Bioorganic & Medicinal Chemistry Letters 18 (2008) 5601–5604

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Small, non-peptide C5a receptor antagonists: Part 1

Julian Blagg [a], Charles Mowbray [a], David C. Pryde [a,*], Gary Salmon [b], Esther Schmid [b], David Fairman [b], Kevin Beaumont [c]

[a] Department of Discovery Chemistry, Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK
[b] Department of Discovery Biology, Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK
[c] Department of Pharmacokinetics, Distribution and Metabolism, Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

ARTICLE INFO

Article history:
Received 28 July 2008
Revised 26 August 2008
Accepted 28 August 2008
Available online 31 August 2008

Keywords:
C5a receptor
Complement
Inflammation
Antagonist

ABSTRACT

The optimisation of a series of amides for C5a receptor binding and functional activity, and physicochemical properties is described. The initial hit, **1** (IC_{50} 1 μM), was discovered during high throughput screening, from which highly potent C5a receptor antagonists (e.g. **14**, IC_{50} 5 nM) were developed.

C5a is a 74-amino acid peptide cleaved from C5 at sites of inflammation or infection during activation of the complement system.[1] C5a has been implicated in several inflammatory conditions. For example, C5a is a potent chemoattractant of leukocytes and phagocytes and stimulates via cell surface receptors (C5aR) both the upregulation of integrins on the cell surface, and cell degranulation at the source of inflammation, leading to endothelial damage.[2] Blocking the action of C5a on its receptor should provide an effective treatment for a variety of inflammatory diseases.

A C-terminal analogue of C5a has been reported to show potent agonism of the C5aR,[3] while conformationally restricted cyclic analogues are antagonists.[4] Few non-peptidic C5a receptor ligands have been reported in the literature.[5] Of these, both agonists,[6] and isolated reports of antagonists[7] are represented.

Our own efforts towards a small, orally bioavailable C5a receptor antagonist began with the hit, **1** (IC_{50} 1 μM, Figure 1), from a high throughput radiolabelled C5a receptor binding screen of the corporate compound file. **1** was then elaborated using parallel chemistry[8] to the potent (IC_{50} 31 nM), although lipophilic and poorly soluble project lead **CP-447,697**, **2** which fails the Lipinski rule-of-5[9] on two counts (MWt. > 500 and Log*P* > 5) and would be expected to be poorly absorbed and rapidly metabolized (see Table 4 below). In the benzothiophene and aniline groups, **2** also contains two potentially toxic functional groups,[10] the replacement of which was a priority.

This paper describes our attempts to improve the binding affinity of **2**, while also improving its physicochemical properties towards a compound suitable for oral dosing. The targeted parameters were IC_{50} < 100 nM, Log*D* < 4.0 and a MWt. < 500, in a series devoid of any potentially toxic functional groups.

Lead compound **2** and direct piperidine analogues were synthesized in a straightforward manner according to Scheme 1. Cyclohexane derivatives were synthesized from cyclohexan-1,4-dione monoethylene ketal according to Scheme 2. After reductive amination and acylation, ketal deprotection revealed the ketone, which

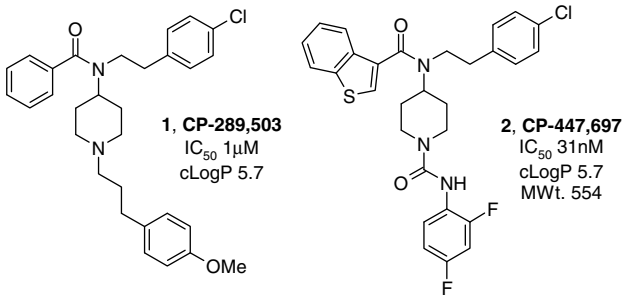

Figure 1. The HTS hit, **1**, and the project lead **2**, obtained following extensive library modification of **1**.

* Corresponding author.
E-mail address: David.Pryde@pfizer.com (D.C. Pryde).

