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# Design and optimization of aniline-substituted tetrahydroquinoline C5a receptor antagonists

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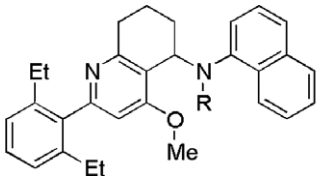
ABSTRACT

A series of aniline-substituted tetrahydroquinoline C5a receptor antagonists were discovered. A functionality requirement of *ortho* substitution on the aniline was revealed. Secondary anilines, in general, outperformed tertiary analogs in inhibition of C5a-induced calcium mobilization. Further enhancement of activity was realized in the presence of an *ortho* hydroxyalkyl side chain. The functional $IC_{50}$ of selected analogs was optimized to the single-digit nanomolar level.



Complement component C5a, a pro-inflammatory serum protein, is generated during activation of the complement cascade by proteolysis of C5. Its interaction with the membrane bound G-protein coupled C5a receptor (C5aR) mediates anaphylatoxic and chemotactic effects. Excessive complement activation may contribute to many inflammatory and autoimmune conditions.[1,2] Preventing the formation of C5a (as well as C5b) using antibodies that bind to and block proteolysis of complement component C5 has been clinically validated as a therapeutic strategy with the recent marketing approval of Soliris™ (eculizumab) by the U.S. Food and Drug Administration as the first therapy approved for paroxysmal nocturnal hemoglobinuria.[3] C5aR has also been an important therapeutic target for complement modulation.[2] The search for C5aR antagonists has resulted in the discovery of different types of ligands, including peptides and peptidomimetics, as well as non-peptidic small molecules.[2,4]

Recently, we have disclosed a tetrahydroquinoline series of small molecule C5aR antagonists.[5] A key feature of the series was the existence/requirement of a tertiary amine substituent at the 5-position of the tetrahydroquinoline scaffold, as illustrated by the activities of tertiary aminonaphthalenes **1** and **2** as contrasted to the less active secondary amine **3** (Fig. 1). Optimization of the aminonaphthalene portion was challenging, as only a limited number of aminonaphthalenes were accessible and activity was




**1**, R = Et, $IC_{50}$ = 0.10 μM
**2**, R = Me, $IC_{50}$ = 0.17 μM
**3**, R = H, $IC_{50}$ > 10 μM

**Figure 1.** Representative aminonaphthalene-substituted tetrahydroquinoline C5aR antagonists.[5]

sensitive to naphthalene ring modifications. We considered anilines as truncated aminonaphthalenes, with the potential advantage that their wide accessibility would offer more opportunity for optimization. Here, we report our exploration of anilines as 5-position substituents within the series. This subseries has revealed distinct structure-activity relationships (SAR) as compared to the aminonaphthalenes and has yielded analogs with excellent potency as C5aR antagonists.

A general synthetic approach to the secondary (2°) and tertiary (3°) aniline analogs is outlined in Scheme 1. The well-optimized 2,6-diethyl/dimethyl phenyl group was selectively installed in **5** via Suzuki coupling of dichloroquinolinone **4** with the corresponding phenylboronic acids. Substitution of the 4-chloro group in **5** with a sodium alkoxide, reduction of the ketone with $NaBH_4$, and treatment of the alcohol with $SOCl_2$ resulted in chloride **6**. Alkylation of primary anilines with chloride **6** yielded 2° anilines.[6] Targeted 3° anilines were generated by reductive methylation of the

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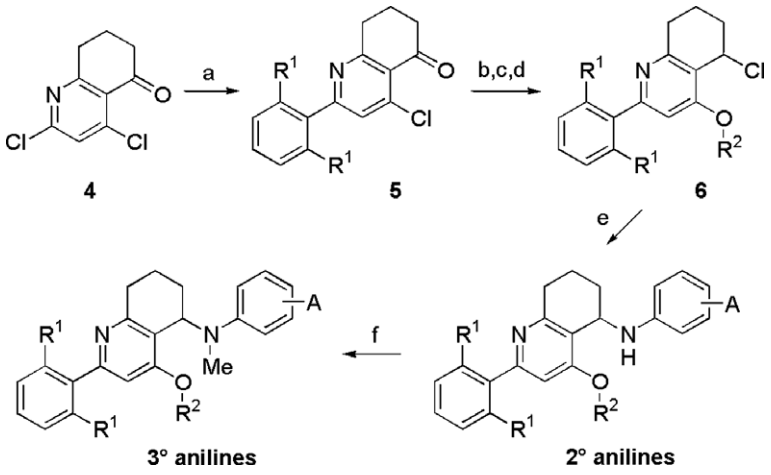
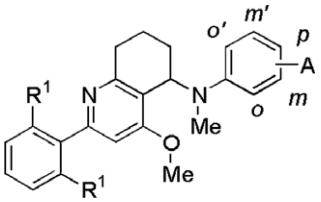



