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Discovery and SAR studies of a novel series of noncovalent cathepsin S inhibitors

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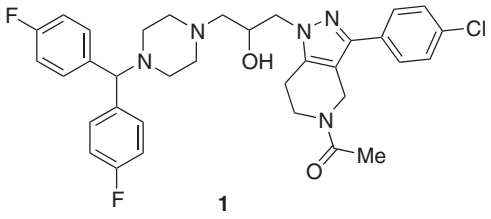
Abstract—A novel series of competitive, reversible cathepsin S (CatS) inhibitors was discovered and optimized. The 4-(2-keto-1-benzimidazolinyl)-piperidin-1-yl moiety was found to be an effective replacement for the 4-arylpiperazin-1-yl group found in our earlier series of CatS inhibitors. This replacement imparted improved PK properties as well as decreased off-target activity. Optimization of the ketobenzimidazole moiety led to the discovery of the lead compound JNJ 10329670, which represents a novel class of selective, noncovalent, reversible, and orally bioavailable inhibitors of cathepsin S.

Cathepsin S (CatS) is a cysteine protease of the papain family that is involved in the presentation of antigens to the cell surface of certain antigen-presenting cells (APCs) for recognition by $CD4^+$ T-cells. The main target of the proteolytic activity of CatS is the invariant chain (Ii), a chaperone molecule for major histocompatibility complex class II molecules (MHCII).[1–3] Inhibition of CatS activity slows the removal of Ii and attenuates antigen presentation to $CD4^+$ T-cells. The only selective CatS inhibitors disclosed to date, such as the dipeptidyl vinyl sulfone LHVS,[4] rely upon the formation of a covalent adduct at the active site cysteine of CatS to achieve potent inhibition.[5–8]



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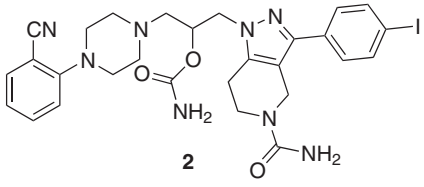

2

Our discovery and initial optimization of the screening hit **1** leading to the potent (IC_{50} = 20 nM) and selective noncovalent cathepsin S inhibitor **2** have recently been disclosed.[9]

While compound **2** and analogues are completely selective against other proteases tested, including the related cathepsins B, E, F, K, and L, several analogues were found to have affinity for the $\alpha 1_a$ adrenergic receptor (Table 1). In addition, although several of the arylpiperazine inhibitors are active in a secondary cellular assay measuring Ii degradation in (human) JY cells, they had limited oral bioavailability.[10] We thus set out to identify new CatS inhibitors with improved selectivity and bioavailability compared to the arylpiperazines.

During a survey of commercially available aryl-substituted piperazines and piperidines, we discovered that a 4-(2-keto-1-benzimidazolinyl)-piperidine was a suitable replacement for the 4-arylpiperazine moiety. Reported

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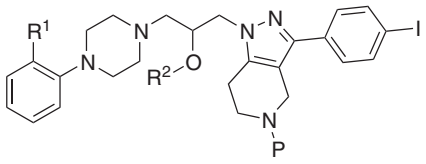
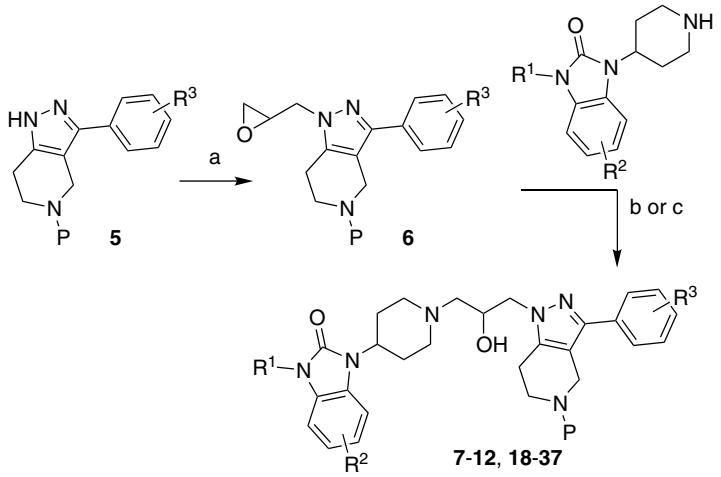
Table 1. Arylpiperazine cathepsin S inhibitors[a]



