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Synthesis and characterization of 5,6,7,8-tetrahydroquinoline C5a receptor antagonists

J. Kent Barbay,[a,*] Yong Gong,[a] Mieke Buntinx,[b] Jian Li,[a] Concha Claes,[b] Pamela J. Hornby,[a,†] Guy Van Lommen,[b] Jean Van Wauwe[b] and Wei He[a,‡]

[a]*Drug Discovery, Johnson & Johnson PRD, East Coast RED, Spring House, PA 19477-0776, USA*
[b]*Drug Discovery, Johnson & Johnson PRD, RED EU, Turnhoutseweg 30, B-2340, Beerse, Belgium*

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Abstract—A novel series of substituted 2-aryl-5-amino-5,6,7,8-tetrahydroquinoline C5a receptor antagonists is reported. Synthetic routes were developed that allow the substituents on the tetrahydroquinoline core to be efficiently varied, facilitating determination of structure–activity relationships. Members of the series display high binding affinity for the C5a receptor and are potent functional antagonists.

The complement system plays an important role in the body's immune defense.[1] Activation of complement triggers a cascade involving proteolysis of serum proteins to active peptides. The anaphylatoxin C5a, formed by proteolysis of complement component 5 (C5), is a potent pro-inflammatory mediator involved in recruitment and activation of leukocytes. The functional effects of C5a are mediated by interaction with the G-protein coupled C5a receptor (C5aR, CD88) expressed by target cells.[2]

Inappropriate and/or excessive complement activation has been implicated in the pathology of a number of autoimmune and inflammatory diseases, and therapeutic agents capable of attenuating the complement response have been widely sought.[3] Eculizumab (Soliris™, Alexion Pharmaceuticals, Inc.), the first marketed complement directed therapy, recently received FDA approval for treatment of paroxysmal nocturnal hemoglobinuria (PNH).[4] This C5-directed antibody blocks C5 proteolysis, inhibiting the formation of C5a and C5b. Considerable effort has also been directed toward the discovery of small molecule drugs capable of blocking the complement response,[5] and in particular C5aR signaling.[6]

3-Substituted-6-arylpyridine C5aR antagonists (general structure **1**, Fig. 1) were discovered by scientists at Neurogen Corporation.[7] Aided in part by molecular modeling, we hypothesized that these antagonists could be biased toward their proposed bioactive conformation by the addition of a cyclic tether (converting the pyridine core to a 5,6,7,8-tetrahydroquinoline).[8] We report here the synthesis, C5aR binding and functional antagonism, and pharmacokinetic properties of the members of the resulting series.

In the first phase of this study, the amino group at the 5-position was varied. The preferred synthetic route for varying this substituent incorporates the amino group

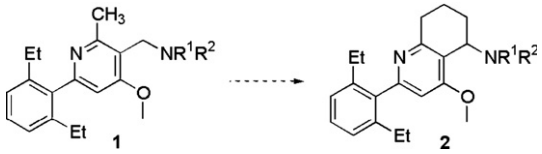

Figure 1. Generalized structures of disclosed 3-substituted-6-aryl pyridine C5a receptor antagonists[7] (**1**) and proposed 5,6,7,8-tetrahydroquinolines (**2**).

Keywords: C5a receptor antagonist; C5aR; C5a; Complement; Tetrahydroquinoline.
* Corresponding author. Tel.: +1 215 628 5033; fax: +1 215 628 4985; e-mail addresses: kbarbay@prdus.jnj.com; kentbarbay@comcast.net
† Present address: Centocor, 145 King of Prussia Road, Radnor, PA 19087, USA.
‡ Present address: Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, USA.

