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Discovery of new C3aR ligands. Part 1: Arginine derivatives

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Abstract—The synthesis and in vitro binding of several new arginine-containing C3aR ligands are reported. DMPK properties and functional activities of selected compounds have been evaluated. One compound is shown to be active in an in vivo model of airway inflammation after aerosol administration.

The complement system is at the heart of the inflammatory process. This complex system encompasses approximately 30 components working together to recruit phagocytes for the destruction of pathogens. It is activated by the immune complex and complements C1 activators. This results in C3 being cleaved to release C3a, which in turn leads to the cleavage of C5 into C5a and C5b. The latter binds together with C6–C9 to form the membrane attack complex, responsible for cell lysis. When left unchecked this very efficient system favors the development of chronic inflammatory diseases such as asthma, rheumatoid arthritis, multiple sclerosis, etc. C3a in particular has been linked to various pro-inflammatory processes linked to allergic airway diseases, such as contraction of human parenchymal strips,[1] increase of vascular permeability,[2] and release of vasoactive amines.[3] Moreover, C3aR knock-out mice are characterized by decreased airway hyperresponsiveness[4] and KO guinea pigs show decreased allergic response.[5] In humans, asthmatic patients have elevated levels of C3a in the broncho-alveolar fluid[6] and in the plasma.[7]

This central role of C3a makes it an ideal drug target. C3a is a 77-amino acid peptide which activates a G-protein coupled receptor called C3aR, widely expressed in the periphery and the brain. It has been shown that the C-terminus LPLPR sequence is essential for C3aR activation.[8] Disrupting this interaction seems a good way to block the inflammatory processes linked to C3a.

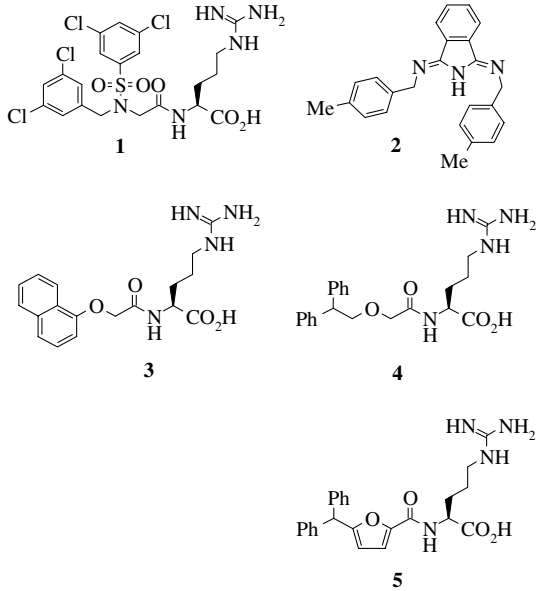

Figure 1. Small molecule ligands of the C3a receptor.

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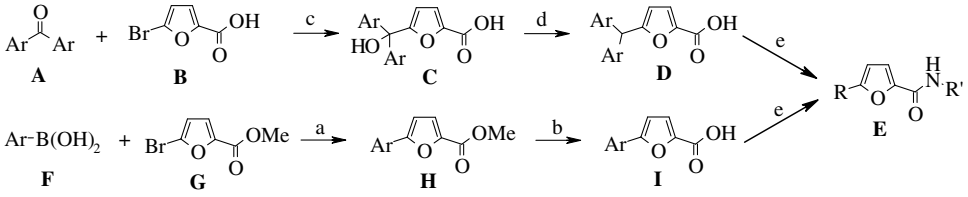
This approach has been followed by several others before us, and as a result, all known ligands feature an arginine (compounds **1**, **3**, **4**, and Fig. 1)[9] or a bioisostere in the case of **2**.[10] We selected **4** (pIC_{50} = 6.3) as a starting point and we reasoned that we could gain some affinity by rigidifying its ether chain. We were delighted to see that compound **5** in which the ether chain is locked within a furan ring had a much improved affinity for C3aR indeed, with a pIC_{50} of 7.2.

With this good starting point in hand we decided to investigate its structure–activity relationships.

'Western' substitution was varied starting from 5-bromofuran-2-carboxylates (**B** or **G**, Scheme 1). The ester **G** was coupled under Suzuki conditions with a range of aryl boronic acids **F**. It was then hydrolyzed and coupled with arginine. On the other hand, carboxylic acid **B** was treated with three equivalents of *tert*-butyllithium and the resulting dianion was reacted with substituted benzophenones **A**. The resulting tertiary alcohols **C** were then reduced with triethylsilane and finally coupled with arginine.

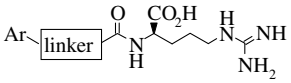
'Eastern' substitution (data not shown) arose from chemical modifications of protected amino-acids and coupling with the carboxylic acid of interest (**D** or **I**, Scheme 1).

