **19** in a combined 78% yield. This substituted benzyl bromide **19** was heated with a series of phenols using potassium carbonate in acetonitrile at 60 °C for 6 h (Scheme 2). In this library, hydrolysis of the *tert*-butyl ester was performed using base, because a cleaner impurity profile resulted than with acidic deprotection. The success rate of the library, after purification, was 64% with a purity of >85% as determined by ELSD and UV/vis.

Library 3: A search of the Pfizer monomer collection revealed the availability of a commercial compound, Spinaceamine **20**. Alkylation of the imidazole was readily achieved after protection of the amine to give the carboxylic acid motif and then acylation to give the diversity.[13]



Scheme 2. Reagents and Conditions: (i) $BrCH_2CO_2Me$, K_2CO_3, DMF, rt 14 h; (ii) $NaBH_4$, ethanol, 0 °C, 4 h; (iii) CBr_4, PPh_3, THF, rt 3 h; (iv) K_2CO_3, CH_3CN, 60 °C, 16 h; (v) NaOH (1 M), MeOH/THF (4:1), rt, 4 h.

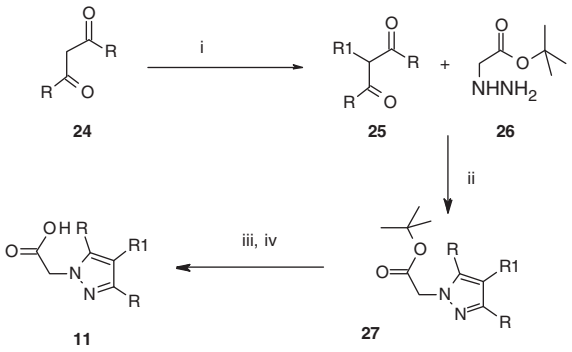


Scheme 3. Reagents and conditions: (i) Benzylchloroformate, dichloromethane, Hünig's base, rt, 16 h; (ii) $BrCH_2CO_2Me$, Cs_2CO_3, DMF, rt 13 h; (iii) Pd/C (20 mol %), EtOH, NH_4OAc, 50 °C, 4 h; (iv) HATU, CH_3CN, Et_3N, DMF; (v) trifluoroacetic acid, dichloromethane, rt, 8 h.

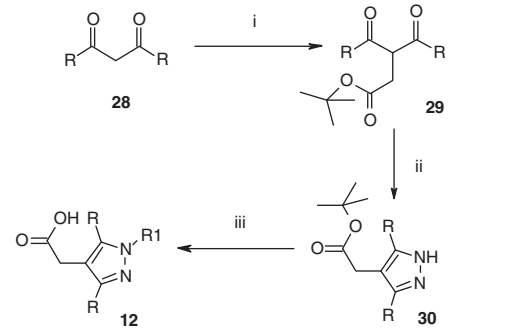
Spinaceamine **20** was protected as the benzyl carbamate (Cbz) compound **21** in quantitative yield by reaction with benzyl chloroformate in dichloromethane and Hünig's base at room temperature, overnight. Deprotonation of the imidazole nitrogen with sodium hydride in tetrahydrofuran (THF) at 0 °C and then alkylation with *tert*-butyl bromoacetate gave approximately a 1:1 mixture of regioisomers **22** in a combined 89% yield (Scheme 3).

The two regioisomers **22** were separated by column chromatography and the Cbz group was then removed by phase transfer hydrogenation using catalytic Pd/C in refluxing ethanol for 4 h. Both regioisomers of the alkylated spinaceamine **23** were used as templates in the library to add diversity. The library synthesis consisted of an amide coupling using HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetra-methyluronium hexafluorophosphate) in acetonitrile at room temperature. Trifluoroacetic acid (TFA) mediated deprotection of the *tert*-butyl ester gave the final carboxylic acids **10**. After purification a library success rate of 69% was obtained with purities of >85% as determined by ELSD and UV.

