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Design and Synthesis of 6-Substituted Amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one Derivatives as Cysteine Proteases Inhibitors

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Abstract—A series of 6-substituted amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one compounds was designed and synthesized as a new class of inhibitors for cysteine proteases cathepsins B, L, K, and S. One compound (5*S*,6*S*)-6-(*N*-benzyloxycarbonyl-L-phenylalanyl) amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one showed excellent cathepsin L and K inhibition activity with IC_{50} at a low nanomolar 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 functionality have served as the central pharmacophore for cysteine proteases inhibitors,[3] such as aldehydes,[4] nitriles,[5] α-ketocarbonyl compounds,[6] halomethyl ketones,[7] acyloxymethyl ketone,[8] epoxide,[9] vinyl sulfones,[10] cyclopropenone,[11] and cyclohexanone.[12] We have reported that 3-substituted-4-oxa-1-azabicyclo[3,2,0]heptan-7-one derivatives exhibited good cysteine protease inhibitory activity.[13] Further to optimize and enhance the activity, we studied the interaction of these inhibitors with the papain and cathepsin B enzyme crystal structures. Molecular modeling studies suggested that the 1-N atom in 4-oxa-1-azabicyclo[3,2,0]heptan-7-one ring can be involved in hydrogen-bonding to a protonated Histidine in the active site of cysteine proteases (Fig. 1). This binding may weaken the lactamic bond and activate the four member ring towards acylation of the thiol of the cysteine residue in cysteine proteases. Since papain has a high specificity for phenylalanine at the P2 position,[14] the Cbz-Phe fragment was selected as a substituent at the C6 position of the 4-oxa-1-azabicyclo[3,2,0]heptan-7-one ring. We have found that the Cbz-Phe substitution at position 6 of 4-oxa-1-azabicyclo[3,2,0]heptan-7-one enhances the S2 and S3 subsite interaction with the papain enzyme. The side chain of Phe occupies the S2 subsite and the Cbz group interacts with the S3 subsite. A comparison of the modeling structure of (5*S*,6*S*)-6-(*N*-benzyloxycarbonyl-L-phenylalanyl) amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one (Fig. 1) as a tetrahedral intermediate complex with papain and the X-ray structure of a substrate (Cbz-Phe-Ala-NH-methyl) complex with papain showed good superposition.

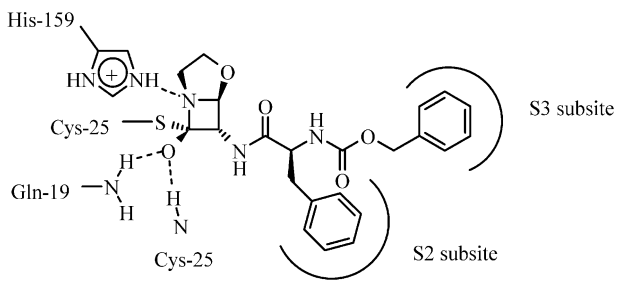

Figure 1. Schematic presentation of the modeling interactions of compound **3** as a tetrahedral intermediate with papain.

