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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF BENZOPHENONES AS INHIBITORS OF CATHEPSIN D

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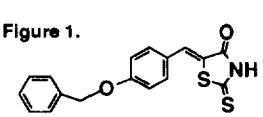
Abstract. Non peptide inhibitors of cathepsin D, an aspartyl protease that has been implicated in many disease states including Alzheimer's disease, were prepared and evaluated. The most potent inhibitor of cathepsin D in this series was found to be (Z)-5-[[4-(4-benzoyl-3-hydroxy-2-propylphenoxy) methylphenyl]methylene]-2-thioxo-4-thiazolidinone (**3f**, IC_{50} = 210 nM). Copyright © 1996 Elsevier Science Ltd

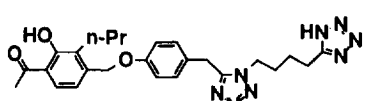
Cathepsin D, a soluble lysosomal enzyme, is an aspartyl endopeptidase that is a member of the pepsin family.[1] Cathepsin D has been implicated in a number of disease processes including Alzheimer's disease, tumor invasion, metastasis, and joint destruction. High levels of cathepsin D immunoreactivity in the senile plaques found in the brain of Alzheimer's patients suggests that cathepsin D may be involved in the pathology of this disease.[2,3] Studies have shown that, in some circumstances, cathepsin D can produce cleavages in amyloid precursor protein (APP) to produce Aß, the 40-43 amino acid fragment that has been implicated in the pathology of Alzheimer's disease.[4,5] Cathepsin D is overproduced and hypersecreted by breast cancer cells. In vivo studies in nude mice suggest that the overexpression of cathepsin D may be responsible for metastasis in breast cancer.[6] The enzyme may also be involved in the progression of other neoplastic diseases.[7] Cathepsin D has been shown to be fibrinolytic[8] and may possess a role in the regulation of cellular insulin-like growth factor (IGF) action by altering the structure or function of insulin-like growth factor-binding protein-3 (IGFBP-3)[9] and in the regulation of cysteine protease activity.[10] The design and synthesis of potent and selective non peptide inhibitors of cathepsin D could provide a mechanism to study the role of the enzyme in these and other human disease states. While some peptide inhibitors of cathepsin D are known, potent small molecule inhibitors have not been discovered.

Our goal was to develop a small molecule inhibitor of cathepsin D. A large number of small organic molecules were screened with **1** and **2** (Figure 1) achieving greater than 50% inhibition at the screening

Figure 1.



1

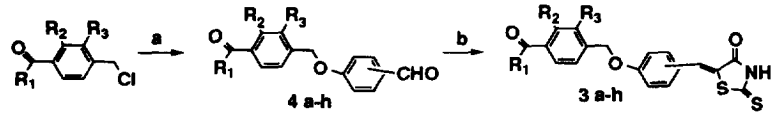


2

concentration of 8.3 µg/mL. Similarity studies of existing compounds led to the evaluation of **3a** (LY197466) which exhibited inhibitory activity against cathepsin D with an IC_{50} of 0.64 µM. To develop the SAR of this molecule, modifications were made in the lipophilic propyl side chain, the hydrophobic heterocycle and phenol, and the acetyl group.

Chemistry. To study the SAR of **3a**, the appropriate benzyl chloride[11] (Scheme 1) was reacted with 3 or

Scheme 1



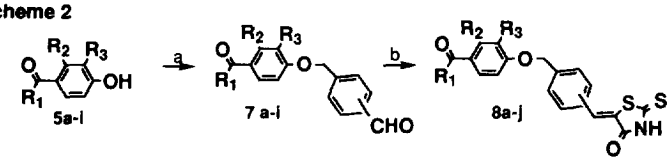
	a	b	c	d	e	f	g	h
R_1	CH_3	CH_3	CH_3	CH_3	C_2H_5	C_6H_5	C_6H_5	CH_3
R_2	OH	H	OH	OH	OH	OH	H	OH
R_3	C_3H_7	H	H	C_2H_5	C_3H_7	C_3H_7	H	C_3H_7

