Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 7, pp. 907-912, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00124-8

NEW NON PEPTIDIC C5a RECEPTOR ANTAGONISTS

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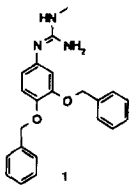
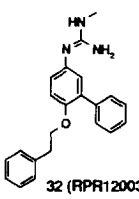
Abstract : A series of phenylguanidines which bind to the C5a receptor has been developed. The lead compound **1** (IC_{50}=30μM), discovered through random screening, has been modified to provide **32** (RPR121154) with submicromolar activity. This compound was shown to further elicit functional antagonism in a human neutrophil C5a stimulated respiratory burst assay. © 1997 Elsevier Science Ltd.

Introduction

The complement component C5a is a 74-amino acid glycoprotein generated upon activation of the complement system. C5a is an anaphylatoxin involved in recruitment of leukocytes to sites of inflammation. It causes degranulation and release of respiratory burst products from neutrophils and also stimulates adhesion molecule expression on both leukocytes and endothelial cells. Consequently, antagonists of the C5a receptor may have therapeutic potential in pathological conditions involving leukocyte recruitment and activation.[1] Although a number of peptides binding to the C5a receptor have been published,[2-4] very few non peptidic ligands have been disclosed.[5-7] Reported here is the discovery and optimisation of a new series of substituted phenylguanidines that bind to the C5a receptor.



1



32 (RPR120033)

Results and discussion

Preliminary screening of a randomly selected set of 10000 compounds led to the identification of **1** with an IC_{50} of 30μM in the C5a binding assay. Systematic modification of the structure was then undertaken with the guanidine moiety at R1 considered first. Replacement of the methyl guanidine with unsubstituted guanidine **2** and ethyl guanidine **3** led to a loss in activity. Replacement of the guanidine group with amidines **5-7** provided inactive compounds, as did the extended guanidine at R1 **4** and replacement with other simple basic groups as in the aniline **8** or the benzylamine **9**. The optimum group at R1 remained the methyl guanidine (Table 1).

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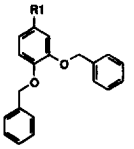
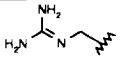
Table 1. Binding affinity to C5a receptor. Modification of R1:

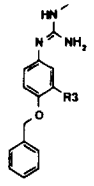


Compd.	R1	IC_{50}	Compd.	R1	IC_{50}	Compd.	R1	IC_{50}
1		30 μM	**4**		IA[1]	**7**		IA
2		>100 μM	**5**		IA	**8**		IA
3		40% @ 30μM	**6**		IA	**9**		IA

[1] IA: inactive, i.e. activity < 20% @30 μM.

Retaining R1 as a methyl guanidine the R3 and R4 groups were independently modified. Shortening of the R3 benzyloxy group of **1** to a phenoxy group as in **10** resulted in improvement in activity. In contrast elongation of the chain to give the phenethyloxy derivative **11** abolished all activity. Further shortening of the chain to the biphenyl derivative **13** provided a 15 fold increase in activity when compared to **1**. However, substitution of the phenyl ring with ortho or para groups **16,19-21** abolished or substantially decreased activity, whilst meta substituents **17,18** decreased activity significantly. Other aromatic rings, such as 1-naphthyl **14**, or 2-thiophenyl **15**, also gave reduced activities. Replacement of the phenyl group with a cyclopentyl group led to a loss in activity (compare **10** and **12**), implying the interacton with the receptor at R3 is aromatic. The unsubstituted phenyl was found to be the optimum group at this position. The results are summarised in Table 2:

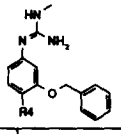
Table 2. Binding affinity to C5a receptor. Modification of R3:

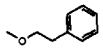
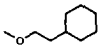
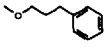
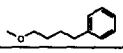


Compd.	R3	IC_{50} (μM)	Compd.	R3	IC_{50} (μM)	Compd.	R3	IC_{50} (μM)
10		4	**14**		4.5	**18**		8
11		IA	**15**		9	**19**		54
12		25	**16**		IA	**20**		20% @30μM
13		2	**17**		9	**21**		50

Modification of the R4 benzyloxy group of **1** is summarised in Table 3. In contrast to R3, shortening of the chain to give the phenoxy derivative **22** led to a loss of activity whilst elongation **23-25** resulted in a beneficial effect. Furthermore, the phenyl group could be replaced by a cyclohexyl group **26** and **27** without loss of activity. A n-butyl group **28** gave activity similar to benzyl and the simple methyl group **29** showed some weak activity. The conclusion from these results is that the interaction with the receptor at this position is hydrophobic.

