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SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF NOVEL SMALL MOLECULE CATHEPSIN D INHIBITORS

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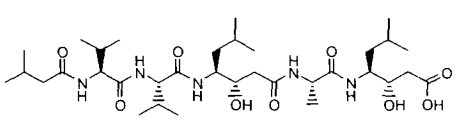
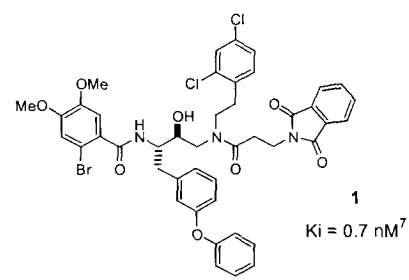
Abstract: Cathepsin D, a lysosomal aspartyl protease, has been implicated in the pathology of Alzheimer's disease as well as breast and ovarian cancer. A weakly active cathepsin D inhibitor was identified by high throughput screening. Subsequent optimization led to the discovery of a new class of small molecule inhibitors of this enzyme, culminating with the sulfonamide **13** (IC_{50} = 250 nM).

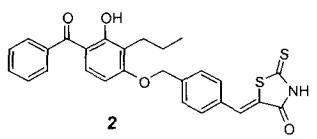
Cathepsin D, an aspartyl protease primarily located in lysosomes, has been implicated in the proteolysis of the Amyloid Precursor Protein (APP) in neurons, a key element of Alzheimer's disease pathology.[1] We have previously established[2] that cathepsin D can cleave APP *in vitro* to produce Aβ, the main component of amyloid plaques found in brains from Alzheimer's disease patients. Moreover, cathepsin D is secreted by breast and ovarian cancer cell lines,[3] and might play a role in tumor metastasis and invasion.[4] Known inhibitors of cathepsin D include peptides[5a] and phosphinic acid-based peptide isosteres.[5b] The discovery of pepstatin,[6] an *N*-acylated pentapeptide obtained from culture filtrates of actinomycetes, provided a useful peptidomimetic lead to drug discovery efforts. Therefore, numerous small molecule statin-based inhibitors of cathepsin D have been reported in the literature, some of them, such as **1**, prepared using a combinatorial chemistry approach.[7]



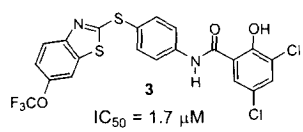
Pepstatin[6]
Cathepsin D IC_{50} = 2 nM



1
Ki = 0.7 nM[7]



2
IC_{50} = 340 nM[8]



3
IC_{50} = 1.7 μM

Statin-based analogs can be extremely potent inhibitors of this enzyme, and therefore provide excellent medicinal chemistry tools. This class suffers, however, from poor pharmacokinetic and oral absorption profiles. In contrast, very few examples of non-peptidomimetic aspartyl protease inhibitors can be found in the literature. Recently, Eli Lilly reported non-statin-based cathepsin D inhibitors with sub-micromolar activity[8] (**2**). As part

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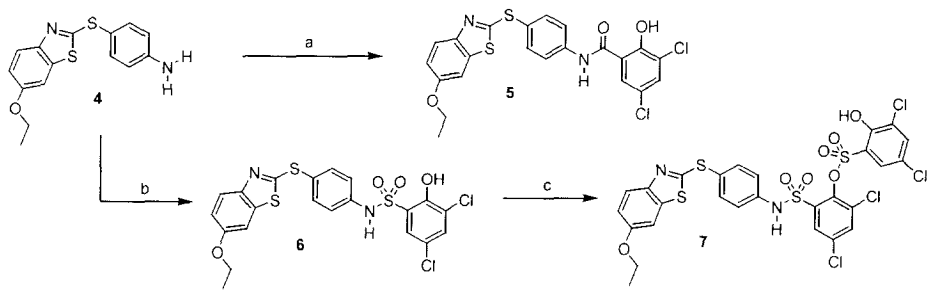
of our medicinal chemistry programs, we screened human cathepsin D, and identified the primary hit **3** (IC_{50} = 1.7 μM, n = 19) as a reversible, competitive (as evidenced by a Lineweaver-Burke plot) inhibitor. Our goal was to improve the potency of this lead, and eliminate the 2,4-dichlorosalicylamide moiety, known in the literature to cause mitochondrial uncoupling in the low micromolar range.[9]

