Journal of Medicinal Chemistry

Volume 47, Number 20 September 23, 2004

Letters

Nonpeptidic, Noncovalent Inhibitors of the Cysteine Protease Cathepsin S

Robin L. Thurmond,[†] Mary Pat Beavers,[‡] Hui Cai,[†] Steven P. Meduna,[†] Darin J. Gustin,[†] Siquan Sun,[†] Harold J. Almond,[§] Lars Karlsson,[†] and James P. Edwards*,[†]

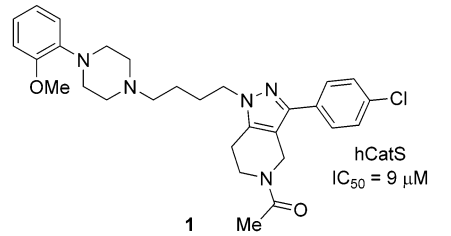
Johnson & Johnson Pharmaceutical Research and Development L.L.C., 3210 Merryfield Row, San Diego, California 92121; Johnson & Johnson Pharmaceutical Research and Development L.L.C., Route 202, P.O. Box 300, Raritan, New Jersey 08869; Johnson & Johnson Pharmaceutical Research and Development L.L.C., Welsh and McKean Roads, Spring House, Pennsylvania 19477

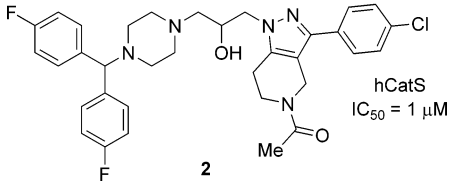
Received May 26, 2004

Abstract: The first nonpeptidic, noncovalent inhibitors of the cysteine protease cathepsin S (CatS) are described. Electronic database searching using the program DOCK generated a screening set of potential CatS inhibitors from which two lead structures were identified as promising starting points for a drug discovery effort. Lead optimization afforded potent (IC_{50} < 50 nM) and selective inhibitors of CatS demonstrating cellular activity and reversibility of enzyme inhibition.

The cysteine protease cathepsin S (CatS) has received much recent attention as a target for therapeutic intervention in a range of diseases of the immune system.[1] CatS is expressed mainly in antigen-presenting cells[2] and has been implicated in the presentation of antigens to $CD4^+$ T-cells through the use of both knockout animals[3] and systemic administration of a peptidic, irreversible cysteine protease inhibitor, LHVS.[4] The CatS inhibitors reported to date[5] rely on covalent attachment of an electrophilic peptide-derived ligand to the active site thiol to achieve potent enzyme inhibition. Very recently, noncovalent peptidic inhibitors of the related cysteine protease cathepsin K have been disclosed.[6] Herein we report the discovery and initial lead optimization of the first nonpeptidic, noncovalent inhibitors of human CatS.

Chart 1. Initial CatS Screening Hits





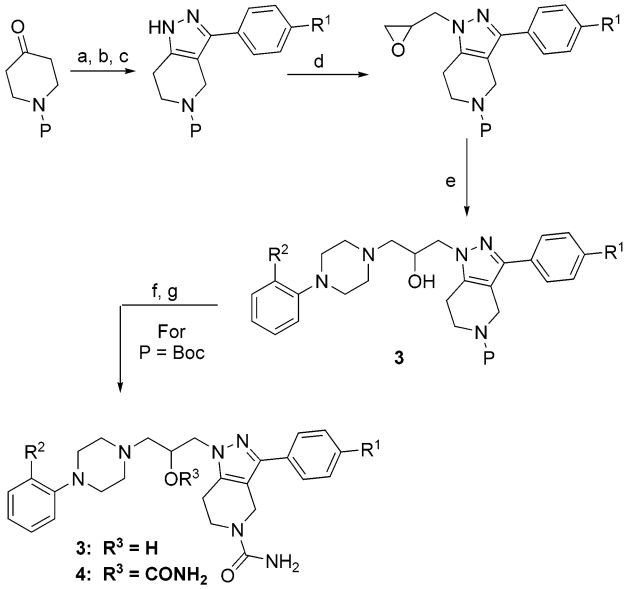
Using the coordinates from the X-ray crystal structure of the related cysteine protease cathepsin K,[7] a theoretical model of CatS was generated. A subset (~248 000 compounds) of the J&JPRD compound database was virtually screened against the predefined active site of CatS using DOCK.[8] Of the 1000 top-scoring electronic hits, 926 were screened against recombinant human CatS at a concentration of 20 μM and 19 were found to have inhibitory activities in the 58−100% range (2% hit rate).