Compound	R^1	R^2	P	CatS IC_{50}, nM	$\alpha1_a$ K_i, nM	JY Cell (Ii deg.) IC_{50}, μM
2	CN	$CONH_2$	$CONH_2$	20	12	0.88
3	CN	H	Ac	120	ND	3.9
4	CN	H	$CONH_2$	50	36	1.3

$\alpha1_a$ K_i values were determined by MDS PanLabs.
[a] CatS IC_{50} and JY Ii degradation assay IC_{50} values are the mean of $n \geqslant 3$ runs and determined as described previously.[9,11]



Scheme 1. Reagents and conditions: (a) epichlorohydrin (4–6 equiv), Cs_2CO_3 (2 equiv), DMF, rt; (b) amine, 3–10% $Yb(OTf)_3$, CH_2Cl_2, rt, 0 °C; (c) amine, EtOH, reflux.

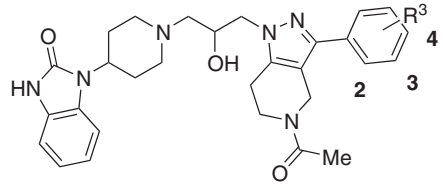
herein is an SAR study of ketobenzimidazole-containing CatS inhibitors that led to the preparation of potent, orally bioavailable inhibitors of CatS.[11]

The epoxide intermediates **6**, prepared as described previously,[9] provided the ability to quickly survey 'head group' piperidine inputs and to assess the corresponding structure–activity relationships (Scheme 1). The addition of the piperidines to the epoxides was promoted either thermally (EtOH, 80 °C) or by 3–10% $Yb(OTf)_3$ (CH_2Cl_2, rt) to afford the secondary alcohols **7**–**12** and **18**–**37** in 50–90% yields.

As shown in Table 2, the parent ketobenzimidazole afforded analogues with excellent CatS inhibition when paired with a variety of substituted pyrazoles. The 4-chlorophenyl pyrazole derivative **7** inhibited CatS with an IC_{50} of 0.46 μM, comparable to the level of activity observed previously for **1** (1.0 μM). The addition of a *meta*-substituent to the phenyl pyrazole (**8**) or replacement of the chlorine atom with more lipophilic groups (**9**–**12**) improved inhibitory activity by threefold or better. These new CatS inhibitors also had sub-micromolar affinity for $\alpha1_a$.

Given the encouraging CatS activity of these analogues, we next set out to prepare derivatives with substitutions

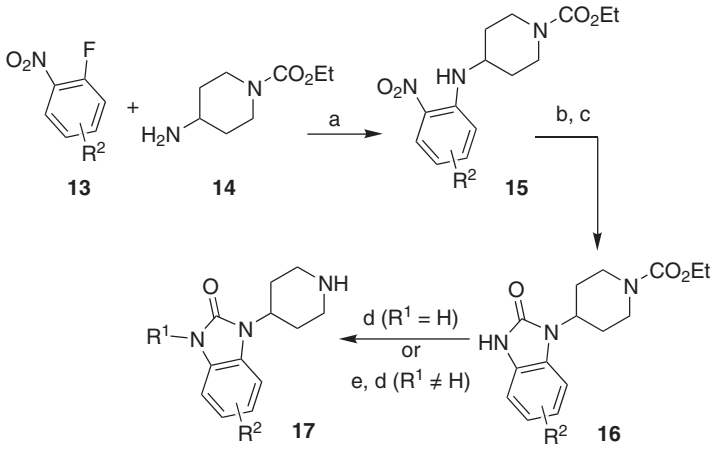
Table 2. Ketobenzimidazole CatS inhibitors[a]



Compound	R^3	CatS IC_{50}, nM
7	4-Cl	460
8	3-Me, 4-Cl	130
9	4-CF_3O	130
10	4-I	120
11	4-Br	80
12	4-CF_3	100

[a] See Table 1 for details.

on the ketobenzimidazole aromatic ring and the distal nitrogen atom with the aim of reducing $\alpha1$ affinity and improving bioavailability. The requisite piperidine intermediates were prepared by the route depicted in Scheme 2.[12] Condensation of a 2-fluoronitrobenzene **13** with the 4-aminopiperidine **14** afforded the nitroanilines **15**. Reduction of the nitro group by transfer hydrogenation and subsequent treatment with triphosgene afforded the ketobenzimidazoles **16** in good yields, providing



