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Discovery of small molecule human C5a receptor antagonists

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ABSTRACT

A novel series of small molecule C5a antagonists is reported. In particular, in vitro metabolic studies and solution based combinatorial synthesis are demonstrated as useful tools for the rapid identification of antagonists with low in vitro clearance. Members of this series specifically inhibited the binding of ^{125}I-labeled C5a to human recombinant C5a receptor (C5aR). In functional cell assays these compounds displayed surmountable antagonism against C5a and did not demonstrate any detectable agonist activity.

The complement system is comprised of a cascade of interrelated proteases that are activated in response to immunoglobins binding to a foreign antigen. Activation of the complement systems leads to a stepwise hierarchy of proteolytic cleavage events ultimately leading to the release of bioactive fragments (C3a, C4a and C5a) known as anaphylatoxins. C5a is recognized as a prominent mediator of inflammation through recruitment of inflammatory cells to the site of injury or infection.

The functional effects of C5a are mediated by specific interaction with the G-protein coupled receptor C5aR, expressed on a variety of cells including mast cells and neutrophils.[1] Consistent with its proinflammatory properties, C5a has been implicated in the pathology of a wide range of disorders, including rheumatoid arthritis, asthma, respiratory distress syndrome, sepsis and inflammatory bowel disease.[2] Significant progress has been made in recent years on the understanding of the complement system and several non-peptidic C5a antagonists have been recently reported in the literature (Fig. 1).[3]

Interest in this area is driven by the potential therapeutic application of a C5aR antagonist in the treatment of inflammatory diseases such as arthritis, asthma and COPD. We report in this paper the discovery of a novel series of small molecule C5aR antagonists.

High throughput screening of the AstraZeneca compound collection followed by a hit to lead (HtL) program identified a number of chemical series with clear structure activity relationships (SAR). The most promising of these was the bisamide series, as exemplified by compounds **1** and **2**.

The bisamide series was identified following an HTS using a whole cell binding Fluorescent Microvolume Assay Technology (FMAT™) assay,[6,10] which measured the inhibition of Alexa-647 labeled C5a binding to human recombinant C5aR (hrC5aR) expressed in HEK-293 cells with G-protein Gα16.[10] The compounds were subsequently confirmed as potent functional C5aR antagonists by determining their inhibition of C5a-stimulated intracellular calcium mobilization in the hrC5aR HEK cells (see Table 1). Activity against the native receptor was also confirmed by measuring compound mediated inhibition of C5a-stimulated calcium mobilization in human neutrophils.[16] For example, Compound (**2**) had a pA_2 value of 7.4 in this neutrophil assay. Activity against the related C5a binding receptor C5L2 was determined using an FMAT binding assay[10] and the series was found to be selective. For example compound (**2**) tested up to 30 μM displayed no inhibition of Alexa-647 labeled C5a binding to C5L2. In additional selectivity profiling, compound (**2**) was inactive at GPCRs FPR, CXCR2, CXCR4 and CCR3, when tested up to 10 μM (data not shown). Species selectivity of the bisamide series was examined in recombinant receptor cell calcium mobilization assays and was demonstrated to have apparent selectivity for the human C5aR (see Table 2), similar to previous reports on small molecule C5aR antagonists.[7]

In order to further characterize the functional antagonism of the bisamide series we measured the ability of exemplars from this series to inhibit C5a stimulated GTPγS binding in HEK cell membranes expressing the hrC5aR.[10] Concentration response curves to C5a were performed following pre-treatment of membranes