The position of the rhodanine is para for **a-g** and meta for **h**. (a) 4-hydroxybenzaldehyde; 5N NaOH, NaH or NaOEt; EtOH or DMF (b) rhodanine, NaOAc, HOAc.reflux

4-hydroxybenzaldehyde in the presence of base in ethanol or DMF. The aldehyde (**4a-h**) was condensed with rhodanine by refluxing in HOAc with NaOAc. The rhodanines (**3a-h**) are in the Z configuration.[12]

Many of the phenols (**5a-f**, Scheme 2) necessary to investigate the effect of reversing the oxygen and

Scheme 2

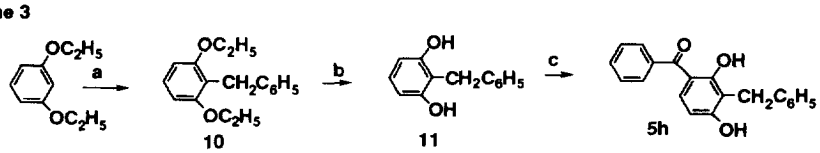


	a	b	c	d	e	f	g	h	i	j
R_1	CH_3	CH_3O	CH_3	C_6H_5	C_6H_5	C_6H_5	C_6H_5	C_6H_5	(2-OH)C_6H_4	OH
R_2	OH	OH	OH	OH	H	OH	H	OH	OH	OH
R_3	C_3H_7	C_3H_7	C_3H_7	C_3H_7	H	H	C_3H_7	$CH_2C_6H_5$	C_3H_7	C_3H_7

The position of the rhodanine is para for **a,b,d-j** and meta for **c** (a) 3 or 4 hydroxymethylbenzaldehyde (**6a and 6b**), K_2CO_3, methyl ethyl ketone, reflux (b) rhodanine, NaOAc, HOAc, reflux.

methylene in the link between the two phenyl rings have been previously reported.[13] The phenol **5g** was synthesized by O-alkylation of 4-hydroxybenzophenone with allyl bromide and potassium carbonate to produce 5-allyloxybenzophenone (**9**) followed by a Claisen rearrangement and subsequent catalytic hydrogenation. The phenol **5h** was prepared (Scheme 3) by alkylation of the lithium salt of diethoxybenzene (formed with n-butyl lithium) with benzylbromide, followed by deprotection with HBr in HOAc. Friedel Crafts acylation of **11** with benzoyl chloride gave **5h**. The phenols (**5a-i**) were reacted with 3 or 4-bromomethylbenzaldehyde (**6a-b**) followed by condensation with rhodanine (Scheme 2) to yield **8a-i**. The acid, **8j**, was prepared by hydrolysis of the ester, **8b**.

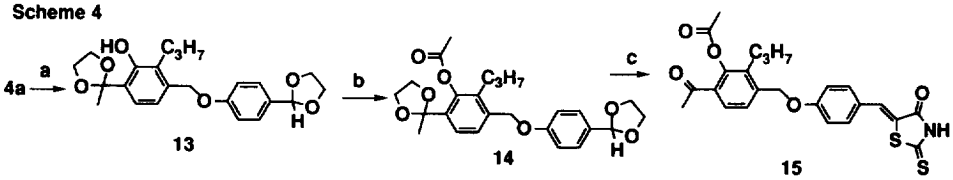
Scheme 3



(a) benzyl bromide, n-BuLi, THF (b) HBr, HOAc (c) Benzoyl chloride, $AlCl_3$, CH_2Cl_2

Compound **15** (Scheme 4), the O-acetyl analog of **3a**, was prepared by forming the diacetal of **4a** (ethylene glycol in toluene with a catalytic amount of p-toluenesulfonic acid) to yield **13**, followed by acylation with acetyl chloride and sodium hydride in DMF (**14**). The protecting groups were removed during