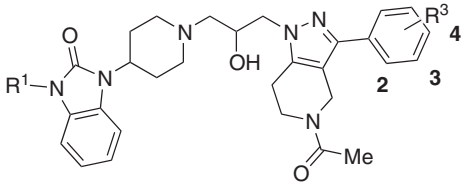
Scheme 2. Reagents and conditions: (a) DMF, 60 °C; (b) Pt/C, NH_4HCO_2, EtOH, rt; (c) triphosgene, Et_3N, CH_2Cl_2, rt; (d) 1 N NaOH, EtOH, reflux; (e) KHMDS, R^1X, THF.

prolonged handling of the unstable phenylene diamines was avoided. Basic hydrolysis afforded the piperidines **17** (R^1 = H). Alternatively, *N*-substitution of the ketobenzimidazole nitrogen could be effected prior to removal of the ethoxycarbonyl protecting group. Basic hydrolysis then afforded the *N*-substituted ketobenzimidazoles **17**. Conversion to the target molecules proceeded as shown in Scheme 1.

Several *N*-alkylated derivatives of **8** and **12** were prepared and tested for their ability to inhibit CatS activity (Table 3).

For the 3-methyl-4-chlorophenyl pyrazole derivative **8**, addition of an *N*-methyl group resulted in a half-log loss in activity (**18**) that could be regained by more lipophilic *N*-substitutions (**19**–**22**). On the other hand, *N*-methylation of the 4-trifluoromethyl phenylpyrazole **12** afforded an inhibitor (**23**) with equivalent potency. Although *N*-substitution on the ketobenzimidazole moiety had only a moderate effect on CatS activity, qualitatively the solubility of these analogues was greatly improved, making them easier to handle in many of the biological assays. Unfortunately, screening against $\alpha 1_a$ showed little improvement in selectivity.

Table 3. Effect of *N*-substitution on CatS activity[a]



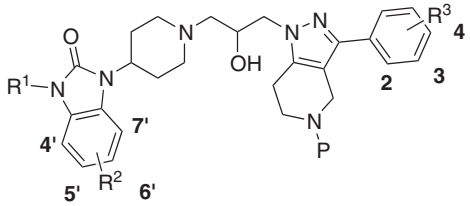
Compound	R^1	R^3	CatS IC_{50}, nM
8	H	3-Me-4-Cl	130
12	H	4-CF_3	100
18	Me	3-Me-4-Cl	500
19	Et	3-Me-4-Cl	310
20	*n*-Bu	3-Me-4-Cl	140
21	*i*-Pr	3-Me-4-Cl	100
22	CF_3CH_2	3-Me-4-Cl	90
23	Me	4-CF_3	110

[a] See Table 1 for details.

We next turned our attention to inhibitors with nonhydrogen substituents on the aromatic ring of the ketobenzimidazole, and the CatS inhibitory activity for these derivatives is tabulated in Table 4, with the data from the parent inhibitors **8**, **10**, **11**, and **12** shown for comparison.

Substitutions were generally well tolerated, particularly at the 5′ and 6′ positions of the aromatic ring, affording no more than 2-fold changes in activity versus the parent structures. For the 4-bromophenylpyrazole **11**, for example, chloro (**26**), methoxy (**28**), or methyl (**30**)

Table 4. Substituted ketobenzimidazoles[a]



Compound	R^1	R^2	R^3	P	CatS IC_{50}, nM
8	H	H	3-Me, 4-Cl	Ac	130
10	H	H	4-I	Ac	120
11	H	H	4-Br	Ac	80
12	H	H	4-CF_3	Ac	100
24	H	5′-Me	3-Me, 4-Cl	Ac	100
25	H	6′-F	4-I	Ac	230
26	H	6′-Cl	4-Br	Ac	120
27	H	5′,6′-diCl	4-Br	Ac	110
28	H	5′-OMe	4-Br	Ac	140
29	H	7′-Me	4-Br	Ac	210
30	H	5′-Me	4-Br	Ac	90
31	H	H	3-Me, 4-Cl	$MeSO_2$	60
32	H	6′-Cl	3-Me, 4-Cl	$MeSO_2$	50
33	H	H	4-CF_3	$MeSO_2$	50
34	H	6′-Cl	4-CF_3	$MeSO_2$	60
35	H	6′-Cl	4-Br	$MeSO_2$	50
36	Me	6′-Me	4-CF_3	$MeSO_2$	30
37	Me	6′-Cl	4-CF_3	$MeSO_2$	80

