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Identification of a potent and selective non-basic cathepsin K inhibitor

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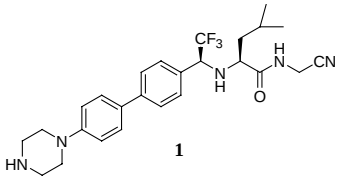
Abstract—Based on our previous study with trifluoroethylamine as a P2–P3 amide isostere of cathepsin K inhibitor, further optimization led to identification of compound **22** (L-873724) as a potent and selective non-basic cathepsin K inhibitor. This compound showed excellent pharmacokinetics and efficacy in an ovariectomized (OVX) rhesus monkey model. The volumes of distribution close to unity were consistent with this compound not being lysosomotropic, which is a characteristic of basic cathepsin K inhibitors.

Cathepsin K (Cat K) is a lysosomal cysteine protease believed to be the principal enzyme responsible for degradation of Type I collagen in osteoclastic bone resorption. Thus, an inhibitor of Cat K may be effective as an anti-resorptive for treatment of osteoporosis.[1] Recently, we have reported trifluoroethylamine as an excellent amide isostere for the P2–P3 amide bond of Cat K inhibitors.[2] When compared to the corresponding dipeptide inhibitors, the trifluoroethylamine derivatives provided enhanced potency for cathepsin K and increased selectivity over other cathepsins. Compound **1** was identified as a 5 pM inhibitor of Cat K with a selectivity of >10,000-fold over other cathepsins. This compound contains a biphenyl piperazine P3 substituent, which has been found to be critical for both potency and selectivity in other series of Cat K inhibitors.[3,4] However, we have also noted that basic inhibitors are often lysosomotropic, which results in increased potency in off-target cell-based enzyme occupancy assays.[5] Indeed, when compound **1** was tested in the whole cell enzyme occupancy assays,[6] we observed much greater potency against cathepsins B, L, and S than was observed in the corresponding enzyme assays (Table 1).[7] Similar potency increases in the functional cathepsin K bone resorption assay[8,9] using rabbit osteoclasts were not observed.[5] Therefore, the high selectivity of **1** observed in enzyme assays is reduced when the compound is evaluated in these cell-based assays. This reduction of selectivity could increase the potential for adverse events in clinic due to inhibition of off-target proteases. Nevertheless, the enhanced potency and selectivity provided by the trifluoroethylamine amide isostere offered an opportunity to re-visit SAR at the P3 region. Our goal was to identify a potent, selective, and orally bioavailable Cat K inhibitor containing a non-basic P3 substituent which would be devoid of the lysosomotropic properties observed with basic Cat K inhibitors.



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Keywords: Bone resorption; Osteoporosis; Cysteine protease; Cathepsin K; Cathepsin K inhibitor; Non-basic cathepsin K inhibitor; L-873724.
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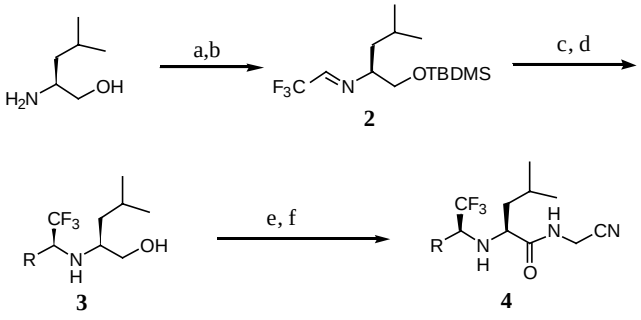
Table 1. Functional, whole cell, and enzyme activity of compound **1**

IC_{50} (nM)			
Enzyme occupancy assays			Functional Cat K
HepG2 Cat B	HepG2 Cat L	Ramos Cat S	Rabbit bone res
82	16[a]	7	12[a]
Enzyme assays			
Cat B	Cat L	Cat S	Rabbit Cat K
1111	47	451	0.01[b]

[a] This value likely represents the lower limit of the assay.
[b] IC_{50} was determined at a concentration of 10 pM enzyme.

