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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2200–2204

A novel series of arylsulfonylthiophene-2-carboxamidine inhibitors of the complement component C1s

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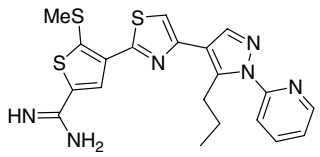
Received 18 November 2005; revised 10 January 2006; accepted 11 January 2006
Available online 3 February 2006

Abstract—Inhibiting the classical pathway of complement activation by attenuating the proteolytic activity of the serine protease C1s is a potential strategy for the therapeutic intervention in disease states such as hereditary angioedema, ischemia–reperfusion injury, and acute transplant rejection. A series of arylsulfonylthiophene-2-carboxamidine inhibitors of C1s were synthesized and evaluated for C1s inhibitory activity. The most potent compound had a K_i of 10 nM and >1000-fold selectivity over uPA, tPA, FX_a, thrombin, and plasmin.

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. 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] C1s is a trypsin-like serine protease that is present as a proenzyme within the first component of complement in the classical pathway.[8] Under normal physiological conditions activated C1s is inhibited by its endogenous inhibitor, C1 esterase inhibitor (C1-INH). Pathological conditions result in excessive activation of complement that is not sufficiently inhibited by C1-INH. Furthermore, C1-INH can be degraded by other proteases that are released during complement-mediated inflammation. A small molecule inhibitor of C1s would be a useful therapeutic agent in the treatment of complement-mediated disease.[9]

We have previously reported the discovery of a novel series of thiopheneamidine C1s inhibitors (**1**).[10] These compounds, while having very good selectivity over thrombin, plasmin, and FX_a, had poor selectivity over the serine protease urokinase plasminogen activator (uPA). Because the thiopheneamidine moiety appeared to be a good S1 binding fragment for C1s, several compound libraries containing this moiety were screened for C1s inhibitory activity. Compound **2** was identified as an 11 μM inhibitor of C1s, with 2-fold selectivity over uPA. Substituting the isopropyl residue with a phenyl residue provided compound **3** with $K_i = 0.75$ μM for C1s and 13-fold selectivity over uPA.



1 $K_{i\,(uPA)} = 0.135$ μM; $K_{i\,(C1s)} = 0.065$ μM

The observed selectivity profile for **1** and **3** may be related, at least in part, to a difference at Lys614 (Gln192; C1s numbering, followed by uPA/chymotrypsinogen

Keywords: Classical pathway; Complement; C1s; Complement inhibitor; C1s inhibitor; Classical pathway inhibitor.
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doi:10.1016/j.bmcl.2006.01.036

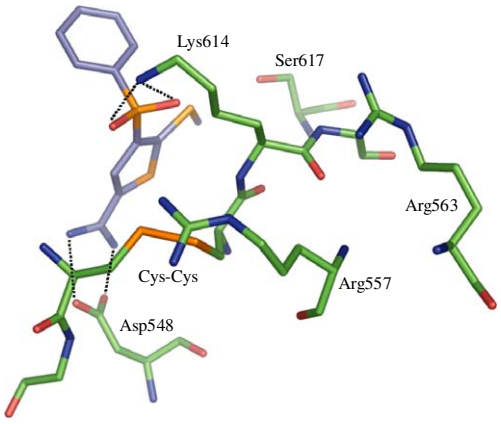


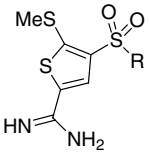
Figure 1. Model of sulfone **3** bound to C1s. This figure shows selected residues of C1s (PDB ID 1ELV; residue numbering is according to 1ELV; Ser617 is the catalytic serine, Asp548 is the canonical S1 acid residue of trypsin-like serine proteases), with superposition of the thiophene amidine as described previously.[10] Cys-Cys indicates the cystine disulfide formed by Cys613 and Cys644, and the dashed lines indicate key intermolecular electrostatic contacts of the binding model (see text). In this model, the side-chain conformation of Lys614 has been adjusted from that described in the published crystal structure,[11] to favor interaction with the sulfone moiety of **3**. The distance between Lys614:NZ and the two inhibitor sulfone oxygen atoms is 3.0 and 3.5 Å, respectively.

