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Trifluoromethylphenyl as P2 for ketoamide-based cathepsin S inhibitors

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ABSTRACT

The trifluoromethylphenyl P2 motif from previously reported heteroarylnitrile series has been successfully applied for the design and synthesis of highly potent novel ketoamide-based cathepsin S inhibitors. The key in this process is the change of the torsion angle between the P2 phenyl ring and the attached secondary amide by adding a small Cl, F, or Me group at the 2-position.

Cathepsin S has been a target of extensive studies by the pharmaceutical industry.[1] A variety of inhibitor subtypes have been patented,[2] published and reviewed.[3] We have recently published heteroarylnitrile based cathepsin K and S inhibitors with trifluoromethylphenyl as a distinctive P2 group, such as compound **1** (Fig. 1).[4–9]

As part of an effort for identifying non-heteroarylnitrile based cathepsin S inhibitors of diminished peptidic elements, we reasoned that it might be possible to convert the pyrimidine ring of compound **1** (Fig. 1) to the corresponding aliphatic nitrile based inhibitor of compound **2**. Disappointingly compound **2** showed no inhibitory activity against both human cathepsins S and K. Computer-aided docking studies suggest that the trifluoromethylphenyl group is not in the correct conformation while the cyanomethyl amide part overlays well with known cyanomethyl amide based cathepsin S inhibitors.[10] As the thioimidate formed between the nitrile war-head and the active site cysteine thiol adopts a flat sp^2 structure, it was hoped that an aldehyde war-head might fit better to the binding site as the thiol hemiacetal formed from the covalent binding has a tetrahedral sp^3 structure. Again, compound **3** is inactive. Encouragingly, when a small P1[11,12] ethyl group was attached, compound **4**[13] showed ca. 1 μM inhibitory activity against human cathepsin S. With a view to increasing the torsion angle between the trifluoromethylphenyl ring and the amide group for a potentially better binding conformation, a chlorine atom was inserted at the 2-position of the phenyl ring to give compound **5**. This chlorine atom boosted the human cathepsin S inhibitory activity about 30-fold to 31 nM. An X-ray structure of compound **5** bound to human cathepsin S was obtained (Fig. 2).

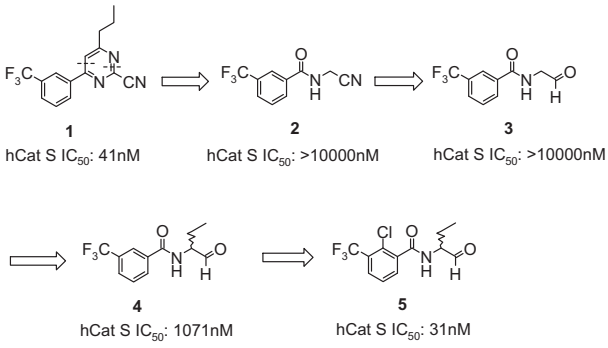

Figure 1. Evolution from arylnitrile to aldehyde based human cathepsin S inhibitors using trifluoromethylphenyl as P2.

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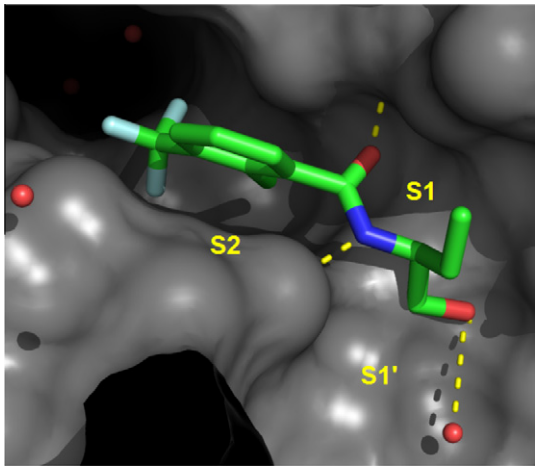


Figure 2. Crystal structure of compound **5** covalently bound to human cathepsin S enzyme, solved at 2 Å resolution (PDB code: 3OVX and RCSB code: rcsb061638).