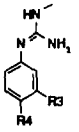
Table 3. Binding affinity to C5a receptor. Modification of R4:

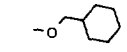
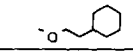


Compd.	R4	IC_{50} (μM)	Compd.	R4	IC_{50} (μM)
22		32% @ 30 μM	**26**		8
23		7.5	**27**		9
24		6	**28**		30
25		4	**29**		20% @ 30μM

Combination of optimum groups at R1, R2 and R3 resulted in the preparation of the most active compound **32** with an IC_{50} of 0.8 μM . Results are summarised in Table 4:

Table 4. Binding affinity to C5a receptor. Modification of R3 and R4:



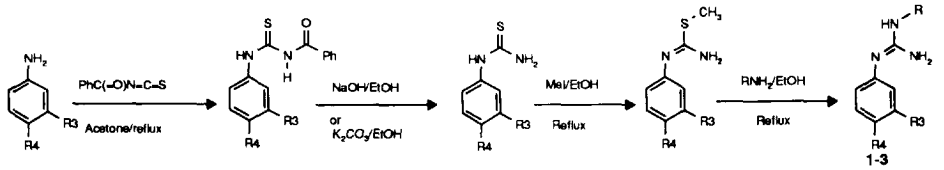
Compd.	R3	R4	IC_{50} (μM)	Compd.	R3	R4	IC_{50} (μM)
30			3	**32**			0.8
31			1	**33**			1

Scatchard analysis of **32** revealed binding to be competitive with C5a. When examined in a human neutrophil C5a stimulated respiratory burst assay the compound exhibited functional antagonist activity (see biology).

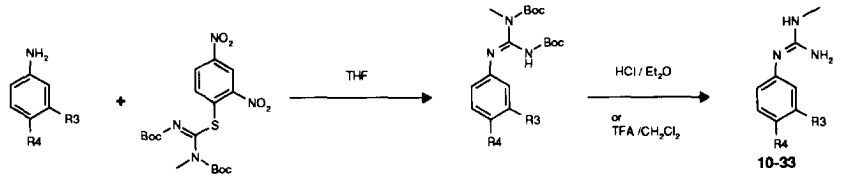
Chemistry

The general synthesis for the phenyl guanidine derivatives **1-3** was performed following literature methodology in 4 steps (scheme 1).[8] The lengthy nature of this procedure prompted the development of a new guanylating agent which permitted the synthesis of the guanidine derivatives **10-33** in two steps from the appropriate aniline (scheme 1-bis).[9]

Scheme 1

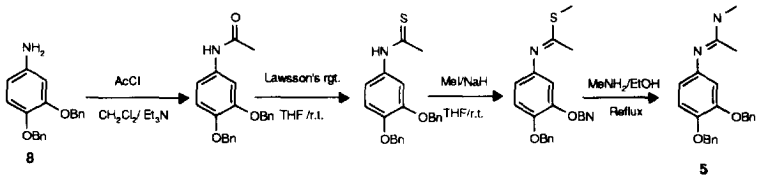


Scheme 1-bis



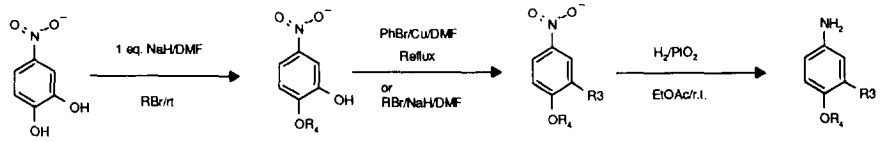
Compound **4** was synthesised by reacting the benzylamine **9**, obtained by reduction of the corresponding nitrile with borane-dimethyl sulfide,[10] with cyanamide following literature method.[8] Compound **5** was prepared following scheme 2. The amidines **6** and **7** were prepared in one step from the corresponding nitrile, using methylchloroaluminium methylamide.[11] The benzylnitrile precursor for **6** was prepared from the commercially available 3,4-dibenzyloxybenzyl chloride in one step from treatment with acetone cyanohydrine,[12] whilst the arylnitrile precursor for **7** was prepared in one step from the commercially available 3,4-dibenzylbenzaldehyde by treatment with $Cu^{(0)}$ and ammonium chloride in pyridine.[13]

Scheme 2

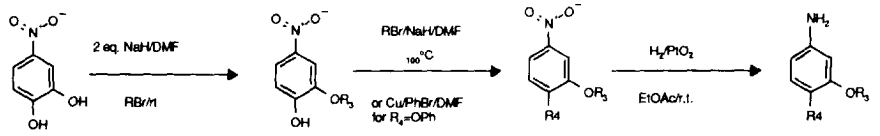


The difference in pKa between the two phenolic groups of 4-nitrocatechol allowed independant modification of R3 and R4. Treatment with one equivalent of base followed by one equivalent of alkylating agent permitted selective introduction of an alkyl group at R4 (Scheme 3). However, repetition of the reaction but with two equivalents of base resulted in selective alkylaton at R3 (Scheme 4). The subsequent phenol in each case was alkylated with different bromoalkanes or reacted with bromobenzene under Ullman conditions to obtain the phenyl ethers before reduction of the nitro group to the corresponding aniline.