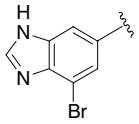
numbering in parentheses). In the original description of the C1s structure, Gaboriaud et al.[11] noted the potential of a role for Lys614 in binding specificity. Lys614 of C1s is adjacent to S1 and is positioned so that it could play a role in restricting access to S1 and/or the catalytic region. Our binding models (Fig. 1),[10,12] with inhibitors modeled into S1 of the apo C1s structure, are consistent with the previously hypothesized importance of Lys614 in active-site binding. We extend this hypothesis to propose that Lys614 of C1s and the corresponding uPA residue Gln192 are involved in defining C1s selectivity for compound **3**. Figure 1 details some of the key features of our proposed C1s binding model for **3**. Arg557 and Arg563 are positioned to constrain the side chain of Lys614 to point in the direction of S1, and Lys614:NZ can form a hydrogen bond with one or possibly both of the sulfone oxygen atoms of **3** as it is positioned in our model. Such interactions cannot be readily modeled for **1**. Additionally, Gln192, the corresponding residue in uPA, is too short to achieve a similar interaction and also appears to be restricted from leaning into S1 due to a hydrogen bonding interaction with Lys143 (not shown). Therefore, it seems reasonable to propose that C1s shows an increased preference for **3** due to hydrogen bonding interactions with Lys614:NZ.

Several other aryl- and alkyl-sulfonyl derivatives (Table 1) were synthesized in order to identify a core fragment with significantly potent inhibitory activity toward C1s. The benzimidazole **9** was chosen as a starting point for further lead optimization. In this paper, we describe structure–activity relationships and in vitro biological results for this series of C1s inhibitors.

Compounds **3**–**8** and **10** (Table 1) were synthesized according to Scheme 1. Treating 4-Br-5-NO_2-thiophene-2-carboxylate **36**[13] with an appropriate thiol at −78 °C resulted in substitution at the 4-position to give the corresponding thioether, which was oxidized to the sulfone **37** by treating with MCPBA in refluxing DCM. Treating the sulfone **37** with sodium thiomethoxide at −78 °C affords primarily the nitro-displacement product, which was converted to the amidine **38** by treating with trimethylaluminum and ammonium chloride in refluxing toluene.[14] When the thiomethoxide addition was performed at room temperature, mixtures of nitro and sulfone displacement products were observed.