**Scheme 1.** Reagents and conditions: (a) 2,6-di-$R^1$-PhB$(OH)_2$ ($R^1$: Me or Et), Pd$(PPh_3)_4$, $Na_2CO_3$, $H_2O$/toluene, reflux; (b) $R^2ONa/R^2OH$, reflux ($R^2$: Me or $CHMe_2$); (c) $NaBH_4$, MeOH; (d) $SOCl_2$, $CH_2Cl_2$; (e) A-$PhNH_2$, $K_2CO_3$, MeCN or DMF; (f) paraformaldehyde, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, μW, 120 °C, 10 min.

corresponding 2° anilines. Phenylether analogs **49** and **50** shown in Fig. 3 were prepared similarly by alkylation of phenols with chloride **6**.

Previously demonstrated SAR within the aminonaphthalene series suggested the importance of a tertiary amine substituent at the 5-position of the tetrahydroquinoline core.[5] Accordingly, a set of tertiary anilines, listed in Table 1, were prepared initially and tested in a C5a-induced calcium mobilization functional assay.[7] Unsubstituted aniline analog **7** only offered weak activity ($IC_{50}$: 7.9 μM), and was much (46-fold) less potent than aminonaphthalene analog **2**. However, certain substituted anilines offered improved activities. For instance, the *ortho*-methyl-substituted compound **8** improved potency over **7** by 10-fold to 0.79 μM and an additional methyl or methoxy group at the *m*′-position was favorable (**11**: $IC_{50}$ 0.69 μM and **12**: $IC_{50}$ 0.56 μM, respectively). Combining the preferred *o*-methyl, *m*′-methoxy aniline group with a 2,6-dimethylphenyl substituent at the tetrahydroquinoline 2-position resulted in a modest decrease in activity relative to **12** (**16**: $IC_{50}$ 3.2 μM); therefore the 2,6-diethylphenyl group was generally retained in subsequent analogs.

**Table 1**
C5a-induced calcium mobilization results for tertiary anilines

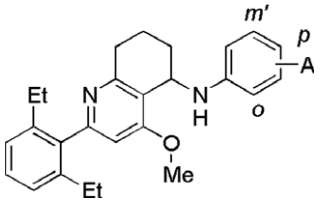


| Compound[a] | $R^1$ | A | $Ca^{2+}$ flux Inhibition $IC_{50}$ (μM) |
|---|---|---|---|
| **7** | Et | H | 7.9 |
| **8** | Et | *o*-Me | 0.79 |
| **9** | Et | *o*-Me, *m*′-Cl | 2.6 |
| **10** | Et | *o*-Me, *m*′-Ph | >10 |
| **11** | Et | *o*-Me, *m*′-Me | 0.69 |
| **12** | Et | *o*-Me, *m*′-OMe | 0.56 |
| **13** | Et | *o*-Me, *p*-OMe | 6.2 |
| **14** | Et | *o*-OMe, *m*′-OMe | 2.6 |
| **15** | Et | *o*-Cl, *m*′-OMe | 0.95 |
| **16** | Me | *o*-Me, *m*′-OMe | 3.2 |
| **17** | Me | *o*-Me, *m*-OMe | >10 |
| **18** | Me | *o*-Me, *o*′-Me | >10 |

[a] All compounds were tested as racemates.

