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Pyrazole-based arylalkyne Cathepsin S inhibitors. Part III: Modification of P4 region

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ABSTRACT

Novel classes of tetrahydropyrido-pyrazole thioether amines and arylalkynes that display potency against human Cathepsin S have been previously reported. Here, key pharmacophoric elements of these two classes are merged, and SAR investigations of the P4 region are described, in conjunction with re-optimization of the P5 and P1/P1′/P3 regions. Identification of *meta*-substituted arylalkynes with good potency and improved solubility is described.

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The lysosomal cysteine protease Cathepsin S (CatS) mediates cleavage of major histocompatibility class II (MHC II)-associated invariant chain (Ii), a key step in the immune response to an antigen.[1] The invariant chain prevents loading of the antigen into the MHC II binding groove for subsequent presentation on the cell surface to CD4+ T-cells. By preventing the degradation of the invariant chain, inhibition of CatS reduces antigen presentation. CatS inhibitors have thus been proposed for treatment of various autoimmune disorders and other inflammatory diseases. Both covalent-binding active site modifiers and noncovalent inhibitors of CatS have been disclosed.[2–5]

In preceding papers,[5] the synthesis and biological evaluation of novel classes of noncovalent thioether amine- and arylalkyne-containing tetrahydropyrido-pyrazoles led to the identification of two series of molecules (e.g., **1** and **2**, Fig. 1) with good in vitro potency against human CatS, as measured in an enzymatic assay (hCatS IC_{50}) and in an invariant chain degradation cellular assay (JY Ii IC_{50}).[3a,3c] Computational studies and preliminary X-ray crystal structure analysis have given insight into the potential binding mode of these compounds. The alkyne portion of molecules such as **2** is hypothesized to access the S1/S1′ binding pocket of the enzyme, and the oxamide substituent of compounds such as **1** is believed to make beneficial interactions with the S4 region of the enzyme.

Thioether molecules illustrated by compound **1**, though quite potent, are limited by poor oral bioavailability in rat PK studies. On the other hand, arylalkyne molecules such as **2** tend to display good PK properties but possess sub-optimal cellular potency. In general, both series suffer from poor equilibrium solubility at pH 7, complicating potential development of molecules within either series. Additional investigation into the SAR of the arylalkynes was undertaken, specifically through incorporation of various P4

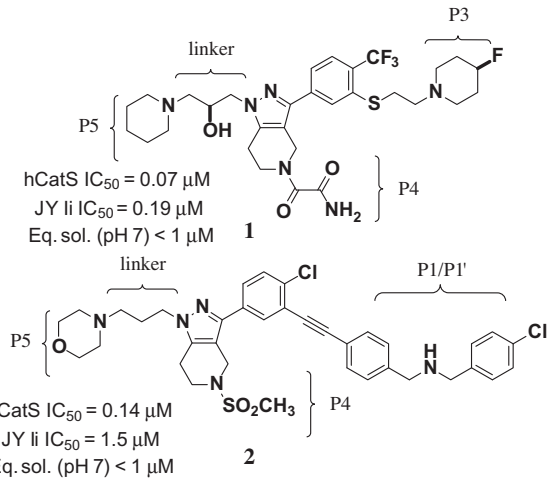

Figure 1. Reported tetrahydropyrido-pyrazole cathepsin S inhibitors.[5]