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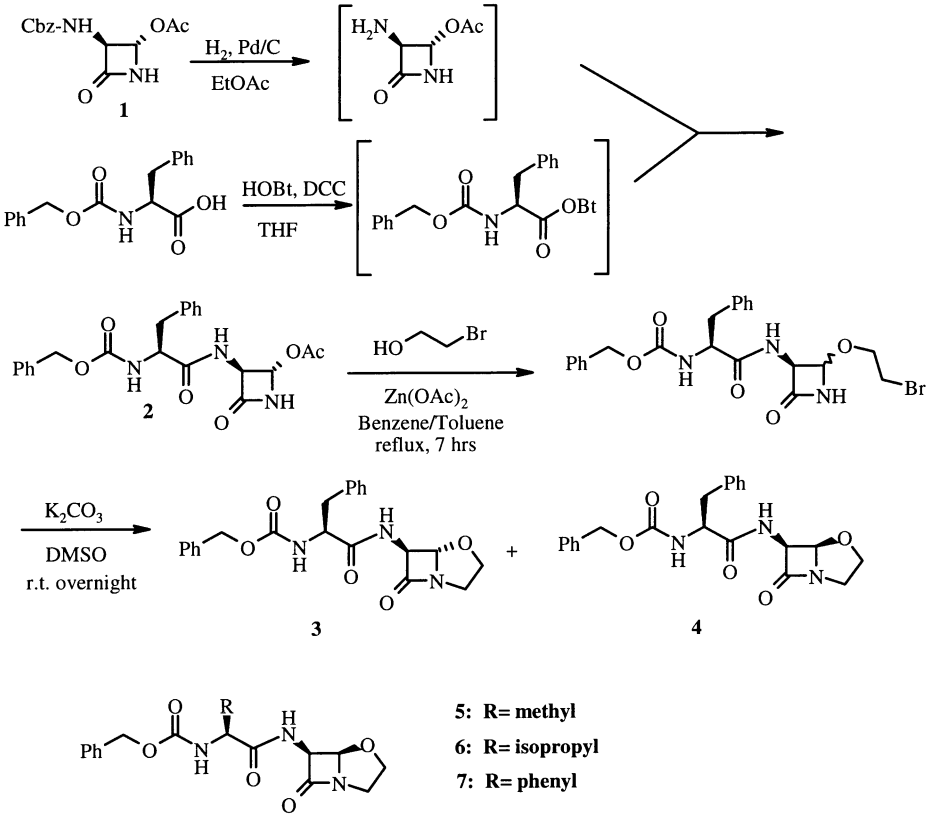
The synthesis of 6-substituted amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one compounds is shown in Scheme 1. The intermediate **1** was prepared according to the known method[15] from 6-aminopenicillanic acid. (3*S*)-3-Amino-4-acetoxy-azetidin-2-one was obtained by hydrogenation of compound **1** in the presence of Pd/C catalyst and without purification, directly coupled with Cbz-Phe-OH via an active ester to give compound **2**. Substitution of the acetate **2** with bromoethanol in the presence of zinc acetate in benzene–toluene with the azeotropic removal of water resulted in the 4-bromoethoxy-azetidin-2-one derivative. Cyclization of the 4-bromoethoxy-azetidin-2-one gave two optically pure isomers (**3**: in 0.6% yield for two step reactions and **4**: in 4% yield for two step reactions) after silica gel chromatography.[16] By a similar method, compounds **5** in 2.4%, **6** in 1.5% and **7** in 0.7% yield (for three-step reaction) were also prepared. The (5*S*,6*S*) isomers of compound **5**, **6** and **7** were not obtained during the purification due to either very low formation or poor stability of such isomers.

The inhibitory activities of these compounds against cathepsin B, L, K, and S were determined according to the procedure described in the literature[17] 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. Compound **3** showed excellent cathepsin L and K inhibitory activities with IC_{50} values at low nanomolar level. The better activity of compound **3** in comparison to compound **4** indicated the importance of the 5*S* stereo configuration at C5 position. Molecular modeling suggested that in the 5*S* isomer, the bridge nitrogen of oxapenam skelton forms a hydrogen bond with His159 of papain whereas the bridge nitrogen in the 5R isomer is too far from His159 to form any hydrogen bonding. Replacement of Phe with Ala (**5**) or phenylglycine (**7**) resulted in loss of activities. Phe is generally well accepted at the P2 position for papain type of cysteine proteases. By comparing the activity of compound **4** and **6**, the Leu residue is more preferred for cathepsin K than Phe. These results confirmed the molecular modeling predictions that the C6 substitution interacts with the S subsite and Phe binds at the S2 subsite.

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

Compd	IC_{50} (μM)			
	Cath B	Cath L	Cath K	Cath S
3	12.2	0.004	0.005	0.077
4	1.76	0.085	1.63	0.43
5	>50	38	>2.5	>2.5
6	30	0.6	0.1	0.26
7	>50	1.83	n.d.[a]	n.d.[a]

[a]n.d., not determined.



Scheme 1.

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16. For **3**: mp: 175–176 °C; $[\alpha]_D^{22} = -7°$ (*c* 1.85 mg/mL, $CHCl_3$); FAB-MS: 432 ($M + Na^+$), calcd for $C_{22}H_{23}N_3O_5$ 409; IR (KBr, cm^{-1}): 3285, 1779, 1683, 1659, 1525; ^1H NMR (DMSO-d_6) α 2.72–2.97 (3H, m), 3.76 (1H, m), 4.00 (2H, m), 4.23 (1H, m), 4.53 (1H, d, $J = 7.7$ Hz), 4.96 (2H, s), 5.05 (1H, s), 7.20–7.35 (10H, m), 7.62 (1H, d, $J = 8.5$ Hz), 8.95 (1H, d, $J = 7.7$ Hz). For **4**: mp: 144–145 °C; $[\alpha]_D^{22} = +7°$ (*c* 2 mg/mL, $CHCl_3$); FAB-MS: 432 ($M + Na^+$), calcd for $C_{22}H_{23}N_3O_5$ 409; IR (KBr, cm^{-1}): 3280, 1780, 1608, 1647, 1526, 1219; ^1H NMR (DMSO-d_6) α 2.67–3.14 (3H, m), 3.72 (1H, m), 4.05 (2H, m), 4.39 (1H, m), 4.93 (2H, s), 5.23(1H, d, $J = 2.8$ Hz), 5.42 (1H, dd, $J = 2.8$ and 9.1 Hz), 7.20–7.35 (10H, m), 7.58 (1H, d, $J = 8.8$ Hz), 8.67 (1H, d, $J = 9.1$ Hz).
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