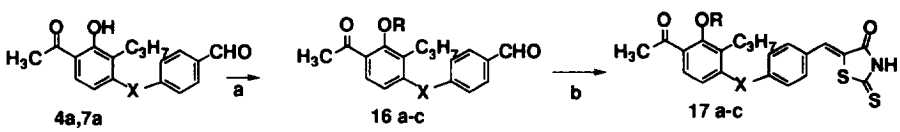
Scheme 4



(a) ethylene glycol, pTsOH (cat), toluene, reflux (b) CH_3COCl, NaH, DMF (c) rhodanine, NaOAc, HOAc, relfux

the condensation reaction with rhodanine. Other analogs, **17a-c**, (Scheme 5) substituted by methyl or acetyl on the hydroxyl group of **4a** or **7a** were prepared by acylation or alkylation of the corresponding benzaldehyde (**4a** or **7a**) with MeI or CH_3COCl to yield **16a-c** and subsequent condensation with rhodanine.

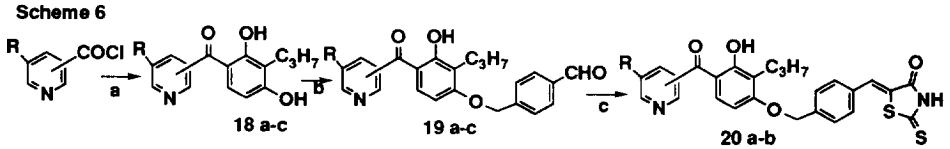
Scheme 5



a: X = OCH_2, R = CH_3 **b**: X = OCH_2, R = CH_3CO **c**: X = CH_2O, R = CH_3
(a) NaH, CH_3COCl or CH_3I, DMF (b) rhodanine, HOAc, NaOAc

To prepare heterocyclic analogs (Scheme 6) of the benzophenone **8d** (**20a** and **20b**), nicotinyl or isonicotinyl chloride was used in a Friedel-Crafts acylation of 1,3-dihydroxy-2-propylbenzene to produce

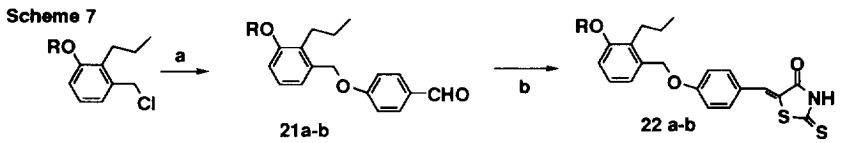
Scheme 6



a: 3-pyridyl, R = H **b**: 4-pyridyl, R = H **c**: 3-pyridyl, R = CO_2H (a) $AlCl_3$, 1,3-dihydroxy-3-propylbenzene, CH_2Cl_2
(b) 4-hydroxymethylbenzaldehyde (**6a**),K_2CO_3, KI, methyl ethyl ketone (c) rhodanine, NaOAc, HOAc, reflux

phenols **18a** and**18b** which were then converted as before to the aldeydes **19a** and **19b**, and then to **20a** and **20b**. To prepare an analog of **20a** substituted on the pyridine ring with a carboxy group, the requisite 5-carbomethoxypyrid-3-ylcarbonylchloride was prepared from the monoester of 3,5-pyridinedicarboxylic acid and converted by a similar synthesis to **20c**. The ester was hydrolized before condensation to the rhodanine.

