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Biphenylsulfonyl-thiophene-carboxamidine inhibitors of the complement component C1s

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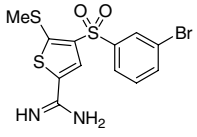
Abstract—Complement activation has been implicated in disease states such as hereditary angioedema, ischemia-reperfusion injury, acute respiratory distress syndrome, and acute transplant rejection. Even though the complement cascade provides several protein targets for potential therapeutic intervention only two complement inhibitors have been approved so far for clinical use including anti-C5 antibodies for the treatment of paroxysmal nocturnal hemoglobinuria and purified C1-esterase inhibitor replacement therapy for the control of hereditary angioedema flares. In the present study, optimization of potency and physicochemical properties of a series of thiophene amidine-based C1s inhibitors with potential utility as intravenous agents for the inhibition of the classical pathway of complement is described.

The complement cascade is a major component of the innate immune system in mammals and other vertebrate species.[1] It plays a major role in the destruction of invading microorganisms and the clearance of immune complexes. However, unregulated complement activation leading to acute inflammation and tissue damage has been implicated in the pathology of many disease states.[2] Activation of the classical pathway has been implicated in humorally-mediated graft rejection,[3] ischemia-reperfusion injury (IRI),[4] hereditary angioedema (HAE),[5] vascular leak syndrome,[6] and acute respiratory distress syndrome (ARDS).[7] C1-esterase inhibitor (C1-Inh) has been used for many years as a replacement therapy for patients with hereditary angioedema caused by a deficiency of C1-Inh.[8] Although C1 esterase inhibitor has been the only complement inhibitor approved for clinical use, a humanized monoclonal antibody (eculizumab) against terminal complement protein C5 has recently been approved for the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH).[9]

Small-molecule complement inhibitors have yet to enter the marketplace. Compstatin, a synthetic 13 amino acid cyclic peptide that blocks all three known complement activation pathways, is currently undergoing clinical development for the treatment of age-related macular degeneration (ARMD).[10] We have previously reported the discovery of a novel series of arylsulfonylthiophene-2-carboxamidine inhibitors[11] of the complement component C1s, a trypsin-like serine protease that is present as a proenzyme within the first component of complement in the classical pathway.[12] We have continued to optimize the potency and physicochemical properties of these compounds in order to identify a candidate compound suitable for dosing as an intravenous agent for the treatment of acute complement mediated diseases such as IRI and ARDS.



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Our previous work identified compound **1**, which has a K_i of 0.36 μM for C1s.[11] Further substitution at the 3-position of the phenyl ring provided compound **3** with

Keywords: C1s; Complement; Protease; Inhibitor; Amidine; Pharmacokinetic; Suzuki.
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a K_i of 0.26 μM. This observation led to more extensive exploration of the structure–activity relationship (SAR) at the 3-position of the phenyl ring. Molecular modeling studies using the published C1s structure[13,14] suggested two possible binding modes for substituents originating from the 3-position of the phenyl ring A (Fig. 1). To further explore the SAR at this position, a small library of biaryl compounds was synthesized via parallel synthesis (Table 1).[15] Compound **1** was treated with *tert*-butyloxycarbonyl anhydride in THF to protect the amidine moiety. The protected amidine **2** (Scheme 1) was coupled to a series of aryl and heteroaryl boronic acids using Suzuki coupling conditions to give compounds **3**–**18**.[16] Compound **19** was prepared by treating compound **1** with 3-methyl-2-pyridylzinc bromide. Replacement of bromo with phenyl to give compound **3** provides only a marginal improvement in activity. Further substitution on compound **3** involving the introduction of small hydrophobic or hydrophilic groups at the 3-position (**5**, **8**, **11**) or the 4-position (**6**, **9**, **12**) of the distal aryl ring (ring B) did not have a significant effect on activity. However, addition of small hydrophobic groups at the 2-position of ring B generated compounds with significantly improved potency. For example, as shown in Table 1, when R^1 is 2-methyl (**7**) or 2-chloro (**14**) there is approximately 9- and 7-fold enhancement in activity, respectively, in comparison to compound **3**.

Molecular modeling suggests a preference for binding conformations in which ring B is orthogonal to the proximal aryl ring (ring A), and this applies to both possible binding modes (Fig. 1). The improved potency observed with compounds **7** and **14** is likely due to both a conformational effect and enhanced binding contacts. Having a substituent at the *ortho* position of ring B (**4**, **7**, **10**, **13**, and **14**) can result in the biphenyl dihedral being closer to the predicted bound conformation than in the unsubstituted case, **3**. In either of the plausible binding modes the *ortho* substituent could be directed toward the protein surface, or away from it and out toward solvent (Fig. 1). Modeling studies also suggested that a small hydrophobic residue at the 2-position can make a favorable interaction with the protein surface and this is consistent with the improved potency observed with compounds **7** and **14**, compared to the unsubstituted compound **3**. However, larger hydrophobic residues would make this an unfavorable binding conformation due to steric-clash with the protein surface. Introducing larger hydrophobic residues (**4** and **15**) or hydrophilic residues (**10** and **13**) at this position has little effect on activity, fully consistent with a binding mode in which these substituents can be directed toward the solvent.