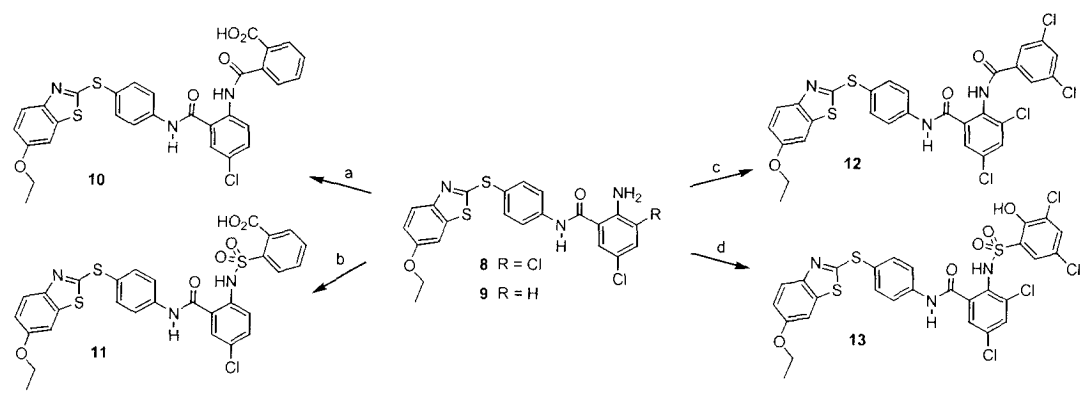
Chemistry

An aromatic nucleophilic substitution between easily available 2-mercapto-benzothiazoles and 4-fluoro nitrobenzene (K_2CO_3, DMF, 75 °C), followed by a nitro-group reduction (Fe, AcOH, water, room temp.) creates the requisite skeleton for the aniline fragments such as **4**. Subsequent coupling of the anilines with acid chlorides or sulfonyl chlorides is performed as follows:



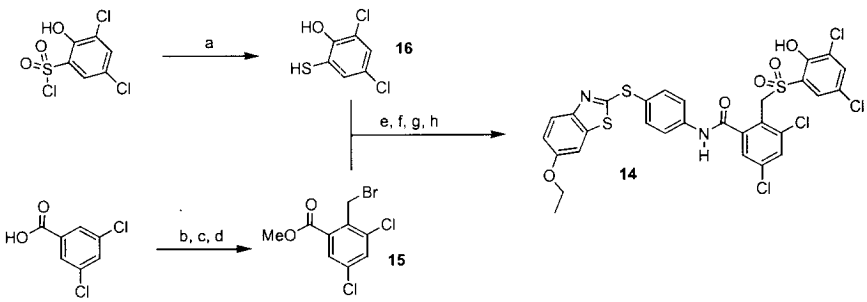
(a) 3,5-dichloro-2-hydroxybenzoylchloride, NEt_3, DMAP, CH_2Cl_2, 6 days, 39%. (b) 3,5-dichloro-2-hydroxy-benzenesulfonyl chloride, pyridine, DMAP, CH_2Cl_2, rt, 52%. (c) 3,5-dichloro-2-hydroxybenzenesulfonyl chloride, NEt_3, CH_2Cl_2, rt, 21%.

Aniline **4** is cleanly monosulfonylated with pyridine as base, affording the sulfonamide **6**. This compound can be sulfonylated a second time, by the use of a stronger base such as triethylamine, providing access to the sulfonate **7** as shown above.



