Bioorganic & Medicinal Chemistry Letters 18 (2008) 5605–5608

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Small, non-peptide C5a receptor antagonists: Part 2

Julian Blagg [a], Charles Mowbray [a], David Pryde [a,*], Gary Salmon [b], David Fairman [b], Esther Schmid [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
Antagonists
Inflammation

ABSTRACT

Starting from **2**, several highly potent C5a receptor antagonists were synthesised through α-amide substitution. Attempts to increase the polarity of these compounds through the introduction of basic centres or incorporation into weakly basic heterocycles is described.

C5a is a 74-amino acid peptide cleaved from C5 at sites of inflammation or infection during activation of the complement system.[1] Inappropriate activation of this mechanism can lead to the C5a-mediated chemoattraction and activation of granulocytes leading to the production of oxygen respiratory burst products through degranulation, and ultimately tissue damage.[2] C5a has been implicated in a variety of inflammatory diseases, including rheumatoid arthritis, and antagonists of the C5a receptor are of significant therapeutic interest.

The two letters in this series describe our attempts to develop a series of small, non-peptide C5a receptor antagonists with sub-100 nM potency suitable for oral delivery. Several series of C5a receptor antagonists are known in the literature,[3] but there are only isolated reports of orally active compounds being progressed into the clinic.[4]

In the preceding letter,[5] we described our efforts to improve the potency and physical properties of a high-throughput screen-derived lead compound **1** towards a profile commensurate with oral dosing (Fig. 1). The targeted properties were IC_{50} <100 nM, a Log *D* of below 4 and a molecular weight below 500, in a series devoid of any potential toxicophores. While we were able to make progress on all these fronts e.g., with compound **2**, primarily by downsizing, the compounds which emerged from this work were still quite lipophilic and resisted all attempts at introducing more polar functionality and retaining potency. They consequently suffered from

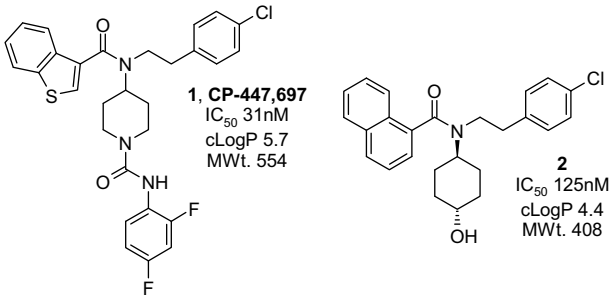


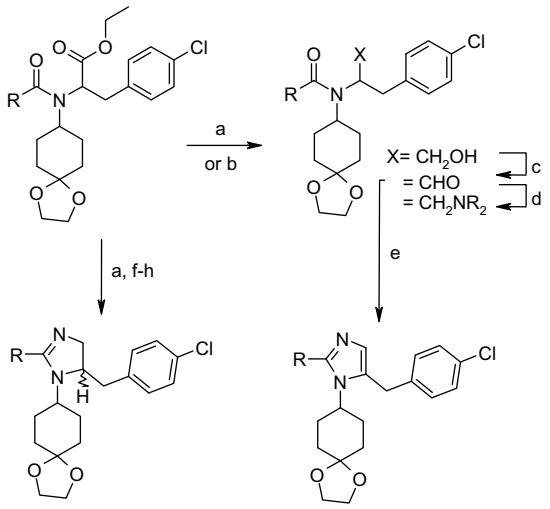
Figure 1. Project lead **1**, and initial progress from the first phase of the project, summarised in compound **2**.

unacceptable systemic clearance and short in vivo $T_{1/2}$, unsuitable for oral administration.

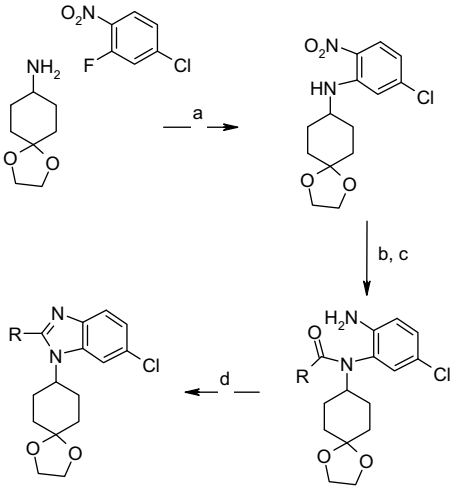
In this letter, we describe our attempts to take the learning obtained en route to **2** and drive down lipophilicity further through the synthesis of basic, i.e., charged, compounds. The syntheses of **1** and **2** have been described already.[5] α-Substituted amides were prepared according to Scheme 1 from readily available α-amino esters, en route to the derivative imidazoles and imidazolines. Notable synthetic transformations in the sequence include the ammonium acetate mediated cyclisation of α-amido aldehydes to imidazoles,[6] and the intramolecular cyclisation of an iminophosphorane derived from a β-azido amide to prepare imidazolines.[7] Analogous benzimidazoles were synthesised (Scheme 2) by an initial fluoro displacement of a fluoro-nitro-benzene, followed by