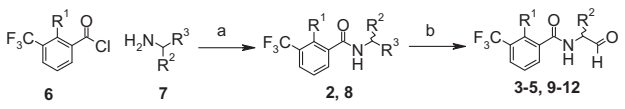
The Cl atom interacts with the backbone of Gly69, Met71, and Asn165, and the torsion angle between the phenyl ring and the amide is 70°, much larger than the one expected for the unsubstituted phenyl (normally <40°). This suggests that the function of the chlorine might be to increase binding to the protein but also to keep the trifluoromethylphenyl ring in an optimal orientation for binding to S2. The X-ray structure also shows the ethyl group interacting with the backbone of Asn67-Gly68.

Compounds **2**–**5** and further aldehyde analogs were synthesized according to Scheme 1. Direct amide coupling between acyl chlorides **6** and amines **7** gave compound **2** and other alcohol intermediates **8**. Oxidation of alcohols **8** with Dess–Martin periodinane gave the expected aldehydes in high yield. The cathepsin inhibitory results are summarized in Table 1.

As shown in Table 1, compounds **9** and **10** with F, Me at 2-position of the phenyl ring are as active as compound **5**. However compound (**11**) is much less potent, presumably because an intramolecular H-bond between the methoxy oxygen and amide (H) could alter the required active conformation. The methoxy group could also be too big to fit in the S2 pocket. Methyl as P1 (**12**) is slightly less active than the corresponding ethyl group.

With these results in hand, we turned our attention to the more drug-like ketoamide as war-head using the newly identified 2-chloro-3-trifluoromethylphenyl as the P2 group. It should also be possible that interactions with the S1′ pocket from this ketoamide could afford higher binding affinity. The general synthesis of the ketoamide inhibitors is shown in Scheme 2. Amide coupling between carboxylic acids **13** and β-aminoesters **14** gave intermediates **15** which were then converted to the corresponding carboxylic acids **16** in high yields. Another amide formation between **16** and amines using HATU as coupling reagent gave the secondary alcohol intermediates **17** which were then converted to the desired final ketoamides **18**–**32** by treating with EDCI in a mixed solvent of DMSO and toluene.

The carboxylic acid **38** was synthesized using the route shown in Scheme 3. Nitration of 2-chloro-6-fluorobenzyltrifluoride **33** gave intermediate **34** which was then treated with 2-pyridylmethanol and sodium hydride in THF to afford compound **35**. Reduction

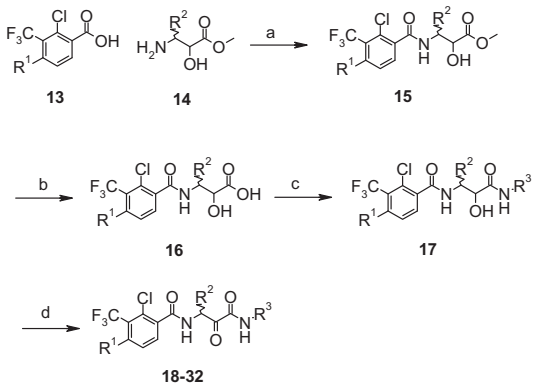


Scheme 1. Reagents and conditions: (a) $NaHCO_3$, H_2O–THF, rt, 1 h; (b) Dess–Martin periodinane, DCM, rt, 3 h.