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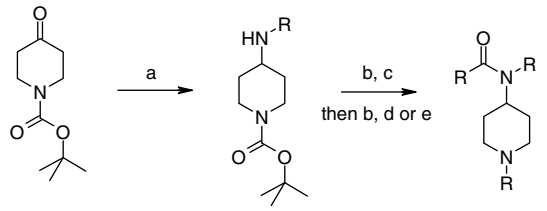
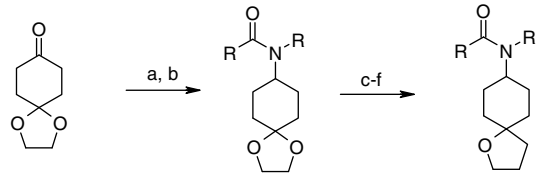
doi:10.1016/j.bmcl.2008.08.106



Scheme 1. Preparation of **2** and analogues. Reagents and conditions: (a) RNH_2, $Na(AcO)_3BH$, DCM, rt, 18 h. (b) RCO_2H, $(COCl)_2$, 1 drop DMF, DCM, rt, 1 h. (c) TFA, DCM, rt, 6 h. (d) RNCO, Et_3N, DCM, rt, 5 h. (e) RHal, K_2CO_3, MeCN, reflux, 16 h.



Scheme 2. Preparation of cyclohexane derivatives of compound **2**. Reagents and conditions: (a) RNH_2, $Na(AcO)_3BH$, DCM, rt, 18 h. (b) RCO_2H, $(COCl)_2$, 1 drop DMF, DCM, rt, 1 h. (c) 2 N HCl, THF, 60 °C, 3 h. (d) AllylMgBr, THF, 0 °C, 4 h. (e) 9-BBN, THF, reflux, 4 h then H_2O_2, NaOH, rt, 3 h. (f) PPh_3, CBr_4, DCM, rt, 16 h.

was reacted with a hydride source or a Grignard reagent.[11] In the case illustrated in Scheme 2, allylation of this ketone, followed by olefin hydroboration/oxidation and cyclisation led to the tetrahydrofurans. Heterocyclic derivatives of the piperidine/cyclohexane were prepared by simple displacement of halo-substituted heterocycles with an amine (according to step (e) in Scheme 1 below), followed by acylation.

This simple, modular chemistry was especially amenable to parallel chemistry in 96-well plate format, allowing rapid modification of all 3 substituents around the central amide N atom through simple variation of the amine, acyl or cycloalkyl-/heteroaryl component.

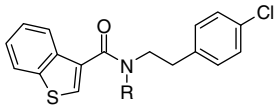
Biological activity was assessed initially using a radiolabelled C5a binding assay in U937 cells to generate compound IC_{50}s.[12] A secondary, functional screen assessed the inhibition of C5a-induced elastase release from human neutrophils.[13] Table 1 summarises the activity and lipophilicity of some piperidine analogues of **2**. Simple amide and amine derivatives, **3**–**5**, were significantly less potent than the parent urea. An early breakthrough came with the discovery that the entire piperidine substituent could be excised, with the ketone **6** showing good activity at 350 nM. Further encouragement came with the *trans*-alcohol **7**, some threefold more active than its *cis* counterpart, and with the synthetically very accessible ketal **9**, equipotent with **7**. Heterocyclic derivatives **10** and **11** were inactive, suggesting lipophilic groups to be favoured. This was borne out by the good activity of the cyclohexyl **12** and benzyl **13** analogues. The most potent cyclohexyl substituent proved to be the *cis* tetrahydrofuran (THF) **14**. When tested in the functional screen, both **7** and **14** lost some 15-fold activity, with the ketal **9** losing some fourfold in potency. The origin of these drop offs in functional potency was unclear, but could be due to non-specific binding of these lipophilic compounds to membranes or variability in neutrophil sensitivity to a C5a challenge.

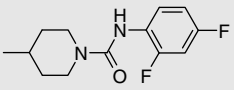
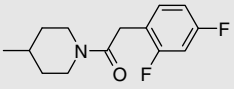
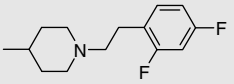
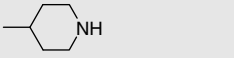
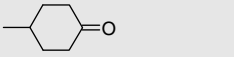
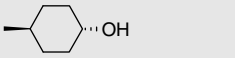
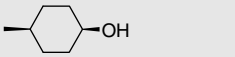
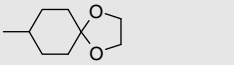
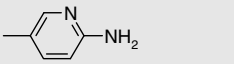
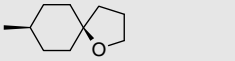
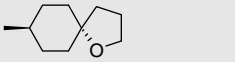
Due to ease of synthetic accessibility, the ketal was chosen as the most suitable group around which to explore further SAR's, despite its obvious acid lability (see Table 4 below). The tetrahydrofuran and *trans* alcohols offered more robust functionality to swap at a later stage.

