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3-Acylamino-azetidin-2-one as a Novel Class of Cysteine Proteases Inhibitors

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Abstract—A new class of inhibitors for cysteine proteases cathepsin B, L, K and S is described. These inhibitors are based on the β-lactam ring designed to interact with the nucleophilic thiol of the cysteine in the active site of cysteine proteases. Some 3-acylamino-azetidin-2-one derivatives showed very potent inhibition activities for cathepsins L, K and S at the nanomolar or subnanomolar IC_{50} values.

The cysteine proteases cathepsin B, L, K and S are involved in diseases[1] such as osteoporosis, cancer metastasis, rheumatoid arthritis, and infectious diseases. These proteases may be important targets for the development of inhibitors as therapeutic agents.[2] Several types of chemical functionalities served as the central pharmacophore for cysteine proteases inhibitors,[3] such as aldehydes,[4] nitriles,[5] α-ketocarbonyl compounds,[6] ketones[7] (halomethyl, acyloxymethyl, mercaptomethyl, cyclic), epoxide,[8] vinyl sulfones,[9] and aryaminoethyl amides.[10] To explore new classes of pharmacophores for cysteine proteases inhibitors, a series of 4-substituted-3-Cbz-Phe-β-lactam compounds are identified as a novel class of cysteine proteases inhibitors. The design, synthesis and activities of these inhibitors are reported in this communication.

The β-lactam skeleton is a well known pharmacophore for antibiotics[11] and for inhibitors of serine proteases.[12] The chemical reactivity of the β-lactam ring can be adjusted by substitution at the C3 and C4 positions. In addition, substituents at C3 and C4 of β-lactam skeleton may extend interactions into both the S and S′ subsites which may increase potency and specificity. Known substrate selectivity for the papain type of cysteine proteases provided us with the template for exploration of a new pharmacophore. Since papain has a high specificity for phenylalanine at the P2 position,[13] the Cbz-Phe fragment was selected as the substituent at C3 of the β-lactam ring to explore novel pharmacophore.

The synthesis of the β-lactam compounds reported in this communication is shown in Scheme 1. The intermediate **1** was prepared according to a known method[14] from D-Ser. Using a similar method; its 3*S*-isomer **3** was also prepared. Compounds **2**, **4**, and **6** were prepared by coupling Cbz-Phe-OH with (3*R*)-3-amino-monobactam **1**, (3*S*)-3-amino-monobactam **3** or (3*S*)-3-amino-4-acetoxy-monobactam[15] **5**, respectively, via the unsymmetrical anhydride method or via an active ester. The compounds **7** and **8** were obtained by reacting **6** with the carboxylic acids in presence of NaOH. Only *trans* lactam products **9**–**12** were obtained by refluxing of the acetate **6** with the corresponding alcohols in the presence of zinc acetate dihydrate in benzene–toluene with the azeotropic removal of water.[16] Substitution of the acetate **6** with phenol or thiophenol in presence of NaOH gave both *trans* β-lactam compound **13** and **16** and its *cis*-isomer **14** and **15**, respectively. The *trans*- and *cis*-isomers were separated by silica gel column chromatography. The *trans*-isomer **13** and **16** could also be prepared by reacting **6** with phenol or thiophenol in the presence of zinc acetate dihydrate in benzene–toluene in low yield. The sulfone compound **17** was obtained by oxidation of **16** with $KMnO_4$ in acetic acid.

The inhibitory activities of these compounds against cathepsins B, L, K, and S were determined according to the procedure described in the literature[17] by using Cbz-

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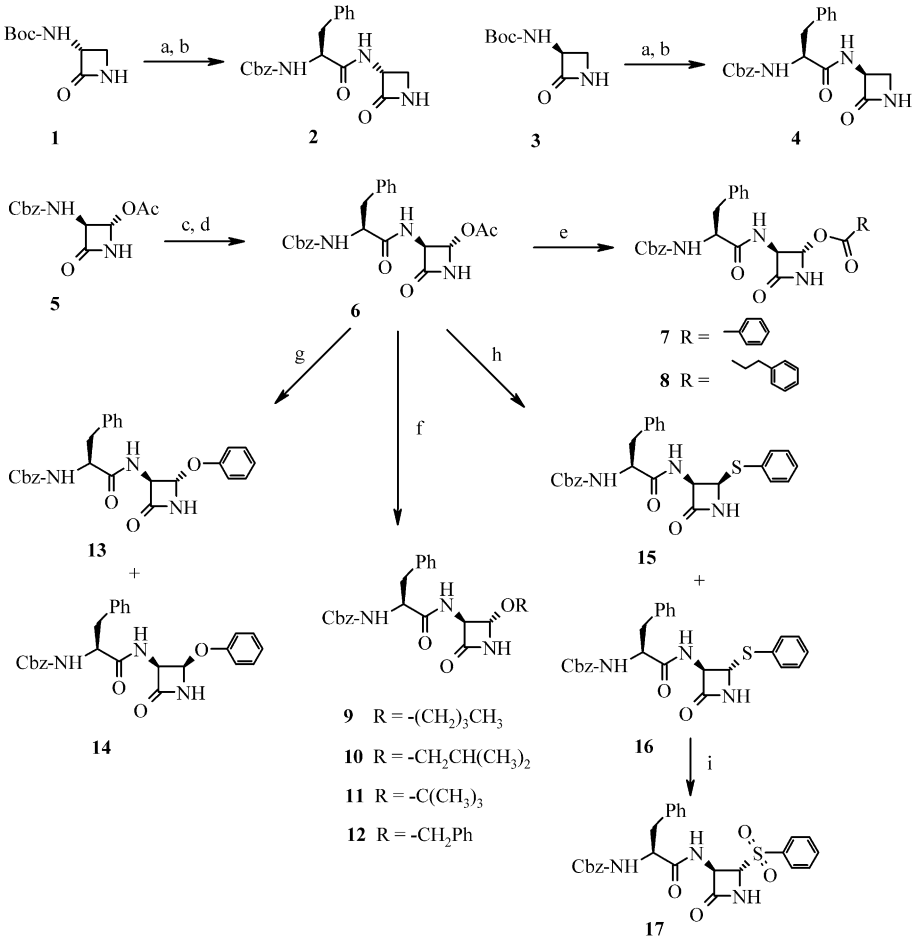