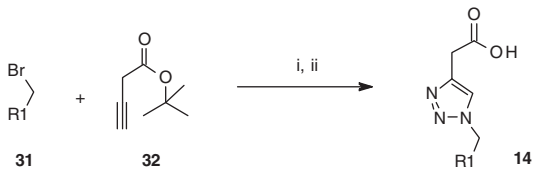
Library 4: The design of this library was based on a functionalised pyrazole template **11**. Alkylation of a 1,3-diketone with an alkyl halide using potassium carbonate in acetone at room temperature for 6 h gave the alkylated diketone **25**. This was not isolated but used directly in the next step (Scheme 4).



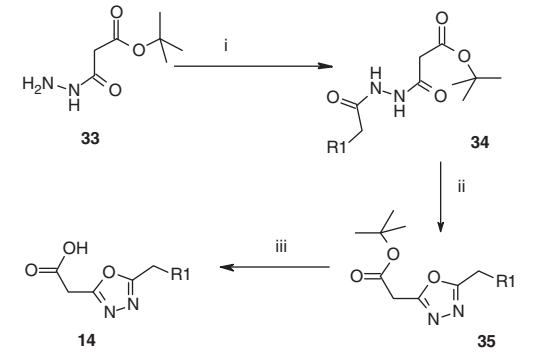
Scheme 4. Reagents and conditions: (i) $BrCH_2CO_2Me$, K_2CO_3, acetone, 0 °C-rt 6 h; (ii) ethanol, 70 °C, 14 h; (iii) NaOH (1 M aqueous), methanol; (iv) HCl in dioxane, rt 2 h.



Scheme 5. Reagents and conditions: (i) $BrCH_2CO_2Me$, Cs_2CO_3, DMF, rt 14 h; (ii) NH_2NH_2, EtOH, 80 °C, 19 h; (iii) alkyl bromide, NaH, DMF, 0 °C to rt (for 2° alkyl halides); Cs_2CO_3, THF, 30 °C, 48 h (for 1° alkyl halides), 8 h; (iv) trifluoroacetic acid, MeCN, rt 14 h.



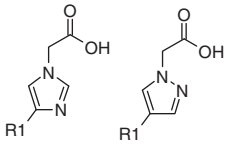
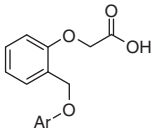
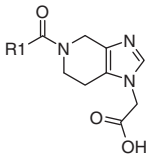
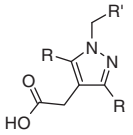
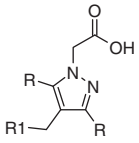
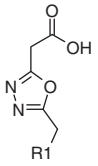
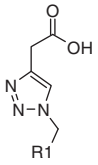
Scheme 6. Reagents and conditions: (i) NaN_3, $CuSO_4{\cdot}5H_2O$ (20 mol %), DMSO, rt 12 h; (ii) trifluroacetic acid, dichloroethane, rt 8 h.



Scheme 7. Reagents and conditions: (i) HATU, Et_3N, R^1CO_2H, dichloromethane, 30 °C, 16 h; (ii) CBr_4, PS-PPh_3, dichloromethane, 0 °C, 2 h; (iii) trifluoroacetic acid, dioxane, 30 °C, 3 h, rt.

Cyclisation of the diketone intermediate **25** with the commercially available *tert*-butyl hydrazinoacetate **26** by heating in

Table 1
Summary of biological data for library compounds by series

Number	Compound structure	Number of compounds made (% success rate)	Number of compounds with >40% inhibition at 2.8 μM
7,**8**		129 (60)	4
9		529 (64)	105
10		529 (69)	0
11		148 (87)	0
12		89 (36)	5
13		145 (31)	16
24		150 (67)	0

ethanol at 70 °C overnight gave the pyrazole products **27**. Base mediated ester hydrolysis with sodium hydroxide gave, after work-up, the carboxylic acids **11** with an overall success rate of 36%. The majority of compounds lost in this library were at the pyrazole cyclisation and/or ester hydrolysis steps (33%).[14] Attempts at acid mediated ester deprotection gave complex mixtures and so base hydrolysis was used on these particular templates.