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

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.08.101



Scheme 1. Preparation of α-amido substituents, imidazoles and imidazolines: (a) $LiBH_4$, THF, rt, 6 h; (b) K_2CO_3, MeOH, H_2O then carbonyl diimidazole, THF then $NaBH_4$, EtOH, H_2O, rt, 4 h; (c) $(COCl)_2$, DMSO, DCM, Et_3N, −78 °C rt, 1 h; (d) R_2NH, $Na(OAc)_3BH$, DCM, AcOH, rt, 16 h; (e) NH_4OAc, AcOH, 85 °C, 16 h ; (f) MsCl, DCM, Et_3N, 0 °C, 1 h ; (g) LiN_3, DMF, 100 °C, 16 h ; (h) Ph_3P, THF, H_2O, reflux, 1 h.

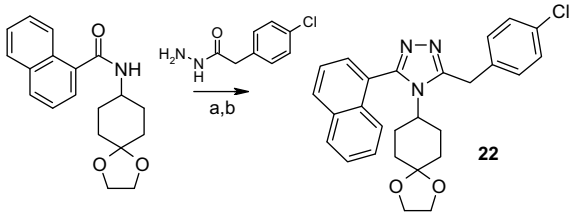


Scheme 2. Preparation of benzimidazoles: (a) Et_3N, MeCN, rt, 6 h; (b) Fe, $CaCl_2$, EtOH, H_2O, reflux, 2 h; (c) RCOCl, DCM, Et_3N, rt, 1 h; (d) AcOH, 85 °C, 16 h.

amide formation, mild Fe-mediated nitro reduction[8] and cyclisation.

Triazole **22** was prepared by modification of a known literature method,[9] from a thioamide and cyclising with an acyl hydrazide (Scheme 3).

Compound IC_{50} values were generated using a radiometric binding assay in cyclic-AMP differentiated U937 cells.[5] Two screens were used to assess functional potency. The majority of



Scheme 3. Preparation of triazoles: (a) Lawesson's reagent, THF, rt, 5 h; (b) $Hg^{II}O$, $^{n}BuOH$, reflux, 16 h.

compounds were assessed for their ability to inhibit C5a-induced release of elastase from human neutrophils during degranulation using a *p*-nitroanilide substrate.[5] A more accurate estimate of the functional potency of compounds in whole human blood was also obtained from a second screen in which the concentrations of selected compounds required to shift the dose–response curve by 10-fold for C5a-induced upregulation of the cell-surface integrin CD11b in whole blood was measured.[10]

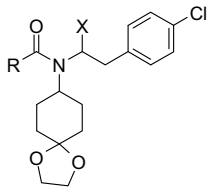
In the preceding letter, reducing the lipophilicity of the lead compound **1** proved to be difficult in a neutral series through size reduction or especially by the introduction of polar functionality.[5] In the second phase of the project, we sought a region of the molecule where a full basic centre could be tolerated, to drive down lipophilicity. In the Merck diaminoquinoline series, positively charged ligands were tolerated,[3a] albeit weakly in what was believed to be the transmembrane region of the receptor where we assumed our series bound, offering some evidence that this approach might work.

