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Pyrazole-based arylalkyne cathepsin S inhibitors. Part II: Optimization of cellular potency

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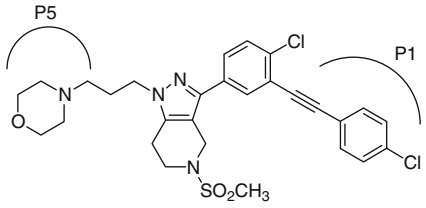
ABSTRACT

Basic lipophilic substituents dramatically improved the cellular potency of a previously disclosed series of pyrazole-based arylalkyne cathepsin S inhibitors. The incorporation of substituted benzylamines in the *para* position of the arylalkyne maintained enzymatic activity (hCatS IC_{50} = 80–420 nM) and imparted cellular potency (IC_{50} = 0.8–4.0 μM). Further refinement of the morpholine portion of the pharmacophore enabled the identification of bicyclic piperidines with enhanced affinity for CatS (IC_{50} = 10–30 nM) and sub-micromolar cellular potency (JY Ii IC_{50} = 200–720 nM).

The cysteine protease cathepsin S (CatS) resides in the lysosome of certain antigen presenting cells, as well as the extracellular matrix of activated macrophages. Due to its central role in both antigen and invariant chain (Ii) processing, CatS is an important mediator of MHC class II related immune responses.[1] CatS knockout mice exhibit impaired invariant chain degradation, as well as a reduced susceptibility to collagen-induced arthritis compared to wild type animals.[2] This suggests that inhibition of CatS may prove beneficial for the treatment of certain immunological disorders. Aberrant CatS activity has been implicated in other diseases as well.[3] Many CatS inhibitors are peptidic compounds that covalently bind to the active site cysteine, but recently several non-covalent inhibitors have been disclosed as well.[4–7]

We have previously reported pyrazole-based CatS inhibitors containing arylalkynes as P1 binding elements.[7f] A representative example from this series, 4-chlorophenyl alkyne **1** (Fig. 1), demonstrated activity against both human and mouse forms of CatS, exhibited selectivity for CatS over several closely related cysteine proteases, and reached systemic circulation following oral dosing in rats (% F = 21). However, this compound and numerous closely related alkyne analogs failed to display any appreciable activity (JY Ii IC_{50} >10 μM) in a secondary cellular assay measuring invariant chain degradation in human JY cells. In this Letter, we describe how the incorporation of lipophilic basic substituents on either the P1 alkyne or P5 morpholine portions of the molecule can dramatically improve the cellular potency of this series of inhibitors without compromising enzymatic activity.

Although a morpholine ring was initially selected as a solubilizing group for alkyne **1**, its modest enzymatic activity and lack of cellular potency prompted us to consider alternate heterocycles. Since earlier work related to non-alkyne pyrazole-based CatS inhibitors established that substituted piperazine[7b] or piperidine[7e] groups in the P5 region of the pharmacophore enhanced enzymatic and cellular activity, we evaluated these six-membered rings as potential morpholine replacements within the alkyne series (Scheme 1). To enable rapid evaluation of the left-hand side of the molecule, cyclic amines were introduced via a two-step process involving regioselective alkylation of pyrazole **2**[7f] with epichlorohydrin, followed by



1

hCatS = 200 nM; mCatS = 690 nM
hCatB, hCatF, hCatL > 20 μM
JY Ii IC_{50} > 10 μM

Figure 1. Profile of **1**.