Scheme 3

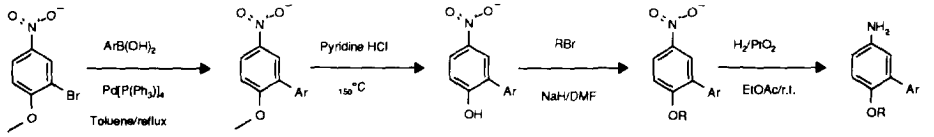


Scheme 4



The 3-aryl derivatives were prepared *via* a Suzuki reaction using commercially available arylboronic acids. The starting material used in this synthesis was the readily available 2-bromo-4-nitroanisole, which was sequentially arylated, demethylated, alkylated and reduced to the corresponding aniline (Scheme 5).

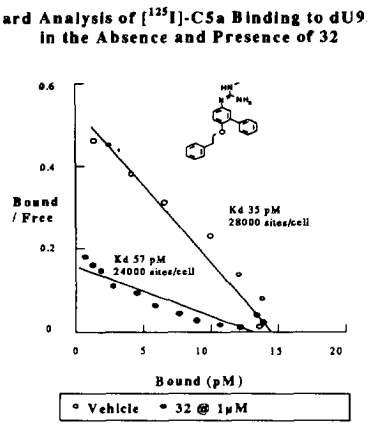
Scheme 5

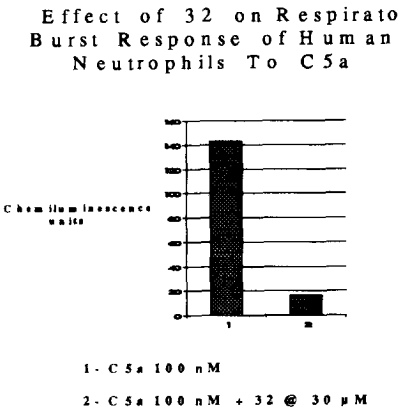


Biology

Primary screening. (*In Vitro* C5a binding). The assay was performed on human monocyte cell line U937. To stimulate C5a receptor expression the cells were cultured for 2 days in 1 mM dibutyryl cAMP. The cells were harvested and frozen at -20°C for use in the binding assay. The assay buffer contained 50mM Hepes, 5mM $MgCl_2$, 0.5% BSA and 0.1% Bacitracin. The assay was performed in a 96 well filtration plate (Millipore) with each well containing 50pM ^{125}I-C5a, the compound and 75000 cells. The final DMSO concentration was 3%. The incubation time was 2 hours at 4°C. The test solutions were filtered and washed and the plate left to dry for 30 minutes. 25µl Microscint 0 was added to each well before counting (Canberra Packard Topcount). The non specific binding was 20nM C5a.

Secondary screening. (*In Vitro* C5a functional assay). Upon activation by C5a, neutrophils release hydrogen peroxide (in a concentration dependant manner), a phenomenon known as the respiratory burst. Human peripheral neutrophils were isolated from fresh whole blood using a Metrizamide density gradient centrifugation method. The neutrophils were counted in a Neubauer chamber and resuspended in Hanks Balanced Salt Solution (HBSS: Sigma) at a concentration of approximately $5x10^7$ cells/ml. Neutrophil suspensions were kept on ice until required for the assay. The respiratory burst was quantified by measuring hydrogen peroxide using a chemiluminescent assay. Approximately $1x10^6$ human neutrophils in a volume of 200µl HBSS were added to 3.5ml Sarstedt tubes containing 200 µml Luminol (Sigma) and the mixtures were preincubated at 37°C for 2 minutes, followed by the addition of C5a or compound and C5a. Light emission was measured in an LKB 1250 luminometer maintained at 37°C and linked to a chart recorder.





Conclusion: We have identified a new series of phenyl guanidines that bind to the C5a receptor. SAR studies on the lead compound **1** (IC_{50} = 30 μM) provided **32** with a submicromolar IC_{50}. Compound **32** proved competitive as shown by the Scatchard analysis, and behaved as a functional antagonist of C5a in the respiratory burst assay.

Acknowledgement: We are grateful to B. Wyman, C. T. Howells, I. Ahmed, J. Archer, S. Aldous, D. Neighbourgh, A. Al-Shaar, R. Petheram, B. Pedgrift and D. Chatterjee for technical suport. We are also grateful to M. Wong and A. Stoppard for the *in vitro* assays.

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(*Received in Belgium* 27 *January* 1997; *accepted* 4 *March* 1997)