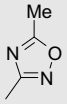
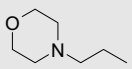
Extensive modification of the acyl substituent or the piperidine group in **1** afforded some improvements in potency and some small reductions in lipophilicity.[5] The chlorophenethyl region of **1**, however, appeared to offer a useful handle for diverse modification. Introducing conformational locks produced gains in potency. One such lock was an α-ester function, which offered excellent potency, viz. compounds **3** and **5**. Both compounds were still lipophilic, but it became apparent that a range of functionality could be tolerated in this position (Table 1). Reduction of the esters to the hydroxymethyl **4** lost all potency, while hydrolysis to the carboxylic acid **6** retained some activity at 350 nM. Ester isosteres such as the oxadiazole **7** and the methoxymethyl analogue **8** were equipotent with the esters. The methyl ketone **9** was slightly less potent than the original esters. This early SAR was very promising in delivering several sub-15 nM compounds, including several with sub-50 nM activity in the functional screen. However, these potent compounds remained lipophilic, and we had not made inroads into reducing the overall Log*D* of the series, which the early results taught us was crucial for an acceptable oral agent. Several amines were synthesised in the α-amido position in an attempt to drive Log*D* down. The first amines **10** and **11** were encouragingly active in binding and almost equipotent in the functional assay. However, we were surprised to find that both compounds had higher than expected measured Log*D*s. Upon measuring the pK_as for **10** and **11**, they had a much lower than expected basicity, at pK_a only 7.4, due to steric crowding of the N atom (cf. values in Table 2).

Greater basicity would be needed if the strategy of polarity through basicity were to be successful. Extending the basic group away by a methylene to provide a more basic N atom, viz. **12**, lost all potency. This potency drop was somewhat ameliorated by alteration of the basicity through incorporation into a morpholine ring **13**, but even here, the potency was only 200 nM. Table 2 shows the pK_as for a range of compounds from Table 1. We began to explore other regions where a full (pK_a > 8) basic centre would be tolerated. From our earlier work,[5] ethers were known to be tolerated in the *ortho* position of a benzoyl substituent, and this seemed a likely area to incorporate a more exposed amine. The best of these was compound **14**, which did show the expected basicity and encumbent drop in Log*D* to 3.3, but again, this polarity had compromised potency, suggesting that a pK_a of 8 and above was poorly tolerated.

Also shown in Table 2 are some human plasma protein binding data for selected compounds, which illustrates the highly bound nature of these lipophilic bases. Non-specific membrane binding was one possible explanation for the variable drop in functional activity seen for many of the compounds. This would be expected to be particularly pronounced in a whole blood environment, where high plasma protein binding would reduce free drug levels available for receptor binding even further. In an alternative

Table 1
Binding affinity for α-amido analogues of **2** and related compounds



Compound	R	X[b]	^{125}I binding affinity IC_{50}[a] (nM)	Elastase release functional activity IC_{50}[a] (nM)	*C*Log*P* (Log*D*[c])
3	1-Naphthyl	CO_2Et	3	32	5.6
4	3-Benzo-thiophene	CH_2OH	>1000	nd	4.4
5	3-Benzo-thiophene	CO_2Me	15	172	5.0
6	2-Ethyl-phenyl	CO_2H	350	nd	4.8
7	2-Ethyl-phenyl		8	40	3.9 (>5)
8	3-Benzo-thiophene	CH_2OMe	12	27	5.0
9	3-Benzo-thiophene	C(O)Me	45	nd	4.8
10	1-Naphthyl	CH_2NMe_2	13	22	5.1 (4.6)
11	2-Ethyl-phenyl	CH_2NMe_2	27	25	5.0 (>5)
12[d]	2-Ethyl-phenyl	(*S*)-$CH_2CH_2NMe_2$	>1000	nd	3.5
13[d]	2-Ethyl-phenyl	(*S*)- 	200	nd	3.8 (3.4)
14	2-(2-Dimethylaminomethyl)-phenyl	H	500	nd	3.6 (3.3)

[a] Values are means of at least two experiments.
[b] Compounds are racemates unless indicated otherwise.
[c] Log*D* measured at pH 7.4 in octanol/neutral buffer. nd, not determined.
[d] These compounds were made in the *des*-chloro-phenyl series. The *S* enantiomers are approximately two orders of magnitude more potent than the *R*.

Table 2
Potency and physical property data for selected compounds

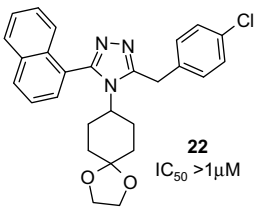
Compound	^{125}I binding affinity IC_{50}[b] (nM)	pK_a	Human ppb (%)	CD11b upreg. functional activity (free)[b,c] (nM)	Binding/ functional translation[b,d] (free) (nM)
10	13	Est. 7.4[a]	nd	nd	nd
11	27	7.4	99.6	4800 (17)	178 (0.63)
12	>1000	Est. 8.5[a]	nd	nd	nd
13	200	Est. 7.1[a]	97.3	4400 (119)	22 (0.60)
14	500	8.4	99.6	>10,000 (>40)	nd

[a] Estimated using ACD labs prediction software.
[b] Values are means of at least two experiments.
[c] Expressed as both the total and free drug concentrations required to produce a 10-fold shift in the C5a-induced CD11b upregulation dose–response curve.
[d] Based on the CD11b upregulation functional potency. nd, not determined.