Secondary anilines were originally prepared as precursors for the targeted tertiary anilines. Some of these precursors were selected and tested in the functional assay in comparison to tertiary analogs. Surprisingly, those 2° anilines were not only active, but in most cases were even more potent than their 3° counterparts (see Table 2). For example, 2° aniline **25** was 4-fold more potent than the corresponding 3° aniline **12**. SAR trends in the 2° anilines were similar to those in the 3° anilines. For instance, the synergistic

**Table 2**
C5a-induced calcium mobilization results for secondary anilines



| Compound | A | $Ca^{2+}$ flux Inhibition $IC_{50}$ (μM) | $IC_{50}$ Ratio ($NCH_3$/NH)[a] |
|---|---|---|---|
| **19** | *o*-Me | 0.91 | 0.87 |
| **20** | *m*′-OMe | 7.4 | |
| **21** | *o*-Me, *m*′-Cl | 1.5 | 1.7 |
| **22** | *o*-Me, *m*′-F | 1.4 | |
| **23** | *o*-Me, *m*′-Ph | 2.5 | >4 |
| **24** | *o*-Me, *m*′-Me | 0.43 | 1.6 |
| **25** | *o*-Me, *m*′-OMe | 0.14 | 4.0 |
| **26** | *o*-Me, *m*′-$CF_3$ | 0.29 | |
| **27**[b] | *o*-Me, *m*′-OH | >10 | |
| **28** | *o*-Me, *m*′-$CH_2OH$ | >10 | |
| **29** | *o*-Me, *p*-OMe | 2.4 | 2.6 |
| **30** | *o*-OMe, *m*′-OMe | 0.42 | 6.2 |
| **31** | *o*-Cl, *m*′-OMe | 0.38 | 2.5 |
| **32** | *o*-Ph, *m*′-OMe | >10 | |
| **33** | *o*-Et | 0.73 | |
| **34** | *o*-$CH_2NH_2$ | 5.0 | |
| **35** | *o*-$CO_2H$ | 0.41 | |
| **36** | *o*-$CONH_2$ | >10 | |
| **37** | *o*-$CO_2Me$ | >10 | |
| **38** | *o*-COMe | >10 | |
| **39** | *o*-$CF_3$ | >10 | |
| **40** | *o*-CN | 4.9 | |

[a] Values are $IC_{50}$ ratios between tertiary anilines in Table 1 and corresponding secondary aniline counterparts in Table 2.

[b] Compound **27** was prepared by treatment of **25** with $BBr_3$ in $CH_2Cl_2$.

effect of *o*-methyl and *m*′-methoxy substitutions on activity was again observed (disubstituted aniline **25**: $IC_{50}$ 0.14 μM). While holding the *ortho*-position constant as methyl, in addition to the methoxy group at the *m*′-position, an optionally trifluorinated methyl group was also preferred, halo and phenyl groups were tolerated, and hydroxy and hydroxymethyl substituents reduced activity (compounds **21**–**28**). An initial screen of additional 2° *o*-monosubstituted anilines failed to identify compounds offering significant further potency increases (compounds **33–40**).

The introduction of functional groups on the anilines was intended to pick up additional interactions between ligand and binding site (as discussed later) to improve potency. Although initial results with *m*′-hydroxy in **27** and *m*′-hydroxymethyl in **28** were disappointing, a significant advancement in potency was observed in the case of *o*-hydroxyalkyl anilines (Table 3). *o*-Hydroxymethyl analog **41** possessed impressive activity with an $IC_{50}$ of 0.12 μM. The potency was further improved to double-digit nanomolar with either additional substituents on the aniline ring (compounds **42**–**45**), or homologation to *o*-hydroxyethyl analog **46**. A single-digit nanomolar $IC_{50}$ was achieved when the methoxy group on the tetrahydroquinoline core in **46** was replaced by *i*-propoxy in **47**.

Compound **47** was docked into a homology model of human C5aR.[8] Fig. 2 depicts the putative ligand binding site[9] and the proposed binding mode of **47**.[10] In this binding mode, the diethylphenyl group sits in the hydrophobic pocket close to residues Ile116 and Val286, which have been proposed to be involved in triggering activation of the receptor.[9] The *i*-propoxy oxygen in the central part of the molecule forms a hydrogen bond to Tyr258. The *o*-hydroxyethyl-phenyl moiety is involved in two interactions: a cation-π interaction between Arg206 and the phenyl ring, and a direct hydrogen bond between Ser138 and the hydroxyl group in the *o*-hydroxyethyl chain. This binding mode can serve as a starting point to understand the SAR in this series of compounds.