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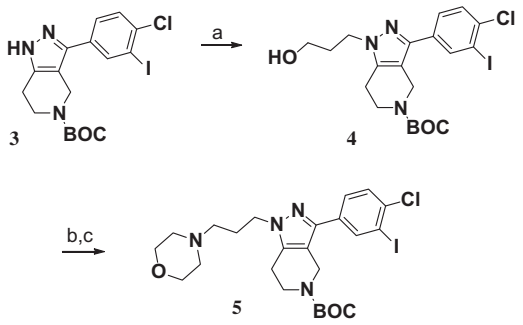
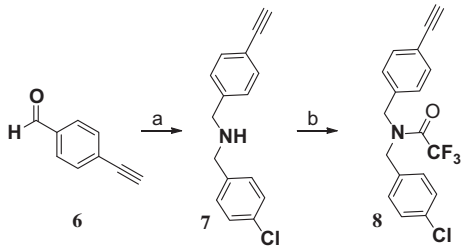
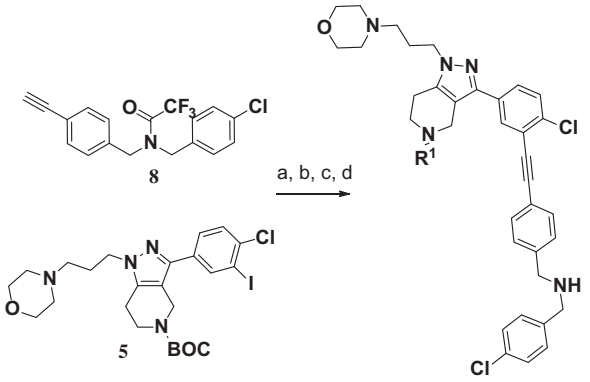


Scheme 1. Reagents and conditions: (a) 1-bromopropanol, Cs_2CO_3, DMF, 0 °C to rt (74%); (b) Dess–Martin, CH_2Cl_2, rt; (c) morpholine, AcOH, CH_2Cl_2, then $NaBH(OAc)_3$ after 30 min, rt (69% from **4**).



Scheme 2. Reagents and conditions: (a) benzyl amine, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt (75%); (b) TFAA, iPr$_2$NEt, CH_2Cl_2, rt (95%).



Scheme 3. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, rt (69%); (b) 3:1 CH_2Cl_2:TFA, 0 °C to rt; (c) R^1-X, Cs_2CO_3, DMF, rt or R^1-OH, HATU, HOAT, iPr$_2$NEt, DMF, 80 °C or TMSNCO, CH_2Cl_2 or R^1NCO, CH_2Cl_2, rt; (d) aq K_2CO_3/MeOH, rt.

binding elements in an attempt to improve potency and physical properties.

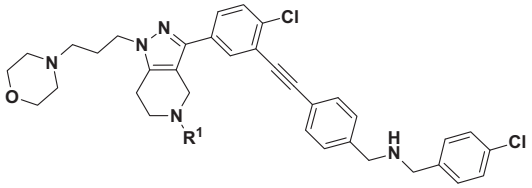
Incorporation of P4 changes into the arylalkyne series was accomplished using the synthetic route shown in Scheme 1, from intermediate **3** (reported previously).[5c] Alkylation with bromopropanol was followed by an oxidation/reductive amination sequence to install the morpholine substituent of intermediate **5**. The protected diaryl alkyne fragment **8** was prepared as shown in Scheme 2 via a reductive amination protocol. Sonogashira conditions were used to couple iodide **5** and alkyne **8** as shown in Scheme 3. Removal of the BOC protecting group allowed introduction of the P4 substituent, and removal of the trifluoroacetamide protecting group under basic conditions subsequently afforded the target compounds.

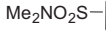
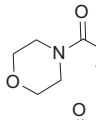
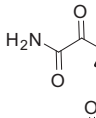
This route allowed for facile modification of the P4 region, and a selection of the analogs prepared is shown in Table 1. Whereas removal of the substituent altogether (**9**) leads to a loss of potency, a primary urea (**10**), a sulfonyl urea (**13**), substituted acetamides (**15** and **16**), and oxamides (**17** and **18**) afford comparable enzymatic potency. As is further evident from these data, the primary oxamide (**17**) and urea (**10**) substituents additionally offer improved cellular potency and were chosen for further optimization studies.