Library 5: Alkylation of the 1,3-diketo ester **30** with *tert*-butyl bromoacetate and then cyclisation with hydrazine in ethanol at 80 °C for 19 h gave the pyrazole templates **30** in >60% yields. This was the basis of the route for the fifth library, again using a pyrazole core (Scheme 5).

N-alkylation of the pyrazole templates **35** was carried out using one of two sets of conditions depending on whether primary or secondary alkyl halides were used. For primary halides, alkylation was effected by stirring with cesium carbonate in tetrahydrofuran (THF) at room temperature for 48 h. For secondary halides, the pyrazoles **35** were deprotonated using sodium hydride in dimethyformamide (DMF) at 0 °C, followed by addition of the alkyl halide and left stirring at 30 °C for 8 h. This gave, after deprotection and

Table 2
Summary of hCRTH2 binding and functional data for most potent library compounds

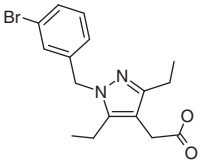
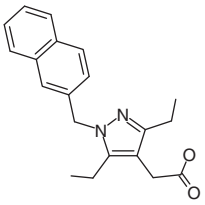
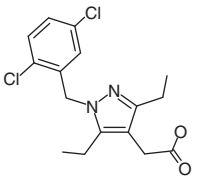
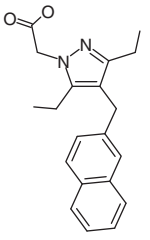
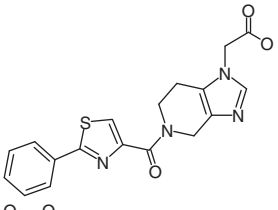
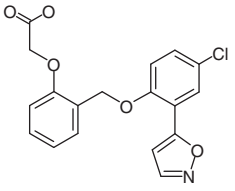
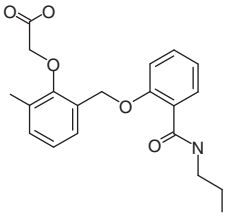
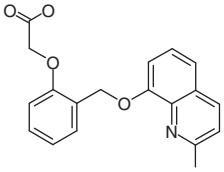
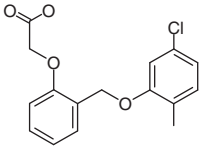
	R/compound	hCRTH2 binding K_i[a] (nM)
36		563
37		574
38		623
39		631
40		752
41		247
42		333
43		492
44		520

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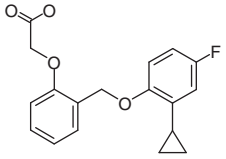
Table 2 (*continued*)

R/compound		hCRTH2 binding K_i[a] (nM)
45		591

[a] The Cheng and Prussof relationship uses the IC_{50}s determined by SiGHTS in conjunction with the K_D and ligand concentration supplied by the analyser to calculate the K_i. K_i are the geometric mean of three or more independent determinations.

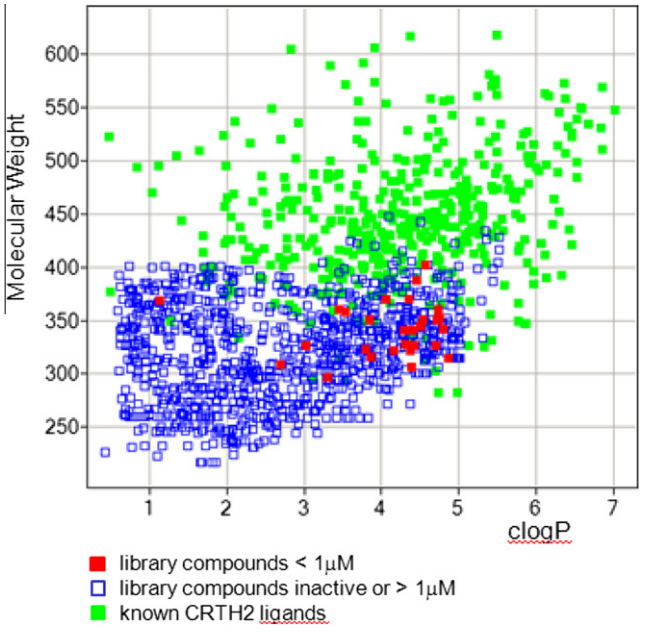


Figure 4. A molecular weight versus *c*log*P* plot of known CRTH2 ligands and library compounds.

purification, an excellent library success rate of 87% (>85% purity). As the pyrazoles **30** have a plane of symmetry, alkylation on either nitrogen gave the same product.