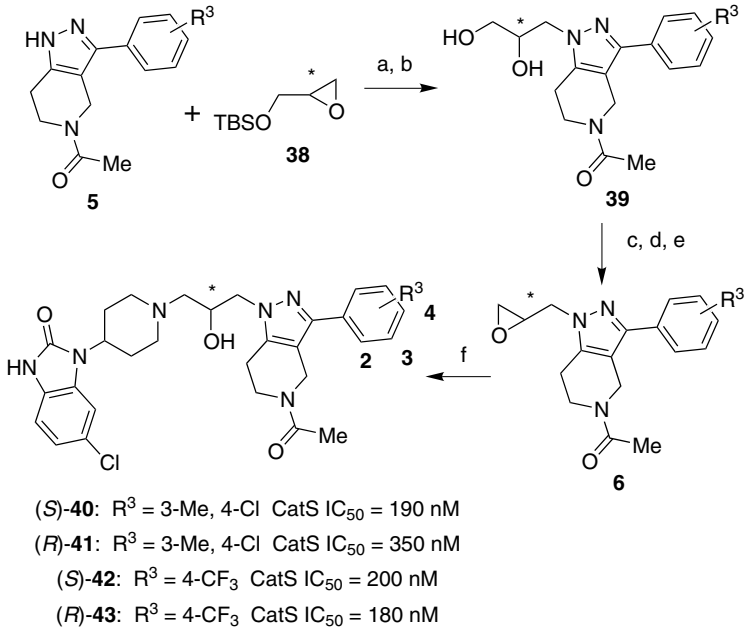
[a] See Table 1 for details.

substituents at 5′ or 6′ afforded inhibitors with activity comparable to **11**. Dichloro substitution (**27**) was tolerated equally well.

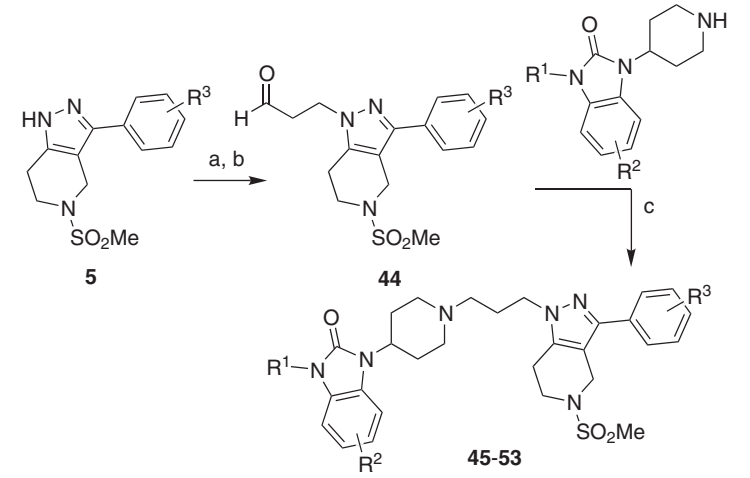
An interesting effect regarding the nature of tetrahydropyridine *N*-substituent on CatS activity was also observed. Replacement of the acetamide with a methylsulfonamide resulted in a modest 2-fold improvement in potency for both the 3-methyl-4-chlorophenylpyrazole (compound **8** vs compound **31**); and 4-trifluoromethylphenylpyrazole (**12** vs **33**). This trend was maintained with a 6′-chloroketobenzimidazole (**26** vs **35**). In addition to the modest improvements in CatS potency, $\alpha 1_a$ selectivity of certain analogues was greatly improved. For example, compound **37** had a K_i of 3 μM against the $\alpha 1_a$ adrenergic receptor.

In order to understand the effect of the linker hydroxyl on activity, two pairs of enantiomers containing the 6-chloroketobenzimidazole were synthesized (Scheme 3). The pyrazole **5** was condensed with *t*-butyldimethylsilyl-protected glycidols (*R*)- or (*S*)-**38** (>98% ee), followed by cleavage of the TBS ether with CSA. The diols (*S*)- and (*R*)-**39** were converted[13] to the epoxides (*S*)- or (*R*)-**6** and then to the desired analogues **40**–**43**. As shown by the CatS inhibitory activity (Scheme 3), the absolute stereochemistry of the inhibitors had little effect on CatS potency.

