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Structure–activity relationships (SAR) and structure–kinetic relationships (SKR) of bicyclic heteroaromatic acetic acids as potent CRTh2 antagonists II: Lead optimization

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ABSTRACT

Extensive structure–activity relationship (SAR) and structure–kinetic relationship (SKR) studies in the bicyclic heteroaromatic series of CRTh2 antagonists led to the identification of several molecules that possessed both excellent binding and cellular potencies along with long receptor residence times. A small substituent in the bicyclic core provided an order of magnitude jump in dissociation half-lives. Selected optimized compounds demonstrated suitable pharmacokinetic profiles.

Small molecules which antagonize the pro-inflammatory effects of PGD_2 mediated by CRTh2 on key cell types associated with allergic inflammation (basophils, eosinophils and Th2 lymphocytes) should have a potential benefit in related pathologies. As a consequence there has been considerable interest of late in the search for CRTh2 antagonists.[1]

In the preceding article,[2] the design and synthesis of a number of potent bicyclic heteroaromatic acetic acids (Fig. 1) as CRTh2 antagonists, was described.

In this work, the optimization of this series is described resulting in the discovery of orally available compounds possessing long receptor residence times.

An exhaustive study of the SAR of a variety of the bicyclic templates previously discovered[2] began with the investigation of the effects of ring substitution on potency in the indazole series (compounds **1**–**12**, Table 1). A general synthetic route used to access such derivatives has been previously described[2] and full experimental details can be found elsewhere.[3]

In addition to binding potency, the optimization of the receptor dissociation half-life was of particular interest given that: (a) drug-target residence time is a factor which, depending on the particular pharmacokinetic properties of a drug, may be important when considering the duration of a pharmacological effect in vivo[4] and (b) at the onset of this research, only relatively short receptor residence half-lives ($t_{½}$ <2 h) had been reported for optimized carboxylic acid-containing CRTh2 antagonists.[5] In order to maximize the possibility of the discovery of an acidic antagonist compatible with a once-daily dosing regimen, a molecule possessing a long receptor residence time ($t_{½}$ >12 h) was targeted.

Biological results for indazoles **2**–**12** are presented in Table 1. It was quickly found that small substituents in R^1 led to moderate increases in both binding potency and dissociation half-life (Table 1), fluorine being the preferred group (compound **3**). Substitution in R^2 (compounds **7**–**9**) led to only minimal changes in binding potency although compound **8** demonstrated the highest potency in eosinophil shape change (ESC) functional assay of the whole indazole series. Incorporation of the substituents which gave the best improvements with respect to residence time in R^1 (F, compound **3**) and R^2 (Cl, compound **7**) in a single molecule led to compound **10** with a significantly improved receptor residence half-life (dissociation $t_{½}$ 10.5 h). Comparison of this result with compound **1** clearly demonstrated that binding potency was not the only factor governing the receptor residence time. The incorporation of even the small fluorine substituent in R^3 led to a significant loss of potency (compound **12**) so no further derivatives

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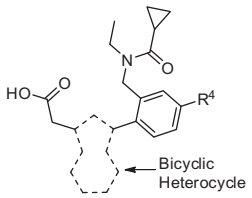
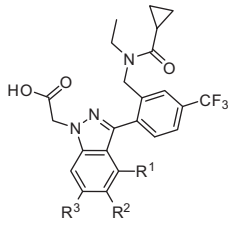


Figure 1. Bicyclic heteroaromatic CRTh2 antagonists.

Table 1
Ring substitution in the indazole series[a]



Compd	R^1	R^2	R^3	GTPγ binding IC_{50} (nM)[6]	ESC whole blood IC_{50} (nM)[6]	hCRTh2 dissociation $t_{½}$ (h)[b]
1[2]	H	H	H	19	47	1.6
2	Cl	H	H	6.3	160	3.2
3	F	H	H	4.3	23	5.0
4	OMe	H	H	23	27	2.4
5	Me	H	H	17	108	2.3
6	CN	H	H	33	103	1.2
7	H	Cl	H	16	23	2.8
8	H	Me	H	9	13	1.8
9	H	F	H	15	34	1.4
10	F	Cl	H	15	18	10.5
11	Cl	F	H	10	71	3.3
12	H	H	F	127	ND	ND

[a] All mean data values reported are derived from at least two experiments.
[b] For details of the biological assays, see Ref. 6. ND = not determined.

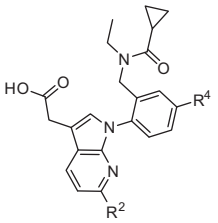
were prepared. Although only slight potency gains had been achieved in this series, it was now evident that the desired residence half-life target of >12 h was likely to be achieved.