(a) phthalic anhydride, toluene, reflux. (b) 2-carboxymethylbenzenesulfonylchloride, pyridine, cat. DMAP, 60 °C, 82%; then NaOH, MeOH, water, rt, 62%. (c) 3,5-dichlorobenzoyl chloride, toluene, 100 °C, 22%. (d) 3,5-dichloro-2-hydroxybenzenesulfonyl chloride, pyridine, 50 °C, 10%.

Aniline **4** also condenses smoothly with isatoic anhydrides (xylenes, reflux, 18 h) to afford anthranylamides such as **8** and **9**, which can be acylated, sulfonylated, or reacted with anhydrides. We also prepared an example of the sulfone linker (**14**) using a different 8-step protocol. Benzyl bromide **15** is obtained in 3 steps by dianion alkylation followed by esterification and NBS-bromination. Hydroxythiol **16** is separately prepared from the corresponding sulfonyl chloride (commercially available). The two intermediates are coupled, and the sulfide oxidized to a sulfone. The ethoxybenzothiazole side-chain **4** is attached in the last step. Interestingly, the phenolic group of **14** never needs protection during this synthesis.



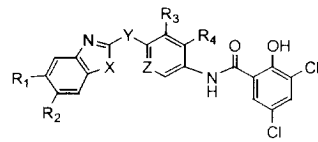
(a) $LiAlH_4$, THF, reflux, 87%. (b) *t*-BuLi, TMEDA, THF then MeI, 45%. (c) MeI, K_2CO_3, acetone, reflux, 96%. (d) NBS, carbon tetrachloride, reflux. (e) CH_2Cl_2, $N(iPr)_2Et$, 0 °C, 62% (two steps). (f) *m*CPBA, CH_2Cl_2, rt, 87%. (g) NaOH, MeOH, water, 83%. (h) oxalyl chloride, CH_2Cl_2, then **4**, toluene, 29%.

Results and Discussion

The left side of **3** consists of two aromatic entities linked by a heteroatom. We varied both of these entities, keeping the rest of the molecule constant (Table 1). The benzothiazole substitution seems to have little effect on the activity. The heteroatom linker between the two rings can be either sulfur or oxygen, while the more hydrophilic benzoxazole **18** appears less potent. Replacing the benzothiazole with its quinoline isostere **22** shows little impact on the activity. Substitution of the middle ring results in slight increases in activity when a lipophilic substituent (chlorine, methyl, trifluoromethyl) is added ortho to the heteroatom linker. The overall potency of these analogs seems to track with the lipophilicity of the side-chain. For the variation of the salicylate portion, we used ethoxybenzothiazole **5** as our reference compound (Table 2). The dichlorophenol group is important for the activity. Methylation of the phenol as in **40** results in an inactive compound. Deletion of either or both chlorine atoms results in a dramatic loss of activity, suggesting that the pKa of the phenol is important for activity. This finding is confirmed by the fact that the dimethyl analog **39** (similar lipophilicity, but different pKa) appears totally inactive in the assay. However, replacement of the amide by a sulfonamide as in **6** does not affect potency. Replacement of the hydroxy group by an amino group (**8**) decreases potency. The sulfonamide-sulfonate 7 (Table 3) was an unexpected finding. This compound is sensitive to both basic and acidic hydrolysis, but its inhibition of cathespin D is not time dependent. The best compound of this new series is the amide-sulfonamide **13** (IC_{50} = 250 nM, n = 5), which is stable to hydrolysis. Replacement of the sulfonamide by a sulfone (compound **14**) does not improve activity. In the extended series, the 2,4-dichloro-substitution remains optimal, but in this case, deletion of one of the chlorine atoms as in **43** does not impact the potency to a large extent, while decreasing the lipophilicity of our analogs. Interestingly, the phenolic group of **41** can be deleted without potency impact (**12**). We also established that the acidic phenol