Variation of the P5 amine architecture was subsequently explored, in conjunction with these two P4 moieties using the revised synthetic route shown in Scheme 4. This modified approach involved incorporation of the P4 substituent earlier in the synthetic sequence. Deprotection of intermediate **4** was followed by introduction of the P4 substituent. Sonogoshira coupling of the biaryl alkyne fragment proceeded in good yield, and the desired P5 amine substituent was incorporated via an oxidation/reductive amination sequence.

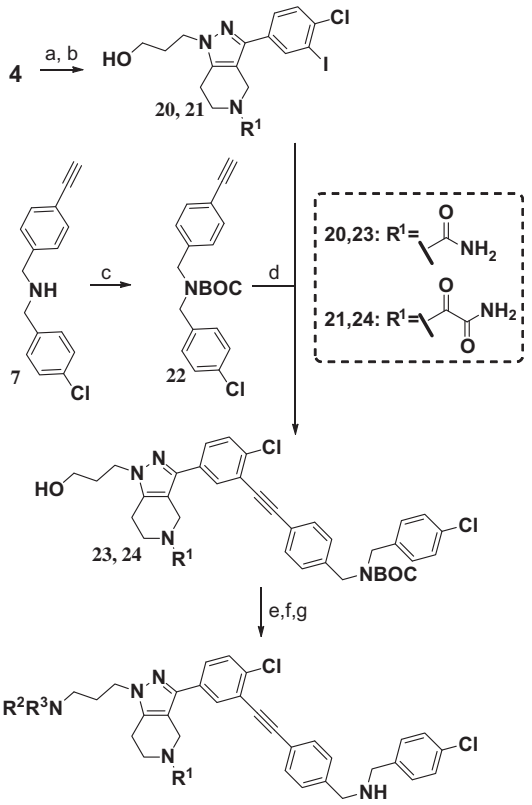
An assortment of P5 substituents were used in conjunction with these two P4 substituents, with representative data shown in Table 2. Generally, enzymatic and cellular potency were unchanged across a variety of P5 structures, including unsubstituted

Table 1
Arylalkynes: variation of P4 substituent



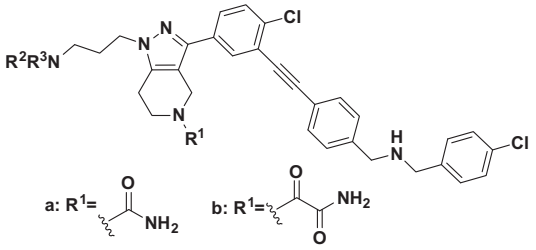
Entry	R^1	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a,b] (μM)	Eq. Soln (pH 7, μM)[b,c]
2		0.14	1.5	nd
9		2.5	nd	nd
10		0.09	0.44	nd
11		0.79	nd	nd
12		4.56	nd	nd
13		0.18	5.0	nd
14		1.63	nd	nd
15		0.26	nd	nd
16		0.16	1.1	nd
17		0.06	0.51	<1
18		0.11	1.75	<1

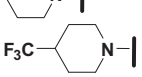
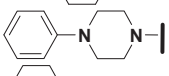
[a] CatS IC_{50} and JY Ii degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[3a,c] All IC_{50} values were within a twofold range.
[b] 'nd' Denotes data not determined.
[c] pH 7 Phosphate buffer equilibrium solubility determination using LCMS quantification.



Scheme 4. Reagents and conditions: (a) 3:1 CH_2Cl_2:TFA, rt (89%); (b) TMSNCO, CH_2Cl_2, rt or H_2NCOCO_2H, HATU, HOAT, iPr_2NEt, DMF, 80 °C; (c) $(BOC)_2O$, CH_2Cl_2, rt; (d) $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, rt (88%); (e) (b) Dess–Martin, CH_2Cl_2, rt; (f) HNR^2R^3, AcOH, CH_2Cl_2, then $NaBH(OAc)_3$ after 30 min, rt; (g) 3:1 CH_2Cl_2:TFA, 0 °C to rt.