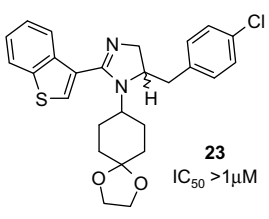
assessment of functional potency, a small subset of compounds from Table 2 were assessed in a complementary assay in which the concentrations of compound required to shift the dose–response curve for the C5a-induced upregulation of the neutrophil cell-surface integrin CD11b in human whole blood by a factor of 10 was measured.[10] The CD11b potency of compounds **11**, **13** and **14** expressed as total drug showed a common large drop off from binding activity. Expressing these functional potencies as free drug levels gives an excellent correlation with binding and elastase release data.

If only mildly basic centres were tolerated, our next plan was to attempt to cyclise some of the α-amido compounds from Table 1 to form a series of more compact, and weakly basic heterocyclic cores. Through cyclising and reducing rotational freedom, it was hoped that gains in potency could also be made. This strategy would also provide the option to explore substitution at the equivalent of the α-amide position, which we already knew tolerated much functionality. Table 3 shows the key data for a selection of heterocycles based on this premise, the majority of which were imidazoles or benzimidazoles. Pleasingly, the naphthyl imidazole **15** had good activity at 90 nM, and equally gratifyingly, the Log*D* had come down to 4.1. Surprisingly, the benzothiophene analogue **16** was significantly less active, but the replacement of chlorophenyl with cyclohexylmethyl **17** restored potency.

A more polar 2-imidazo substituent such as 3-OMe phenyl **18** was inactive, consistent with our earlier findings. Similarly, the benzimidazoles **20** and **19** were quite weak binders, and suffered from very poor aqueous solubility. Interestingly, insertion of a methyl group in the 4-position of the imidazole ring in **21** gave a >5-fold improvement in binding potency, albeit with the Log*D* of this compound creeping above 4.5 again.

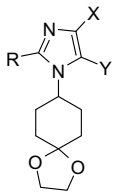


22
IC_{50} >1μM



23
IC_{50} >1μM

Table 3
Activity and lipophilicity data for heterocyclic templates



Compound	R	X	Y	^{125}I binding affinity IC_{50}[a] (nM)	Functional activity IC_{50}[a] (nM)	Log*D*[b]
15	1-Naphthyl	H	CH_2(*p*-Cl-phenyl)	90	877	4.1[c]
16	3-Benzo-thiophene	H	CH_2(*p*-Cl-phenyl)	500	nd	>4
17	3-Benzo-thiophene	H	CH_2-cyclo-hexyl	47	850	3.8[d]
18	3-Methoxy-phenyl	H	CH_2(*p*-Cl-phenyl)	>1000	nd	4.0
19	3-Benzo-thiophene	3,4-diCl-phenyl	790	nd	>5	
20	3-Benzo-thiophene	3-Cl-phenyl	325	nd	nd	
21	3-Benzo-thiophene	Me	CH_2(*p*-Cl-phenyl)	90	nd	4.6

[a] Values are means of at least two experiments.
[b] Log*D* measured at pH 7.4 in octanol/neutral buffer.
[c] pK_a estimated as 6.9 using the ACD Labs prediction software.
[d] pK_a estimated as 7.3 using the ACD Labs prediction software. nd, not determined.

Both the triazole **22** and the much more basic imidazoline **23** were inactive. The heterocyclic core compounds were not as potent as hoped for, and the only way to install higher potency into them would be to increase their size and lipophilicity. We had obtained no encouragement that a basic centre could rescue the physical properties of the compounds and achieve our target goal of Log*D* <4. The compounds which came closest to meeting these criteria were **13** and **15**. The payoff in installing polarity into these structures was only modest potency. Sub-10 nM potency was only achieved in compounds which had a Log*D* >4. Our conclusions from this piece of work were similar to those after the initial investigations described in the previous paper, that the C5a receptor did not tolerate strong polarity or basicity in the transmembrane region where we assumed our molecules were binding. As such, it would be difficult to achieve an appropriate balance of potent C5a receptor binding potency and physical properties commensurate with oral dosing in this series.