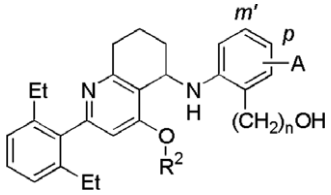
Further SAR extension was briefly explored, as represented by the examples in Fig. 3. Aminopyridine **48** was about 30-fold less potent than aniline analog **41**. Replacement of the *N*-linkage in tertiary aniline **12** or secondary aniline **25** with an *O*-linkage in phenylether **49** offered comparable submicromolar activity. For aniline **46**, the phenylether linked analog **50**, however, lost potency by about 80-fold, which indicated SAR differences between anilines and phenylether analogs.

Representative compounds were also tested in a [$^{125}$I]C5a radioligand binding assay.[11] The results are listed in Table 4. Selected compounds showed activity in this assay, with $K_i's$ ranging from submicromolar (unsubstituted tertiary aniline **7**) to low double-digit nanomolar (substituted secondary anilines **25**, **45**, and **46**). Further in vitro characterization, including receptor and species selectivity, will be published separately.

Compound **25**[12] was selected for a pharmacokinetic (PK) study in rats and results are presented in Table 5. Upon intravenous dosing at 2 mg/kg, compound **25** had a fairly long terminal elimination half-life of over 6 h. Volume of distribution and systemic clearance were relatively high. When compound **25** was dosed orally at 10 mg/kg, systemic exposure was achieved with $t_{1/2}$ over 5 h and bioavailability of 21%.[13]

In summary, a novel aniline-substituted tetrahydroquinoline series of C5aR antagonists were discovered. Distinct SAR was revealed as compared with those of the previously disclosed aminonaphthalene-substituted antagonists. Micromolar to submicromolar $IC_{50}$s of initial tertiary anilines were improved with secondary aniline analogs. Further enhancement of activity was realized with the introduction of an *ortho* hydroxyalkyl side chain. The receptor binding affinity, putative binding mode, and PK prop-

**Table 3**
C5a-induced calcium mobilization results for *o*-hydroxyalkyl anilines




| Compound | $R^2$ | *n* | A | $Ca^{2+}$ flux Inhibition $IC_{50}$ (μM) |
|---|---|---|---|---|
| **41** | Me | 1 | H | 0.12 |
| **42** | Me | 1 | *m*′-Cl | 0.026 |
| **43** | Me | 1 | *m*′-$CF_3$ | 0.022 |
| **44** | Me | 1 | *p*-F | 0.053 |
| **45** | Me | 1 | *p*-Cl | 0.071 |
| **46** | Me | 2 | H | 0.030 |
| **47** | $CHMe_2$ | 2 | H | 0.007 |

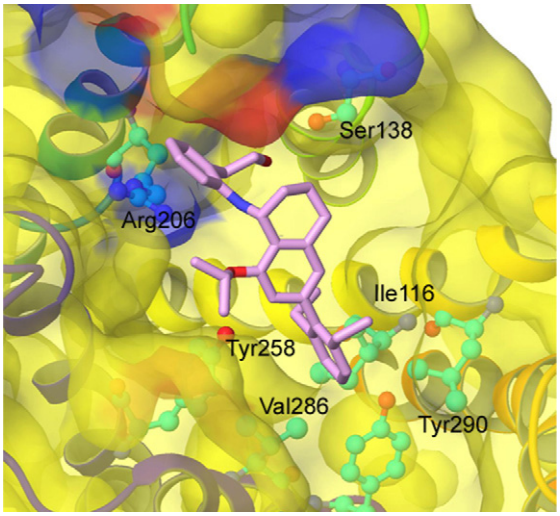



**Figure 2.** Binding mode of **47** in human C5aR homology model.

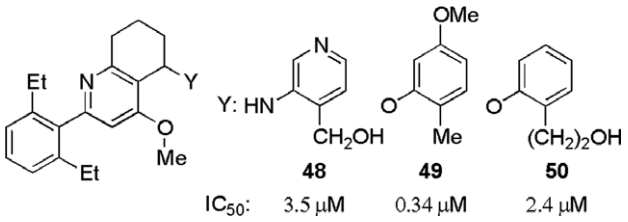



**Figure 3.** Aminopyridine and phenylether analogs.

**Table 4**
[$^{125}$I]-C5a binding assay results for selected compounds

| Compound | **7** | 25 | **45** | **46** |
|---|---|---|---|---|
| $K_i$ (μM) | 0.68 | 0.010 | 0.013 | 0.021 |