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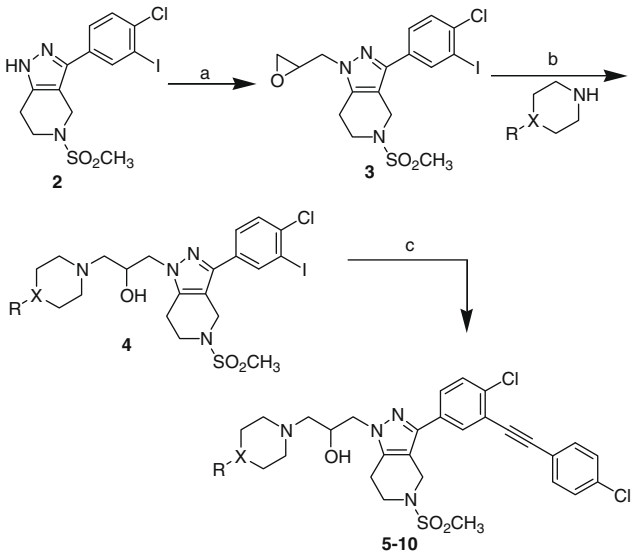


Scheme 1. Reagents and conditions: (a) epichlorohydrin, Cs_2CO_3, DMF (73%); (b) EtOH, 80 °C (68–89%); (c) 4-chloroethynylbenzene, 10% $Pd(PPh_3)_2Cl_2$, 10% CuI, Et_3N, THF, rt (68–83%).

thermal ring-openings of racemic epoxide **3** with the desired amines. Late-stage incorporation of the 4-chlorophenylalkyne P1 moiety under standard Sonogashira conditions provided the targeted heterocyclic analogs **5–10** of morpholine **1**.

As shown in Table 1, the presence of a hydroxyl group in the linker had a minimal effect on enzymatic activity, as morpholine **5** exhibited comparable activity to its des-hydroxy analog **1** (CatS IC_{50} = 140 and 200 nM, respectively). Although piperazine replacements **6–7** for the morpholine group did not improve enzymatic activity, piperidine **8** demonstrated a twofold enhancement in CatS affinity. In general, substitution at the 4-position of the piperidine ring was tolerated, and methyl ester **9** exhibited an eightfold improvement in enzymatic activity compared to the parent piperidine **8**. Thus, it is clear that small changes to the P5 region of the pharmacophore can dramatically affect CatS affinity. Unfortunately, enhanced enzymatic activity did not translate to an improvement in cellular potency, and suggested that the lack of cellular activity for this class of inhibitors might be attributed to poor cellular permeability.[8]

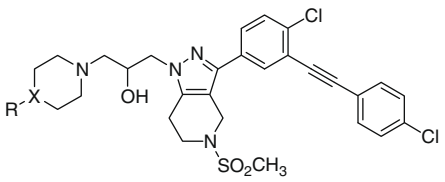
Concurrent with our work on left-hand side modifications, we attempted to improve cellular activity by altering the P1 arylalkyne moiety in the morpholine series. Since previous SAR for the arylalkynes established that substituents in the *para* position of the aromatic ring were preferred for optimal enzymatic activity, a variety of polar groups (i.e., alcohols, amines, acids, etc.) were introduced in the 4-position and evaluated in our cellular assay (data not shown). In addition to altering the lipophilicity and solubility of the alkyne inhibitors, it was anticipated that these functional groups would provide a synthetic handle upon which to append further substitution. However, among all of these motifs, derivatized benzylamines **15** demonstrated the only hint of cellular activity (Table 2).

Initially, the substituted benzylamines **15** were prepared using one of two routes (Schemes 2 and 3). The parent benzylamine **14** was assembled from the previously described terminal alkyne **11**[7f] by a two-step process involving Sonogashira coupling with (4-iodo-benzyl) carbamic acid *tert*-butyl ester **12** and subsequent acid-mediated deprotection (Scheme 2). Although sulfonylation and acylation of benzylamine **14** proceeded smoothly under standard conditions, reductive aminations provided only moderate yields of the desired mono-alkylated benzylamines **15** due to extensive over-alkylation of the amine. In an attempt to circumvent this problem and also access unsymmetrical N,N-disubstituted benzylamines, benzaldeyhde **17** was prepared and subjected to reductive aminations with primary and secondary amines (Scheme 3). Although it was possible to isolate the desired benzylamines **15**, reduction of the alkyne was frequently a competing side reaction.