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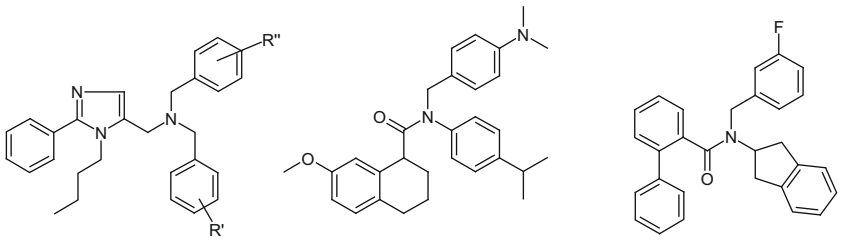
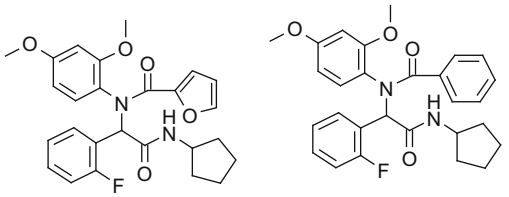


Figure 1. A selection of literature C5a receptor antagonists.

Table 1
Table of bisamides



(**1**) (**2**)

Compound	M_W	Log D (pH 7.4)	C5a[a] R pIC_{50}	Human mics[b]	Rat heps[c]
1	467	3.1	6.9	>150	>150
2	477	3.9	7.6	>150	>150

[a] Human C5a receptor HEK calcium mobilization antagonist assay. Values are means of at least two experiments.
[b] Human microsome metabolism intrinsic clearance (μL/min/mg).[4]
[c] Rat Sprague–Dawley hepatocyte metabolism (μL/min/10^6 cells).[5]

Table 2
Compound species selectivity

Compound	Human rC5a[a] R pIC_{50}	Mouse rC5a[b] R pIC_{50}	Rat rC5a[c] R pIC_{50}	Dog rC5a[d] R pIC_{50}
1	6.9	NA	NA	NA
2	7.5	NA	NA	NA

Inhibition of C5a-stimulated intracellular calcium mobilization in HEK cells transfected with recombinant [b]mouse, [c]rat and [d]dog C5a receptors. Values are means of at least two experiments. NA: not active at 30 μM.

with increasing concentrations of the antagonists (see Fig. 2 for example). The results demonstrated that all C5a antagonists tested inhibited C5a in an apparent surmountable fashion.

The two-state model of GPCR activation assumes that the receptors exist in an equilibrium between inactive, R and active, R* states.[8] Previous studies using functional [^{35}S]GTPγS binding assays have demonstrated a role for Na^+ in stabilizing the inactive form of the receptor.[9] Consistent with these studies we found that by removing Na^+ from the buffer we were able to detect significant levels of constitutive activity of the hrC5aR.

Analysis of **2** in this system demonstrated a clear reduction in basal [^{35}S]GTPγS binding by 63% with a pEC_{50} of 6.91 (Fig. 3). In contrast rhC5a, a full agonist, increased basal [^{35}S]GTPγS binding by 50% with a pEC_{50} of 8.7. These data are consistent with **2** having inverse agonist activity. Furthermore, while whole cell binding and functional (intracellular calcium mobilization) potencies were broadly equivalent for this series, the bisamide compounds displayed only partial inhibition of hrC5a in hrC5aR membrane-binding assays,[10] with relatively low potencies in comparison to whole cell binding. Full inhibition and increased potencies were subsequently obtained when the membrane-binding assay was supplemented with 10 μM GTPγS. (see Table 3). These data are consistent with the two-state model of GPCR activation whereby the inclusion of GTPγS in the system would be expected to favor the dissociation of G-protein from the receptor complex (i.e., form the inactive, R state) thereby increasing the apparent affinity of rhC5a receptor for inverse agonists.

The bisamide compounds **7** were obtained using a choice of two synthetic routes, dependent upon the availability of commercial starting reagents. Scheme 1 involved a one-pot synthesis via an Ugi multicomponent reaction, where a mixture of a carboxylic acid **3**, aniline **4**, isonitrile **5** and aldehyde **6** were stirred together in methanol.[11] Where the isonitrile component was unavailable (Scheme 2) the amide bond was constructed using a conventional coupling reaction with an appropriate amine and the carboxylic acid intermediate **8**. The acid **8**[12] was in turn prepared from a 4 component Ugi reaction using the convertible isonitrile **5**.[13]