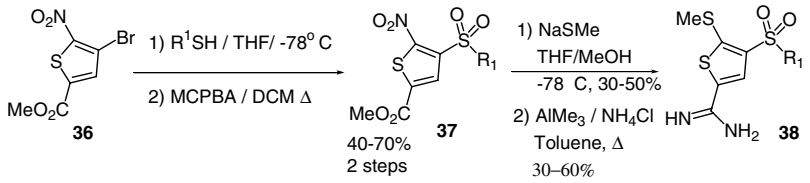
Table 1. SAR of sulfone substitution



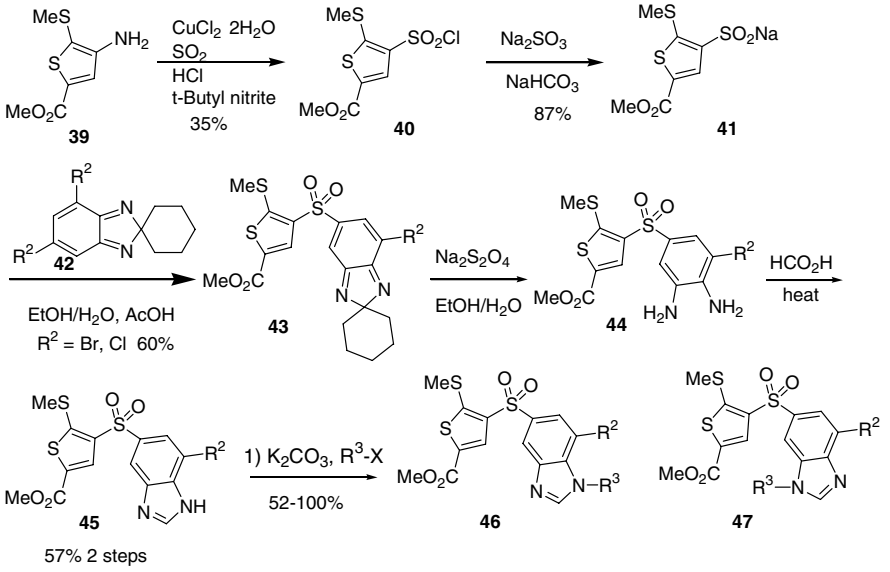
Compound	R	C1s inhibition K_i (μM)
2	$CH_2(CH_3)_2$	11.0
3	Phenyl	0.75
4	2-Naphthyl	0.41
5	2-Pyridyl	2.29
6	1-Methylimidazol-2-yl	1.88
7	2-Methylfuran-3-yl	1.79
8	2-Thiopheneyl	2.24
9		0.40
10	Benzyl	2.0

Compounds **9**, **11**, **12**, **34**, and **35** were synthesized according to Scheme 2. The diazonium salt of amine **39**[15] was treated with sulfur dioxide in the presence of cupric chloride to give the sulfonyl chloride **40**.[16] Sulfonyl chloride **40** was reduced with sodium sulfite to give the sulfinate **41**.[17] Addition of **41** to 5,7-dihalo-benzimidazole (**42**) afforded the sulfone **43**.[18] Sulfone **43** was reduced with sodium dithionite to **44**, which upon heating with formic acid gave the benzimidazole **45**. Benzimidazole **45** was directly converted to the amidine to give compound **9**. Alkylation of **45** with the appropriate alkylhalide to give the regioisomers **46** and **47**, followed by amidination, gave compounds **11**, **12**, **34**, and **35**. To overcome the poor yields associated with this method of benzimidazole synthesis, an alternative route (Scheme 3) was also pursued.

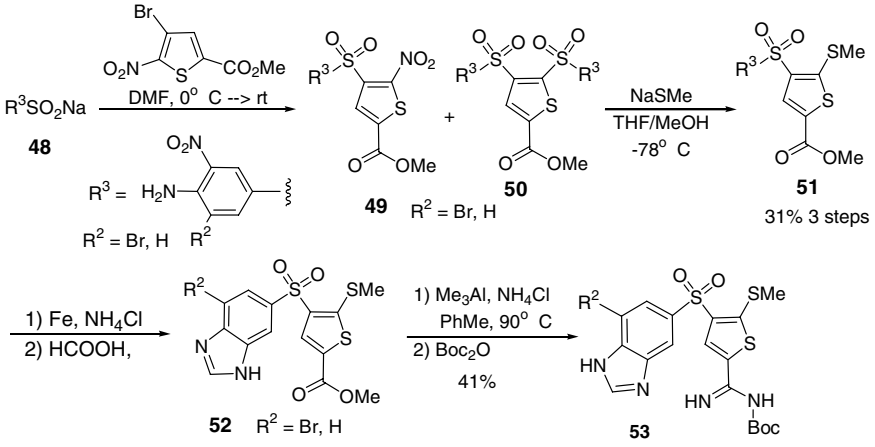
The sulfinate **48** (R^2 = Br) was obtained by converting the corresponding sulfonamide[19] to the sulfonyl chloride by treating with chlorosulfonic acid,[19] followed by reduction with sodium sulfite. Sulfinate **48** (R^2 = H) was obtained by reducing the commercially available 4-acetylamino-3-nitrobenzenesulfonyl chloride, followed by hydrolysis of the acetamide. Addition of **48** to the thiophene **36** gave a mixture of **49** and **50**. Thio-



Scheme 1.



Scheme 2.