**Table 5**
Rat PK results[a] for compound **25**

| IV (2 mg/kg) | PO (10 mg/kg) |
|---|---|
| $t_{1/2}$: 6.3 (±1.2) h<br>Vss: 4.5 (±0.2) L/kg<br>Cl: 21 (±9) mL/min/kg | $t_{1/2}$: 5.3 (±1.8) h<br>$C_{max}$: 0.29 (±0.3) mg/mL<br>AUC: 42 (±12) min · mg/mL<br>F: 21 (±6)% |

[a] Values are means of four rats, standard deviation is given in parentheses.

erties of representative compounds were also explored. We hope that these studies may aid in the eventual development of orally bioavailable C5aR antagonists capable of modulation of the complement response.

## Acknowledgments

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6. For anilines containing other nucleophilic functional groups (e.g., $CH_2OH$, $CO_2H$, and $CH_2NH_2$), no protections were necessary in the initial quick aniline screening, as the desired products could be separated from undesired isomers. An attempt to make an *o*-$CH_2CO_2H$ analog was complicated by the cyclization of 2-aminophenyl acetic acid to the oxindole.
7. The calcium mobilization assay was performed using the human monocytic cell line U937 (ATCC CRL-1593). For details, see Ref. 5.
8. The human C5aR homology model was constructed using bovine rhodopsin X-ray crystal structure (PDB code 1L9H) as template. Both model construction and molecular docking were done using Maestro 6.0, module Prime and Glide (Schrodinger LLC, Portland, OR).
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10. Compound **47** was a racemate. Both (*R*)- and (*S*)-enantiomers were docked to the model. The binding mode of (*R*)-enantiomer was favored over (*S*)-enantiomer and was illustrated in Fig. 2. Chiral separation/synthesis was planned for further profiling.
11. The [$^{125}$I]C5a radioligand binding assay was conducted using dibutyryl cAMP-differentiated U937 cells. For details, see Ref. 5.
12. To 5-chloro-2-(2,6-diethylphenyl)-4-methoxy-5,6,7,8-tetrahydroquinoline (**6**, 0.13 g, 0.40 mmol) in DMF (1 mL) were added 5-methoxy-2-methylaniline (0.22 g, 1.6 mmol) and potassium carbonate (0.22 g, 1.6 mmol). The suspension was stirred at ambient temperature for five days, and then heated at 40 °C for another day. The mixture was purified by HPLC (10–90% acetonitrile in water containing 0.05% TFA). The combined pure fractions were partially concentrated, basified with saturated sodium bicarbonate, and extracted with dichloromethane. The organic extract was washed with water, dried over magnesium sulfate, filtered, and concentrated, yielding aniline **25** as a white foam (0.12 g, 70% yield). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.28 (t, 1H, $J$ = 7.5 Hz), 7.14 (d, 2H, $J$ = 7.2 Hz), 6.98 (d, 1H, $J$ = 8.7 Hz), 6.63 (s, 1H), 6.50 (d, 1H, $J$ = 1.8 Hz), 6.24 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$ = 8.1 Hz), 4.92 (s, 1H), 3.83 (s, 3H), 3.76 (s, 3H), 3.66 (br, 1H), 3.06 (dd, 1H, $J_1$ = 5.1 Hz, $J_2$ = 18 Hz), 2.84 (m, 1H), 2.26–2.46 (m, 5 H), 2.06 (s, 3H), 1.90–2.03 (m, 2H), 1.64 (m, 1H), 1.10 (m, 6H). MS $m/z$ 431.27 $(MH)^+$. Anal. calcd for $C_{28}H_{34}N_2O_2{\cdot}0.5\ H_2O$: C, 76.50; H, 8.03; N, 6.37. Found: C, 76.48; H, 7.96; N, 6.16.
13. The plasma level of **27**, a potential des-methyl metabolite, was also found to be minimal.