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A NONPEPTIDIC AGONIST LIGAND OF THE HUMAN C5A RECEPTOR: SYNTHESIS, BINDING AFFINITY OPTIMIZATION AND FUNCTIONAL CHARACTERIZATION.

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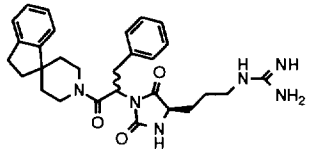
Abstract: The structural optimization for binding affinity and attempted modification of agonist function of a nonpeptide ligand of the human C5a receptor is described.

Introduction

C5a, a 74 amino acid peptide, is generated at sites of inflammation by proteolytic cleavage from the complement factor C5 following initiation of the complement cascade by immune complexes, foreign proteins, or polysaccharides such as those derived from bacterial cell wall fragments.[1] C5a is a chemotaxin that attracts and promotes the degranulation of granulocytes and macrophages at the tissue site where the cascade has been activated. This process, when appropriately initiated, is a protective mechanism that recruits phagocytic cells, stimulates complement receptor bearing cells of the immune system and causes invading cell membrane disruption and lysis. Inappropriate activation has been implicated as a mediator in many inflammatory diseases such as rheumatoid arthritis.[2] Antagonists of C5a are, therefore, of therapeutic interest. Peptidic agonist ligands of the C5a receptor (C5ar) (a precoupled G-protein linked seven helix membrane spanning receptor), as small as six amino acids derived from the C-terminus have been identified.[3,4] A peptidic antagonist of C5a (CO89: NMeF-K-P-dCha-W-dR-OH) was recently reported.[5] A nonpeptidic cationic antagonist of C5a is believed to bind to the acidic N-terminal extra-membrane portion of the hypothetical C5ar structure.[6]

A C5ar ligand lead **1** was identified from the Merck sample collection by screening for species able to displace ^{125}I-C5a from a human neutrophil membrane preparation. The poor affinity of **1** for the receptor (60% displacement at 30 uM) precluded functional characterization of the lead. The optimization of binding affinity and functional characterization of this lead is described below.