Although a number of potent bisamides were identified, issues relating to their metabolic instability had to be addressed. It is likely that these DMPK issues arise from high lipophilicity, leading to high measured intrinsic clearances in rat hepatocytes and human liver microsomes. The main objective of this HtL programme

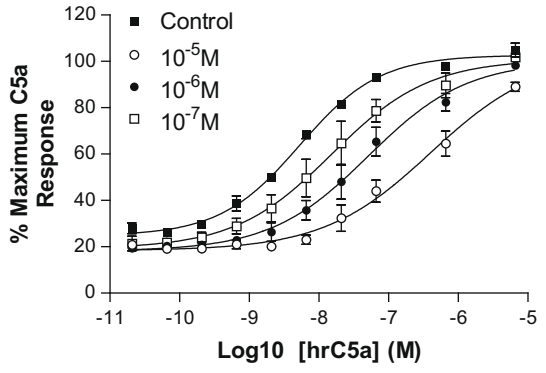


Figure 2. Inhibition of rhC5a-stimulated [35S]GTPγS binding by compound **2**. Values are the means of at least two independent experiments.

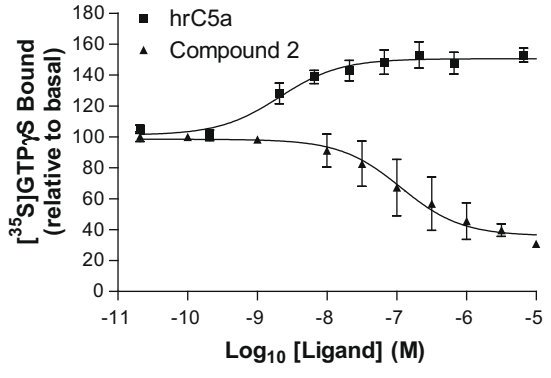
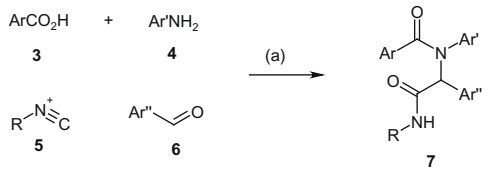


Figure 3. Compound **2** and rhC5a effects on basal [^{35}S]GTPγS binding activity in rhC5a-HEK cell membranes. Values are the means of at least two independent experiments.

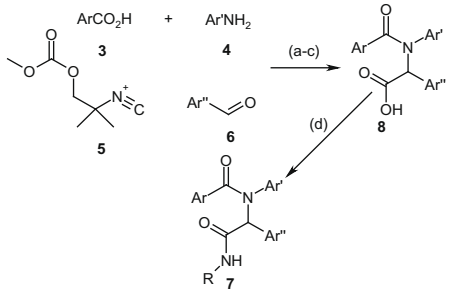
Table 3
Compound biological activities

Compound	C5a[a] R whole cell binding pIC_{50}	C5a[b] R Ca^{2+} pIC_{50}	C5a[c] R membrane-binding pIC_{50}	C5a[d] R membrane-binding + GTPγS pIC_{50}
1	7.1	6.9	5.5	6.6
2	7.4	7.5	6.1	7.5

Values are means of at least two experiments.
[a] Human C5a receptor HEK whole cell [Alexa^{647}]C5a competition binding assay.
[b] Human C5a receptor HEK calcium mobilization antagonist assay.
[c] Human C5a receptor HEK membrane ^{125}I-labeled C5a competition binding assay.
[d] Human C5a receptor HEK membrane ^{125}I-labeled C5a competition binding assay in presence of 10 μM GTPγS.[10]



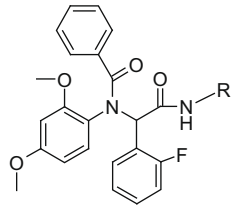
Scheme 1. (a) Methanol, 40 °C, 4 h.

