Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3043–3047
Bioorganic & Medicinal Chemistry Letters

A novel series of potent and selective small molecule inhibitors of the complement component C1s

Nalin L. Subasinghe,[a,*] Farah Ali,[a] Carl R. Illig,[a] M. Jonathan Rudolph,[a] Scott Klein,[a] Ehab Khalil,[a] Richard M. Soll,[a] Roger F. Bone,[a] John C. Spurlino,[a] Renee L. DesJarlais,[a] Carl S. Crysler,[a] Maxwell D. Cummings,[a] Philip E. Morris, Jr.,[b] John M. Kilpatrick[b] and Y. Sudhakara Babu[b]

[a]*3-Dimensional Pharmaceuticals Inc., 665 Stockton Drive, Exton, PA 19341, USA*
[b]*BioCryst Pharmaceuticals, Inc., 2190 Parkway Lake Drive, Birmingham, AL 35244, USA*

Received 26 March 2004; revised 13 April 2004; accepted 13 April 2004

Abstract—Activation of the classical pathway of complement has been implicated in disease states such as hereditary angioedema, ischemia-reperfusion injury and acute transplant rejection. The trypsin-like serine protease C1s represents a pivotal upstream point of control in the classical pathway of complement activation and is therefore likely to be a useful target in the therapeutic intervention of these disease states. A series of thiopheneamidine-based inhibitors of C1s has been optimized to give a 70 nM inhibitor that inhibits the classical pathway of complement activation in vitro.
© 2004 Elsevier Ltd. All rights reserved.

The complement cascade is a major component of the innate immune system in mammals and other vertebrate species.[1] Complement activation plays a major role in the destruction of invading microorganisms and the clearance of immune complexes. Complement is activated by three pathways, the classical pathway, the alternative pathway, and the mannan-binding lectin (MBL) pathway. The classical pathway is primarily activated by immunoglobulin (Ig) antigen complexes binding to C1q, a component of the macromolecular C1 complex that also includes C1s and C1r. Antigen-Ig complex binding to C1q results in the auto-activation of C1r, which in turn activates C1s. Activated C1s cleaves its natural substrates C2 and C4 to give fragments C4b and C2a, which combine to form the classical pathway C3 convertase (C4bC2a). The remaining steps in the activation pathway are common to all three pathways and are initiated by C3 convertase of the corresponding pathway cleaving C3 to release the anaphylatoxin C3a. The remaining C3 fragment (C3b) combines with C3 convertase to form C5 convertase, which cleaves C5 and releases the potent anaphylatoxin C5a. The resulting C5b fragment, while still attached to C5 convertase, sequentially combines with C6 and C7 to form the C5b-7 complex, which is released into the fluid phase. The C5b-7 complex inserts into the lipid bilayer of the cell membrane and combines with C8 and several copies of C9, to form a transmembrane pore (Membrane Attack Complex, MAC) resulting in cytolysis.

The alternative pathway is in a state of continuous low-level activation due to spontaneous hydrolysis of the thioester residue in native C3, which results in a form of C3 that can bind to factor B to produce a C3 convertase (C3H2OBb).[2] Low-level production of C3b is maintained by proteolysis of C3 by C3H2OBb. Binding of C3b to surfaces of pathogens that do not contain complement inhibitors results in the amplification of the alternative pathway following a series of steps that leads to the formation of the alternative pathway C3 convertase (C3Bb). The mannan-binding lectin pathway is activated by mannose-binding lectin (MBL) binding to carbohydrate polymers on pathogenic surfaces.

Under normal physiological conditions, complement activation is regulated by a series of membrane-bound and soluble complement control proteins. Unregulated complement activation leading to acute inflammation

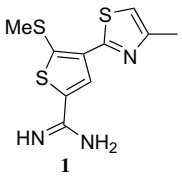
Keywords: C1s; Complement; Inhibitor; Small; Molecule; Thiopheneamidine; Amidine; Classical.
* Corresponding author. Tel.: +1-610-458-6066; fax: +1-610-458-8249; e-mail: nsubasin@prdus.jnj.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.034

and tissue damage has been implicated in the pathology of many disease states.[3] Activation of the classical pathway has been implicated in humorally mediated graft rejection,[4] ischemia-reperfusion injury (IRI),[5] hereditary angioedema (HAE),[6] vascular leak syndrome,[7] and ARDS.[8] Treatment with C1 inhibitor (C1-INH) a plasma serpin inhibitor of C1s has proven beneficial in animal models of IRI.[9] Life-threatening mucosal edema in HAE, a condition resulting from C1 deficiency, is effectively managed with C1-INH replacement therapy.[10] C1-INH has also proven to be beneficial in IRI,[11] vascular leak syndrome following bone marrow transplantation[12] and acute pancreatits[13] in small groups of patients. However, it is difficult to specifically ascribe the therapeutic effect observed with C1-INH to classical pathway inhibition due to its ability to inhibit kallikrein, plasmin, and the MBL pathway enzyme MASP-2. Even though classical pathway-selective inhibitors have not entered into clinical use, a peptidomimetic C1s-selective inhibitor has shown efficacy in a rabbit model of IRI.[14]