In parallel, structural activity relationships in an alternative series based upon the 7-azaindole template (Table 2) was under investigation. Previously described compound **13**, unsubstituted in the central core, had shown superior activity and pharmacokinetic properties when compared to the equivalent indazole derivative **1**.[2] Compounds synthesised (**14**–**26**) and biological results are given in Table 2 and a representative synthesis,[7] as exemplified by the preparation of **14** is shown in Scheme 1.

6-Methyl-7-azaindole **28** was prepared from the known chloro azaindole **27**[8] via a Suzuki coupling with trimethylboroxine followed by base-promoted hydrolysis of the carbamate protecting group. Intermediate **28** was then subjected to a copper-catalyzed coupling reaction with aryl bromide **29** to give **30** in good yield. Bromination of the azaindole nucleus went smoothly with NBS to give **31** which was subjected to a palladium-catalyzed Negishi-type coupling reaction with a commercially available organozinc derivative (^{t}BuO$_2$CCH$_2$ZnCl) to install the acetic ester side chain. Finally, deprotection with trifluoroacetic acid led to the compound **14**.

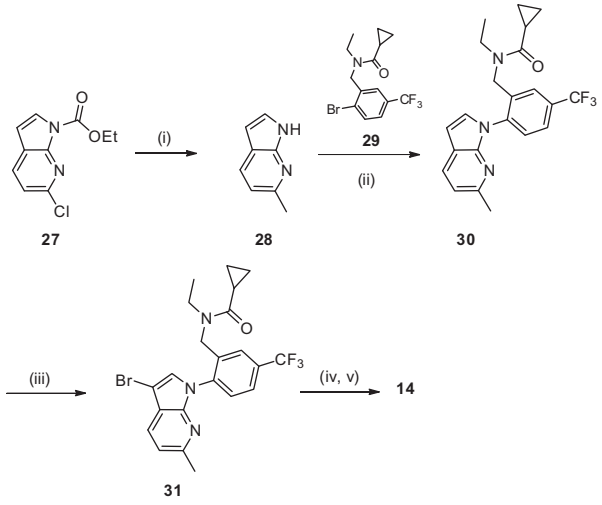
In the 7-azaindole series, keeping the substituent at R^4 constant as a trifluoromethyl residue (Table 2, compounds **13**–**19**), it was found that placement of small substituents at R^2 led to major changes in receptor dissociation half-life (compounds **14** and **17**), although binding affinities were, in general, little affected. The most striking difference was seen between compounds **13** and **14**

Table 2
SAR 7-azaindole derivatives



Compd	R^2	R^4	GTPγ binding IC_{50}[a] (nM)	ESC whole blood IC_{50}[a] (nM)	hCRTh2 dissociation $t_{½}$[a] (h)
13[2]	H	CF_3	9	34	2.3
14	Me	CF_3	10	7.8	21
15	Et	CF_3	10	ND	9.9
16	cPr	CF_3	49	ND	5.4
17	CHF_2	CF_3	11	8.0	**29**[b]
18	CF_3	CF_3	54	ND	ND
19	OMe	CF_3	135	ND	ND
20	H	F	17	11	1.3
21	Me	F	4.7	6.4	11
22	Me	H	5.0	9.5	8.8
23	Me	Cl	5.2	18	**31**[b]
24	Me	Me	5.9	13	24
25	Me	OCF_3	5.8	6.7	16
26	Me	SO_2Me	40	ND	ND

Bold numbers for dissociation indicate $t_{½}$ >12 h.
[a] All mean data values reported are derived from at least two experiments.
[b] Extrapolated from remaining % inhibition at last time point after washout.



Scheme 1. Reagents and conditions: (i) trimethylboroxine (1.2 equiv), $PdCl_2$·dppf·DCM (5 mol %), K_2CO_3 (3 equiv), 1,4-dioxane, argon, 120 °C, 18 h then NaOH, MeOH, rt, 2 h, 80%; (ii) bromide **29** (1 equiv), CuI (10 mol %), *trans*-N^1,N^2-dimethylcyclohexane-1,2-diamine (15 mol %), K_3PO_4 (2 equiv), 1,4-dioxane, argon, 130 °C, 18 h, 72%; (iii) NBS (1 equiv), EtOAc, 0 °C, 1 h, 78%; (iv) ^{t}BuO$_2$CCH$_2$ZnCl (3 equiv), QPhos (5 mol %), Pd(dba)$_2$ (5 mol %), THF, argon, reflux, 1 h, 84%; (v) TFA, DCM, rt, 2.5 h, 78%.