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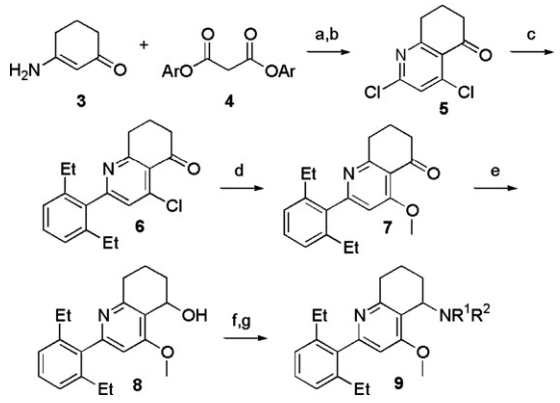
at the last step (Scheme 1). Cyclocondensation of 3-amino-2-cyclohexen-1-one (**3**) with malonic acid bis(2,4,6-trichlorophenyl) ester (**4**) and chlorination of the crude bis-phenol intermediate with phosphorus(V) oxychloride afforded **5**. Suzuki cross-coupling with 2,6-diethylphenylboronic acid occurred predominantly at the 2-position. Refluxing intermediate **6** with sodium methoxide in methanol resulted in substitution of the 4-chloro group. Installation of the amino group at the 5-position was accomplished by reduction of the ketone, activation of alcohol **8** as the corresponding chloride, and displacement with an amine. The displacement reactions tended to be sluggish; excess amine and potassium carbonate (4 equiv each) were used to drive the reaction and decrease the amount of elimination byproduct formed. The reactions were typically conducted at ambient temperature using an extended reaction period (1–3 d); higher temperatures promoted elimination. In several instances in which it was desired to independently vary the two amino substituents, or due to commercial availability of the amine starting material, a primary amine was used in the displacement reaction and the second alkyl group (Me or Et) was subsequently installed by reductive amination (compounds **28**, **33**–**34**, **36**–**37**, and **42**, Table 2).

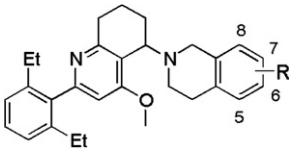
In order to efficiently prepare analogs that varied at the 2- and 4-position, syntheses were explored in which each of these substituents was installed at the last step.[9] These routes proved more challenging than that of Scheme 1; displacements of the chlorides prepared from alcohols **11** and **14** with *N*-ethyl-1-naphthylamine required heating and were less efficient than those using the chloride derived from **8**. Nevertheless, preparation of 2-chlorotetrahydroquinoline **12** allowed installation of the 2-position aryl substituent in the final step by a microwave-accelerated Suzuki reaction, allowing rapid generation of structure–activity relationships (Scheme 2a). Two analogs varying at the 4-position were produced by the route shown in Scheme 2b (**60**–**61**), while others (**62**–**63**) were prepared using the route shown in Scheme 1, with alternative nucleophiles substituted for sodium methoxide.



Scheme 1. Reagents and conditions: (a) PhBr, reflux, Ar = 2,4,6-trichlorophenyl; (b) $POCl_3$, reflux; (c) 2,6-DiEtPhB$(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, toluene, reflux; (d) NaOMe, MeOH, reflux; (e) $NaBH_4$, MeOH; (f) $SOCl_2$, CH_2Cl_2; (g) R^1R^2NH, K_2CO_3, CH_3CN.

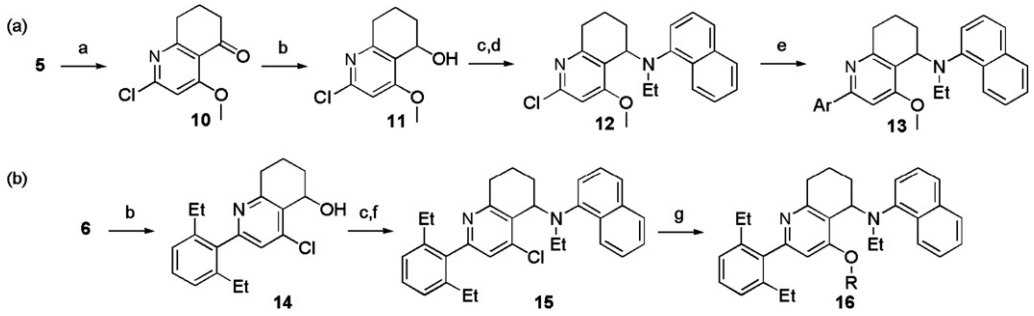
Compounds were screened for their ability to block C5a-induced intracellular calcium mobilization in the human monocytic cell line U937.[10] An initial set of analogs that revealed potential for C5a receptor antagonist activity within the series incorporated 1,2,3,4-tetrahydroisoquinolines at the 5-position (Table 1). The unsubstituted tetrahydroisoquinoline analog **17** had an IC_{50} of 1.1 μM; a brief screen of substitutions achieved