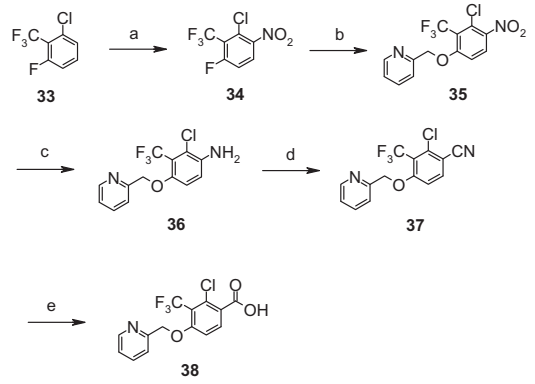
Table 1
Compounds inhibitory activity against human cathepsins S and K

Compds	R1	R2	IC_{50} (nM)	
			CatS	CatK
1			41	33
2			>10,000	>10,000
3	H	H	>10,000	>10,000
4	H	Et	1070	na
5	Cl	Et	31	32
9	F	Et	36	na
10	Me	Et	18	na
11	MeO	Et	692	na
12	Cl	Me	110	na

For assay conditions see Ref. 7; na = not available.



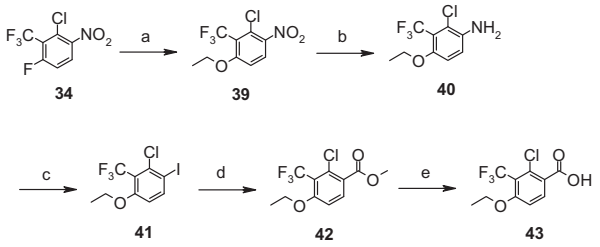
Scheme 2. Reagents and conditions: (a) HBTU, TEA, DCM, 5 h, 70–90%; (b) LiOH, THF–H_2O (1:1), 3 h, 100%; (c) HATU, DIPEA, R3NH_2, rt, 3 h; (d) EDCI, DMSO–toluene (1:1), rt, 3 h, 20–80%.



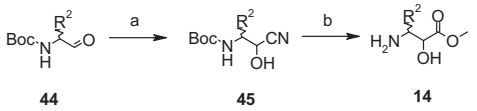
Scheme 3. Reagents and conditions: (a) HNO_3–H_2SO_4, 0 °C to rt, 1 h, 80%; (b) 2-pyridylmethanol, NaH, THF, rt, 5 h, 90%; (c) $SnCl_2$, EtOH, rt, 3 h, 90%; (d) (i) $NaNO_2$, H_2SO_4 (aq 10%); (ii) CuCN, NaCN, Na_2CO_3, 80 °C, 2 h, 30%; (e) NaOH (aq 1 M), 120 °C, 3 days, 100%.

of compound **35** with tin(II) chloride provided aniline compound **36** which was then converted to the arylnitrile **37** by means of a Sandmeyer reaction. The nitrile group of compound **37** was then hydrolyzed to the carboxylic acid **38** by heating with 1 M sodium hydroxide at 120 °C for 3 days.

Carboxylic acid **43** was synthesized according to Scheme 4. Treatment of compound **34** with sodium ethoxide in ethanol gave intermediate **39** which was then reduced by tin(II) chloride to the corresponding aniline **40**. This aniline was converted to the aryl iodide **41** by using the Sandmeyer reaction. The methyl ester **42** was obtained in high yield by treating the iodide with carbon monoxide in methanol using palladium acetate as catalyst at 50 °C. The final



Scheme 4. Reagents and conditions: (a) EtONa–EtOH, rt, 3 h, 79%; (b) $SnCl_2$, EtOH, rt, 3 h, 100%; (c) (i) $NaNO_2$, HCl, 30 min; (ii) KI, rt, 8 h, 59%; (d) CO (6 bar), DMF-MeOH (1:1), TEA, $Pd(OAc)_2$ (cat.), 50 °C, 6 h, 98%; (e) LiOH, THF–H_2O, rt, 3 h, 100%.



Scheme 5. Reagents and conditions: (a) acetone cyanohydrin, TEA, DCM, rt, 20 h, 100%; (b) HCl/MeOH, reflux, 6 h, 100%.

carboxylic acid **43** was obtained by hydrolyzing the methyl ester with aqueous lithium hydroxide.