Library 6: This library was synthesised by a one-pot three-component coupling reaction utilising copper(I)-catalysed triazole formation chemistry. In-situ reduction of copper(II) to copper(I) by DMSO catalyses the reaction.[15] Commercially available *tert*-butyl-but-3-ynoate **37** was reacted with sodium azide and a series of alkyl and benzyl halides **36** and the resulting compounds purified using preparative HPLC. Acid mediated deprotection afforded the disubstituted triazole-carboxylic acids **14** with a success rate of 67%. The final compounds were isolated in >85% purity as determined by ELSD and UV (Scheme 6).

Library 7: In order to increase diversity in both structure and in the chemistry, a library was designed in which an oxadiazole **14** was formed by the coupling of two carboxylic acid groups[16] (Scheme 7).

Acyl hydrazide **33** was first coupled with a set of carboxylic acids to give the unsymmetrical diacyl hydrazides **34**. Initial attempts at cyclisation to the desired oxadiazoles **35** were hampered by low yields, in addition to triphenylphosphine oxide residue contamination of the products after purfication. Optimised conditions were subsequently developed which utilised polymer-supported triphenylphosphine (typically 10 equiv) and carbon tetrabromide in dichloromethane at 0 °C for 2 hours. Yields in the route validation work were in the 40-88% range. During the final acid-mediated deprotection of the *tert*-butyl esters **35**, decarboxylation of the product acids was sometimes seen, this being the major reason for the low library success rate of 31%.

In total, across all the libraries, >1700 compounds were synthesised with an average library success rate of 59%, an excellent success rate considering the diversity of the chemistry.

Biological activity against human CRTH2 was determined in a single point binding assay at 2.8 μM. The K_i values were determined for 130 (7.5% of total library set) active compounds showing >40% inhibition[17] (Table 1).

Full dose response screening yielded 10 compounds with K_i<1 μM (Table 2). Thus, novel series were identified with potencies which ranged from 247 to 752 nM.

While the potency of these compounds is still modest, new leads distributed in three distinct classes were identified.

The goal of the project was to design potent compound libraries which had some features present in known CRTH2 ligands, but that were sufficiently different to be clearly outside the patent scope of known ligands and have improved physicochemical properties. The molecular weight versus *c*log*P* plot shown in Figure 4 illustrates that the known CRTH2 ligands are quite large and lipophilic, a particular problem since they are also acidic. In contrast the designed library and active library compounds are generally smaller and less lipophilic suggesting that they may constitute better starting points for the discovery of orally active CRTH2 inhibitors.

The libraries described thus gave rapid access to novel lead CRTH2 ligands despite the backdrop of congested prior art demonstrating the power of parallel synthesis. Even though the probability of any one of these compounds being active was relatively low, the efficiency of parallel synthesis made the cost/benefit ratio of this experiment desirable and resulted in the identification of novel series for further lead optimisation.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.041.