The recently reported high-resolution crystal structure of C1s[15] established that the protease domain adopts the classic chymotrypsin-like fold.[16] C1s exhibits trypsin-like substrate specificity, and recognition of physiological substrates involves an extended recognition surface comprising elements from multiple modules of the C1 complex. Unfortunately there is currently no C1s structure available with a bound active-site ligand. Thus, structural analyses and modeling studies based on the structural information presently available may only yield a partial picture of biologically relevant protein–ligand binding interactions.

A broad screen of small molecule libraries containing compounds with basic residues that could potentially bind to the C1s specificity pocket resulted in the identification of thiopheneamidine **1** (3.5 μM), a weak inhibitor of C1s.[17]



1

Modeling studies with the deposited[18] C1s crystal structure[15] and compound **1** indicate that when the amidine moiety is docked into the S1[19] pocket such that it forms a salt bridge with Asp 611(189) of C1s (C1s numbering, followed by chymotrypsinogen numbering in parentheses, see also Fig. 1), the thiophene ring fully occupies the binding pocket. Based on proprietary structures of similar small molecules bound to related serine proteases (not shown) this aspect of our binding model seems unequivocal. The uncertainty of the binding model increases with distance from S1.

The 5-methylthio substituent on the thiophene ring occupies a small pocket near the catalytic triad. Of the substituents tested at this position, the 5-methylthio group is optimal (Table 1). Removal results in a significant loss of activity, as does replacement with smaller or larger hydrophobic groups. The methylthio group was replaced with electron withdrawing substituents in an attempt to reduce the pK_a of the amidine, and these compounds had significantly reduced activity as well.

Subsequent structure–activity studies were directed toward the thiazole ring. A series of substituted thiazoles was synthesized and tested in the C1s enzyme assay (Table 2). Replacing the methyl group at R_2 of compound **1** with hydrophobic aromatic groups provides a 5–8-fold improvement in activity (e.g., **11**–**13**, **16**, **19**, and **21**). Similar substitution at R_3 (**20**) did not change activity. In general these results are consistent with our favored binding model, which indicates that substituents

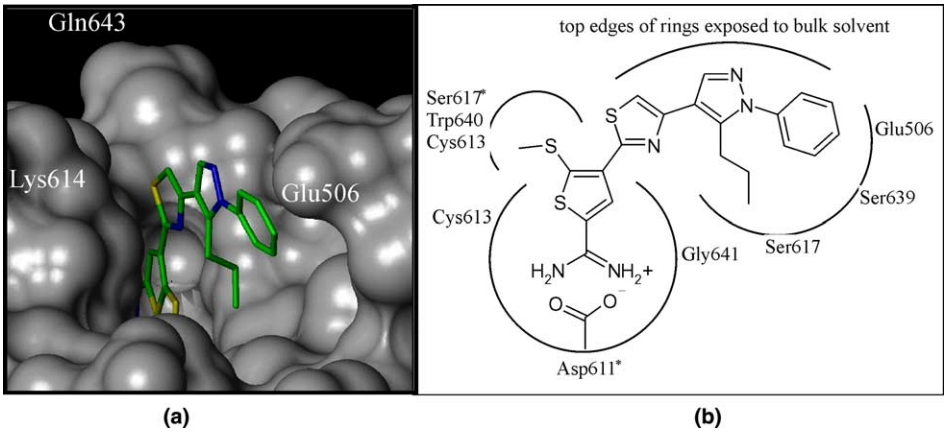
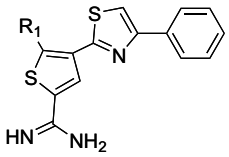


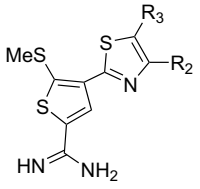
Figure 1. (a) Binding model of **49** in the active site of C1s. This model is derived from the crystal structure of an analog of **49** bound to a C1s homolog (unpublished results). The partially disordered side chain of Lys614(192)[15] has been adjusted to a different rotamer from that in the deposited structure, to accommodate the modeled inhibitor. Figure shows the C1s crystal structure[15] with a Connolly surface[24] prepared with InsightII (Accelrys). (b) Schematic of C1s binding model of **49**. Residue names denote key interactions (e.g., Asp611(189)) or residues closest to indicated moiety of bound inhibitor (e.g., Trp640(215), Glu506(970)). *Residue numbers are according to the deposited C1s crystal structure,[15] followed by chymotrypsinogen numbering in parentheses.