The β-amino esters of Scheme 2 were synthesized according to Scheme 5. The Boc protected aldehydes **44** were treated with acetone cyanohydrin and triethylamine in DCM at room temperature for 20 h to give the cyanohydrin intermediates **45** in quantitative yield. Heating at reflux of the cyanohydrin intermediates in 5 M hydrochloride in MeOH for 6 h provided the β-amino esters **14** in quantitative yield.

The biological results of compounds **18**–**32** are shown in Table 2.

In comparison with aldehyde **12**, the ketoamide compound **18** is slightly more potent. In order to improve interactions with the S1′ pocket, a set of two dozen amides was synthesized. Of these compounds, only 2-aminopyrazole derivatives **19**–**21** were identified to have better cathepsin S inhibitory activity than the original primary amide **18**. As with the aldehyde series, ethyl as P1 is also better than methyl in this ketoamide series, for example, compound **22** is sixfold better than compound **19**. Attachment of an ethoxy group at the 4-position of the P2 phenyl ring[6–8] increases cathepsin S inhibitory potency two- to five-fold, such as compound

Table 2
Compounds inhibitory activity against human cathepsins S, K, B, L, V, and human JY cell cellular activity (Lip10)

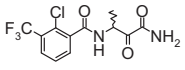
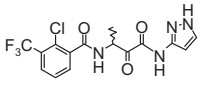
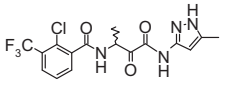
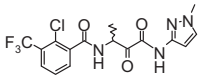
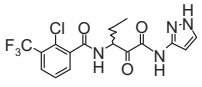
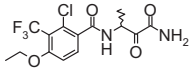
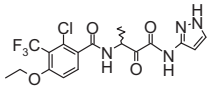
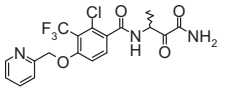
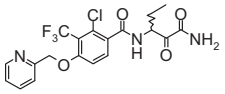
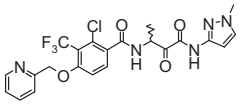
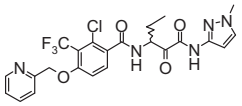
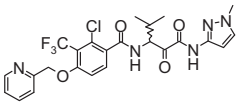
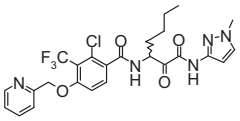
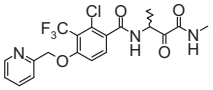
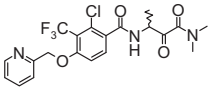
Compds	Structure	IC_{50} (nM)					Lip10
		CatS	CatK	CatB	CatL	CatV	IC_{50} (nM)
18		30.9	107.2	na	na	na	na
19		14.1	51.3	na	na	na	na
20		10.5	26.3	na	na	na	na
21		8.5	33.1	na	na	na	na
22		2.3	11.7	5754	891	29.5	1300
23		11.0	195	na	na	na	na
24		2.9	91.2	na	na	na	na
25		4.8	37.2	na	na	na	na
26		1.1	8.7	na	na	na	1925
27		1.0	12.9	15,136	479	36.3	na
28		1.1	20.9	3236	141	16.6	78

Table 2 (*continued*)

Compds	Structure	IC_{50} (nM)					Lip10
		CatS	CatK	CatB	CatL	CatV	IC_{50} (nM)
29		2.3	11.5	na	na	na	na
30		1.9	47.9	na	na	na	na
31		19.5	46.8	>10,000	>10,000	1122	na
32		>10,000	>10,000	>10,000	>10,000	>10,000	na

For assay conditions see Ref. 7; na = not available.