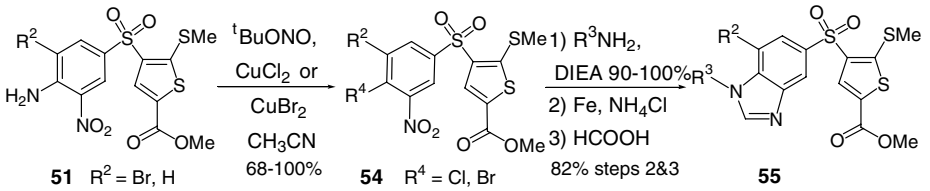
Scheme 3.

methoxide addition occurs selectively at the 5-position of both **49** and **50** to give compound **51**.

Reduction of the nitro group followed by heating in formic acid provided the benzimidazole **52**.

Benzimidazole **52** (R^2 = Br) was converted to the corresponding amidine and BOC-protected to provide a common scaffold that was alkylated with the respective alkylhalide and deprotected to provide compounds **15**–**20** and **23**–**30**. Benzimidazole **52** (R^2 = H) was alkylated with benzylbromide and converted to the amidine directly to give compounds **31** and **32**.

Compounds **14**, **21**, **22**, and **33** were regioselectively accessed (Scheme 4) starting from intermediate **51**. Compound **51** was diazotized and halogenated to give compound **54**. Displacement of the halogen with an appropriate amine followed by reduction and cyclization in formic acid gave the benzimidazole **55**. Compound **45** (R^2 = Br) was treated with benzeneboronic acid in the presence of copper(II)acetate to provide the *N*-phenyl derivative,[20] which was treated with trimethylaluminum and ammonium chloride in refluxing toluene to give compound **13**. Arylation occurs regioselectively at the less hindered nitrogen of the benzimidazole.

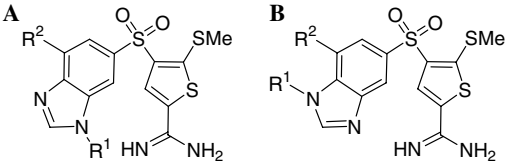


Scheme 4.

Table 2 lists the K_i values for the inhibition of C1s.[21] N-methylation at either nitrogen (**11**,**12**) has minimal effect on affinity, suggesting that these nitrogens are not involved in a hydrogen bond. Interestingly, when R^2 is bromine the effect of N-alkylmethyl and N-arylmethyl substitution on affinity varies depending on the nitrogen that is substituted.

When the N-benzyl substituent is on the nitrogen proximal to the bromine (**20**), there is a 13-fold improvement in activity, whereas similar substitution at the distal nitrogen (**19**) provides no enhancement in activity. This pattern holds true for all the N-alkylmethyl and N-arylmethyl substituents, where compounds **16**, **18**, **20**, **24**, **26**, **28**, and **30** provide 6- to 40-fold improvement in activity, while the corresponding regioisomers **15**, **17**, **19**, **23**, **25**, **27**, and **29** show only marginal enhancement in activity. Unfortunately, some of the key pieces of information required to establish a single plausible binding model that explains the observed SAR is lacking. Most significantly, a structure of an inhibitor or substrate complex is not available, and key specificity determining regions of the available apo structure are disordered.[11] Our modeling studies suggest that bound inhibitors could be making hydrophobic interactions with the region of loop 3[22] that is disordered in the apo structure.[11] Nevertheless, our results suggest that there is a hydrophobic binding site for the N-alkylmethyl and N-arylmethyl substituents that is proximal to the bromine. Even though further substitution on the aryl