As shown in Table 2, derivatization of primary amine **14** had a minimal effect on enzymatic activity. Both sulfonamide **15a** and amide **15b** retained affinity for CatS but did not inhibit invariant chain degradation in the cellular assay. Although a small alkyl substituent **15c** was not favored, the addition of aromatic groups **15d–15f** was tolerated. More importantly, despite their modest enzymatic activity, the benzyl and phenethyl amines **15d** and **15f** displayed a glimmer of cellular activity, and provided a clue that the key to optimizing cellular potency might lie in balancing the basicity and lipophilicity of the substituents appended to the amine.

In order to test this theory and further improve the cellular activity of benzylamine **15d**, we profiled a small set of substituted benzylamines **19** (Table 3). Given the problems associated with the previous synthetic routes, most of these compounds were constructed using an alternate method in which terminal alkynes **18** were first assembled via a reductive amination between functionalized benzylamines and 4-ethynylbenzaldehyde, and then coupled to iodoarene **16** using conventional Sonogashira conditions (Scheme 4). Gratifyingly, reductive aminations with 4-ethynylbenzaldehye

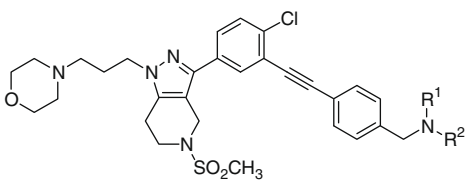
Table 1
SAR of morpholine/piperazine/piperidine CatS inhibitors[a]



Compound	X	R	CatS IC_{50} (μM)	JY Ii IC_{50} (μM)
5	O	—	0.14	>10
6	N	Boc	0.15	>10
7	N	H	0.38	>10
8	CH	H	0.08	>10
9	CH	CO_2Me	0.02	>10
10	CH	$CONH_2$	0.11	>10

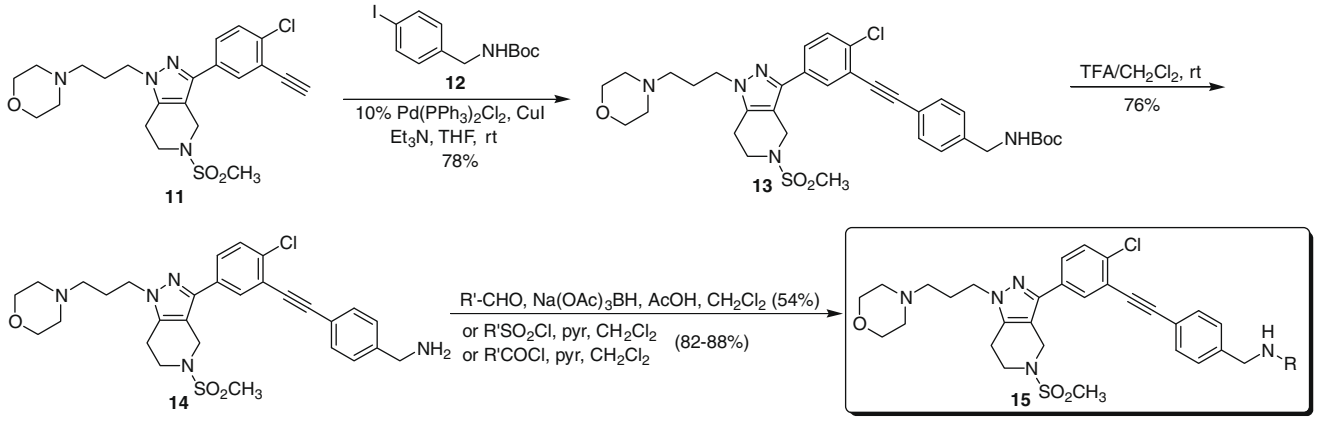
[a] CatS IC_{50} and JY Ii degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[6a]

