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6-Acylamino-penam Derivatives: Synthesis and Inhibition of Cathepsins B, L, K, and S

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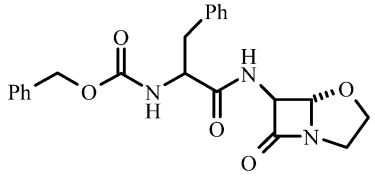
Abstract—The synthesis of a new series of 6-acylamino penam derivatives and their inhibition of cysteine proteases cathepsins B, L, K, and S is described. The 6-acylamino-penam sulfone compounds showed excellent cathepsin L, K, and S inhibition activity with IC_{50} values in the nanomolar and subnanomolar range.

The cysteine proteases cathepsin B, L, K, and S may be involved in diseases such as osteoporosis, cancer metastasis, rheumatoid arthritis, and infectious diseases.[1] These proteases are implicated as an important targets for the development of inhibitors as potential therapeutic agents.[2] Several types of chemical functionalities served as the central pharmacophore for cysteine proteases inhibitors, such as aldehydes, nitriles, α-keto-carbonyl compounds, halomethyl ketones, acyloxymethyl ketone, epoxide, vinyl sulfones, cyclopropenone and cyclohexanone.[3,4]



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We have reported on the rational design and synthesis of a series of 6-substituted-4-oxa-1-azabicyclo[3,2,0]heptan-7-one derivatives, **1**, as cysteine protease inhibitors.[5] Although potent inhibition of cathepsin L and K was achieved with IC_{50} values in the nanomolar range (for compound **1**; IC_{50} is 4 and 5 nM for cathepsin L and K, respectively), one concern with such inhibitors was their potential toxicity, since the 4-oxa-1-azabicyclo[3,2,0]heptan-7-one skeleton is reported to show cytotoxic activity.[6] The alternative structure related to 4-oxa-1-azabicyclo[3,2,0]heptan-7-one skeleton is the penam which is a well known pharmacophore for antibiotics and is widely used in clinic.[7] Therefore, a new series of 6-acylamino-penam derivatives was synthesized and their inhibitions with cathepsins B, L, K, and S were evaluated. The synthesis of 6-acylamino-penam compounds is outlined in Scheme 1. The intermediate 4-acetoxy-3-acylamino-azetdin-2-one 2 was prepared according to our previous report[8] from 6-aminopenicillanic acid. Substitution of the acetate **2** with mercaptoethanol in the presence of NaOH gave 4-hydroxyethylthio-azetidin-2-one. Treatment of the hydroxy compound with toluenesulfonyl chloride under standard conditions gave toluenesulfonyloxyethylthio-azetidin-2-one, which was converted to 4-bromoethylthio-azetidin-2-one, **3**, with lithium bromide. Without further purification, cyclization of 4-bromoethylthio-azetidin-2-one **3** with base gave two optically pure isomers of the penam derivatives 4 and 5 in high yield after silica gel column chromatography. A careful and controlled oxidation of compound 4 with hydrogen peroxide in acetic acid[9] gave a mixture of 4β- and 4α-penam sulfoxides **6** in ratio of 3:1.[10] Complete oxidation of **4** or **5** with $KMnO_4$ resulted in penam sulfones **7** or **8**, respectively. For comparison, two cephem sulfone derivatives **9** and **10** were also prepared by a similar method as described above. Using a similar method, compounds **11**–**15** were prepared as well, where phenylalanine was replaced by cyclohexylalanine, 2-naphthylalanine, 2-thiophenalanine, 3-fluorophenylalanine, or 3-pyridylalanine, respectively.