Our initial synthesis of the trifluoroethylamine moiety was not diastereoselective.[2] Subsequently an efficient diastereoselective method was developed (Scheme 1). Commercially available (*S*)-leucinol was converted to imine **2** and then reacted with alkyl, aryl, or heteroaryl lithium reagents in a diastereoselective manner.[10] Deprotection of the silyl group gave the desired (*S*,*S*)-amino alcohol intermediate **3** with diastereoselectivities ranging from 5:1 to 20:1; the accompanying (*R*,*S*)-diastereomer could be partially removed by chromatography in many cases. Oxidation with periodic acid in the presence of catalytic chromium trioxide[11] provided the carboxylic acid intermediate.[12] The diastereomeric purity of this acid could be further improved by trituration in diethyl ether or by crystallization of the dicyclohexylamine salt. Amide bond formation between the acid intermediate and aminoacetonitrile then afforded **4**.[13]



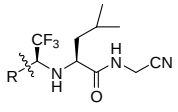
Scheme 1. Reagents and conditions: (a) *tert*-Butyldimethylsilyl chloride, Et_3N, cat. DMAP, CH_2Cl_2, room temperature, 2 h; (b) trifluoroacetaldehyde methyl hemiacetal, benzene, reflux with a Dean–Stark trap, overnight, 85% for two steps; (c) R-Li, −78 °C, 2 h; (d) TBAF, THF, HOAc, room temperature, 2 h, 15–75% for two steps; (e) H_5IO_6/CrO_3, Na_2HPO_4, CH_3CN, 0 °C, 1 h; (f) aminoacetonitrile hydrochloride, HATU, Et_3N, DMF, room temperature, 2 h, 10–55% for two steps.

Early in the SAR study, it was quite clear that simple P3 substituents would not provide sufficient potency and selectivity (Table 2). Compound **8** lacking a P3 substituent (R = H) exhibited low potency against all the tested cathepsins. Introduction of a small alkyl substituent such as the butyl group in **9** improved the activity, while the phenyl analog **10** provided a dramatic improvement in potency against cathepsins K, L, and S. The 4-bromo-substituted analog **11** showed a further improvement in potency of Cat K. This beneficial effect of para substitution on the phenyl group of **10** prompted us to pursue our SAR studies in this direction.

Our strategy for obtaining a wide range of 4-substituted analogs was to use rapid analog synthesis based on a Suzuki coupling reaction with readily available boronic acids or arylhalides. For these subsequent studies, the bromide **5** and boronate **6** serve as convenient intermediates for Suzuki coupling reactions to provide biaryl inhibitors as represented by compound **7**. Bromide **5** was obtained from imine **2** as described in Scheme 1, where the lithium reagent used was derived from 1,4-dibromobenzene (Scheme 2). In the cases where the substitituent in the Ar group of compound **7** could be further transformed, an appropriate reaction would be used to complete the conversion such as methyl sulfide to methyl sulfone by oxidation reaction.

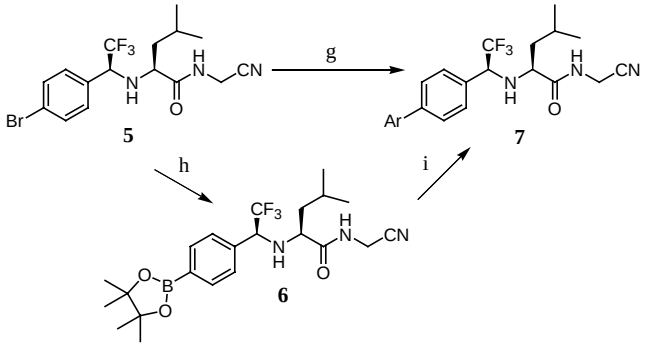
The results of representative examples are summarized in Table 3. The replacement of the bromo substituent in **11** with a phenyl group (**12**) resulted in the improvement of Cat K potency and selectivity against Cat L and Cat S, and thus confirmed the beneficial effect of para substitution at the proximal phenyl ring. Further substitutions on the distal phenyl ring were generally tolerated. However, there was no clear trend for the SAR on potency and selectivity. Most compounds showed selectivities of >5000-fold for cathepsin B, >1000-fold for cathepsin L, and >500-fold for cathepsin S based on the enzyme assays. Mono-substitution with halo (**13**–**15**), alkyl (**18**), electron-donating (**19** and **21**),