Table 2
Effect of amine substitution on enzymatic and cellular activity

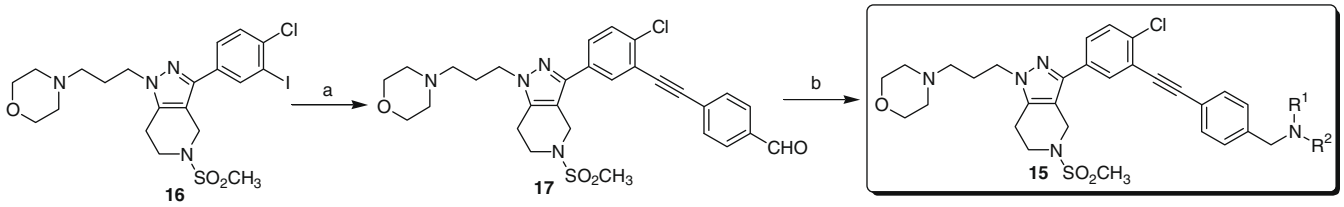


Compound	R_1	R_2	Route	CatS IC_{50} (μM)	JY Ii IC_{50} (μM)
14	H	H	A	0.49	10
15a	H	SO_2Ph	A	0.16	>10
15b	H	COPh	A	0.35	>10
15c	H	CH_3	B	1.30	>10
15d	H	CH_2Ph	A	0.42	4.2
15e	CH_3	CH_2Ph	B	0.60	>10
15f	H	CH_2CH_2Ph	B	0.58	5.7

[a] See Table 1, footnote a for details.

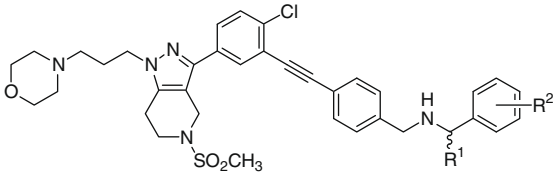


Scheme 2. Route A: general synthesis of N-substituted benzylamines **15**.



Scheme 3. Route B: alternate synthesis of N-substituted benzylamines **15**. Reagents and conditions: (a) 4-ethynylbenzaldehyde, 10% $Pd(PPh_3)Cl_2$, 10% CuI, Et_3N, THF, rt (92%); (b) R_1R_2NH, $Na(OAc)_3BH$, AcOH, CH_2Cl_2 (11–68%).

Table 3
SAR of *N*,*N*-dibenzyl cathepsin S inhibitors[a]



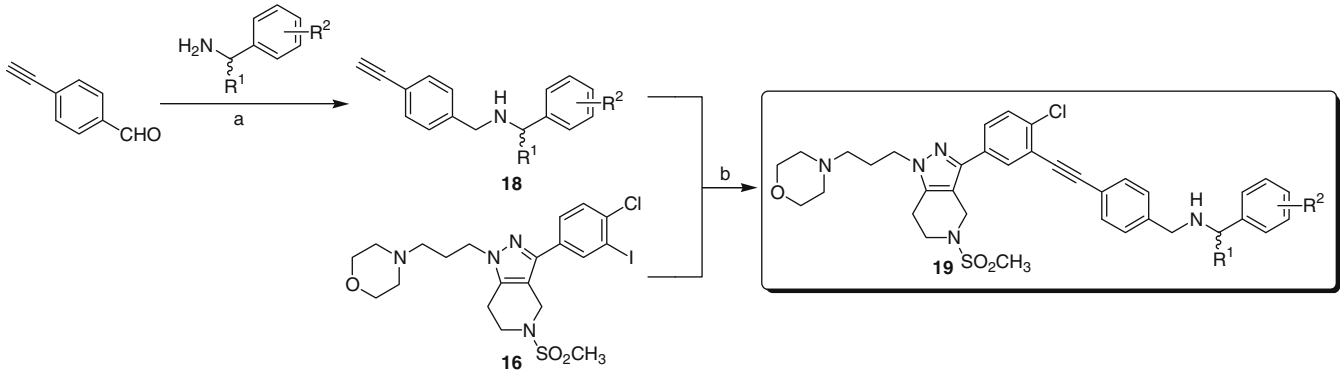
Compound	R_1	R_2	Route	CatS IC_{50} (μM)	JY Ii IC_{50} (μM)
15d	H	H	A	0.42	4.2
19a	H	4-CH_3	B	0.35	1.1
19b	H	4-OCH_3	C	0.23	0.82
19c	H	4-Cl	C	0.14	1.5
19d	H	3,4-diCl	C	0.08	1.8
19e	CH_3 (±)	H	C	0.19	1.0
19f	CH_2OH (R)	H	C	0.10	1.7
19g	CO_2CH_3 (R)	H	C	0.10	>10
19h	CO_2CH_3 (S)	H	C	0.12	>10