TABLE 1

Lipophilic side chain variation

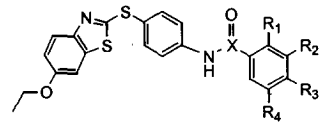


R_1	R_2	X	Y	Z	R_3	R_4	MP (°C)	MS (FAB)	#	Cat D IC_{50} (μM)
OCF_3	H	S	S	CH	H	H	80	531 (M+H), 343	**3**	1.7 ± 0.43
H	H	S	S	CH	H	H	168	447 (M+H), 185	**17**	3.0 ± 1.4
H	H	O	O	CH	H	H	261	415 (M+H), 381, 227	**18**	7.2 ± 4.4
H	H	O	S	CH	H	H	212	431 (M+H), 305, 185	**19**	3.2 ± 1.4
H	H	S	O	CH	H	H	175	431 (M+H), 185, 93	**20**	2.8 ± 0.28
OEt	H	S	S	CH	H	H	173	491 (M+H), 185, 133	**5**	1.8 ± 0.34
H	Cl	S	S	CH	H	H	170	483 (M+H), 293, 185	**21**	1.2 ± 0.15
H	H	CH=CH	S	CH	H	H	>300	443 (M+H), 369, 277	**22**	2.5 ± 0.7
H	OCH_3	S	S	CH	H	H	107	479 (M+H), 289, 196	**23**	2.0 ± 0.35
OCH_3	H	S	S	CH	H	H	86	477 (M+H), 289, 196	**24**	5.0 ± 2.1
H	H	S	S	CH	Cl	H	192	483 (M+H), 185, 133	**25**	1.8 ± 0.49
H	H	S	S	CH	CH_3	H	210	461 (M+H), 185, 133	**26**	2.0 ± 0.35
H	H	S	S	CH	CF_3	H	169	515 (M+H), 161, 133	**27**	1.1 ± 0.14
H	H	S	S	N	H	H	136	448 (M+H), 260, 133	**28**	2.5 ± 0.63
H	H	S	S	CH	CN	H	261	472 (M+H), 369, 277	**29**	1.6 ± 0.07
H	H	S	S	CCl	Cl	H	148	517 (M+H), 461, 269	**30**	1.3 ± 0.16
H	H	S	S	CH	OCH_3	H	134	477 (M+H), 289, 185	**31**	2.0 ± 0.07
H	H	S	S	CH	H	CH_3	145	461 (M+H), 427, 369	**32**	1.3 (n = 2)
H	H	S	S	CH	H	CN	255	472 (M+H), 284, 185	**33**	2.4 ± 0.56

Assay Conditions: The cathepsin D assay was performed in a Millipore CytoFluor 96-well plate reader. Human liver cathepsin D (480 ng/mL, Calbiochem) was incubated for 10 min at room temperature with the test compound in the presence of 160 mM of sodium acetate buffer pH 4.5, and 120 mM of sodium chloride. The reaction was initiated with the addition of a fluorogenic substrate *N*-succinyl-R-P-F-L-L-V-Y-7-amido-4-methylcoumarin (AMC, Sigma) to a final concentration of 5 μM. After incubation for 45 min at 37 °C, the reaction was terminated with the addition of Tris-HCl, pH 8.0 to a final concentration of 330 mM, together with leucine aminopeptidase (Sigma), which was added to a final concentration of 200 mU/mL. The background fluorescence (360 nm excitation/460 nm emission) was determined immediately after the addition of leucine aminopeptidase, the fluorescence which reflects cathepsin D activity was measured following an incubation at 37 °C for 120 min. IC_{50} values were interpolated from a sigmoidal fit of mean percent inhibition (n = 3) versus log of inhibitor concentration. At least two independent IC_{50} determinations were performed on each compound, and the mean value is reported with standard deviation. Pepstatin (IC_{50} = 2 nM) and **3** were used as references.