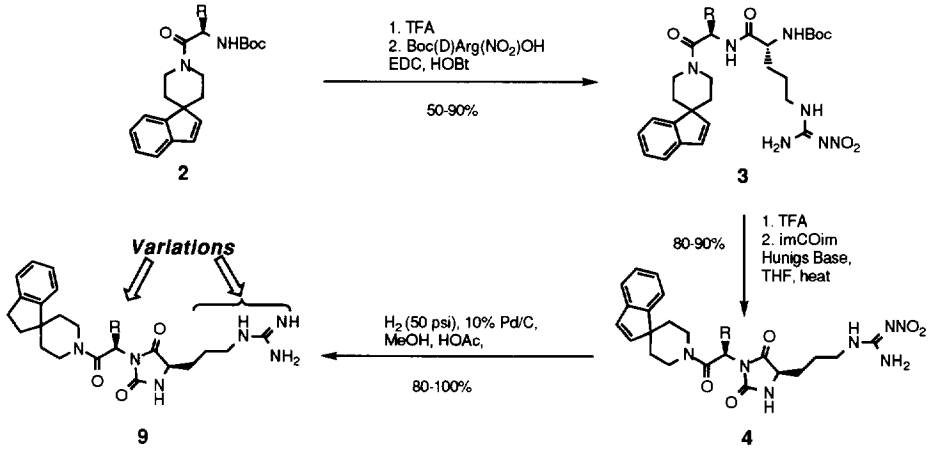


1

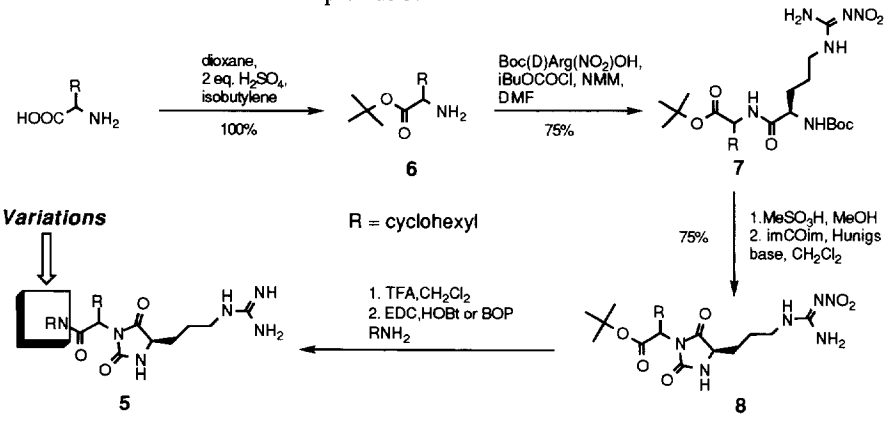
Chemistry

A general synthetic method for the preparation of analogs that incorporate modifications of the central amino acid residue as well as the hydantoin side chain (with appropriate modifications to the sequence) is illustrated below.[7]

The amino acid analog **2** was deprotected and coupled to give **3** Removal of the BOC group followed by cyclization with carbonyl diimidazole gave the hydantoin **4.** Hydrogenolytic removal of the nitro protecting group gave **1** (**9-30**), followed by hydantoin preparation gave **8**. Deprotection of the *t* -butyl ester gave the desired carboxylic acid, ready for derivitization at the C-terminus.



Rapid synthesis of C-terminal variants **5** (**31-39**) required the preparation of a fully elaborated hydantoin carboxylic acid. The preferred central amino acid was converted to the *t* -butyl ester **6.** Mixed anhydride coupling with Boc-D-Arg(NO_2)OH gave **7**. Methane sulfonic acid catalysed selective hydrolysis of the Boc group[8] followed by hydantoin preparation gave **8**. Deprotection of the *t* -butyl ester gave the desired carboxylic acid, ready for derivatization at the C-terminus to provide **5**.



Biological Evaluation

In Vitro C5a Binding Assay.[9]

a. Ability of test compound to compete with ^{125}I-CO13 (I^{125}-Y-F-K-A-Cha-G-L-dF-R) (a C5a C-terminal peptide analog) for binding to human neutrophil membrane preparations.

b. Ability of test compound to compete with ^{125}I-C5a for binding to human neutrophil membrane preparations.

In Vitro Functional Assay.[10]

Ability of test compound to block C5a induced myeloperoxidase (MPO) release from neutrophils (for antagonists) or induce MPO release (for agonists). Results expressed as % of maximal increase in MPO release induced by the compound compared to that induced by CO13 at 1μM.

Results

Variation of the central amino acid (Table 1).

Compounds were initially evaluated for binding affinity to the C5ar. C5a is believed to bind to its receptor at two locations: a receptor extracellular binding domain that interacts with the globular core of C5a and an interhelical binding site for the C-terminal 8 amino acids.[11] I^{125}-CO13, a nonapeptide C5a C-terminal analog, was utilized in a radioligand binding study to evaluate the ability of ligands to interact with the interhelical site. Competition of test compound with ^{125}C5a provided a measure of the overall affinty of the nonpeptide ligand for the C5a receptor. As expected, the binding affinity of the ligands, when tested against the endogenous peptide C5a, were approximately 10-fold less due to the additional binding interaction of C5a with the extracellular binding domain. Compounds of sufficient receptor affinity (C5a IC_{50}= <2.0 μM) were evaluated for functional activity.

Compound **1**, although of marginal affinity for the C5a receptor, was considered to be an exciting lead because of its small size, nonpeptidic character and the presence of a guanidine group derived from arginine. An attractive hypothesis could be advanced that the guanidine group of **1** was interacting at the receptor site responsible for binding the guanidine group at the C5a C-terminus: His^{67}LysAspMetGlnLeuGly**ArgOH**. The affinity of **5** was too low for functional evaluation. Thus, the research plan was to optimize affinity for the receptor so that the function of the ligand could be evaluated.