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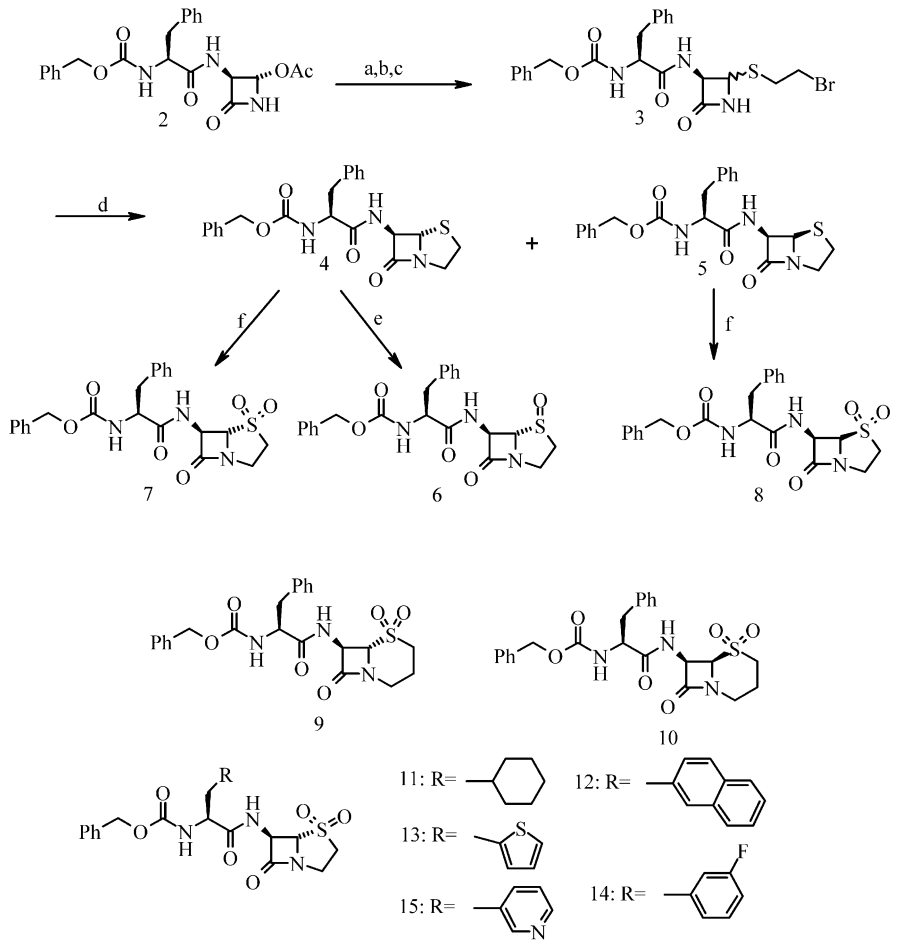

Scheme 1. Reagents and conditions: (a) $HS(CH_2)_2OH$, 1 equiv NaOH, THF/H_2O, rt 1 h; (b) TsCl, pyridine, 5 °C, 6 h; (c) LiBr, HMPA, 60 °C, 2 h; (3) 45% for three step reaction; (d) K_2CO_3, DMSO, rt overnight; (4) 65%; (5) 25%; (e) 1.5 equiv H_2O_2, 4 equiv AcOH, DCM, rt 2 days; (6) 50%; (f) $KMnO_4$, AcOH, H_2O, rt, 1 h; (6) 65%; (7) 45%.

The inhibitory activities of these compounds with cathepsin B, L, K, and S were determined according to the procedure described in the literature[11] by using Cbz-Phe-Arg-AMC for cathepsin B, L, and K and Cbz-Val-Val-Arg-AMC for cathepsin S. The inhibitory activities of the compounds are shown in Table 1. The 6-acylamino-penam sulfone derivatives are most potent inhibitors of cathepsin L, K, and S with IC_{50} in subnanomolar or low nanomolar range. It appears that activity correlates strongly with an oxidation state of the penam ring (compare the activity of compound **4** with compounds **6** and **7**). The sulfones are more active than sulfoxide and sulphide, may be due to the increased chemical reactivity of azetidin-2-one skelton. The 5*S* stereo isomer compound **7** is preferred for better cysteine proteases inhibitory activity over the 5*R* stereo isomer compound **8** as in our previous finding.[5] In contrast, six-membered ring cepham sulfones do not exhibit any inhibitory activity. Different aromatic and heterocyclic substitutions at R, mimicking presumably P2 portion revealed lack of selectivity (**11**, **13**, **14**, and **15**), with exception of a naphthyl substituent which is not tolerated well with cathepsin K, but better with other cathepsins (**12**). Penam sulfoxides and sulfides are fully reversible inhibitors, while sulfones are slow reversible and time dependent inhibitors.

Table 1. In vitro inhibitory activity of azetidin-2-one derivatives with cysteine proteases

Compd	IC_{50} (μM)			
	Cath B	Cath L	CathK	Cath S
4	>50	6.68	>2.5	n.d.[a]
6	16.4	0.068	0.035	0.07
7	0.35	0.0014	0.0005	0.01
8	6.01	0.0096	0.0075	0.21
9	>50	10.6	>2.5	>2.5
10	>50	>50	>2.5	>2.5
11	0.43	0.0007	0.0076	0.0007
12	0.39	0.003	0.4	0.003
13	0.43	0.003	0.0018	0.0046
14	1	0.003	0.006	0.008
15	1.3	0.0017	0.0025	0.058

[a]n.d., not determined.

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