All compounds were tested in a C3a binding assay. Various aryl (Table 1) or diarylmethine groups (Table 2) were introduced on the Western side. In the first series, it quickly became apparent that the 3-substitution of the phenyl gave the best improvements in affinities compared to 2- and 4-substituted phenyls (data not shown). After some experimentation, a chlorine atom (**12**) was found optimal. Additional substituents did not yield any improvements (**13**–**15**). Attempts at replacing the furan linker with a benzene (**18** and **19**) or a thiophene (**20**) highlighted the essential role of the oxygen atom. In the second series, the linker seemed as critical as in the first series, as highlighted by close analogues of **5**: **21** and **22**. However, here again, the introduction of halogen atoms at the 3-position of phenyls gave rise to more potent ligands **23** and **24**. The most active compounds were profiled internally against a panel of 20 different GPCRs and showed no additional affinity.



Scheme 1. Synthetic route toward Western side modifications. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, 100 °C; (b) LiOH, THF/H_2O; (c) *t*-BuLi (3 equiv), THF, −70 to 22 °C; (d) TFA, Et_3SiH, DCM (Ar = Ph, 42% two steps); (e) $(COCl)_2$, THF then $R'NH_2$, K_2CO_3, THF/H_2O.

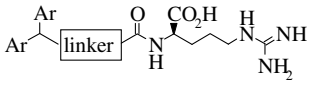
Table 1. SAR data for biaryl-substituted arginines



Compound	Aryl	Linker	C3a pIC_{50}[a]
6	3-Fluorophenyl	2,5-Furyl	6.5
7	3-Methylphenyl	2,5-Furyl	6.9
8	3-Methoxyphenyl	2,5-Furyl	6.5
9	3-Ethoxyphenyl	2,5-Furyl	6.7
10	3-Hydroxymethylphenyl	2,5-Furyl	6.1
11	3-Cyanophenyl	2,5-Furyl	6.7
12	3-Chlorophenyl	2,5-furyl	7.1
13	3-Chloro-4-fluorophenyl	2,5-Furyl	6.7
14	3-Chloro-6-methylphenyl	2,5-Furyl	6.1
15	3,6-Dichlorophenyl	2,5-Furyl	5.8
16	3-Benzothiophenyl	2,5-Furyl	6.5
17	6-Dibenzofuryl	2,5-Furyl	5.4
18	3-Chlorophenyl	1,4-Phenyl	4.0
19	3-Chlorophenyl	1,3-Phenyl	6.1
20	3-Chlorophenyl	2,5-Thienyl	5.2

[a] Values are means of three experiments. Assays were performed in 96-well format using wheat germ agglutinin SPA beads (GE). Various concentrations of compounds were added, followed by ^{125}I-C3a (2200 Ci/mmol, PE) at 0.02 nM in 120 μl of 50 mM Tris/2 mM $MgCl_2$ gC/0.5% BSA. Non-specific binding was determined using C3a at 1 nM. Pre-coupled beads and membranes prepared from HMC-1 cells (stably expressing both Aequorin and the human C3a receptor) diluted in 80 μl buffer were then added. Plates were sealed and incubated at rt for 3 h. Bound C3a was determined by scintillation counting on a Wallac Beta Trilux scintillation counter.

Table 2. SAR data for triarylmethine-substituted arginines



Compound	Diarylmethine	Linker	C3a pIC_{50}[a]
21	Phenyl	1,4-Piperazinyl	4.3
22	Phenyl	1,4-Phenyl	6.1
5	Phenyl	2,5-Furyl	7.2
23	3-Fluorophenyl	2,5-Furyl	7.5
24	3-Chlorophenyl	2,5-Furyl	7.8
25	4-Chlorophenyl	2,5-Furyl	6.8

[a] Values are means of three experiments.

On the other side of the molecule, it proved impossible to replace the original arginine while maintaining a good affinity: the three-carbon chain, the chirality, and the carboxylic acid were all found essential for C3aR recognition. Moreover, all modifications of the guanidine function to make it less basic also led to compounds with drastically reduced affinities.

The functional activity of our best ligands was then investigated (Table 3). In the first series, most of the ligands behaved as antagonists, such as **19** and **20**. However, **12**, our best ligand in this series, turned out to be a full agonist. To our dismay this tendency was confirmed in the other series, where our strongest ligand **24** showed agonistic properties, while its *p*-dichloro analogue **23** was a partial agonist. Compound **5** was the best example of this series to keep an antagonistic profile.

Our compounds were also evaluated for their in vitro DMPK properties. Compound **5**, representative of the chemical family, showed low in vitro metabolic clearance (Clint of 4 μL/min/mg prot in NADPH-fortified rat liver microsomes and 5 μL/min/10^6 cells in rat hepatocytes) and low potential to inhibit human CYP2D6/3A4 (12 and 0% inhibition at 50 μM, respectively). However, **5** showed a low apical-to-basolateral permeability in Caco-2 assay (0.4×10^{-6} cm/s). In line with this finding, the oral bioavailability in male rats was found to be ca. <1% (single oral and iv dosing at 1 mg/kg). The measured concentrations in the portal vein and the vena cava further confirmed that only a small fraction of the dose is absorbed, while the hepatic extraction is minimal. Overall this suggests that all our arginine compounds suffer from restricted intestinal absorption, presumably because of high hydrogen bonding and/or high basicity. The high PSA (above 130 Å^2 on average) of all these compounds might also account for their low permeability[11] (Fig. 2).