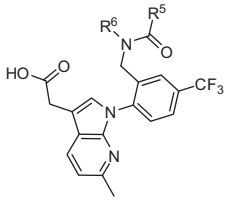
which differ by only a single methyl group—whilst both compounds had a very similar binding potency, the latter compound showed an improvement in dissociation half-life by an order of magnitude (dissociation $t_{½}$ >20 h).[9] This is in marked contrast to the equivalent pair of indazole derivatives compounds **1** and **8** (Table 1) in which no difference was seen with regard to residence time. The same trend was seen when comparing derivatives where R^4 had been modified—the incorporation of the methyl substituent at R^2 dramatically improved residence time (compounds **20** and

21) in the 7-azaindole series. When R^2 was fixed as a methyl substituent, a variety of groups could be placed at R^4 and the resultant derivatives (compounds **14**, **21**–**26**) maintained excellent binding potency and residence times (residence time ranking from low to high H < F < OCF_3 < CF_3 < Me < Cl). The exception was the sulfone derivative **26** which was significantly less potent than the unsubstituted compound **22**.

In general, the incorporation of a substituent (either Me or CF_3) in the same relative position as the 6-position of the 7-azaindole series in a variety of other bicyclic heterocycles[6] led to similar dramatic increases in receptor residence time (Table 3, compounds **32**–**42**). Clear differences were seen comparing the dissociation half-lives of substituted compounds with their un-substituted counterparts.[10] The origin of this general effect remains unexplained as no attempts to dissect the kinetic features of receptor binding with radiolabelled ligands[11] were made.

Results of the preliminary investigations into the SAR of the amide portion of the molecule in the 7-azaindole series are presented in Table 4 (compounds **43**–**55**). A typical synthetic route is illustrated by the preparation of **44** (Scheme 2). Compound **28** was coupled with the Boc-protected derivative **56** to give **57**. Bromination of **57** followed by installation of the ester side chain using the protocols described previously for the synthesis of **14** gave the intermediate **58** in good yield. Exhaustive deprotection of **58** followed by re-esterification of the acid moiety proceeded in quantitative yield to give key intermediate **59** which was used for the synthesis of several derivatives. For example, treatment of **59** with cyclobutane carbonyl chloride followed by ester hydrolysis provided **44** in good yield.

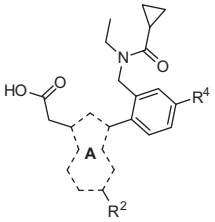
Table 4
SAR of amide region in the 7-azaindole series[a]

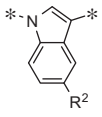
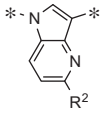
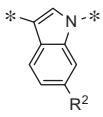
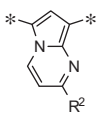
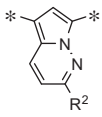


Compd	R^5	R^6	GTPγ binding IC_{50} (nM)	ESC whole blood IC_{50} (nM)	hCRTh2 dissociation $t_{½}$ (h)
14	cPr	Et	10	7.8	21
43	Me	Et	10	ND	6.4
44	cBu	Et	10	8.5	24
45	CH_2cPr	Et	7	29	22
46	CH_2OMe	Et	9	5	**21**[b]
47	CH_2Ph	Et	6	25	**24**[b]
48	2-pyridyl	Et	97	ND	ND
49	$NHCH_2Ph$	Et	5	4.7	26
50	CH_2NMe_2	Et	163	ND	ND
51	cPr	^{i}Pr	28	ND	9.2
52	cPr	CH_2cPr	11	22	**32**[b]
53	cPr	CH_2CH_2OPh	4	60	**40**[b]
54	cPr	CH_2CH_2OMe	4	ND	7.0
55	cPr	CH_2CH_2OH	14	ND	7.8

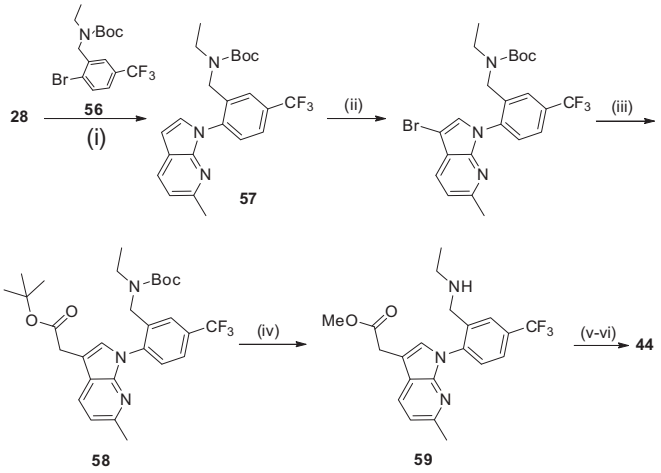
Bold numbers for dissociation indicate $t_{½}$ >12 h.
[a] All mean data values reported are derived from at least two experiments.
[b] Extrapolated from remaining % inhibition at last time point after washout.