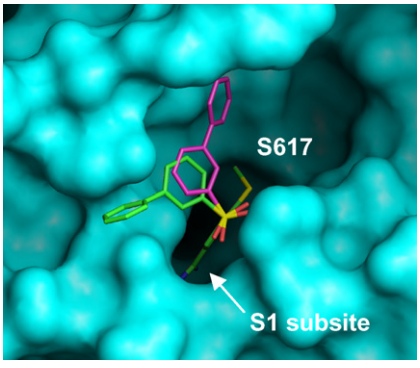
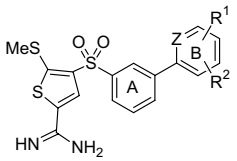


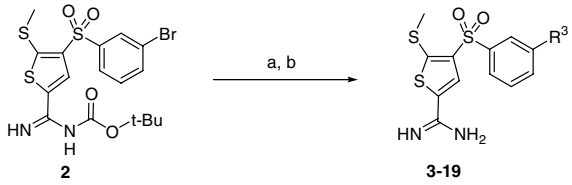
Figure 1. Binding models of **2** in the active site of C1s. These models are derived from the crystal structure of an analog of **2** bound to a C1s homolog (unpublished results), which shows that the thiophene amidine occupies the S1 subsite. The C1s crystal structure[14] is depicted with a Connolly surface[19] (cyan) and the two binding models with green and magenta carbons, respectively. The figure was prepared with PyMOL.[20]

Table 1. SAR from Suzuki coupling library



Compound	Z	R^1	R^2	K_i[a] (nM)
3	C	H	H	260
4	C	2-OCH_3	H	610
5	C	3-OCH_3	H	490
6	C	4-OCH_3	H	350
7	C	2-CH_3	H	30
8	C	3-CH_3	H	1850
9	C	4-CH_3	H	250
10	C	2-OH	H	460
11	C	3-OH	H	150
12	C	4-OH	H	190
13	C	2-CH_2OH	H	120
14	C	2-Cl	H	40
15	C	2-$CHCH_2$	H	140
16	C	2-CH_3	6-CH_2OH	50
17	C	2-CH_3	6-CO_2H	150
18	C	2-CH_3	NH_2	20
19	N	2-CH_3	—	40

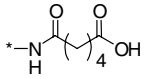
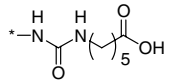
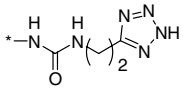
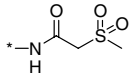
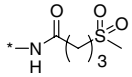
[a] K_i values were determined as described in Ref. 11. Between-run coefficient of variation was <24%.



Scheme 1. Reagents and conditions: (a) $R^3B(OH)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3, EtOH, toluene, 80 °C, 12 h or R^3ZnBr, $Pd(PPh_3)_4$, THF, 80 °C, 1 h; (b) 50% TFA/DCM.

Compounds **3**–**15** did not have sufficient aqueous solubility at physiological pH to be evaluated as potential intravenous agents. To mitigate the poor aqueous solubility of compound **7**, the most potent in the series, a pyridyl ring was introduced instead of the phenyl ring to give compound **19**. While compound **19** had the required solubility properties, it had poor pharmacokinetic properties, a poor in vitro safety profile,[17] and potent hERG K channel blocking in the patch-clamp assay[18] (Table 2). In an alternative approach, **7** was further substituted with hydrophilic residues at the

Table 2. Amine substituted derivatives of compound **18**: potency, solubility, and in vitro safety

Compound	R^5	Z	K_i[a] (nM)	Aqueous solubility (μM)	Activity in SEP (% inhibition at 10 μM)[b]	Inhibition of hERG (% inhibition at 10 μM)[18]
19	H	N	45	22	*A* = 69; *B* = 74; *C* = 95; *D* = 76; *E* = 83	66.0
30		C	42	40	*A* = 2; *B* = 1; *C* = 9; *D* = 11; *E* = 17	0.5
31		C	22	39	*A* = 0; *B* = 15; *C* = 0; *D* = 26; *E* = 5	5.8
32		C	64	—	*A* = 7; *B* = 5; *C* = 9; *D* = 21; *E* = 11	1.0
33		C	12	43	*A* = 23; *B* = 47; *C* = 31; *D* = 51; *E* = 73	0.9
34		C	14	35	*A* = 9; *B* = 28; *C* = 40; *D* = 54; *E* = 53	18.0

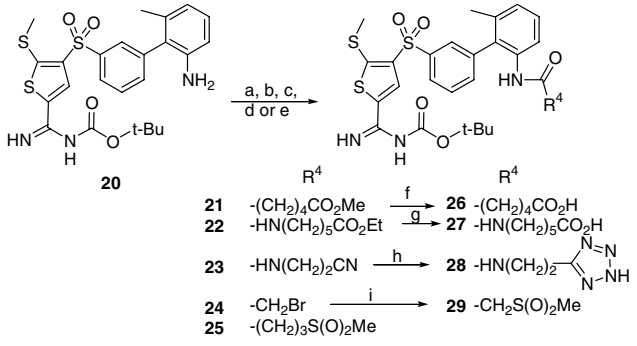
NovaScreen uses a criteria of 50% inhibition or greater to qualify a compound as active. *A* = adrenergic, alpha 1, non-selective; *B* = dopamine transporter; *C* = muscarinic, non-selective, peripheral; *D* = opiate, non-selective; *E* = sodium, site 2.