Table 1. SAR of thiophene C-5 substitution



Compound	R_1	K_i, μM
2	SCH_3	0.56
3	H	>20
4	SCH_2CH_3	>20
5	SBnz	>20
6	CH_3CH_2	3
7	CH_3	6
8	SO_2CH_3	>28
9	CF_3	12.5
10	OCH_3	14.4

Table 2. SAR of thiazole substitution



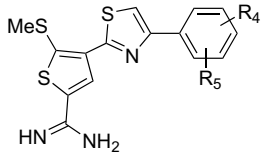
Compound	R_2	R_3	K_i, μM
11	Benzothiophen-3-yl	H	0.45
12	2-Naphthyl	H	0.49
13	Thiophene-2-yl	H	0.70
14	2-Cl-3-pyridyl	H	1.02
15	4-Cl-3-pyridyl	H	0.87
16	$PhOCH_2$	H	0.52
17	Cyclohexyl	H	4.7
18	$PhCH_2$	H	1.06
19	Ph	H	0.56
20	H	Ph	3.25
21	*N*-Methylpyrazol-4-yl	H	0.46

at R_3 are likely to be more fully solvent exposed, while substituents at R_2 are more likely to yield binding contacts with the protein (Fig. 1).

Substitution around the phenyl ring of compound **19** did not provide a useful SAR (Table 3). 4-Ph substitution (**24**) results in a 28-fold loss in activity suggesting a steric-limit to 4-substitution. Modeling studies were less useful in understanding the SAR derived from these analogs, due to the possibility of multiple potential binding modes for these more distal regions of the inhibitors.

A series of analogs around compound **21** was synthesized according to Scheme 1 (Table 4).[17] Pyrazolylbromomethyl ketone **34** was treated with the thioamide **35** in refluxing acetone to give the corresponding thiazole, which was converted to the corresponding amidine **36** by treating with chlorodimethylaluminum ammonia complex.[20] Pyrazolylbromomethyl ketones were synthesized according to Scheme 2. An appropriately

Table 3. SAR of phenyl substitution for compound **19**



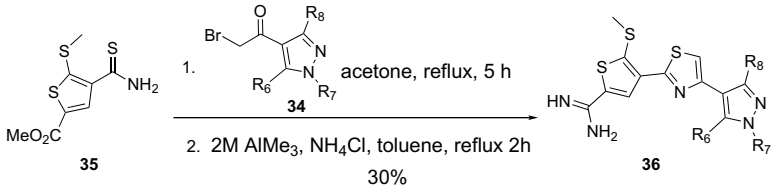
Compound	R_4	R_5	K_i, μM
22	4-CH_3	H	0.42
23	4-Cl	H	0.436
24	4-Phenyl	H	15.4
25	4-NO_2	H	1.0
26	4-CH_3O	H	0.9
27	2-CH_3O	H	0.85
28	2-CH_3	H	0.85
29	3-CH_3O	H	0.47
30	3-CH_3	H	0.96
31	2-CH_3O	4-CH_3O	0.64
32	2-Cl	4-Cl	3.0
33	3-NO_2	4-Cl	0.86

substituted ketoester **37** was condensed with *N*,*N*-dimethylaminoformaldehyde dimethylacetal to give the corresponding enamine **38**. Treatment of the enamine **38** with appropriately substituted hydrazine in refluxing ethanol provided the pyrazole ester **39**.[21] The pyrazole ester was hydrolyzed to the acid **40**, which was then converted to the corresponding acid chloride **41**. The acid chloride **41** was treated with trimethylsilyldiazomethane to give the corresponding silyldiazoketone, which was treated with hydrogen bromide in acetic acid to give the corresponding bromomethylketone **42**.[17] Several of the bromomethylketones were prepared from commercially available pyrazole carboxylic acids. The bromomethylketones required to accesses 3-*O*-alkyl-containing pyrazoles (**50**–**52**) were synthesized via Mitsunobu chemistry according to Scheme 3.[22] The commercially available pyrazole **43** was alkylated under Mitsunobu conditions to give the ether **44**. Compound **44** was hydrolyzed to the acid **45** and converted to the bromomethylketone according to Scheme 2.