Scheme 7

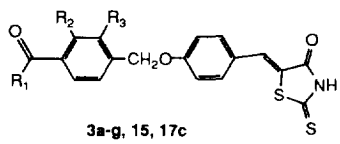


a: R = H, **b**: R = CH_3 (a) Na, EtOH, 4-hydroxybenzaldehyde, NaI (b) rhodanine, NaOAc, HOAc, reflux

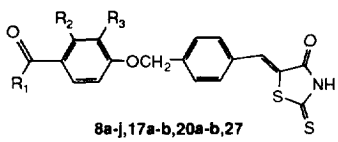
Analogs lacking an acetyl or benzoyl group appended on the benzene ring, **22a** and **22b,** were prepared by reacting the appropriate benzyl chloride[11](Scheme 7) with 4-hydroxybenzaldeyde to afford conversion to the aldehydes **21a** and **21b**. The thiohydantoin and thiazolidinedione derivatives (**22a** and **22b**) were prepared by the condensation of the aldehyde **7a** with 2.4-thiazolidinedione or thiohydantoin, respectively.

Results and Discussion. The compounds were evaluated for their ability to inhibit cathepsin D with the results reported in Table 1. Each result is the mean of four determinations with, except in rare occasions, less than 20% variablility with the results for each assay. The standard enzyme level of cathepsin D for the inhibition assay was 25 ng/well. In the assays for **8d, 8g, 8h**, and **20b,** all or part of the experiments were run with an enzyme level of 50 ng/mL. Comparisons for SAR purposes were only made with results from assays using the same enzyme level. One analog (**8d**) was tested 3-5 times at each enzyme level to provide a basis forcomparison. In order to check for potential false positives, the compounds were evaluated for their ability to inhibit an aminopeptidase, leucine amino peptidase (LAP) from releasing 7-amido-4-methylcourmarin (AMC) from Leu-Val-Tyr-AMC. Samples were also analyzed to check for background fluorescence before the release of the fluorescent AMC. The background fluorescence of each compound is reported as the percent of quenching of fluorescence before the release of AMC.[14]

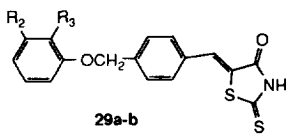
Table 1. Physical Data and in Vitro Activity of Acetophenones and Benzophenones.



3a-g, 15, 17c



8a-j,17a-b,20a-b,27



29a-b

no.	R1	R2	R3	MS	mp°C.	Yield[a]	Cat D[b]	LAP[c]	quench[d]
3a	CH_3	OH	C_3H_7	427	218-19	38	0.64±0.13(5)	15.0±1.7(5)	2.5±1.2(5)
3b	CH_3	H	H	369	250 dec	59	22%	5	1
3c	CH_3	OH	H	385	268-70	24	14.1±3.0(4)	7.0±1.6(4)	15.7±1.3(4)
3d	CH_3	OH	C_2H_5	413	270-72	70	17.6±8.6(3)	7.2±2.8(4)	10.5±5.6(3)
3e	C_2H_5	OH	C_3H_7	441	239-40	50	3.69±1.48(5)	4.4±1.9(5)	18.4±5.8(5)
3f[f]	C_6H_5	OH	C_3H_7	490	221-24	4	0.21(1)	9(1)	9(1)
3g	C_6H_5	H	H	431	252-54	47	1.31(1)	12 (1)	NT
3h[g]	CH_3	OH	C_3H_7	427	212-13	52	6.64-9.60	20.3±6.4(3)	6.0±4.6(3)
8a[h]	CH_3	OH	C_3H_7	427	236-38	16	2.56±0.43(4)	9.6±2.8(4)	8.2±3.8(4)
8b	OCH_3	OH	C_3H_7	443	227-29	39	9.74±3.59(3)	6.3±14.1(3)	8.0±1.0(3)
8c[g]	CH_3	OH	C_3H_7	427	213-15	47	53% (1)	9 (1)	2 (1)
8d	C_6H_5	OH	C_3H_7	489	229-30	84	0.34±0.11(5) 1.30±0.33(3)[e]	29.0±5.5(3) 0-13[e]	11.0±3.0(3) 4-14(2)[e]
8e	C_6H_5	H	H	431	244-46	66	0.78(1)	8 (1)	NT
8f	C_6H_5	OH	H	447	264-66	42	7.2(1)	27(1)	6(1)
8g	C_6H_5	H	C_3H_7	475	191-92	88	18-23%[e]	8(1)	10(1)
8h	C_6H_5	OH	$CH_2C_6H_5$	537	278-80	83	0.82[e]-0.64[e]	-4(1)	4(1)
8i	$(2\text{-}OH)C_6H_4$	OH	C_3H_7	505	218-19	59	0.62 (1)	47(1)	16(1)
8j	OH	OH	C_3H_7	429	240-dec	29	5.13±0.17(3)	2 (1)	6.7±0.7(3)
15	CH_3	OAc	C_3H_7	469	224-25	22	33.3±9.2%(3)	17-1	0-1
17a	CH_3	OCH_3	C_3H_7	441	200-02	25	48.4±18.8(4)	10.0±3.2(4)	5.0±3.0(4)
17b	CH_3	OAc	C_3H_7	469	218-21	43	2.98-4.47	6-11	6-4
17c	CH_3	OCH_3	C_3H_7	441	198-99	44	1.86±0.75(4)	17.2±47(4)	7.7±0.3(4)
20a	3-pyridyl	OH	C_3H_7	491	>225	45	1.46(1)	21(1)	10(1)
20b	4-pyridyl	OH	C_3H_7	492	>225	67	7.94[e]15.84	32(1)	8(1)
27	$5\text{-}CO_2H\text{-}3\text{-}$pyridyl	OH	C_3H_7	534	185-90	87	0.69(1)	19(1)	1(1)
29a	H	OH	C_3H_7	385	190-95	17	2.94 (1)	22	18
29b	H	OCH_3	C_3H_7	399	165-66	66	2.76-2.83	3 (1)	10-30