Table 2
Arylalkynes: variation of P5 substituent



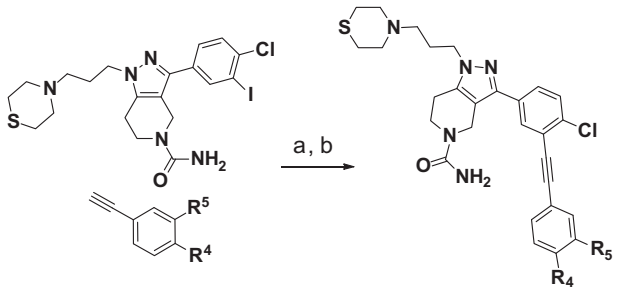
Entry	NR^2R^3	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a,b] (μM)	Eq. Soln (pH 7, μM)[b,c]
25a		0.06	0.20	nd
25b		0.10	0.30	nd
26a		0.06	0.69	<1
26b		0.10	1.77	<1
27a		0.04	0.71	nd
27b		0.01	0.56	<1
28a		0.07	0.87	<1
28b		0.03	0.27	<1
29a		0.11	1.54	3
29b		0.05	0.96	<1

[a] CatS IC_{50} and JY Ii degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[3a,c] All IC_{50} values were within a twofold range.
[b] 'nd' Denotes data not determined.
[c] pH 7 Phosphate buffer equilibrium solubility determination using LCMS quantification.

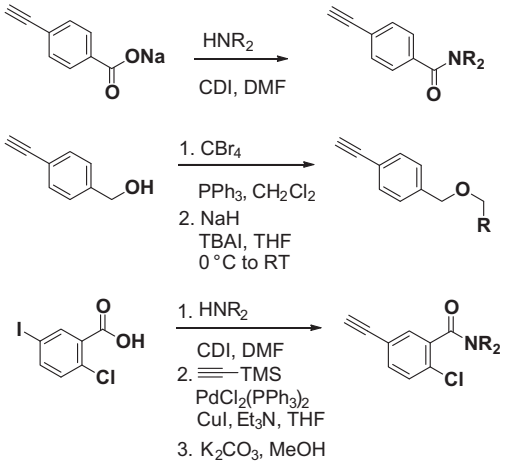
piperidine (**25**), phenyl piperazine (**27**), thiomorpholine (**28**), and methyl-morpholine (**29**). As well, these analogs display only negligible, if any, changes in solubility.

At this stage of the CatS program, analogs **28a** and **29b** were taken into advanced profiling assays, as they provided optimal combinations of in vitro and in vivo potency, suitable rat PK profiles, and minimal hERG binding as measured by a patch clamp assay.[6,7] Importantly, the thiomorpholine analog **28a** displayed comparable in vitro metabolic stability and in vivo rat half-life to the morpholine analog **29b**. Concurrent with those advanced profiling studies, medicinal chemistry efforts were directed towards potentially realizing a more drastic modulation of physical properties. As such, modification to the right-hand side amine portion of analogs **28a** and **29b** was undertaken. Ethereal and amido alkyne fragments were incorporated in both a *para* and *meta* orientation around the phenyl ring relative to the alkyne. Compounds were again prepared utilizing Sonogashira coupling conditions as shown in Scheme 5, incorporating arylalkyne fragments constructed as shown in Scheme 6. The data for a representative selection of these compounds are shown in Table 3; in the interests of brevity, only those analogs derived from analog **28a**, which contains a thiomorpholine P5 and urea P4, are shown, as trends for analogs of both **28a** and **29b** were similar.