In comparison with compound **21**, when R_6 or R_8 is propyl there is approximately 2-fold enhancement in activity (Table 4, **46** and **47**). Replacement of Me with Ph at R_7 (**48**) results in a 3-fold increase in activity. Phenyl at R_7 and propyl at R_6 (**49**) gave the most potent compound in this series. Substituents larger than propyl at R_6 attenuate activity (**51**–**52**). Substitution of phenyl at R_7 (**49**) with a pyridyl residue (**53**) provides a compound with similar potency and marginally improved solubility.

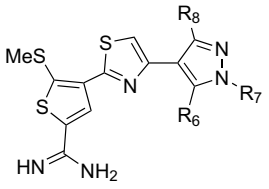
The C1s selectivity of compound **53** was assessed by screening against a panel of related serine proteases (Table 5). Against related complement cascade proteases Fd and C1r, compound **53** has greater than 53-fold and 700-fold selectivity, respectively. Compound **53** has 70-fold or greater selectivity over thrombin, plasmin, and FXa, and also has 3-fold selectivity over trypsin.

Compound **53** was tested in an erythrocyte hemolytic assay[23] and, as expected, proved to be a potent inhibitor

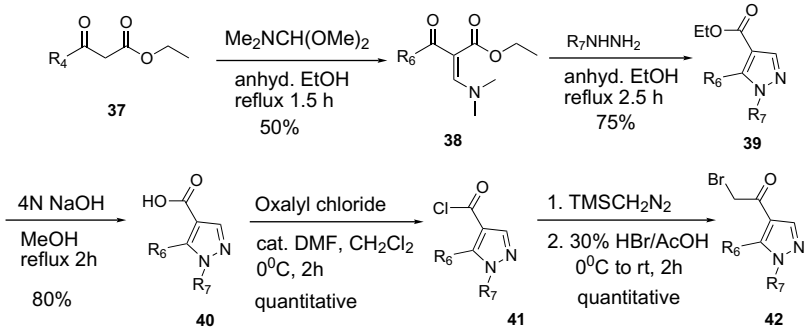


Scheme 1.

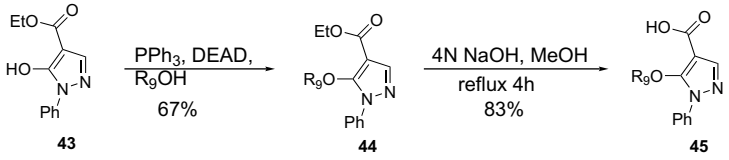
Table 4. SAR of the pyrazole series



Compound	R_6	R_7	R_8	K_i, μM
21	H	CH_3	H	0.46
46	$CH_3CH_2CH_2$	CH_3	H	0.33
47	H	CH_3	$CH_3CH_2CH_2$	0.19
48	H	Ph	H	0.15
49	$CH_3CH_2CH_2$	Ph	H	0.06
50	CH_3CH_2O	Ph	H	0.09
51	$(CH_3)_2CHCH_2CH_2O$	Ph	H	1.5
52	Cyclobutyl-CH_2O	Ph	H	0.53
53	$CH_3CH_2CH_2$	2-Pyr	H	0.07



Scheme 2.



Scheme 3.

Table 5. Protease selectivity for compound **53**

K_i, μM					
C1r	Fd	Fxa	Thrombin	Plasmin	Trypsin
>50[a]	3.7	5.0	>50[a]	5.0	0.18

[a] No observable inhibition at this screening concentration.

of the classical pathway of complement activation ($IC_{50} = 300$ nM).

In conclusion, starting from a weakly active P1 scaffold, we have constructed a novel series of potent and selective inhibitors of human C1s. These compounds inhibit the classical pathway of complement activation in vitro.

Further results from structure-based optimization and in vivo biological results will be reported in due course.