Table 2. In vitro activity of simple P3 analogs



Compound	R	IC_{50}[a] (nM)				
		Cat K[b]	Cat B	Cat L	Cat S	Bone res[c]
8	H	4123	>10,000	>10,000	6183	
9	Butyl	391	>10,000	1950	1308	
10	Phenyl	6.7	>10,000	34	36	200
11	4-Bromophenyl	0.8	2993	13	54	111

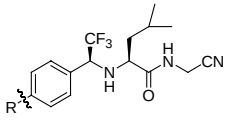
[a] IC_{50}s are an average of at least two independent titrations.
[b] Humanized rabbit enzyme (Ref. 3), see Ref. 6 for assay conditions.
[c] Rabbit bone resorption assay—functional assay, see Ref. 8 for assay conditions.



Scheme 2. Reagents and conditions: (g) $ArB(OH)_2$, $PdCl_2(dppf)$, aq Na_2CO_3, DMF, 80 °C; (h) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc; (i) Ar-(Br or I), $PdCl_2(dppf)$, aq Na_2CO_3, DMF, 80 °C.

and electron-withdrawing (**20**, **22**, **23** and **24**) substituents provided compounds with similar potency against the Cat K enzyme (IC_{50} 0.1–0.9 nM). Disubstitution was also tolerated, but the substitution patterns were critical to potency, as the 3,4-difluoro analog **16** has an IC_{50} of 1.1 nM and the 3,5-difluoro analog **17** is less potent with an IC_{50} of 6 nM against Cat K. Insertion of a spacer atom either as an ether linker (**25**) or a carbonyl (**26**) reduced Cat K potency. Among these biphenyl analogs, the most selective compound appeared to be **13** with >6000-fold selectivity for Cat S and over 16,000-fold for cathepsins B and L. The most potent non-basic biphenyl analog in the rabbit bone resorption assay is the methyl sulfone analog **22**. The SAR with heterocyclic substituents was also studied. Some representative examples are also shown in Table 3. Pyridine **27**, thiophene **28**, thiazole **29**, and indole **32** are very potent against Cat K, while oxadiazole **30** and pyrimidine **31** show reduced potency. In the functional rabbit bone resorption assay, the pyridine **27** has excellent potencies, whereas other heterocyclic analogs are less potent. However, pyridine **27** shows poorer selectivity than the biphenyl analog **22** against Cat L (267-fold vs 1320-fold) and Cat S (206-fold vs 890-fold). The tricyclic P3 analogs **33** and **34** were briefly studied. This modification resulted in poor pharmacokinetics in rats and therefore was not pursued further. Nevertheless, the SAR showed increased selectivity over Cat L and S, but decreased selectivity over Cat B (**33** vs **22** and **34** vs **27**). Among two of the best biphenyl analogs, **13** and **22**, preliminary pharmacokinetic study showed that **22** had superior