The lead **1** was a mixture of diastereomers: R,S at the Phe residue ("a") and R at Arg ("b") (Table 1). The two isomers: SR **9** (S at "a" and R at "b") and RR **10** were prepared. The superior affinity of the RR isomer established the preferred absolute configuration at "a" as R. The RS isomer **11** was 4-fold less potent than the RR isomer **10**, thereby establishing R as the preferred configuration at "b". Replacement of the benzyl group R with other benzylic aromatic systems: substituted phenyl analogs **12** and **13**, aromatic groups of increased size **14** and **15**, homologated and substituted poly-aromatic groups **17** and **18** or a heterocyclic group **19** failed to improve affinity significantly. The cyclohexylmethyl analog **21** was 26 fold (C5a IC_{50} = 0.3 μM) more potent than the benzyl lead **10**. Compound **21** was evaluated for MPO release and found to be an agonist: eliciting 86% of the increase in MPO release at 30 uM that is produced by CO13 at 1 μM. Further modifications of the structure were pursued with the goal of identifying a non-peptide antagonist from the series.

Introduction of smaller alkyl groups **22-27** or more hydrophilic groups **28-30** in place of the cyclohexymethyl group failed to improve or reduced affinity.

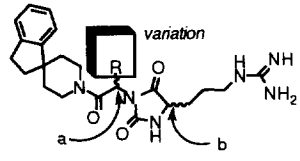
Variation of the C-terminus (Table 2)

Many C-terminal analogs of **20** were prepared (Table 2). No improvements in affinity over **20** were identified. Some of the more interesting aspects of the SAR that was observed are described below. The spiroindene analog **31** of **20** was 5-fold less potent than **20.** The spiroindanone **32** was still less potent as was the ring-opened analog **33**. The affinity could be restored by the simple 4-phenyl piperidine analog **34**. The isosteric piperazine **35** was considerably less potent than **34**. The affinity for the receptor could be restored by

introduction of an ortho methyl substituent **36**. Two ortho methyl groups as in **37** or the introduction of a *p*-methyl group **38** did not affect affinity. However, the 2- isopropyl analog **39** lost almost all affinity for the receptor.

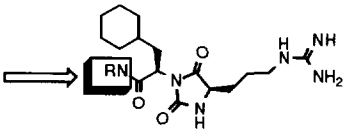
Compounds **34**, **36**, **37**, and **38** were evaluated in the MPO assay and were all found to be agonists. The C5a antagonist C089 was found to block the release of MPO induced by **38**. This result is evidence for the selective agonist properties of this series of C5a ligands.

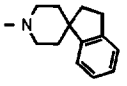
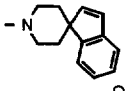
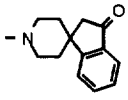
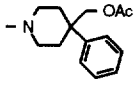
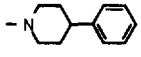
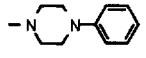
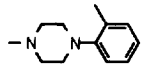
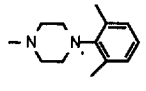
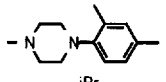
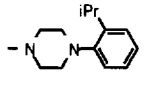
Table 1: Binding affinity and function of analogs of **1**