The next area we looked at was the acyl benzothiophene group. We were particularly concerned about this group being S-oxidised in vivo and resulting toxicity through addition of plasma proteins,[10] and were keen to replace it. As can be seen in Table 2, initial attempts to do this, even in very close analogues, for example, **16** and **17**, were disappointing. It quickly became apparent that an *o*-substituted aryl amide was a crucial pharmacophoric element. This was particularly true if the *o*-substituent were non-polar.

Hence, while *m*-benzoyl, polar *o*-substituents or heterocyclic analogues (**18**, **20**–**22**) were weaker than benzothiophene, 1-naphthyl **19**, was of equivalent potency, and the *o*-alkyl benzoyl deriv-

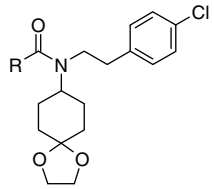
Table 1
C5a receptor binding and functional activity for a series of piperidine analogues of compound **2**

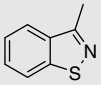
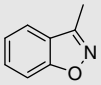
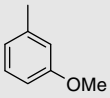
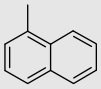
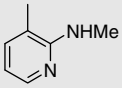
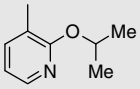
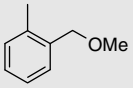
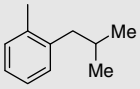
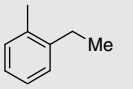
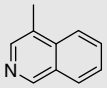
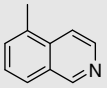


Compound	R	^{125}I Binding affinity IC_{50}[a] (nM)	Functional activity IC_{50}[a] (nM)	*c*Log*P* (Log*D*)[b]
2		31	250	5.6
3		330	nd	5.5
4		200	3000	6.7 (4.1)
5		>1000	nd	3.6
6		350	nd	4.0
7		70	1000	4.4 (4.9)
8		200	nd	4.4
9		75	300	4.9 (5)
10		>1000	nd	5.7
11		>1000	nd	4.0
12	Cyclohexyl	175	nd	6.5
13	Benzyl	370	nd	6.2
14		5	77	5.6
15		200	nd	5.6

[a] Values are means of at least two experiments.
[b] Measured at pH 7.4 in octanol/neutral buffer. nd = not determined.

Table 2
C5a receptor binding and functional activity for a series of benzothiophene analogues of compound **9**

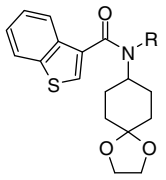


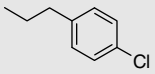
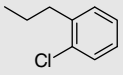
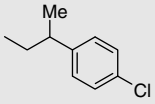
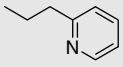
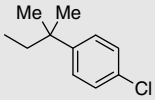
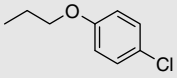
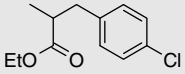
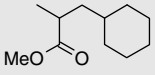
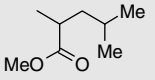
Compound	R	^{125}I Binding affinity IC_{50}[a] (nM)	Functional activity IC_{50}[a] (nM)	cLog*P* (Log*D*)[b]
9		75	300	4.9 (5)
16		>1000	nd	3.7
17		>1000	nd	3.6
18		1000	nd	4.0
19		80	nd	4.9
20		>1000	nd	3.2
21		835	nd	3.9
22		175	nd	3.5
23		1.5	29	5.7
24		18	nd	4.8
25		400	1600	3.8
26		110	1360	3.7 (4.0)

[a] Values are means of at least two experiments.
[b] Measured at pH 7.4 in octanol/neutral buffer. nd = not determined.

atives **23** and **24** were much more active. The latter two compounds were of particularly poor metabolic stability and solubility and were not pursued further. An investigation into introducing polarity into the naphthyl ring through insertion of N atoms was made. The best of these were **25** and particularly **26,** of comparable potency to naphthyl and a log unit more polar.

Table 3
C5a receptor binding and functional activity for a series of *p*-chloro-phenethylamine analogues of compound **9**



Compound	R	^{125}I Binding affinity IC_{50}[a] (nM)	Functional activity IC_{50}[a] (nM)	cLog*P* (Log*D*)[b]
9		75	300	4.9 (5)
27		300	nd	4.9
28		35	150	5.3
29		>1000	nd	2.7
30	Benzyl	395	nd	4.0
31		90	300	5.7
32		435	nd	5.4
33		5	30	5.6
34		89	nd	6.0
35		290	nd	4.9

[a] Values are means of at least two experiments.
[b] Measured at pH 7.4 in octanol/neutral buffer. nd = not determined.