Table 3. In vitro activity of representative P3 analogs



Compound	R	IC_{50}[a] (nM)				
		Cat K[b]	Cat B	Cat L	Cat S	Bone res[c]
1	4-Piperazin-1-yl phenyl	⩽0.005[d]	1111	47	451	12[e]
12	Phenyl	0.2	1605	1473	303	44
13	2-Fluorophenyl	0.1	6086	1670	614	28[*]
14	3-Fluorophenyl	0.5	2476	2792	278	67[*]
15	4-Fluorophenyl	0.6	5939	887	449	29[*]
16	3,4-Difluorophenyl	1.1	8869	1001	189	46[*]
17	3,5-Difluorophenyl	6	>10,000	3002	249	n.d.
18	4-Methylphenyl	0.3	1337	809	282	27[*]
19	4-Methoxyphenyl	0.1	1660	560	196	n.d.
20	4-Cyanophenyl	0.9	7392	344	172	30[*]
21	4-(Methylthio)phenyl	0.2	1400	524	153	50[*]
22[f]	4-(Methylsulfonyl)phenyl	0.2	5239	264	178	13[e]
23	4-(Aminosulfonyl)phenyl	0.2	1561	470	154	52
24	3-(Methylsulfonyl)phenyl	0.3	6984	1480	265	53[*]
25	Phenoxy	2.3	>10,000	771	220	88[*]
26	Benzoyl	1.5	>10,000	1915	254	151[*]
27	Pyridin-4-yl	0.3	2374	80	62	19
28	3-Thienyl	0.2	1155	1475	365	33[*]
29	1,3-Thiazol-2-yl	0.3	4818	878	240	52
30	3-Methyl-1,2,4-oxadiazol-5-yl	2.6	4073	314	436	118[*]
31	Pyrimidin-2-yl	44	5903	1857	1698	93[*]
32	4-(1*H*-Indol-4-yl)	0.4	4699	3047	607	80[*]
33	4′-(Methylsulfony)biphenyl-4-yl	1.2	1873	2422	653	5[e]
34	4-Pyridin-4-yl-phenyl	<0.2	483	342	188	14[e,*]

[a] IC_{50}s are an average of at least two independent titrations unless specified otherwise.
[b] Humanized rabbit enzyme (Ref. 3), see Ref. 6 for assay conditions.
[c] Rabbit bone resorption assay, see Ref. 8 for assay conditions.
[d] $IC_{50}$was determined at a concentration of 10 pM enzyme.
[e] This value likely represents the lower limit of the assay.
[f] L-873724.
[*] $n = 1$. n.d. = not determined.

pharmacokinetics to **13** in rats. Therefore, compound **22**, now designated as L-873724, was selected for further profiling.

L-873724 has good pharmacokinetics in three preclinical animal species (Table 4). The volumes of distribution close to unity are consistent with this compound not being lysosomotropic. In the whole cell enzyme occupancy assays (Table 5), L-873724 showed no significant increases in potency against Cat B, L, and S compared to isolated enzyme potencies, further evidence supporting its non-lysosomotropic character. In addition, L-873724 had excellent potency in the rabbit bone resorption assay. As a result, L-873724 is anticipated to maintain good functional selectivity in vivo.

The important question of whether a non-basic inhibitor L-873724 would show efficacy in an animal model of bone resorption now needed to be addressed. The possibility exists that the lysosomotropic properties associated with basic Cat K inhibitors may help the accumulation of compound in the acidic osteoclast resorption lacunae where cathespin K digests collagen and therefore contribute to efficacy. In this context, one could conclude that a non-basic inhibitor would show reduced efficacy in vivo. Fortunately, in the ovariectomized (OVX) rhesus monkey model,[14] a mean decrease of 68% in urinary uNTx/Cr was observed during the six-day on-treatment phase at 3 mg/kg with once-daily oral dosing, as indicated in Figure 1. Three days following the last dose, uNTx/Cr levels returned to baseline. The response of L-873724 in this model is comparable to that of the basic P2–P3 amide dipeptide Cat K inhibitor CRA-013783/L-006235 which has similar in vitro potency.[15]

An X-ray crystallographic structure of compound **23,** which is a close analog of **22**, with Cat K at 2Å resolution has been obtained (Fig. 2, PDB entry 2FD2). Our previous model structure[2] of trifluoroamine analog into

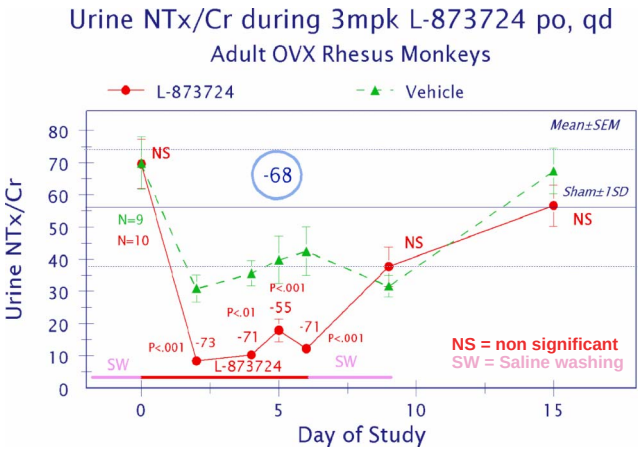


Figure 1. Efficacy of compound **22** (L-873724) in the OVX rhesus monkey model.