Table 1. C5aR antagonist activity of 5-position tetrahydroisoquinolines



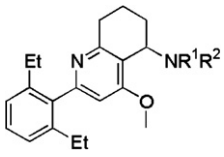
Compound[a]	R	C5a-induced Ca^{2+} flux IC_{50} (μM)
17	H	1.1
18	5-Cl	1.7
19	5-CF_3	>10
20	6-OMe,7-OMe	>10
21	7-Cl	1.4
22	7-F	0.88
23	7-OMe	>10
24	7-CF_3	>10
25	8-CO_2Me	0.84
26	8-CF_3	0.27

[a] Except where noted, compounds were tested as racemates.



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH, 23 °C (44% **10**, 40% separable 2-methoxy-4-chloro regioisomer); (b) $NaBH_4$, MeOH; (c) $SOCl_2$, CH_2Cl_2; (d) *N*-ethyl-1-naphthylamine, K_2CO_3, CH_3CN, reflux; (e) ArB$(OH)_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3, H_2O, CH_3CN, μW 120–160 °C, 10 min; (f) *N*-ethyl-1-naphthylamine, K_2CO_3, CH_3CN, 110 °C (sealed tube); (g) ROH, RONa, μW, 155–175 °C.

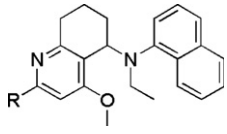
Table 2. C5aR antagonist activity of 5-position naphthylamines and analogs



Compound	R^1	R^2	C5a-induced Ca^{2+} flux IC_{50} (μM)
27	1-Naphthyl	Et	0.10
28	1-Naphthyl	Me	0.17
29	1-Naphthyl	2-OH–Et	0.19
30[a]	1-Naphthyl	Acetyl	6.2
31	1-Naphthyl	H	>10
32	H	Et	>10
33	2-Naphthyl	Me	>10
34	2-Me-1-naphthyl	Me	1.5
35	6-OMe-1-naphthyl	Me	0.18
36	5-OMe-1-naphthyl	Me	>10
37	4-OMe-1-naphthyl	Me	0.53
38	(*S*)-1,2,3,4-Tetrahydro-1-naphthyl[b]	Me	0.82
39	(*S*)-1,2,3,4-Tetrahydro-1-naphthyl[c]	Me	5.5
40	(*R*)-1,2,3,4-Tetrahydro-1-naphthyl[b]	Me	1.9
41	(*R*)-1,2,3,4-Tetrahydro-1-naphthyl[c]	Me	8.1
42	5,6,7,8-Tetrahydro-1-naphthyl	Et	0.22
43	Phenyl	Me	7.9
44	Benzyl	Et	>10
45	$-CH_2$-1-naphthyl	Me	>10
(−)-**27**[d]	1-Naphthyl	Et	0.027
(+)-**27**[d]	1-Naphthyl	Et	>10

[a] Prepared by the reaction of *N*-acetyl-1-naphthylamine with the chloride derived from **8** (NaH, THF, 23 °C).
[b] Less polar diastereomer (SiO_2 TLC, 25% EtOAc:hexanes); tetrahydroquinoline 5-position absolute stereochemistry undetermined.
[c] More polar diastereomer.
[d] Enantiomeric excesses (ee): (−)-**27** >99%, (+)-**27** 98.6% by analytical chiral SFC (chiralcel OD-H column, 0.46 × 50 cm; mobile phase 70:30 CO_2:modifier (0.2% *i*-Pr_2NH in MeOH), flow rate 3 mL/min, 50 °C).