The final area of SAR exploration around **2** was the chlorophenethylamine group (Table 3). Conformational locks in the ethyl chain provided modest gains in potency (**28** and **31**) while shortened/extended linkers (**30** and **32**), chlorophenyl isomers (**27**) or heterocyclic analogues (**29**) of the phenyl group all lost potency.

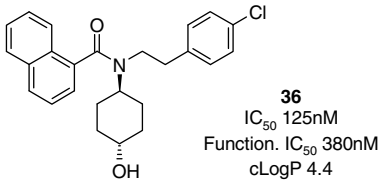
Table 4
Human liver microsome stability and pharmacokinetic data for selected compounds

Compound	cLog*P* (Log*D*[a])	HLM $T_{1/2}$ (mins)	Pharmacokinetic parameters
2	5.6	11	nd
7	4.4 (4.9)	14	Rat: 0.5 mg/kg *iv*, 10 mg/kg *po* $T_{1/2}$ 0.5 h, Cl 67 ml/min/kg, V_d 3 L/kg, *F* 0.5%
26	3.7 (4.0)	10[b]	nd
36	4.3 (>5)	20	Dog: 0.5 mg/kg *iv*, $T_{1/2}$ 1.4 h, Cl 29 ml/min/kg, V_d 3.5 L/kg

[a] Measured at pH 7.4 in octanol/neutral buffer.
[b] Primary metabolite was the alcohol, via hydrolysis and reduction of the ketal.
nd, not determined.

Amino-acid ester derived side chains (**33**), showed good primary and functional potency, but attempts to then saturate/reduce the chlorophenyl group in compounds **34** and **35** were not successful. Overall, the chlorophenyl group appeared to offer the best balance of potency in a relatively small side chain.

At regular intervals throughout the above SAR explorations, progress towards our goal of a compound suitable for oral dosing was assessed. Table 4 summarises some in vitro and in vivo data for a selection of compounds. The benzothiophene analogues **2** and **7** suffered from very short hepatic microsomal half-lives, the latter exhibiting low oral bioavailability in the rat due to high Cl with respect to liver blood flow (67 vs 80 ml/min/kg), combined with a potential for incomplete oral absorption due to poor aqueous solubility. Both of these issues relate to the high lipophilicity. Metabolite identification with the quinolinyl ketal **26** confirmed our expectations of the dioxolanyl group being readily metabolized. The naphthyl group had emerged as one of the more effective benzothiophene replacements, which was combined with a *trans* cyclohexyl alcohol in compound **36**, which at IC_{50} 125 nM, demonstrated comparable potency to its dioxolanyl direct analogue.



36
IC_{50} 125nM
Function. IC_{50} 380nM
cLogP 4.4

Following *iv* administration to dog, **36** exhibited moderate clearance with respect to liver blood flow (29 vs 50 ml/min/kg). However, when combined with its moderate volume of distribution this led to a short elimination $T_{1/2}$. The microsomal half-life suggested that clearance in man would also be moderate to high, with respect to liver blood flow, indicating that **36** would be likely to exhibit moderate to low oral bioavaliability and a short elimination $T_{1/2}$ in man.

In conclusion, no compounds from this investigation had the appropriate combination of functional potency and physicochemical properties to be therapeutically useful oral agents. The data in Table 4 clearly shows a need to reduce lipophilicity and clearance in the series to provide a compound with a reasonable oral pharmacokinetic profile. The above SAR indicates the difficulty in achieving this goal through introduction of polar functionality or downsizing without compromising potency. An alternative approach wherein ionisable groups were targeted, is detailed in the second paper in this series.

Acknowledgments

The authors gratefully acknowledge the technical support of Chris Carr, Kevin Coote, Katherine Grant, Vicky Stubbs, Alan MacInnes, Tony Chuck, Christopher Luckhurst, Paul Glossop, David Beal, Lindsey Sprigens, Fidelma Atkinson and Alison Harper.

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11. Hydride reduction of cyclohexanones forms predominantly *trans* alcohols, while larger nucleophiles favour *cis*. Isomers were separable by chromatography.
12. Bound C5a was measured at 4 °C using dibutyryl cAMP-differentiated U937 cells and a filtration assay using 160pM ^{125}I-labelled r-hC5a (NEN) to measure bound radioligand.
13. Neutrophil degranulation was measured in cytochalasin B-primed isolated human neutrophils at 37 °C by quantification of released elastase by cleavage of the chromogenic MeOSuc-Ala-Ala-Pro-Val-pNA. C5aR antagonists were pre-incubated with neutrophils for 5 min at 37 °C prior to a 1 nM r-hC5a (Sigma) challenge.