Table 2. SAR of the benzimidazole series



Compound	R^2	R^1	C1s inhibition K_i (μM)	
			A	B
11	Br	Me	0.33	
12	Br	Me		0.40
13	Br	Ph	0.18	
14	Br	Ph		0.31
15	Br	Allyl	0.21	
16	Br	Allyl		0.07
17	Br		0.20	
18	Br			0.05
19	Br	Benzyl	0.4	
20	Br	Benzyl		0.03
21	Br	(*R*)-α-Me-Benzyl		0.38
22	Br	(*S*)-α-Me-Benzyl		0.33
23	Br	2,6-Dichlorobenzyl	0.16	
24	Br	2,6-Dichlorobenzyl		0.02
25	Br	2,6-Difluorobenzyl	0.17	
26	Br	2,6-Difluorobenzyl		0.04
27	Br	2,5-Difluorobenzyl	0.24	
28	Br	2,5-Difluorobenzyl		0.01
29	Br	2-Fluoro-5-nitrobenzyl	0.17	
30	Br	2-Fluoro-5-nitrobenzyl		0.03
31	H	Benzyl	0.42	
32	H	Benzyl		0.40
33	H	2-Pyridylmethyl		0.58
34	Cl	2,6-Difluorobenzyl	0.10	
35	Cl	2,6-Difluorobenzyl		0.10

Table 3. Protease selectivity for compound **28**

uPA	tPA	FX_a	Thrombin	Plasmin	Trypsin
K_i (μM)					
>10[a]	>10[a]	11.4	>15[a]	>13[a]	1.4

[a] No inhibition observed at this screening concentration.

group of the arylmethyl substituents does not provide useful SAR information, the diminished activity of compounds **21** and **22** suggests that substituents on the methylene group can prevent the benzyl residue from achieving a conformation that favors a positive interaction with the hydrophobic binding pocket. The observed loss of activity when the bromine was substituted with hydrogen or chlorine (cf. **20**–**32** and **26**–**35**) suggests that this residue is contributing to affinity through intermolecular hydrophobic contacts.

In conclusion, lead optimization studies around the arylsulfonylthiophene-2-carboxamidine template has resulted in a series of *N*-benzylbenzimidazoles with good C1s inhibitory potency and >1000-fold selectivity over uPA (Table 3). Compound **28** also has good selectivity over tPA, FX_a, thrombin, and plasmin.[21]

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21. Dissociation constants were determined at 37 °C in a 96-well format using a Molecular Devices plate reader. Varied concentrations of inhibitors in 10 μL dimethyl sulfoxide were added to wells with 280 μL of assay buffer (pH 7.5) which contained 50 mM HEPES, 0.2 M NaCl, 1% dimethyl sulfoxide, 0.05% *n*-octyl β-D-glucopyranoside, and substrate, and incubated for 15 min at 37 °C. Reactions were initiated by the addition of 10 μL of enzyme in assay buffer without dimethyl sulfoxide and substrate, and the change in absorbance at 405 nm was monitored for 15 min. IC_{50}s were obtained from the inverse slope of plots of the ratio of initial velocity in the absence of inhibitor to initial velocity in the presence of inhibitor as a function of inhibitor concentration. All velocities were corrected for background substrate conversion (no enzyme addition). The dissociation constant (K_i) was calculated using the equation $K_i = IC_{50}/(1 + S/K_m)$ where S is the substrate concentration (Cheng, Y.; Prusoff, W. H. *Biochem Pharmacol.* **1973**, *22*, 3099). K_m values for each enzyme–substrate pair were determined from Hanes–Wolf plots using the same final buffer conditions as K_i determinations. Respective substrate, substrate concentration, and K_m values for each enzyme were: human C1s: benzyloxycarbonyl-Gly-Arg-S-benzyl, 45 μM in 200 μM DTNB (5,5′-dithio-bis-[2-nitrobenzoic acid]); 190 μM; human α-thrombin:succinyl-Ala-Ala-Pro-Arg-*p*-nitroanilide, 100, 320 μM; human factor Xa: benzyloxycarbonyl-D-Arg-Gly-Arg-*p*-nitroanilide, 100, 260 μM; human urokinase: pyro-Glu-Gly-Arg-*p*-nitroanilide, 86, 87 μM; human plasmin: H-D-Val-Leu-Lys-*p*-nitroanilide, 150, 300 μM; human trypsin: benzyloxycarbonyl-D-Arg-Gly-Arg-*p*-nitroanilide, 60, 61 μM; human tPA (2-chain): methlysulfonyl-D-cyclohexyl-Try-Gly-Arg-*p*-nitroanilide, 200, 200 μM. Within-run assay coefficient of variation (CV) was <10%; between-run CV was <20%.
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