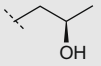
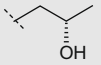
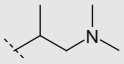


Scheme 2. (a) Methanol, 40 °C, 4 h (b) potassium *t*-butoxide, THF, (c) Lithium hydroxide/water, (d) HATU™, Hunigs base, RNH_2.

was to address the key issues of maintaining potency while reducing lipophilicity and thereby hopefully increasing metabolic stability. Metabolite identification was also used to find the key sites of

Table 5
SAR of compounds marked A and B



Compound	R	C5a R pIC_{50}	Human mics	Rat heps	Log D (pH 7.4)
13 (A)		7.2	104	>150	2.55
14		<7	—	—	—
15 (B)		6.0	135	33	—

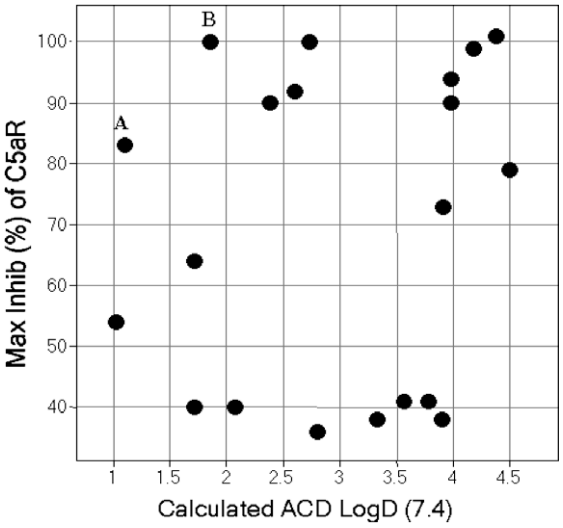
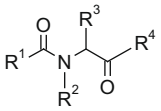
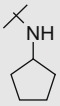
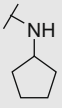
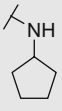


Figure 4. Plot of % Maximal inhibition of C5aR in the calculation mobilization assay using 10 μM compound versus calculated ACD log D (pH 7.4).

Table 4
Compound biological activities



Compound	R^1	R^2	R^3	R^4	C5a R Ca^{2+} pIC_{50}
9	Ph	CH_3	*o*-Fluoro Phenyl		<5
10	Ph	2,4 dimethoxy phenyl	CH_3		<5
11	CH_3	2,4 dimethoxy phenyl	*o*-Fluoro Phenyl		6.1
12	Ph	2,4 dimethoxy phenyl	*o*-Fluoro Phenyl		<5

metabolism of the bisamides. This approach is in line with our lead criteria outlined in previous HtL publications.[14]

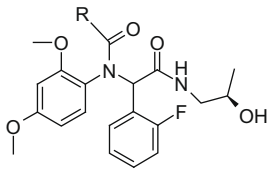
Initial efforts were focused on the SAR of the three-aryl rings (R^1–R^3) (Table 4), replacing each in turn with a methyl group. Replacement at centers R^2 or R^3 with methyl was not tolerated and led to complete loss of activity (**9**, **10**). However replacement of R^1 (**11**) did retain some potency (pIC_{50} 6.1). Replacement of the cyclopentylamide by its parent carboxylic acid (**12**) also resulted in complete loss of activity.

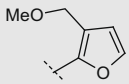
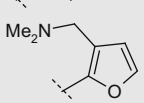
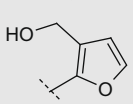
Metabolite analysis highlighted the cyclopentyl motif as the main area of metabolic liability. To address this finding a focused library of cyclopentyl replacements was synthesised. The data from this library is illustrated (Fig. 4) as plot of % maximal inhibition of the C5a receptor in the recombinant HEK calcium mobilization assay[10] against calculated ACDLog $D_{7.4}$[15] for compounds spot tested at 10 μM. Two key compounds emerged as having lower log D and good inhibition (marked as A and B Fig. 4) and these compounds were chosen for further biological analysis (Table 5, **13**, **15**). The diastereomer **13** (A) prepared from the chiral alcohol was found to have a pIC_{50} 7.2 and lower measured log D (relative to cyclopentyl amide) although still had a high intrinsic clearance. Furthermore, the diastereomer **14** prepared from the chiral alcohol of opposite stereochemistry was lower potency.