pairs **23**/**18** and **24**/**19**. Compounds **25** and **27** with pyridyl-2-methoxy as the extended P2 are 6- and 9-fold more potent than the unsubstituted compounds **18** and **21**, respectively. These results indicate that the P2 region shares parallel SAR with the previously reported purine[7] and imidazopyridine[6] analogs. The impact of P1 ethyl relative to the methyl is smaller for compounds with the extended binding interactions, for example, nearly flat activity for compounds **27**–**30**. At least one proton is required for the right-hand amide, for example, compound **32** is totally inactive against all cysteine cathepsin proteases tested. These results agree with the previously reported ketoamide-based cathepsin K inhibitors.[14] Some selected compounds were also tested for cathepsins K, B, L, and V inhibitory activities. In general, this type of inhibitor has low selectivity against human cathepsins K and V. Selectivity over human cathepsin L is generally over 100-fold. Activity against human cathepsin B is quite low. Several compounds from this series were further assessed for cell based Lip10 activity. Most compounds, such as **22** and **26**, have IC_{50} in micromolar level; one of the most active is compound **28** which has a Lip10 IC_{50} of 78 nM.

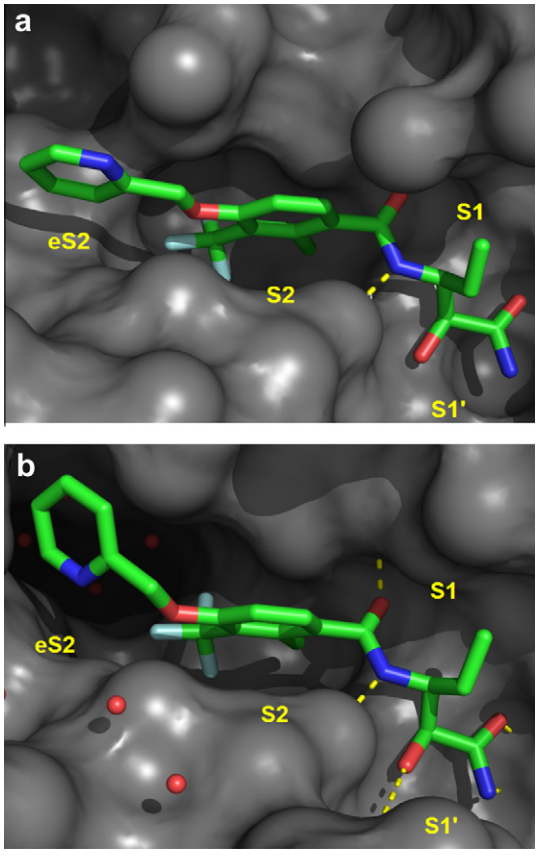


Figure 3. (a) Crystal structure of **26** covalently bound to human cathepsin K enzyme (top), solved at 2.0 Å resolution (PDB code: 3OVZ and RCSB code: rcsb061640). (b) Model of **26** covalently bound to human cathepsin S enzyme.

An X-ray structure of compound **26** bound to human cathepsin K was obtained (Fig. 3a). Based on this structure, compound **26** was then modeled into the active site of human cathepsin S. This model suggests that the pyridyl-2-methoxy group occupies the extended S2 pocket of cathepsin S, interacting with Phe70, Phe211, and Val162 as reported before.[6] 2-Chloro-3-trifluoromethylphenyl binds to the most important S2 pocket with a torsion angle of approximately 70° between the phenyl ring and the secondary amide. The ethyl group interacts with the shallow S1 protein surface. The amide of the ligand forms hydrogen bonding interactions to Gln19, and possibly to solvent, while the hydroxyl group resulting from the reaction of the ketoamide group with Cys25 has a hydrogen bonding interaction to His164 (His162 in cathepsin K). The cathepsin S structure suggests that there is space available in the prime side for a secondary amide, and a pyrazole substituent could potentially have an attractive interaction to Trp186. Tertiary amides, such as compound **32**, are not tolerated, probably because the extra methyl could disrupt the hydrogen bonding interaction to His164.

In summary, the trifluoromethylphenyl P2 motif from previously reported heteroarylnitrile series has been successfully applied for the design and synthesis of highly potent novel ketoamide-based cathepsin S inhibitors. The key in this process is the change of the torsion angle between the P2 phenyl ring and the attached secondary amide by adding a small Cl, F, or Me group at the 2-position. The previously reported extended S2 pocket has also been used to improve human cathepsin S inhibitory potency.

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