[a]Analyses for C. H. N within experimental error. [b] IC^{50} (SEM) μM or % inhibition at 4.15 μg/mL. [c]% inhibition of leucine amino peptidase [d]% inhibition of quench. [e]enzyme level 50 ng/mL. [f]HPLC 30% EtOAc/hexane [g]rhodanine is in the meta position

The structure activity relationship (SAR) of **3a** was investigated by altering the lipophilic propyl group and the hydrophobic rhodanine and hydroxyl groups, which could hydrogen bond to the peptide. The aromatic ring adjacent to the rhodanine will be defined as proximal and the other aromatic ring defined as distal. The character of the acetyl group on the distal aromatic ring was also investigated, replacing this with benzoyl or groups capable of forming additional hydrogen bonds.

Initial SAR studies centered on the acetyl group. In general changing the acetyl to a benzoyl resulted in compounds with IC_{50}s for inhibition of cathespin D slightly lower than the corresponding acetyl analogs comparing **3a** versus **3f** and **8a** versus **8d.** To enhance the potency of cathepsin D inhibitory activity, substituents were added that would allow for additional hydrogen bond interactions. Addition of an ortho-hydroxyl group (**8i**) did not modify cathepsin D activity. Compound **8i** also showed increased inhibition of LAP suggesting a possible trend toward less selective compounds. Replacement of the phenyl with a pyridyl (**20a** and **20b**) resulted in less potent analogs. Addition of an acid group on the 5 position of the 3-pyridyl group in **20a** (**27**) restored the inhibitory activity to the level of the benzoyl derivative (**27** versus **8d**). An acid (**8j**) or methyl ester (**8b**) in place of the acetyl of **8a** resulted in a decrease of activity.

Potential hydrogen bonding groups of **3a** to the enzyme are the hydroxyl and several sites of the rhodanine. Removal of the hydroxyl group from the benzophenone derivative **8d** to give **8g** decreased the affinity for cathepsin D. Removal of both the hydroxy and propyl groups produced a dramatic decrease in activity (**3a** versus **3b**) in the acetophenone series and, based on limited data, a corresponding 6 fold decrease (**3f** versus **3g**) for the benzophenones. In the benzophenone series with the linker reversed (**8a** vs **8e**), the removal of both the hydroxyl and propyl groups did not significantly change the activity. Substitution of the hydroxyl with either a methyl or an acetyl group as in **15** and **17a-c** gave compounds which were less potent than **3a** and **8a** at inhibiting cathepsin D. An exception was the case of the O-acetyl derivative **17b** which had similar or only marginally less activity than **8a**.