The basic amine functionality of analog **28a** was replaced with an amide connection in analog **30**, and cellular potency was eliminated while enzymatic potency remained, in accord with previous findings concerning the role of basic amine functionality in pyrazole-based CatS inhibitors.[5b,d] Neither terminal pyridyl- nor pyrrolidinyl-containing compounds (**31** and **32**), designed to incorporate basicity, were able to restore cellular potency. These results

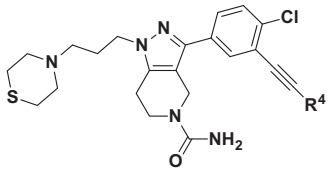


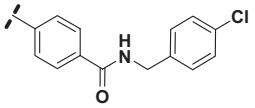
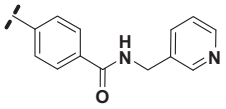
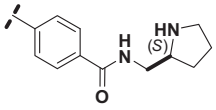
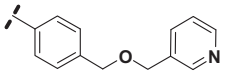
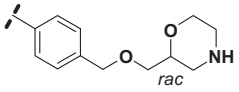
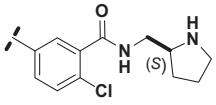
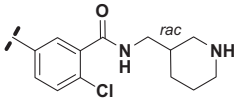
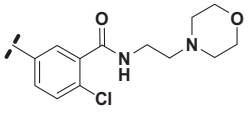
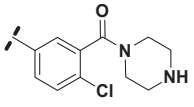
Scheme 5. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF; (b) (only if R^4 or R^5 in starting material contain N-BOC) 3:1 CH_2Cl_2:TFA, 0 °C to rt.



Scheme 6.

Table 3
Arylalkynes: replacement of P1/P1′ amine substituent



Entry	R^4	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a,b] (μM)	Eq. Soln (pH 7, μM)[b,c]
30		0.12	>10	nd
31		0.09	>10	nd
32		0.07	>10	81
33		0.07	>10	nd
34		0.12	>10	46
35		0.03	0.62	175
36		0.03	0.51	nd
37		0.04	0.61	9
38		0.13	0.63	66

[a] CatS IC_{50} and JY Ii degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[3a,c] All IC_{50} values were within a twofold range.
[b] 'nd' Denotes data not determined
[c] pH 7 Phosphate buffer equilibrium solubility determination using LCMS quantification.

highlight a limit to the flexibility in position of the basic amine that had been identified previously.[5d] Ether linkages (**33** and **34**) led to similar results.

Indeed, while accumulation of basic, lipophilic inhibitors of lysosomal cysteine proteases in the acidic lysosomal compartment has been reported, such lysosomotropism typically results in enhanced cellular inhibition relative to the inhibition observed in purified enzyme assays.[4f,8] In the case of the inhibitors reported here, no such disparity is observed; in fact, the cellular IC_{50}s of these inhibitors are typically 3- to 12-fold higher than their enzymatic IC_{50}s. Nonetheless, the possibility that lysosomal accumulation has a role in the cellular potency of these basic inhibitors must be considered, in regard to selectivity over other cathepsins as well as in the potential for phospholipidosis.

Reinforcing the notion that factors beyond lysosomal accumulation may be relevant to potency of these basic inhibitors is the observation that repositioning the amide from the *para* to the *meta* orientation as in **35**–**38** led not only to increased enzymatic potency but also to significant cellular potency. Comparing *para*-compound **32** and *meta*-compound **35** highlights this differential potency based on connectivity. The potency of these *meta* analogs appears unaffected by positioning of the basic amine and extent of basicity. It has been hypothesized that, in contrast to thioethers such as **1** which occupy the S3 pocket and arylalkynes such as **2** which extend into the S1 and S1′ pockets, these *meta* alkynes may interact with both S1 and S3 pockets.

Significantly, many of the analogs in Table 3 are markedly more soluble than the compounds in Tables 1 and 2. This observed improvement may owe to replacing the amine RHS with amido or ethereal functionality or to elimination of the terminal aromatic substituent.

These studies with pyrazole-based arylalkyne CatS inhibitors have provided an expanded understanding of SAR within the P4, P5 and P1/P1′/P3 regions. The *meta*-substituted pyrollidinyl alkyne **35**, containing thiomorpholine P5 and urea P4 moieties, displays not only excellent potency but remarkable solubility improvement. These data warrant further structural optimization studies with the more soluble *meta*-alkyne CatS inhibitors, as well as investigations into the nature of their potential alternative binding mode.

References and notes

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