Given the modest effect of hydroxyl stereochemistry on CatS inhibition, achiral analogues that did not bear the linker hydroxyl group were targeted and prepared as shown in Scheme 4.

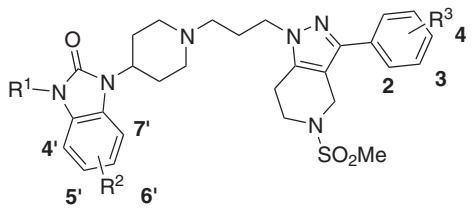


Scheme 3. Reagents and conditions: (a) Cs_2CO_3, DMF, rt; (b) CSA, MeOH; (c) $MeC(OMe)_3$, PPTS; (d) AcBr, MeOH, CH_2Cl_2; (e) KOEt, MeOH; (f) 6-chloro-1-piperidin-4-yl-1,3-dihydro-benzoimidazol-2-one, EtOH, reflux.



Scheme 4. Reagents and conditions: (a) 3-bromopropanol, Cs_2CO_3, DMF; (b) Dess–Martin periodinane; (c) $Na(OAc)_3BH$, AcOH, CH_2Cl_2.

Table 5. CatS inhibition of achiral noncovalent inhibitors[a]



Compound	R^1	R^2	R^3	CatS IC_{50}, nM
45	H	H	3,4-diCl	10
46	H	6′-Cl	4-CF_3	30
47	H	H	4-CF_3	50
48	Me	H	3,4-diCl	50
49	H	H	4-Br	50
50	Me	6′-Cl	4-CF_3	100
51	$NCCH_2$	H	4-CF_3	100
52	Me	H	4-Br	140
53	Me	6′-Cl	3,4-diCl	150

[a] See Table 1 for details.

Regioselective alkylation of pyrazole **5** with 3-bromopropanol in the presence of cesium carbonate followed by oxidation of the primary alcohol with Dess–Martin periodinane afforded the aldehydes **44**. Installation of the piperidines via reductive amination afforded the desired achiral deshydroxy analogs **45**–**53** (Table 5).

Interestingly, removal of the hydroxyl group resulted in compounds with equal or improved activity compared to the hydroxy analogues. For example, the hydroxy compound **34** (Table 4) had an IC_{50} of 60 nM, while the deshydroxy analogue **46** had an IC_{50} of 30 nM (Table 5). Likewise, the deshydroxy version of **11** (CatS IC_{50} = 80 nM) was also an excellent CatS inhibitor (**49**, CatS IC_{50} = 50 nM).

Among the analogues prepared and tested, JNJ 10329670 (**50**, Table 5) was chosen for further pharmacological characterization for several reasons. First, it was selective against the adrenergic receptors $\alpha1_a$, $\alpha1_b$, and $\alpha1_d$ (1.1–1.9 μM). This is a dramatic improvement in selectivity (50–100x) when compared to the unsubstituted ketobenzimidazoles and the arylpiperazines from the original lead series. In addition, JNJ 10329670 has encouraging activity in the secondary cellular assay measuring inhibition of cleavage of the invariant chain in JY cells[9,11] (IC_{50} = 0.60–0.80 μM). Finally, **50** also has good oral bioavailability in rats, dogs, and monkeys (40–75%) and an excellent in vivo half-life (4–14 h). Compound **50** was also tested against cathepsins B, C, D, E, F, K, L, L2, Z, and legumain: IC_{50}'s of greater than 50 μM were observed for these enzymes. The pharmacological characterization of JNJ 10329670 has recently been described.[11]

In conclusion, replacement of the head-group *N*-arylpiperazine in our original series of CatS inhibitors with a ketobenzimidazole piperidine resulted in a new series of potent, noncovalent CatS inhibitors. The incorporation of substituents on the ketobenzimidazole aryl moiety proved useful for improving selectivity over the α1 adrenergic receptors and attaining inhibitors with improved oral bioavailability. In addition, a selective noncovalent reversible inhibitor, JNJ 10329670, was selected for further investigation in order to elucidate the pharmacology of CatS and its potential as a target for immunosuppressive therapies.

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