#	R	*a	*b	IC_{50} μM CO13	IC_{50} μM C5a	MPO
1	CH_2Ph	S,R	R	78% 30 uM	60% 30 uM	-
9	CH_2Ph	S	R	15% 30uM	3% 30 uM	-
10	CH_2Ph	R	R	0.8	8.0	-
11	CH_2Ph	R	S	4.0	39% 30uM	-
12	CH_2Ph-4-F	R	R	0.23	75% 30uM	-
13	CH_2Ph-4-OH	R	R	-	4.0	-
14	CH_2-1-napthyl	R	R	11.0	77% 30uM	-
15	CH_2-2-napthyl	R	R	2.0	67% 30uM	-
16	CH_2Ph-4-Ph	R	R	0.9	2.3	-
17	CH(Ph)Ph	R	R	-	75% 30uM	-
18	$(CH_2)_2Ph$	.12	R	3.0	46% 30uM	-
19	CH_2-3-indoyl	R	R	0.45	5.0	-
20	CH_2-c-hexyl	R	R	0.025	0.3	86%
21	CH_2-c-hexyl-4-c-hexyl	R	R	5.0	26% 30uM	-
22	iPr	R	R	1.2	70% 30uM	-
23	CH(Me)Et	R	R	0.2	1.2	31%
24	n-Pr	R	R	3.0	63% 30uM	-
25	CH_2CH_2SMe	R	R	3.4	57% 30uM	-
26	CH_2CHMe_2	R	R	0.45	2.0	10%
27	n-Bu	R	R	54% 30 uM	20% 30uM	-
28	$CH(OH)CH_3$	R	R	57% 30 uM	14% 30uM	-
29	CH_2COOH	R	R	-	0% 30uM	-
30	$CH_2CON(CH_2)_5$	R	R	52% 30 uM	26% 30uM	-

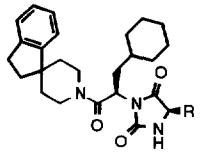
Table 2: Variation of the C-terminus



	R	IC_{50} CO13 (μM)	IC_{50} C5a (μM)	MPO release
20		0.025	0.3	86%
31		0.3	1.5	
32		1.5	58% 30uM	
33		46% 30 uM	35% 30 uM	
34		0.03	0.4	88%
35		1.0	66% 30 uM	
36		0.04	0.35	76%
37		0.04	0.22	78%
38		0.06	0.3	90%
39		14% 30 uM		

Variation of the N-terminus.

The urea analog **40** of the guanidine group of **2q** had negligible affinity demonstrating that the basic guanidine group was essential for potent affinity to the receptor. The primary amine **41**, positioning a basic group in the same locus as the guanidine of **20** was 160-fold less potent. The homologated analog of **20**, **42** was 12-fold less potent. The aniline **43**, a further conformationally restricted analog of **20** also had poor affinity for the receptor.



20	R= $(CH_2)_3NHC(NH)NH_2$	IC_{50} CO13 = 0.02
40	R= $(CH_2)_3NHCONH_2$	IC_{50} CO13 = 58% 30 uM
41	R=$(CH_2)_4NH_2$	IC_{50} CO13 = 4.0
42	R=$(CH_2)_4NHC(NH)NH_2$	IC_{50} CO13 = 0.3
43	CH_2Ph-4-NH_2	IC_{50} CO13 = 75% 30 uM

Discussion

The series of compounds described here constitute selective ligands of the C5a receptor as determined by radioligand binding studies.[13] Our goal had been to improve the affinity and to identify an antagonist of the C5a receptor. Although binding affinity was improved approximately 100-fold from the lead compound (**20** vs **1**), further improvement was not achieved. Analogs of sufficient affinity to the receptor were functional agonists as determined by MPO release from PMNs. The agonist activity of **38** could be blocked by a peptide antagonist of C5a (CO89) thereby demonstrating the selectivity and function of **38**. Other nonpeptidic series of C5a ligands have recently been reported by these laboratories: benzodiazepines[14] and tetrahydroimidazopyridines.[15] Although in these cases some reduction in agonism could be measured, none achieved blockade of the peptidic agonist CO13. It is surprising that no antagonists were found amongst three structurally unrelated ligands, all of which are believed to interact in the interhelical region. The large size of the endogenous ligand that is thought to interact at more than one receptor binding site and the precoupled nature of the receptor combine to make the discovery of C5a antagonists a difficult challenge.

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12. Absolute configuration not known, **18** is the more active of two diastereomers. Less potent diastereomer IC_{50} (CO13)= 72% @ 30 uM; IC_{50} (C5a) = 26% @ 30uM
13. No interaction has been found for this class of compounds with other G-protein coupled receptors following broad screening at concentrations of 1.0 mM.
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