References and notes

1. Hecker, M.; Ullrich, V. *J. Biol. Chem.* **1989**, *264*, 141.
2. (a) Hirai, H.; Tanaka, K.; Yoshie, O.; Ogawa, K.; Kenmotsu, K.; Takamori, Y.; Ichimasa, M.; Sugamora, K.; Nakamura, M.; Takano, S.; Nagata, K. *J. Exp. Med.* **2001**, *193*, 255; (b) Kostenis, E.; Ulven, T. *Trends Mol. Med.* **2006**, *12*, 148.
3. (a) Sandig, H.; Andrew, D. *Abstracts of Papers*, 231st ACS National Meeting, Atlanta GA, 2006.; (b) Barnes, A. A.; Sabroe, I.; Pease, J. *FEBS Lett.* **2006**, *580*, 373; (c) Peter, N. *Expert Opin. Ther. Patent* **1817**, *2005*, 15; (d) Ly, T. W.; Bacon, K. B. *Expert Opin. Invest. Drugs* **2005**, *14*, 769; (e) Armer, R. E.; Ashton, M. R.; Boyd, E. A.; Brennan, C. J.; Brookfield, F. A.; Gazi, L.; Gyles, S. L.; Hay, P. A.; Hunter, M. G.; Middlemiss, D.; Whittaker, M.; Xue, L.; Pettipher, R. *J. Med. Chem.* **2005**, *48*, 6174; (f) Gervais, F. G.; Morello, J.-P.; Beaulieu, C.; Sawyer, N.; Denis, D.; Greig, G.; Malebranche, D. A.; O'Neill, G. P. *Mol. Pharmacol.* **1834**, *2005*, 67.
4. (a) Stubbs, V. E.; Schratl, P.; Hartnell, A.; Williams, T. J.; Peskar, B. A.; Heinemann, A.; Sabroe, I. *J. Biol. Chem.* **2002**, *277*, 26012; (b) Hirai, H.; Tanaka, K.; Takano, S.; Ichimasa, M.; Nakamura, M.; Nagata, K. *J. Immunol.* **2002**, *168*, 981.

5. (a) Sugimoto, H.; Shichijo, M.; Okano, M.; Bacon, K. B. *Eur. J. Pharmacol.* **2005**, *524*, 30; (b) Sugimoto, H.; Shichijo, M.; Iino, T.; Manabe, Y.; Watanabe, A.; Shimazaki, M.; Gantner, F.; Bacon, K. B. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 347–352; (c) Ulven, T.; Kostenis, E. *J. Med. Chem.* **2005**, *48*, 897.
6. (a) Pettipher, R.; Hansel, T. T.; Armer, R. *Nat. Rev.* **2007**, *6*, 313; (b) Ulven, T.; Kostenis, E. *Expert Opin. Ther. Patent* **2010**, *20*, 1505.
7. (a) Ulven, T.; Receveur, J.-M.; Grimstrup, M.; Rist, Ø.; Frimurer, T. M.; Gerlach, L.-O.; Mathiesen, J. M.; Kostenis, E.; Uller, L.; Högberg, T. *J. Med. Chem.* **2006**, *49*, 6638; (b) Frimurer, T. M.; Ulven, T.; Elling, C. E.; Gerlach, L.-O.; Kostenis, E.; Högberg, T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3707.
8. *c*log*D* at pH 7.4 was calculated using ACD/Labs software.
9. Luker, T.; Bonnert, R.; Brough, S.; Cook, A. R.; Dickinson, M. R.; Dougall, I.; Logan, C.; Mohammed, R. T.; Paine, S.; Sanganee, H. J.; Sargent, C.; Schmidt, J. A.; Teague, S.; Thom, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6288.
10. Doherty, S.; Knight, J. G.; Smyth, C. H.; Jorgensen, G. A. *Adv. Synth. Catal.* **1801**, *2008*, 350.
11. Success rate is defined here as the number of library compounds delivered (in sufficient quantity and purity) divided by the number of library compounds attempted.
12. WO2007144127; Pyrrole derivatives with CRTH2 receptor modulator activity; Sandham, D. A.; Leblanc, C.; Adcock, C.; Bala, K. J.; Pipet, M.N.P.
13. Braibanti, A.; Dallavalle, F.; Leporati, E.; Mori, G. *J. Chem. Soc., Dalton Trans.* **1973**, *1*, 323.
14. Compound Library synthesis protocol: see Appendix 1.
15. (a) Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984; (b) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2004**, *2001*, 40; (c) Rostovtsev, V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem.* **2002**, *41*, 2596.
16. Rajapakse, H. A.; Zhu, H.; Young, M. B.; Mott, B. T. *Tetrahedron Lett.* **2006**, *47*, 4827.
17. CRTH2 binding assay: See Appendix 2