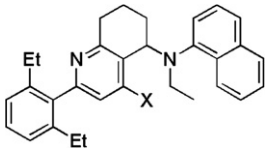
Table 3. C5aR antagonist activity of analogs varying at the 2-position



Compound	R	C5a-induced Ca^{2+} flux IC_{50} (μM)
46	2-Me,6-Me phenyl	0.13
47	2-Me,4-OMe,6-Me phenyl	0.17
48	2-OMe,6-OMe phenyl	1.8
49	2-OMe,6-Cl phenyl	0.11
50	2-Me phenyl	0.35
51	2-Et phenyl	0.65
52	2-*i*-Pr phenyl	1.7
53	2-Ph phenyl	>10
54	2-F phenyl	4.6
55	1-Naphthyl	5.9
56	Phenyl	>10
57	3-Thienyl	>10
58	3-Pyridyl	>10
59	5-Benzo-1,3-dioxolyl	>10
12	Cl	>10

improvement of the potency to 270 nM for the 8-trifluoromethyl substituted antagonist **26**. Screening a variety of additional cyclic and acyclic substituted amines (data not shown) led to the identification of an active 1-naph-

Table 4. C5aR antagonist activity of analogs varying at the 4-position



Compound	X	C5a-induced Ca^{2+} flux IC_{50} (μM)
60	OEt	0.067
61	OCH_2Ph	0.27
62	*O*-Cyclopentyl	0.037
63	SMe	0.082
64[a]	H	>10
15	Cl	2.0

[a] Prepared from 1,2,5,6,7,8-hexahydroquinoline-2,5-dione by the route shown in Scheme 1.

Table 5. C5aR binding assay results

Compound	[^{125}I]-C5a binding K_i (μM)	Compound	[^{125}I]-C5a binding K_i (μM)
15	0.18	**43**	0.68
17	0.035	**48**	1.2
26	0.0097	**51**	0.086
27	0.0089	**54**	3.7
28	0.0073	**55**	0.37
29	0.18	**56**	8.3

Table 6. Human liver microsome (HLM) stability and rat pharmacokinetic profile of compound **27**[a]

Compound	HLM stability ($t_{1/2}$, min)	C_{max}, po ± SD (μM)	Cl, iv ± SD (mL/min/kg)	$t_{1/2}$, po ± SD (h)	F (%)
27	54	1.3 ± 0.3	31 ± 4	1.8 ± 0.2	59

[a] Dosed po at 10 mg/kg, iv at 2 mg/kg, $n = 4$.

thylamine subseries (Table 2). Compound **27**, bearing an *N*-ethyl-1-naphthylamine substituent, had an IC_{50} of 100 nM. Modest changes to the *N*-alkyl group (ethyl to methyl or 2-hydroxyethyl, **28**–**29**) were tolerated, while replacing this group with an acetyl substituent resulted in μM level activity. Removal of the *N*-alkyl group caused a decrease in activity (**31**, >10 μM). The naphthalene group was also required; *N*-ethyl secondary amine **32** did not display significant antagonist activity. Attachment of the amine substituent at the 1-position of the naphthalene ring appeared to be preferred, as 2-naphthyl analog **33** displayed decreased activity. A small set of methoxy-substituted 1-naphthylamines (**35**–**37**) generally displayed reduced activity, with 6-methoxy substitution affording the most active compound (**35**, 180 nM). 1,2,3,4-Tetrahydronaphthalenes **38**–**41** (each tested as a single diastereomer) displayed activities ranging from 820 nM to 8.1 μM, while reduction of the naphthalene to the 5,6,7,8-tetrahydronaphthalene was better tolerated (**42**, 220 nM). Replacement of the naphthalene with an unsubstituted phenyl reduced activity (**43**, 7.9 μM) as did the homologation to the naphthalen-1-ylmethanamine **45** (>10 μM).

In the next phase of the study the *N*-ethyl-1-naphthalene substituent was held constant while the aryl group at the 2-position was varied (Table 3). In general, 2,6-disubstituted analogs (**46**–**49**) were more potent C5a receptor antagonists than 2-monosubstituted compounds (**50**–**54**). Continuing the trend, the unsubstituted phenyl analog **56** did not show significant activity, nor did several heterocycles unsubstituted at the *ortho* carbons (**57**–**59**). These results suggest that maintaining a twisted biaryl conformation is important for activity.