Maintaining the alcohol side chain but addressing the log D further by varying the aryl amide, proved rewarding. Key compounds synthesized following this strategy are highlighted in Table 6. It was demonstrated that the phenyl amide could be replaced by a series of substituted heterocycles. The 2- and 3-furan analogs with a substituent next to the point of attachment (**16**,**17**) were among the most potent but other five membered heterocycles (thienyl, thiazolyl, oxazolyl and isoxazolyl) also had good activity. These compounds had slightly reduced lipophilicity but were still rapidly metabolised in vitro. Metabolite identification on **16** showed the major site of metabolism was oxidation of the methyl substituent on the furan ring. Replacement with other groups either gave good potency and poor metabolic stability (methoxymethyl **18**, chloro **19**, **20**) or good metabolic stability and poor potency (dimethylaminomethyl **21** hydroxymethyl **22**). The diasteromeric mixture of the methoxymethylfuran **18** was separated by HPLC into the two single isomers. All activity resided in one diastereomer of unknown absolute configuration but in vitro clearance was similar for both isomers.

We discovered a series of C5aR antagonists through an FMAT-based C5a binding HTS and characterized them as human C5aR selective inverse agonists. SAR at the four variable positions of the bisamides **1** and **2** was determined rapidly using library synthesis. A large number of compounds with 'lead-like' potency were prepared but these all had poor metabolic stability. Metabolic stability is possible in this series as shown by compounds **20** and **21** but these had poor C5a potency. Other factors that were taken into account when considering this series as potential leads were the high molecular weight and low solubility, as well as lack of species

Table 6
SAR table for aryl amide replacements



Compound	R	C5a R Ca^{2+} pIC_{50}	Human mics	Rat heps	Log D (pH 7.4)	ACD Log D (pH 7.4)[a]
13		7.2	104	> 150	2.6	1.9
16		7.7	126	87	2.0	1.5
17		7.4	134	75	–	1.5
18		7.0	76	35	2.0	0.8
19		6.7	148	59	2.2	1.2
20		6.5	48	33	2.4	1.3
21		5.5	12	<3	–	0.8
22		NA	12	8	1.4	−0.2

[a] ACD log $D_{7.4}$ calculations[15] are approximately 1 log unit lower than measured.

cross over. On the evidence available this series was not progressed into Lead Optimisation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.104.

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10. See online publication supplementary material for detailed methods.
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12. Synthesis of compound (**12**) (in Table 4). To a solution of benzoic acid (0.6 mmol), Carbonic acid, 2-isocyano-2-methylpropyl methyl ester (0.8 mmol) and 2,4-dimethoxyaniline (0.6 mmol) in methanol (10 ml) was added 2-fluorobenzaldehyde (1.0 mmol) and the mixture stirred at 40 deg for 4 h. The mixture was evaporated to dryness and the residue purified by silica chromatography (1:1 ethyl acetate/isohexane). The above intermediate (0.372 mmol) was dissolved in tetrahydrofuran (10 ml) and to this solution was added potassium *t*-butoxide 1.0 M THF (0.5 mmol). Upon completion of reaction the mixture was acidified with glacial acetic acid and evaporated to dryness. The residue was taken up into a mixture of dioxan/water (1:1) and to this solution was added lithium hydroxide (2 equivalents) and stirred overnight. The mixture was acidified and mixture was evaporated to dryness and purified (HPLC, Symmetry®, 0.1% aqueous ammonium acetate:acetonitrile, gradient elution 75:25 to 5–95 over 15 min) to afford **12** 0.073 g (46%) as a colourless solid. MS (APCI) 410 (M + H, 100%). 1H NMR (90 °C) (399.826 MHz, DMSO) δ 7.28–7.10 (m, 6H), 7.05–6.85 (m, 4H), 6.41 (s, 1H), 6.27 (d, J = 7.4 Hz, 1H), 6.02 (s, 1H), 3.59 (s, 3H), 3.29 (s, 3H).
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