References and notes

1. (a) Walport, M. J. *N. Engl. J. Med.* **2001**, *344*, 1058; (b) Walport, M. J. *N. Engl. J. Med.* **2001**, *344*, 1140.
2. Pangburn, M. K.; Muller-Eberhard, H. J. *Biochem. J.* **1986**, *235*, 723.
3. Kirschfink, M. *Immunol. Rev.* **2001**, *180*, 177; Caliezi, C.; Wuillemin, W. A.; Zeerleder, S.; Redondo, M.; Eisele, B.; Hack, C. E. *Pharmacol. Rev.* **2000**, *52*, 91.
4. Pascual, M. A.; Crespo, M.; Tolkoff-Rubin, N. *Nefrologia* **2001**, *21*, 327.
5. D'Ambrosio, A. L.; Pinsky, D. J.; Connolly, E. S. *Mol. Med.* **2001**, *7*, 367.
6. Carugati, A.; Pappalardo, E.; Zingale, L. C.; Cicardi, M. *Mol. Immunol.* **2001**, *38*, 161.
7. Lissoni, P.; Barni, S.; Cattaneo, G.; Archili, C.; Crispino, S.; Tancini, G.; D'Angelo, L.; Magni, S.; Fiorelli, G. *Int. J. Biol. Markers* **1990**, *5*, 195.
8. Langlois, P. F.; Gawryl, M. S. *Clin. Immunol. Immunopathol.* **1988**, *47*, 152.
9. (a) Scherer, M.; Demertzis, S.; Langer, F.; Moritz, A.; Schafers, H. J. *Ann. Thorac. Surg.* **2002**, *73*, 233; (b) Buerke, M.; Prufer, D.; Dahm, M.; Oelert, H.; Meyer, J.; Darius, H. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 429.
10. Nzeako, U. C.; Frigas, E.; Tremaine, W. J.; Nzeako, U. C.; Frigas, E.; Tremaine, W. J. *Arch. Int. Med.* **2001**, *161*, 2417.
11. de Zwaan, C.; Kleine, A. H.; Diris, J. H.; Glatz, J. F.; Wellens, H. J.; Strengers, P. F.; Tissing, M.; Hack, C. E.; van Dieijen-Visser, M. P.; Hermens, W. T. *Eur. Heart J.* **2002**, *23*, 1670.
12. Nurnberger, W.; Heying, R.; Burdach, S.; Gobel, U. *Ann. Hematol.* **1997**, *75*, 95.
13. Schneider, D. T.; Nurnberger, W.; Stannigel, H.; Bonig, H.; Gobel, U. *Gut* **1999**, *45*, 733.
14. Buerke, M.; Schwertz, H.; Seitz, W.; Meyer, J.; Darius, H. *J. Immunol.* **2001**, *167*, 5375.
15. Gaboriaud, C.; Rossi, V.; Bally, I.; Arlaud, G. J.; Fontecilla-Camps, J. C. *EMBO J.* **2000**, *19*, 1755.
16. Perona, J. J.; Craik, C. S. *J. Biol. Chem.* **1997**, *272*, 29987.
17. Syntheses of thiazolylthiopheneamidines are described in: Illig, C. R.; Subasinghe, N. L.; Hoffman, J. B.; Wilson, K. J.; Rudolph, M. J. *Preparation of heteroarylamidines and analogs as protease inhibitors.* PCT Int. Appl. WO0047578, 2000; 326 pp; *Chem. Abstr.* **2000**, *133*, 177162.
18. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucl. Acids Res.* **2000**, *28*, 235.
19. Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1968**, *27*, 157.
20. (a) Garigipati, R. S. *Tetrahedron Lett.* **1990**, *31*, 1969; (b) Sidler, D. R.; Lovelace, T. C.; McNamara, J. M.; Reider, P. J. *J. Org. Chem.* **1994**, *59*, 1231.
21. Menozzi, G.; Mosti, L.; Schenone, P. *J. Heterocycl. Chem.* **1987**, *27*, 1169.
22. Representative procedure: Diethyl azodicarboxylate (Aldrich, 620 mL, 3.94 mmol) was added dropwise to a 0 °C solution of 3-hydroxy-1-phenyl-1*H*-pyrazole-4-carboxylic acid ethyl ester (Maybridge, 832 mg, 3.58 mmol), triphenylphosphine (Aldrich, 1.03 g, 3.93 mmol), and ethanol (Aldrich, 200 proof, 230 mL, 3.94 mmol) in THF (anhydrous, 14 mL). The reaction mixture was stirred at room temperature overnight. The solvents were removed in vacuo to afford a red-colored oil. The crude was purified by MPLC (SiO_2, 10% ethyl acetate in hexanes) to afford 635 mg of 5-ethoxy-1-phenyl-1*H*-pyrazole-4-carboxylic acid ethyl ester as a red oil (68% yield).
23. Szalai, A. J.; Digerness, S. B.; Agrawal, A.; Kearney, J. F.; Bucy, R. P.; Niwas, S.; Kilpatrick, J. M.; Babu, Y. S.; Volanakis, J. E. *J. Immunol.* **2000**, *164*, 463.
24. Connolly, M. L. *J. Mol. Graph.* **1993**, *11*, 139.