Out of these screening hits, two related compounds, containing a tetrahydropyridyl[4,3-*a*]pyrazole pharmacophore, were selected for further study. Compound **1** exhibited an IC_{50} of ~ 9 μM against CatS, whereas compound **2** was a 1.0 μM inhibitor of the target enzyme (Chart 1). Interestingly, these compounds did not resemble any of the known cysteine protease inhibitor chemotypes, nor did they contain any obvious sites susceptible to nucleophilic attack by an active site sulfhydryl group. Compound **2** was selective for CatS, exhibiting no inhibition of the related cathepsins CatB, CatE, CatF, or CatK at 20 μM, although it exhibited moderate inhibition of cathepsin L (IC_{50} ~ 2−5 μM).

[†] California.
[‡] New Jersey.
[§] Pennsylvania.

10.1021/jm0496133 CCC: $27.50
Published on Web 08/20/2004

Scheme 1. Synthesis of CatS Inhibitors[a]



[a] Reagents and conditions: (a) Morpholine, *p*-TSA·H_2O (1−3%), Dean−Stark, △; (b) ArCOCl, Et_3N, CH_2Cl_2, 0 °C to room temperature; (c) H_2NNH_2, EtOH (40−90%, three steps); (d) epichlorohydrin, Cs_2CO_3, DMF, rt (35−65%); (e) 1-arylpiperazine, $Yb(OTf)_3$ (3−10%), CH_2Cl_2, rt (50−80%); (f) TFA, CH_2Cl_2, rt; (g) TMSNCO, CH_2Cl_2, rt (15−35%, two steps).

Compound **1** was inactive against all other cathepsins when tested at concentrations up to 20 μM. Importantly, these compounds were amenable to modular analogue synthesis, which would facilitate rapid investigation of structure−activity relationships.

Examination of screening data from truncated analogues of **1** and **2** demonstrated that each of the major pharmacophoric elements present in the hits was necessary for sub-micromolar CatS inhibition: an aryl-bearing piperazine or piperidine, a three- to four-carbon linker, and a 3-aryl-4,5,6,7-tetrahydropyridyl[4,3-*a*]pyrazole. However, only benzhydryl piperazines exhibited any inhibition of cathepsin L. Further, electron-withdrawing groups at the para-position of the aromatic ring attached to the pyrazole moiety afforded increased CatS inhibition. Finally, analogues of **2** were anticipated to be derived from simple nucleophilic ring-opening of epoxide derivatives. Thus, our preliminary lead optimization effort focused on surveying various arylpyrazoles and 2′-substituted arylpiperazines connected via a three-carbon hydroxyl-bearing linker (Scheme 1).

Starting from the appropriate N-protected 4-piperidone, enamine formation followed by acylation with a benzoyl chloride afforded an intermediate that, upon treatment with excess hydrazine, formed the desired bicyclic pyrazoles. Although the intermediate diketones could be isolated, in our hands this process could be performed efficiently in one pot without isolation of any intermediates, and the desired pyrazoles could be isolated by simple filtration. Alkylation of the pyrazole with epichlorohydrin in the presence of cesium carbonate occurred selectively (5−10:1) at the nitrogen atom distal to the aryl moiety. Although this reaction often required reaction times of 24 h or longer, alternative procedures using stronger bases afforded lower yields and selectivities. Reaction of the resulting epoxide with a variety of *N*-arylpiperazines in the presence of 3−10% $Yb(OTf)_3$ in methylene chloride afforded the desired compounds **3** in good yields. At this stage of the lead optimization project, all compounds were prepared and tested as racemates.

Table 1. Cathepsin S Inhibitory Activity

compound	R^1	R^2	P	CatS IC_{50} (μM)[a]	SD
1	Cl	−	Ac	9.5	0.4
2	Cl	−	Ac	1.0	0.5
3a	Cl	OMe	Ac	2.1	0.8
3b	Cl	Cl	Ac	1.0	0.4
3c	I	OMe	Ac	0.6	0.3
3d	I	CN	Ac	0.12	0.05
3e	I	CN	$CONH_2$	0.05	0.03
3f	I	Me	$CONH_2$	0.14	0.08
4	I	CN	$CONH_2$	0.02	0.01
LHVS	−	−	−	0.005	0.002

[a] IC_{50} of inhibition of human cathepsin S; SD determined from at least two experiments. See Supporting Information for details.