[a] K_i values were determined as described in Ref. 11. Between-run coefficient of variation was <24%.

[b] Compounds were tested in the NovaScreen General Side Effect Profile (SEP).[17]

6-position of the phenyl ring B. Since the 6-position appeared to be solvent-exposed this was a reasonable strategy to follow. While introduction of 6-hydroxymethyl (**16**) and 6-carboxy (**17**) groups reduced potency 2- and 6-fold, respectively, 6-amino (**18**) substitution did not change potency. The 6-amino group was further substituted with a series of hydrophilic groups (Table 2) with the hope of improving solubility as well as the pharmacologic side-effect profile.

The *tert*-butyloxycarbonyl-protected scaffold, **20** was synthesized according to Scheme 1 and functionalized according to Scheme 2 to give compounds **30**–**34**.[15] Compound **20** was treated with 5-chlorocarbonyl-pentanoic acid methyl ester in the presence of base to give the ester **21**. The ester **21** was hydrolyzed to give the acid **26** which was deprotected with 50% TFA in DCM to give the final amidine **30**. Compound **20** was treated with 6-isocyanato-hexanoic acid ethyl ester in the presence of base to give **22**. The ester **22** was hydrolyzed to the acid **27**, which was deprotected with TFA to give targeted compound **31**. Compound **20** was treated with 4-nitrophenyl chloroformate and pyridine to give the 4-nitrophenyl carbamate which was reacted with 3-aminopropionitrile to give the urea **23**. Compound **23** was treated with azidotrimethylsilane and dibutyltin oxide to give the tetrazole **28**, which was converted to the amidine **32** with TFA. Compound **20** was reacted with bromoacetylbromide to give the amide **24**, which was treated with sodium methanesulfinate to give the sulfone **29**. The sulfone **29** was deprotected with TFA to give the amidine **33**. Compound **20** was treated with 4-methanesulfonyl-butyrylchloride to give the sulfone **25**, which was converted to the amidine **34** with TFA.



Scheme 2. Reagents and conditions: (a) $MeO_2C(CH_2)_4COCl$, DIEA; (b) $EtO_2C(CH_2)_5NCO$, TEA, DCM, 3 h; (c) i—*p*-nitrophenyl chloroformate, pyridine, DCM, 4 h; ii—$NC(CH_2)_3NH_2$, DCM, 48 h; (d) $BrCH_2COBr$, DIEA, $CHCl_3$; (e) $CH_3(O)_2S(CH_2)_3COCl$, DIEA; (f) LiOH, 60 °C, 12 h; (g) 1 N NaOH, 2 h; (h) $(CH_3)_3SiN_3$, dibutyltin oxide, toluene, 70 °C, 16 h, 80 °C 4 h; (i) CH_3SO_2Na, EtOH, 16 h.

Incorporating either the carboxylic acid or the sulfone moiety (**30**, **31**, **33**, and **34**) provided compounds with acceptable aqueous solubility. Compound **19** was tested in a 'side-effect' profile panel of 54 target assays from NovaScreen.[17] The compound showed significant activity at 5 receptor targets (Table 2). In contrast, compounds **30**, **31**, and **32** proved to be completely inactive in these assays. Incorporating sulfone moieties provided compounds that had some activity in 2 out of 54 assays. Compounds **30**–**33** were completely devoid of any hERG activity, while compound **34** had weak potency.

Compounds **19**, **31**, and **34** were dosed intravenously in rat pharmacokinetic studies and proved to have very high clearances (2- to 4-fold rat hepatic blood flow) and short half-lives (Table 3).

In summary, we have discovered a novel series of potent and selective C1s inhibitors. These are among the most potent compounds reported to date. More lipophilic compounds in this series such as **19** exhibit significant hERG channel binding activity while more hydrophilic

Table 3. Rat pharmacokinetic properties for selected compounds[a]

Compound	$T_{1/2}$ (h)	V_d (L/kg)	Cl (mL/min/kg)
19	2.0	15	283.0
31	0.2	2.65	228.5
34	1.8	2.03	129.6

[a] Three animals were used for each dosing group. The rats were dosed 2 mg/kg intravenous.

compounds such as **30** are devoid of significant hERG activity. Incorporating hydrophilic residues also improved selectivity against the NovaScreen SEP panel. However, these compounds have poor in vivo pharmacokinetic properties. Further structural modifications to improve in vivo pharmacokinetic properties are in progress.

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