Table 3
Impact of ring substitution on dissociation half-life[a]



Compd	A	R^4	R^2	GTPγ binding IC_{50} (nM)	ESC whole blood IC_{50} (nM)	hCRTh2 dissociation $t_{½}$ (h)
32		F	H	11	11	2.0
33		F	CF_3	14	17	**20**
34		CF_3	H	20	15	1.9
35		CF_3	Me	17	10	**21**
36		CF_3	CF_3	15	82	**25**
37		CF_3	H	37	84	2.5
38		CF_3	Me	119	ND	ND
39		H	CF_3	11	7.7	**27**
40		CF_3	H	13	1.4	2.0
41		CF_3	Me	7.0	24	**19**
42		CF_3	Me	4.2	7.3	**26**

Bold numbers for dissociation indicate $t_{½}$ >12 h.
[a] All mean data values reported are derived from at least two experiments.



Scheme 2. Reagents and conditions: (i) bromide **56** (1 equiv), CuI (10 mol %), *trans*-N^1,N^2-dimethylcyclohexane-1,2-diamine (15 mol %), K_3PO_4 (2 equiv), 1,4-dioxane, argon, 130 °C, 48 h, 23%; (ii) NBS (1.3 equiv), EtOAc, −10 °C, 2 h, 90%; (iii) *t*-BuO_2CCH_2ZnCl (4 equiv), QPhos (5 mol %), $Pd(dba)_2$ (5 mol %), THF, argon, microwave irradiation, 100 °C, 2 h, 68%; (iv) 4 M HCl in dioxane, rt, 18 h, then MeOH, 30 °C, 1.5 h, 100%; (v) cyclobutanecarbonyl chloride (1.9 equiv), Et_3N (3.8 equiv), DCM, rt, 30 min, 81%; (vi) LiOH·H_2O (4 equiv), THF, H_2O, rt, 30 min, 82%.

Table 5
Pharmacokinetic profiles of selected derivatives in Wistar rat (1 mg/kg)[a]

Compd	Terminal $t_{½}$ (h)	C_{max} (ng/mL)	Cl (mL/min/kg)	$AUC_{0-\infty}$ (ng*h/mL)	V_{ss} (L/kg)	MRT (h)
14	4.9	1610	14	1225	1.8	2.3
32	1.9	431	76	222	5.4	1.2
35	0.5	366	134	126	2.7	0.3
39	6.6	1900	16	1074	3.2	3.4
40	4.6	910	26	639	4.2	2.6

[a] Formulations: 40% PEG.

Several conclusions can be drawn from the biological data (Table 4): (i) a variety of changes made at R^5 and R^6 led to compounds which maintained excellent binding potency, including the urea **49**. (ii) Incorporation of a 2-pyridyl ring or a basic centre in R_5 led to a loss in binding potency (compounds **48** and **50**). (iii) In certain cases receptor residence times were adversely affected by small changes in both R^5 (compound **43**) and R^6 (compounds **51** and **54**).

The iv pharmacokinetic profiles in rat were determined for analogues selected on the basis of binding/cellular potencies and residence times and the results are given in Table 5.

As anticipated based on previous observations,[2] the volumes of distribution for all compounds were higher than expected for acidic compounds. Extremely high clearance (⩾hepatic blood flow) was seen for compounds **32** and **35** which resulted in poor to moderate terminal half-lives and low plasma levels. Compounds **14**, **39**, and **40** showed good terminal half-lives and significant plasma levels as a consequence of moderate clearance and high volumes of distribution (for acidic species). Compound **14**, which showed the best overall profile, was also found to have reasonable oral bioavailability in the rat (F = 27%, 10 mg/kg) and was selected for further profiling.

In summary, optimization of the bicyclic heteroaromatic series of CRTh2 antagonists led to the identification of several molecules that possess both excellent binding and cellular potencies along with long receptor residence times. The incorporation of a small substituent (methyl or similar) in the core in many cases led to an order-of-magnitude increase in dissociation half-lives. The successful demonstration that the long drug-target residence times seen in this series translated into sustained in-vivo pharmacological effects will be reported in due course. Further optimization of the binding kinetics of this series is reported in the following article.

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