Scheme 1. Reagents and conditions: (a) TFA, DCM, anisole; (b) Cbz-Phe-OH, Cl–COOEt, TEA, **2**: 70%, **4**: 90%; (c) H_2, Pd/C, EtOAc; (d) Cbz-Phe-OH, DCC, HOBT, THF, **6**: 90%; (e) RCOOH, NaOH, H_2O, acetone, **7**: 20%, **8**: 24%; (f) ROH, $Zn(OAc)_2$–$2H_2O$, benzene/toluene, reflux 4 h, **9**: 14%, **10**: 7%, **11**: 14%, **12**: 26%; (g) Ph–OH, NaOH, H_2O, THF, **13**: 47%, **14**: 25%; (h) Ph–SH, NaOH, H_2O, THF, **15**: 15%, **16**: 33%; (i) $KMnO_4$, AcOH, H_2O, **17**: 70%.

Phe-Arg-AMC as substrate 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.

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

Compd	IC_{50} (μM)				
	Cath B	Cath L	Cath K	Cath S	HLE
2	13.6	37.4	> 5	> 5	> 50
4	2.70	nd	nd	nd	> 50
6	0.47	0.042	0.100	0.207	> 10
7	27.5	0.166	0.100	0.020	> 50
8	9.71	0.047	0.038	0.041	> 50
9	7.01	0.163	0.100	0.355	> 50
10	6.46	0.091	2.30	> 2.5	> 50
11	11.4	0.78	2.5	> 2.5	> 50
12	32.57	3.96	2.5	> 2.5	> 50
13	10.9	0.017	0.100	0.33	> 30
14	0.43	0.0026	0.016	0.0	> 30
15	39.7	0.015	0.072	2.5	> 50
16	10.5	0.0001	0.0084	0.33	> 50
17	7.39	0.0001	0.0065	0.048	> 50

Cath L, K and S are recombinant human enzymes; cath B is recombinant rat enzyme; HLE is human leukocyte elastase as an example of a serine protease; nd, not determined; IC_{50} values were usually derived from single experiments using a range of inhibitor concentration.

All of these β-lactam compounds can selectively inhibit the papain type of cysteine proteases (cathepsins) with no inhibition or non-significant inhibition of serine protease, human leukocyte elastase. They are more potent towards cathepsin L and K, closely related enzymes, than for cathepsin B. The more active compound **4** in comparison to **2** against cathepsin B suggested the importance of the 3S stereo configuration at C-3, which is equal to the configuration of natural L-amino acids, on the interactions with the enzymes. The substitution at C-4 showed a significant effect on inhibitory potency. The substitutions at C-4 such as –OR, –OCOR, –OPh, –SPh and $–S(O)_2Ph$ appear to be necessary for a good inhibitory activity. For the alkoxy substitution, the linear group as in **9** showed better activities against cathepsins K and S than branched groups **10**–**11**. In terms of ether group, phenoxy (**13** and **14**) is much better than alkoxy substitutions (**9**–**12**). However, the substituent at C-4 may not function just as a leaving group, it is also involved in interactions with the S′ subsite of the enzyme. The stereo configuration requirement of C-4 depends upon the nature of the substituents. For the phenoxy substituent, the *cis*-isomer **14** is more active than *trans*-isomer **13**. However, the opposite effect was observed for the phenylthio substituent, the *trans*-isomer **16** being more potent than the *cis*-isomer **15**. The

most potent compounds have the phenylthiol (**16**) or phenylsulfonyl (**17**) group at C-4 with IC_{50} values in the subnanomolar range for cathepsin L.

In summary, we have identified the β-lactam moiety as a new class of pharmacophore for the papain type of cysteine proteases inhibitors. Some of these compounds are potent and selective inhibitor especially against cathepsin L and related cathepsins K and S compared to cathepsin B inhibition. Generally, the inhibitory potency depends on the electro negativity property of substituents and its stereochemistry at C4.

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