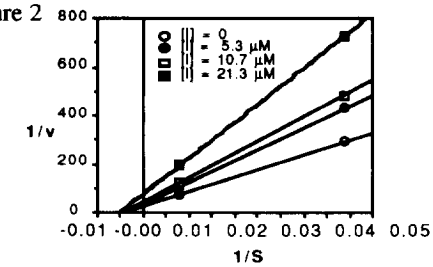
The effect of modifying the lipophilic propyl group was studied. Removal of the group from the acetophenone (**3a**) and the benzophenone (**8d**) decreased activity by 10-20 fold (**3c** and **8f**, respectively). Even a minor decrease in lipophilicity achieved by replacing the propyl with an ethyl substantially decreased activity (**3a** versus **3d**). Replacing the propyl of **8d** with a benzyl increased the inhibition of cathepsin D (**8h**) comparing the IC_{50}s in assays where both had enzyme levels of 50 ng/mL.

A limited SAR of the position and character of the rhodanine demonstrated the importance of the heterocycle for cathepsin D activity. Moving the rhodanine from the para (**3a** and **8a**) to the meta position (**3g** and **8c**) produced a dramatic decrease in activity. Replacement of the rhodanine with a thiohydantoin (**30a**) or a thiazolidinedione (**30b**) resulted in inactive analogs.

Varying the linker between the distal and proximal aromatic rings produced mixed results. Since these changes did not alter the lipophilic or hydrophobic areas on the molecule or dramatically change the position of these groups, these results were not unexpected. (**3f** versus **8d, 3a** versus **8a, 3h** versus **8c**).

The interaction of compound **8d** with cathepsin D was investigated. The Lineweaver-Burk plots for the inhibition of cathepsin D with **8d** were generated (Figure 2). Cathepsin D (0.83 μg/ml) activity was assayed in 0.16 M NaOAc, pH 4.5, containing 0.12 M NaCl and 22.7 μM or 125 μM H-Pro-Thr-Glu-Phe-p-NO_2-Phe-Arg-Leu-OH (Bachem). Rate of proteolysis was monitored at A259 nm and A299 nm at 37 °C. Controls and duplicates were conduted at the same conditions. A259-A299 was ploted against time (min). Initial velocity

Figure 2



(v, A/min) was obtained from the slope of linear curve fit. At each substrate concentration (S,µm), initial velocity was measured in presence of compound of selected oncentrations (I,µm) as indicated. Compound **8d** showed simple noncompetative inhibition of cathespin D.

In conclusion, the best inhibitors were found in the benzophenone series with IC_{50}s of 0.34 µM for (Z)-5-[[4-(4-Benzoyl-3-hydroxy-2-propylphenyl)methoxyphenyl]methylene]-2-thioxo-4-thiazolidinone (**8d**) and 0.21 µM for (Z)-5-[[4-(4-Benzoyl-3-hydroxy-2-propylphenoxy)methylphenyl]methylene]-2-thioxo-4-thiazolidinone (**3f**). The SAR of the series showed that a decrease in activity resulted from the removal of the hydroxy and rhodanine groups which could have important hydrogen bonding to the enzyme. The lipophilic propyl group is essential for good activity with shortening of the propyl side chain resulting in less potent analogs. Reversal of the link between the proximal and distal aromatic rings had little effect on the ability of the compounds to inhibit cathepsin D. The benzophenone series resulted in improved activity in comparison to the acetophenones and propiophenones. These small, non peptide inhibitors can be used to better understand the function of cathepsin D in the disease states where the enzyme is found to be overproduced.

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