Our studies and conclusion on this series of C5a receptor antagonists provides another case to support the increasingly popular view[11] that development candidates and drugs often closely resemble the starting point for the programme and the counterpoint that it is very difficult to turn a fundamentally flawed lead into a quality development candidate.

Acknowledgments

The authors gratefully acknowledge the technical support of Chris Carr, Kevin Coote, Katherine Grant, Vicky Stubbs, Tony Chuck, Christopher Luckhurst, Paul Glossop, Lindsey Sprigens, David Beal, Marie-Blanche Harnois and Alison Harper.

References and notes

1. Goldstein, I. M. In *Inflammation: Basic Principles and Clinical Correlates*; Gallin, J. I., Goldstein, I. M., Snyderman, R., Eds.; Raven Press: NY, 1988.
2. Gerard, C.; Gerard, P. N. *Ann. Rev. Immunol.* **1994**, *12*, 775.
3. For example, see: (a) Lanza, T. J.; Durette, P. L.; Rollins, T.; Siciliano, S.; Cianciarulo, D. N.; Kobayashi, S. V.; Caldwell, C. G.; Springer, M. S.; Hagmann, W. K. *J. Med. Chem.* **1992**, *35*(2), 252; (b) Wong, A. K.; Taylor, S. M.; Fairlie, D. P. *Idrugs* **1999**, *2*(7), 686; (c) Taylor, S. M.; Fairlie, D. P. *Expert Opin. Ther. Pat.* **2000**, *10*(4), 449. see also; d Welfide Corp., WO Patent 0222556, 2002.; e Neurogen Corp., WO Patent 0251414, 2002; WO Patent 082829, 2003.; (f) Sumichika, H.; Sakata, K.; Sato, N.; Takeshita, S.; Ishibuchi, S.; Nakamura, M.; Kamahori, T.; Ehara, S.; Itoh, K.; Ohtsuka, T.; Ohbora, T.; Mishina, T.; Komatsu, H.; Naka, Y. *J. Biol. Chem.* **2002**, *277*, 49403; (g) Allegretti, M.; Moriconi, A.; Beccari, A. R.; Bitondo, R. D.; Bizzarri, C.; Bertini, R.; Colotta, F. *Curr. Med. Chem.* **2005**, *12*, 217; (h) Hutchison, A. J.; Krause, J. E. *Ann. Rep. Med. Chem.* **2004**, *39*, 139–147; (i) Barbay, J. K.; Gong, Y.; Buntinx, M.; Li, J.; Claes, C.; Hornby, P. J.; Lommen, G. V.; Van Wauwe, J.; He, W. *Bioorg. Med. Chem. Lett.* **2008**, *18*(8), 2544; (j) Chemocentryx Inc., WO Patent 07051062, 2007.; (k) Jerini A. G. WO Patent 06128670, 2006.
4. See www.promics.com and related links for information on PMX053.
5. See previous letter in this issue.
6. Beccalli, E. M.; Majori, L.; Marchesini, A.; Torricelli, C. *Chem. Lett.* **1980**, 659.
7. Vaultier, M.; Knouzi, N.; Carrié, R. *Tetrahedron Lett.* **1983**, *24*(8), 763.
8. Njoroge, F. G.; Vibulbhan, B.; Rane, D. F.; Bishop, W. R.; Petrin, J.; Patton, R.; Bryant, M. S.; Chen, K.-J.; Nomeir, A. A.; Lin, C. C.; Liu, M.; King, I.; Chen, J.; Lee, S.; Yaremko, B.; Dell, J.; Lipari, P.; Malkowski, M.; Li, Z.; Catino, J.; Doll, R. J.; Girijavallabhan, V.; Ganguly, A. K. *J. Med. Chem.* **1997**, *40*(26), 4290.
9. Reiter, L. A.; Berg, G. E. *Heterocycles* **1992**, *34*(4), 771.
10. CD11b upregulation was measured in heparinised human blood by flow cytometry following C5a stimulation for 5 min at 37 °C. C5aR antagonists were pre-incubated with blood for 1 min at 37 °C prior to C5a stimulation.
11. Proudfoot, J. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1647.