Replacement of the benzhydryl piperazine in **2** with the 2-methoxyphenylpiperazine moiety of **1** resulted in a slight loss in activity (Table 1, **3a**). Subsequent replacement of the methoxy group on the aryl piperazine with chloride afforded an inhibitor (**3b**) equipotent to **2**. Replacement of the chlorine atom in **3a** with an iodine atom was also well tolerated (**3c**, $IC_{50} = 0.6$ μM). None of these compounds inhibited CatL at concentrations up to 20 μM. Changing the methoxy substituent of **3c** to cyano resulted in an additional half-log improvement in potency (**3d**, $IC_{50} = 0.12$ μM). Further improvements in potency could be achieved by modifications to the tetrahydropyridine N-substituent. Compound **3e**, bearing a urethane moiety in place of the acetyl group of **3d**, was a 50 nM CatS inhibitor. The 2-methylphenylpiperazine derivative **3f** exhibited an IC_{50} of 140 nM, supporting our supposition that these compounds were not binding covalently to the enzyme. Interestingly, during formation of the primary urea **3e** (Scheme 1) a byproduct resulting from carbamoylation of the linker hydroxyl was also isolated (compound **4**, R^1 = I, R^2 = CN).

Compound **4** turned out to be a very potent CatS inhibitor ($IC_{50} = 20$ nM). Compound **4** exhibits reversible enzyme inhibition kinetics, no evidence of covalent modification of the active-site cysteine, and approaches the potency of the peptidic, irreversible CatS inhibitor LHVS (entry 10, $IC_{50} = 5$ nM). Further, compounds **3a**−**f** and **4** did not inhibit any other cathepsins tested at concentrations up to 20 μM.

One of the primary in vivo substrates of CatS is the invariant chain (Ii), which binds MHC class II molecules.[1,3] The cleavage and removal of Ii from MHC II molecules is required for antigen presentation to $CD4^+$ T-cells; inhibition of Ii cleavage results in an attenuated immune response. We have used JY cells (a human B-cell line) to test for cellular activity of our novel CatS inhibitors (see Supporting Information). Compound **4** inhibits Ii processing with an IC_{50} of 0.88 μM in this assay. For comparison, in the same assay LHVS exhibits an IC_{50} of 20 nM.

In summary, we have discovered the first nonpeptidic, noncovalent inhibitors of the cysteine protease, cathepsin S. The lead structures were initially identified using electronic database searching, and modular compound optimization afforded potent inhibitors with encouraging levels of cellular activity. The discovery of

small molecule ligands that inhibit the activity of cysteine proteases without relying upon covalent attachment to the active site thiol represents a major advance toward the development of small-molecule therapeutics inhibiting this important class of protein targets. Such compounds are anticipated to find utility in the treatment of autoimmune diseases and allergies.[9]

Supporting Information Available: Characterization data for compounds **1** and **2**, experimental procedures for the synthesis and characterization of **3a**−**f** and **4**, and descriptions of the enzymatic and cellular assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Villandangos, J. A.; Bryant, R. A. R.; Deussing, J.; Driessen, C.; Lennon-Dumenil, A.-M.; Riese, R. J.; Roth, W.; Saftig, P.; Shi, G.-P. Chapman, H. A.; Peters, C.; Ploegh, H. L. Proteases Involved in MHC Class II Antigen Presentation. *Immunol. Rev*. **1999**, *172*, 109−120. (b) Nakagawa, T. Y.; Rudensky, A. Y. The Role of Lysosomal Proteinases in MHC Class II-Mediated Antigen Processing and Presentation. *Immunol. Rev*. **1999**, *172*, 121−129.

(2) Shi, G.-P.; Webb, A. C.; Foster, K. E.; Knoll, J. H. M.; Lemere, C. A.; Munger, J. S.; Chapman, H. A. Human Cathepsin S: Chromosomal Localization, Gene Structure, and Tissue Distribution. *J. Biol. Chem*. **1994**, *269*, 11530−11536.

(3) (a) Shi, G.-P.; Villadangos, J. A.; Dranoff, G.; Small, C.; Gu, L.; Haley, K. J.; Riese, R.; Ploegh, H. L.; Chapman, H. A. Cathepsin S Required for Normal MHC Class II Peptide Loading and Germinal Center Development. *Immunity* **1999**, *10*, 197−206. (b) Nakagawa, T. Y.; Brissette, W. H.; Lira, P. D.; Griffiths, R. J.; Petrushova, N.; Stock, J.; McNeish, J. D.; Eastman, S. E.; Howard, E. D.; Clarke, S. R. M.; Rosloniec, E. F.; Elliott, E. A.; Rudensky, A. Y. Impaired Invariant Chain Degradation and Antigen Presentation and Diminished Collagen-Induced Arthritis in Cathepsin S Null Mice. *Immunity* **1999**, *10*, 207−217.