Cat K shows excellent agreement with the X-ray crystal structure. The CF_3 group can clearly be seen directed out of the active site, toward water. The hydrogen bond between the NH adjacent to the CF_3 group and the Gly66 carbonyl is well formed and closely resembles the comparable hydrogen bond found in the P2–P3 amide dipeptide series. The phenyl group adjacent to the CF_3 group is coplanar with the glycine shelf (Gly66–Gly65) creating a significant hydrophobic interaction. Due to the sp^3 nature of the trifluoroethylamine group, the orientations of phenyl ring over the glycine shelf and the orientation of the hydrogen bond are preferred. This is in contrast to the P2–P3 amide dipeptide inhibitors where both of these interactions are suboptimal due to the sp^2 nature of the linking amide bond. The sulfonamide substituent at the distal phenyl ring is largely exposed outside the binding pocket and does not have significant interaction at the active site. In addition, one side of the distal phenyl ring is also exposed to solvent and the other side is in the S3 region of the enzyme. This may help to explain why the substi-

Table 4. Pharmacokinetics of **22** (1% methocel as dosing vehicle)

Species	po dose (mg/kg)	% *F*	C_{max} (μM)	$t_{1/2}$ (h)	V_d (L/kg)
Rat	10	40	4.4	3	1.4
Dog	10	65	18	6	0.8
Rhesus monkey	5	24	0.7	2	0.5

Table 5. Functional, whole cell, and enzyme activity of compound **22**

IC_{50} (nM)			
Enzyme occupancy assays			Functional Cat K
HepG2 Cat B	HepG2 Cat L	Ramos Cat S	Rabbit bone res
4807	1221	95	13[a]
Enzyme assays			
Cat B	Cat L	Cat S	Rabbit Cat K
5239	264	178	0.8[b]

[a] This value likely represents the lower limit of the assay.
[b] IC_{50} was determined at a concentration of 10 pM enzyme.

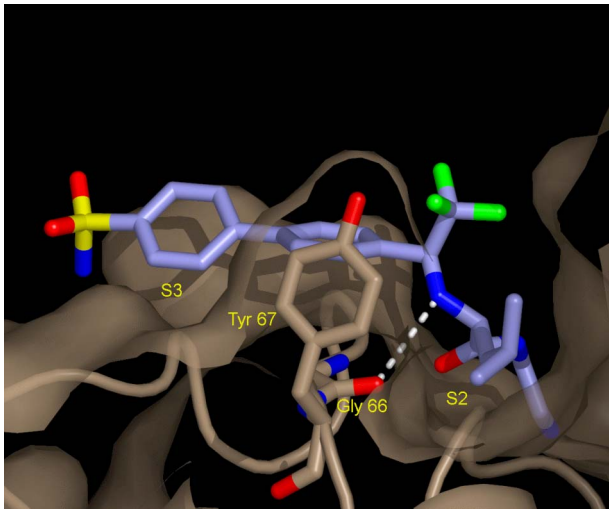


Figure 2. X-ray crystal structure of compound **23** bound to Cat K showing the P2–P3 trifluoroethylamine group and the hydrogen bond it makes to Gly66 (PDB entry 2FDZ).

tution pattern of disubstituted analogs is critical for potency, **16** versus **17**. Overall the binding interactions with compound **23** are essentially identical to that of the P2–P3 amide dipeptide inhibitors (pdb entry 1MEM), without the internal destabilization of the ligand found in the dipeptides.

In summary, we have identified L-873724 as a non-basic, potent, and highly selective cathepsin K inhibitor. This compound is orally bioavailable, non-lysosomotropic, and efficacious in the rhesus monkey bone resorption model.

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