TABLE 2

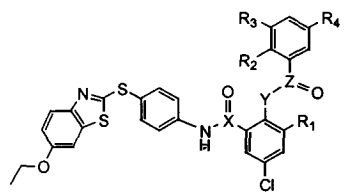
Variation of the salicylate unit



X	R_1	R_2	R_3	R_4	MP (°C)	MS (FAB)	#	Cat D IC_{50} (μM)
C	OH	Cl	H	Cl	173	491 (M+H), 185, 133	**5**	1.8 ± 0.34
SO	OH	Cl	H	Cl	275	565 (M+K), 133	**6**	1.5 (n = 2)
C	NH_2	Cl	H	Cl	177	490 (M+H), 369, 277	**8**	5.5 ± 0.98
C	OH	Cl	H	H	78	457 (M+H), 369, 277	**34**	>10
C	OH	H	CF_3	H	196	491 (M+H), 369, 185	**35**	>10
C	OH	H	H	Cl	188	457 (M+H), 303, 210	**36**	>10
C	OH	CH_3	H	H	66	437 (M+H), 369, 277	**37**	>10
C	OH	H	H	H	182	423 (M+H), 369, 277	**38**	>10
C	OH	CH_3	H	CH_3	105	450 (M+), 302, 273	**39**	>10
C	OCH_3	Cl	H	Cl	155	505 (M+H), 203, 133	**40**	>7

TABLE 3

Analogs of compound **7**



X	Y	Z	R_1	R_2	R_3	R_4	MP (°C)	MS (FAB)	#	Cat D IC_{50} (μM)
SO	O	SO	Cl	OH	Cl	Cl	185	753 (M+H), 527, 369	**7**	0.46 ± 0.11
C	NH	C	Cl	OH	Cl	Cl	148	680 (M+H), 492, 377	**41**	1.5 ± 0.49
C	NH	SO	Cl	OH	Cl	Cl	164	716 (M+H), 490, 277	**13**	0.25 ± 0.09
C	O	SO	Cl	OH	Cl	Cl	181	717 (M+H), 490, 302	**42**	0.49 ± 0.19
C	CH_2	SO	Cl	OH	Cl	Cl	265	700 (M+H), 490, 185	**14**	0.6 (n = 2)
C	O	SO	H	OH	Cl	Cl	191	721 (M+H), 495, 302	**43**	0.7 (n = 2)
C	NH	C	Cl	H	Cl	Cl	243	664 (M+H), 362, 303	**12**	0.78 ± 0.07
C	NH	C	H	SO_3H	H	H	199	640 (M+H), 553, 487	**44**	2.0 ± 0.14
C	NH	C	H	CO_2H	H	H	221	604 (M+H), 553, 461	**10**	3.8 ± 0.7
C	NH	C	H	CH_2-CO_2H	H	H	229	618 (M+H), 277, 185	**45**	2.7 (n = 2)
C	NH	SO	H	H	CO_2H	H	NA	678 (M+K), 447, 403	**11**	2.2 ± 0.28
C	NH	SO	H	CO_2H	H	H	152	640 (M+H), 354, 302	**46**	1.8 ± 1.3

functionality can be replaced by a carboxylic or a sulfonic acid (**10**, **11** and **44** - **46**). Modest cathepsin D inhibition is obtained with this subset, culminating with the amide-sulfonamide **46** (IC_{50} = 1.8 μM).

In conclusion, we wish to report a new class of cathepsin D inhibitors. The phenolic functionality present in the primary hit **3** can be replaced by an amide, as in **12**. Furthermore, the addition of another dichlorophenol unit results in an improvement of the potency, culminating with **13**. Once again, this second phenol function can be replaced by a carboxylic acid unit, albeit with some potency loss (compound **46**, IC_{50} = 1.8 μM). While no non-peptidomimetic cathepsin D inhibitors have yet reached the low nanomolar potency of the best statin derivatives, the existence of this new series provides an important clue that this goal can be realized.

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