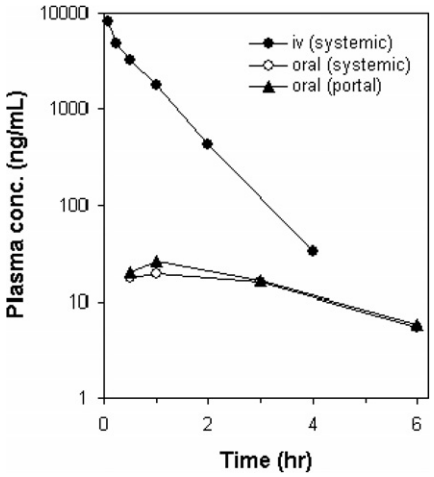


Figure 2. Hepatic extraction of **5** after oral administration (rats).

Compound **5** was also tested in an in vivo model of inflammation.[12] Mice were treated with **5** either intraperitoneally or were exposed to inhale an aerosol of **5** for 30 min. The eosinophil count in the broncho-alveo-

Table 3. Functional activities of selected compounds

Compound	SPA binding pIC_{50}	Antagonist activity[a] pEC_{50}	Agonist activity[b] pEC_{50}
5	7.2	6.8	—
12	7.1	—	6.3
19	6.1	5.4	
20	5.2	4.8	
23	6.4	5.9	—
24	7.9	—	7.7
25	6.8	—	6.9

[a] Following the 2-h incubations with Coelenterazine, the cells were plated and treated with compounds, dilutions (200 μl final volume, 1% DMSO) for 1 h at 37 °C. Fifty microliters of C3a agonist (final concentration of 5 nM) was then injected. Emitted light was immediately recorded for 30 s using Novostar (BMG). Maximal inhibition is determined using **4** as the reference antagonist (20 μM final concentration).

[b] HMC-1 cells stably expressing both Aequorin and the human C3a receptor were loaded with 10 μM Coelenterazine in HBSS/Hepes 20 mM/BSA 0.2%, pH 7.4, for 2 h at 37 °C. They were then diluted four times, placed in 96-well plates (200 μl final volume, 1% DMSO), and incubated for 1 h at 37 °C. Fifty microliters of compound dilutions was then injected to get a final volume of 250 μl at 1% DMSO final concentration. Emitted light was immediately recorded for 30 s using Novostar (BMG). The maximal signal was determined in the presence of C3a agonist (Advanced Research Technologies, 50 nM).

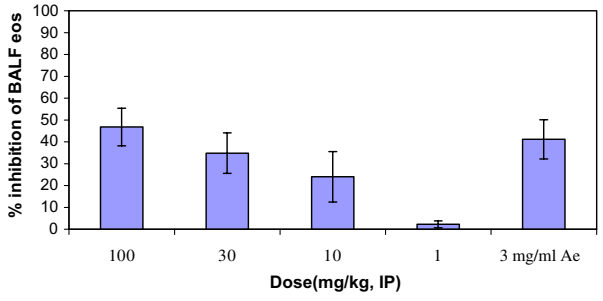


Figure 3. Evaluation of **5** in vivo in an ovalbumin-triggered model of airway inflammation. BalbC mice ($n = 5$) were treated by intraperitoneal injection of ovalbumin (OVA 8 μg + aluminum hydroxide 2 mg per mouse) at day 0 and day 14 and by aerosol (OVA 0.5% for 30 min) at days 21–23. Compound **5** was injected ip 1 h before and 4 h after the aerosol on days 21–23 or inhaled for 30 s at 3 mg/ml. The broncho-alveolar fluid was sampled on day 25 and the eosinophils counted.

lar fluid revealed that inhalation of **5** (3 mg/ml aerosol) was equally effective to a 30-mg/kg dose ip. The measured peak concentrations of **5** in the plasma were of 33,800 and 27 ng/ml for ip and aerosol administration, respectively. Aerosol administration thus leads to a thousandfold reduced plasma exposure with equal effectiveness in preventing airway inflammation. In another study, IL-13 levels in the broncho-alveolar fluid of mice treated with 30 mg/kg of **5** were reduced to levels comparable to mice treated with 10 mg/kg ip of montelukast. This in vivo proof of concept confirmed our hypothesis that C3a was a valid target to treat airway inflammation and that aerosol administration is a promising mode of administration to circumvent the PK issues of this class of compounds (Fig. 3).

In conclusion, rigidification of known scaffold **4** led to the discovery of new potent C3aR ligands. Those compounds had poor bioavailability due to the presence of the arginine side-chain. Attempted modification of this essential part led to almost inactive compounds. Minute structural modifications on the 'Western' part of the molecule were shown to exert a drastic influence on the functional activity. Antagonist **5** was shown to be active in vivo, with aerosol administration leading to much reduced plasma concentrations of active compound. This suggests that such compounds could be useful for the treatment of chronic and acute airway inflammatory diseases.

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