[a] See Table 1, footnote a for details.

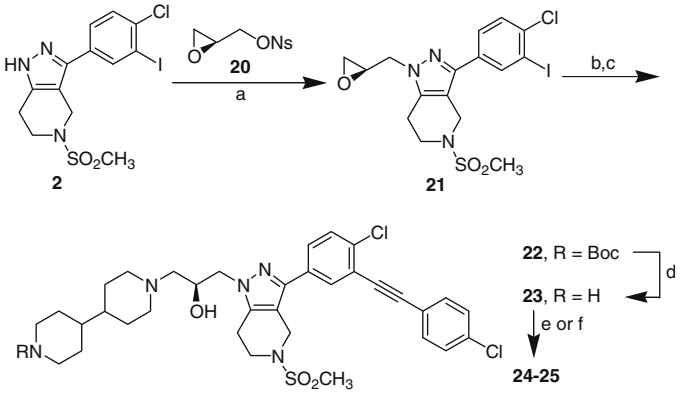
were consistently cleaner than those involving aldehyde **17**. Presumably the absence of a second electron-withdrawing aromatic ring attenuates the susceptibility of the terminal alkyne towards reduction.

As shown in Table 3, lipophilic groups **19a–d** consistently improved both enzymatic and cellular activity relative to the unsubstituted benzylamine **15d**, with each compound typically exhibiting a 5–10-fold difference in activity between the two assays. Substituents adjacent to the nitrogen **19e–h** also improved CatS affinity, and the enzyme did not exhibit a preference for a particular antipode. Interestingly, despite their encouraging enzymatic activity, esters **19g** and **19h** failed to demonstrate any inhibition of invariant chain degradation, further highlighting the potential dependence of cellular potency on the basicity of the benzylamine nitrogen.

In order to assess whether the position of the basic nitrogen substituent is essential for cellular potency, we revisited the P5 morpholine replacements identified in the 4-chlorophenylalkyne series (see Table 1). Since substituted piperidines demonstrated encouraging enzymatic activity, an additional piperidine ring was installed at the 4-position to yield a bicyclic replacement for the morpholine ring (Scheme 5). It was anticipated that further derivatization of the terminal nitrogen would provide an opportunity to tune the basicity and lipophilicity of the piperidine, and hopefully impart cellular potency to this series. To this end, enantiopure bicyclic piperidine **22** was prepared in a manner similar to that described earlier for piperidine **8** (see Scheme 1), except that pyrazole **2** was alkylated with (2*S*)-3-(2-nitrobenzenesulfonyloxy-1,2-epoxypropane **20** (>98% ee) instead of epichlorohydrin. The reactive nosyl group was selected

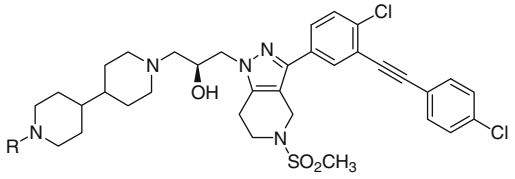


Scheme 4. Route C: optimized synthesis of N,N-disubstituted benzylamines **19**. Reagents and conditions: (a) $Na(OAc)_3BH$, AcOH, CH_2Cl_2 (60–90%); (b) 10% $Pd(PPh_3)_2Cl_2$, 10% CuI, Et_3N, THF, rt (>80%).