Finally, structure–activity relationships at the 4-position were explored by varying this group while holding constant the *N*-ethyl-1-naphthylamine and 2,6-diethylphenyl substituents (Table 4). Alkoxy groups larger than methoxy groups were found to be capable of improving activity (ethoxy, 67 nM; cyclopentoxy, 37 nM). Removal of this substituent, or replacement with a chloro, resulted in lower potency (IC_{50} > 10 and 2.0 μM, respectively). A thiomethyl replacement for the methoxy group was tolerated (82 nM).

The binding affinity of selected compounds to the C5a receptor was assessed by their ability to displace [^{125}I]-C5a from differentiated U937 cells (Table 5).[11] A number of compounds demonstrated binding to the receptor with high affinity (for instance, compound **27**, K_i 8.9 nM). These results provide evidence that the observed functional antagonism of members of the series is a consequence of binding to C5aR (as opposed to a mechanism involving the downstream signaling cascade).

The effect of the stereochemistry of **27** on its C5aR antagonist activity was investigated. The enantiomers of this compound were resolved by chiral supercritical fluid chromatography (SFC). The levorotary enantiomer (absolute stereochemistry undetermined) was shown to be primarily responsible for activity ((−)-**27** IC_{50} 27 nM; (+)-**27** IC_{50} > 10 μM).

The human liver microsome stability and pharmacokinetic profile of racemic **27** in rats were determined (Table 6). The compound displayed 59% oral bioavailability. It had reasonable human liver microsome stability ($t_{1/2}$ 54 min; 88% remaining at 10 min), but displayed moderate clearance (31 mL/min/kg).

In conclusion, a series of substituted 5,6,7,8-tetrahydroquinoline C5aR antagonists was discovered. Structure–activity relationships at the 2-, 4-, and 5-position of the tetrahydroquinoline core were studied. The compounds displayed activity in binding (displacement of [^{125}I]-C5a) and functional (C5a-induced calcium mobilization) assays in a human cell line. These results add to the growing body of evidence that C5aR can be potently antagonized with non-peptidic small molecules. Further exploration of the series will be reported separately.

Acknowledgments

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9. All compounds shown in Table 3 were prepared by the route depicted in Scheme 2a. Except as specifically noted in the text, all other compounds were prepared using the route of Scheme 1.
10. The human monocytic cell line U937 (ATCC CRL-1593) was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 10 mM Hepes, 1 mM pyruvate, 2 mM L-glutamine, 100 IU/mL penicillin G, and 100 μg/mL streptomycin (all Invitrogen). U937 cells were treated with 1 mM dibutyryl cAMP (Sigma) for 48–72 h to induce differentiation prior to assessment of intracellular Ca^{2+} mobilization according to a modified protocol described in Van Lommen et al. (*Bioorg. Med. Chem Lett.* **2005**, *15*, 497). Briefly, Fluo-3-loaded cells (200,000 cells/well) were dissolved in HBSS medium (Invitrogen) supplemented with 10 mM Hepes, 2.5 mM probenecid, and 0.1 % BSA (pH = 7.4) and pre-incubated for 20 min with test compound (1% DMSO) before recombinant human C5a (1.5 nM, Sigma) was added. Changes in intracellular free Ca^{2+} concentration (F_{max}) were measured using FLIPR technology (Molecular Devices). IC_{50} values were calculated using non-linear regression in Graphpad Prism.
11. In C5aR competition binding assays, dibutyryl cAMP-differentiated U937 cells were dissolved in binding buffer consisting of 50 mM Hepes, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% NaN_3, and 0.02% protease-free BSA (pH 7.4). After pre-incubating the cells (2×10^5 cells/well) with test compound (1% DMSO) for 30 min at 25 °C, [^{125}I]-C5a (Bolton & Hunter labeled, Perkin-Elmer, 2200 Ci/mmol, 0.05 nM) was added for 60 min while the cells were kept at 4 °C. Non-specific binding was defined in wells containing 100 nM recombinant C5a. Cells were harvested on GF/B filter plates (presoaked in 0.5% polyethyleneimine) using a Packard cell harvester. The filter plates were washed with binding buffer supplemented with 500 mM NaCl and filter bound radioactivity was determined by liquid scintillation counting on a TopCount NXT™ (Packard). K_i values were calculated using the equation of Cheng and Prusoff (*Biochem. Pharmacol.* **1973**, *22*, 3099) in Graphpad Prism.