(4) (a) Riese, R. J.; Wolf, P. R.; Bromme, D.; Natkin, L. R.; Villadango, J. A.; Ploegh, H. L.; Chapman, H. A. Essential Role for Cathepsin S in MHC Class II−Associated Invariant Chain Processing and Peptide Loading. *Immunity* **1996**, *4*, 357−366. (b) Riese, R. J.; Mitchell, R. N.; Villadangos, J. A.; Shi, G.-P.; Palmer, J. T.; Karp, E. R.; De Sanctis, G. T.; Ploegh, H. L.; Chapman, H. A. Cathepsin S Activity Regulates Antigen Presentation and Immunity. *J. Clin. Invest*. **1998**, *101*, 2351−2363.

(5) (a) Bromme, D.; Smith, R. A.; Coles, P. J.; Kirschke, H.; Storer, A. C.; Krantz, A. Potent Inactivation of Cathepsins S and L by Peptidyl (Acyloxy)methyl Ketones. *Bio. Chem. Hoppe-Seyler* **1994**, *375*, 343−347. (b) Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Bromme, D. Vinyl Sulfones as Mechanism-Based Cysteine Protease Inhibitors. *J. Med. Chem*. **1995**, *38*, 3193−3196. (c) Katunuma, N.; Murata, E.; Kakegawa, H.; Matsui, A. Tsuzuki, H.; Tsuge, H.; Turk, D.; Turk, V.; Fukushima, M.; Tada, Y.; Asao, T. Structure Based Development of Novel Specific Inhibitors for Cathepsin L and Cathepsin S In Vitro and In Vivo. *FEBS Lett*. **1999**, *458*, 6−10. (d) Ward, Y. D.; Thomson, D. S.; Frye, L. L.; Cywin, C. L.; Morwick, T. Emmanuel, M. J.; Zindell, R.; McNeil, D.; Bekkali, Y.; Giradot, M.; Hrapchak, M.; DeTuri, M.; Crane, K.; White, D.; Pav, S.; Wang, Y.; Hao, M.-H.; Grygon, C. A.; Labadia, M. E.; Freeman, D. M.; Davidson, W.; Hopkins, J. L.; Brown, M. L.; Spero, D. M. Design and Synthesis of Dipeptide Nitriles as Reversible and Potent Cathepsin S Inhibitors. *J. Med. Chem*. **2002**, *45*, 5471−5481. (e) Cywin, C. L.; Firestone, R. A.; McNeil, D. W.; Grygon, C. A.; Crane, K. M.; White, D. M.; Kinkade, P. R.; Hopkins, J. L.; Davidson, W.; Labadia, M. E.; Wildeson, J.; Morelock, M. M.; Peterson, J. D.; Raymond, E. L.; Brown, M. L.; Spero, D. M. The Design of Potent Hydrazones and Disulfides as Cathepsin S Inhibitors. *Bioorg. Med. Chem. Lett*. **2003**, *11*, 733−740. For a recent review, see: Leroy, V.; Thurairatnam, S. Cathepsin S Inhibitors. *Expert Opin. Ther. Pat*. **2004**, *14*, 301−311.

(6) (a) Altmann, E.; Renaud, J.; Green, J.; Farley, D.; Cutting, B.; Jahnke, W. Arylaminoethyl Amides as Novel Non-Covalent Cathepsin K Inhibitors. *J. Med. Chem*. **2002**, *45*, 2352−2354. (b) Kim, T.-S.; Hague, A. B.; Lee, T. I.; Lian, B.; Tegley, C. M.; Wang, X.; Burgess, T. L.; Qian, Y.-X.; Ross, S.; Tagari, P.; Lin, C.-H.; Mayeda, C.; Dao, J.; Jordan, S.; Mohr, C.; Cheetham, J.; Viswanadhan, V.; Tasker, A. S. (4-Piperidinylphenyl)aminoethyl Amides as a Novel Class of Non-Covalent Cathepsin K Inhibitors. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 87−90.

(7) McGrath M. E.; Klaus J. L.; Barnes M. G.; Bromme D. Crystal Structure of Human Cathepsin K Complexed with a Potent Inhibitor. *Nat. Struct. Biol*. **1997**, *4*, 105−109.

(8) Kuntz, I. D.; Blaney, J. M., Oatley, S. J.; Langridge, R.; Ferrin, T. E. A Geometric Approach to Macromolecule-Ligand Interactions. *J. Mol. Biol*. **1982**, *161*, 269−288. The DOCK software suite of programs is available from the University of California, San Francisco, CA. DOCK 4.0 was used for this study.

(9) For the pharmacological characterization of a related cathepsin S inhibitor, see: Thurmond, R. L.; Sun, S.; Sehon, C. A.; Baker, S. M.; Cai, H.; Gu, Y.; Jiang, W.; Riley, J. P.; Williams, K. N.; Edwards, J. P.; Karlsson, L. Identification of a Potent and Selective Noncovalent Cathepsin S Inhibitor. *J. Pharm. Exp. Ther*. **2004**, *308*, 268−276.

JM0496133