Scheme 5. Reagents and conditions: (a) Cs_2CO_3, DMF (70%); (b) *N*-Boc-4,4′-bipiperidine, EtOH, 80 °C (90%); (c) 4-chloroethynylbenzene, 10% $Pd(PPh_3)_2Cl_2$, 10% CuI, Et_3N, THF, rt (80%); (d) TFA, CH_2Cl_2 (68%); (e) AcCl, pyridine (61%); (f) aq CH_2O (3 equiv), $Na(OAc)_3BH$, DCE (45%).

Table 4
SAR of piperidine-based cathepsin S inhibitors[a]



Compound	R	CatS IC_{50} (μM)	JY Ii IC_{50} (μM)
22	CO_2tBu	0.01	>10
24	$COCH_3$	0.03	10
23	H	0.01	0.20
25	CH_3	0.01	0.72

[a] See Table 1, footnote a for details.

to avoid an S_N2' racemization process that can occur via ring-opening of the epoxide prior to displacement of the leaving group.[9] Acid-mediated removal of the Boc group provided free piperidine **23**, which was further functionalized to yield the desired analogs **24–25**.

Although all of the bicyclic piperidines **22–25** displayed exquisite activity in the enzymatic assay, their cellular potency varied greatly depending on the group attached to the terminal nitrogen (Table 4). Both of the basic piperidines **23** and **25** exhibited sub-micromolar inhibition of invariant chain degradation, and suggested that a free N–H was not required for cellular potency. However, the presence of electron-withdrawing groups **22** and **24** completely abrogated cellular activity in a manner consistent with the trends observed for the P1 alkynylbenzylamines in the morpholine series (see Table 2). Thus, it appears that a balance of nitrogen basicity and lipophilicity is crucial for achieving activity in the cellular assay, but the position of the basic nitrogen atom is not essential.

The ability of basic, lipophilic substituents to enhance inhibition of lysosomal proteases in cellular assays was first reported by researchers at Merck during their studies on cathepsin K inhibitors.[10] In a series of elegant experiments, they demonstrated that this phenomenon can be attributed to lysosomotropism, the intracellular accumulation of drug in acidic lysosomes, and can result in enhanced cellular inhibition of CatS as well. We have previously reported a series of pyrazole-based CatS inhibitors that appear to display lysosomotropic properties,[7e] but the compounds described in this Letter are distinctly different in that the IC_{50} values obtained from the cellular assay are always 5–10-fold higher than the corresponding IC_{50} observed in the purified enzyme assay. Thus, although it is difficult to rule out lysosomal trapping as the source of the enhanced cellular activity of pyrazole-based arylalkynes bearing basic, lipophilic substituents, additional factors may need to be considered as well (i.e., active cationic transport into cells, intracellular aggregation and/or membrane binding, etc.).[11]

In conclusion, a variety of basic, lipophilic substituents dramatically improved the cellular potency of a series of pyrazole-based arylalkyne cathepsin S inhibitors. The incorporation of substituted benzylamines in the *para* position of the P1 arylalkyne maintained enzymatic activity and imparted cellular potency. Insight gleened from the SAR for this series was used to refine the P5 morpholine portion of the pharmacophore and enabled the identification of bicyclic piperidines with enhanced affinity for CatS (IC_{50} = 10–30 nM) and sub-micromolar cellular potency (IC_{50} = 200–720 nM).

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