Synthesis and evaluation of 3-substituted-4-oxa-azabicyclo [3,2,0]heptan-7-ones as cynteine proteases inhibitors. Part II

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Abstract
The synthesis and inhibitory activity of the 3-substituted-4-oxa-1-azabicyclo[3.2.0]heptan-7-ones, a novel non-peptidyl class of the papain class of cysteine protease inhibitors, is reported.

Keywords: β-Lactams; 3-substituted oxapenam; cysteine protease inhibitor

Introduction

Cysteine proteases of the papain class, such as cathepsin B, L, K and S, are current important targets in medicinal chemistry.[1] They have been implicated in diseases such as osteoporosis, cancer metastasis, rheumatoid arthritis, asthma and infectious diseases.[2] Several types of cysteine proteases inhibitors[3] have been reported, such as peptide aldehydes,[4] nitriles,[5] α-ketocarbonyl compounds,[6] halomethyl ketones,[7] acyloxymethyl ketones,[8] epoxides,[9] vinyl sulfones,[10] cyclopropenone[11] and cyclohexanone.[12] These inhibitors, in general, have a peptidyl affinity fragment and a reactive group towards the thio of the cysteine residue in cysteine proteases. However, the efficacy *in vivo* is not as much as expected on the basis of the reported *in vitro* inhibitory activity and this may be due to the peptidyl fragment negatively affecting the pharmacokinetic properties. Therefore, identifying a low molecular weight nonpeptide inhibitor of cysteine proteases is of significant importance.

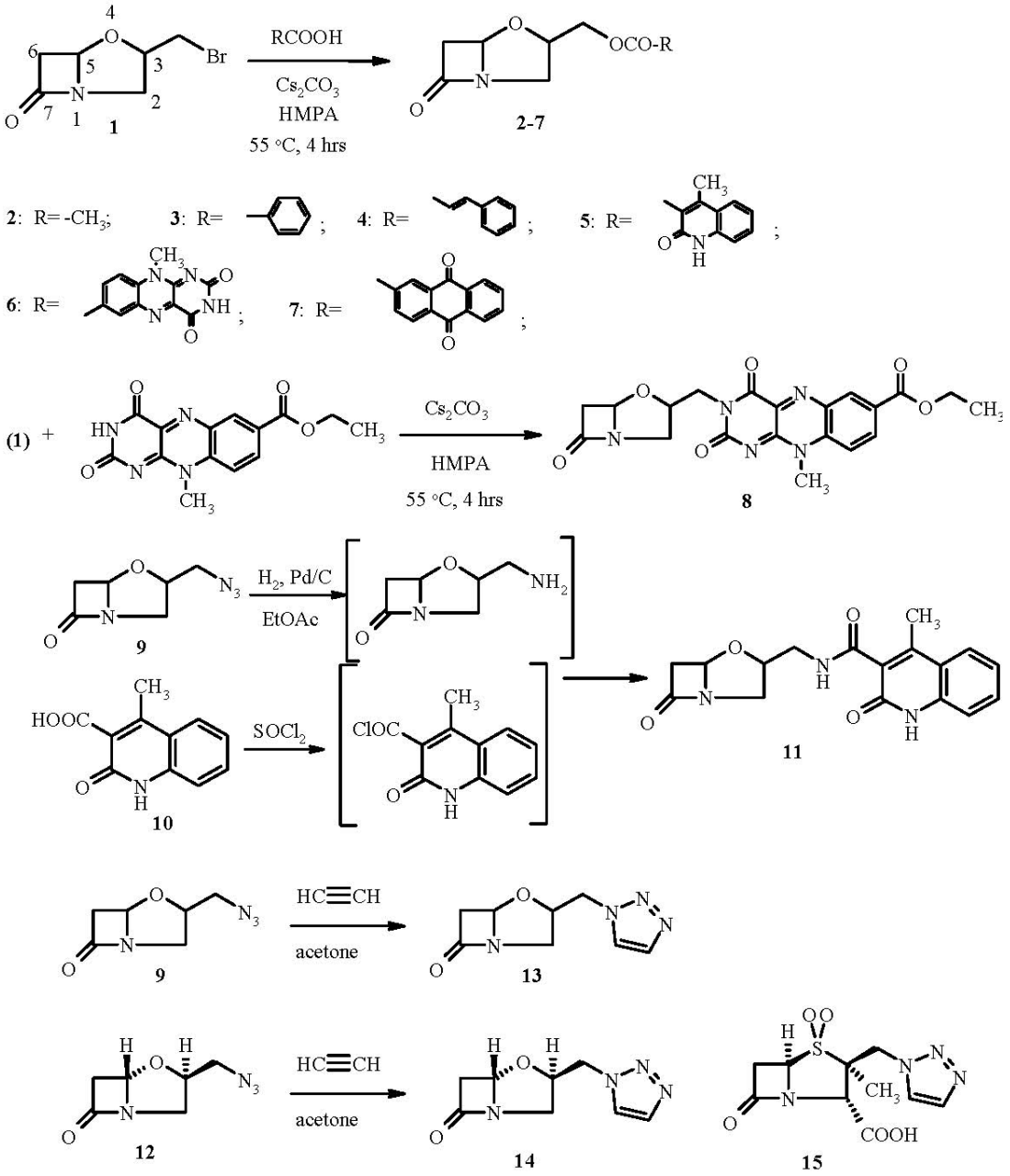
Results and Discussion

In our previous communication,[13] a new cysteine proteases inhibitor of 3-acetamido-4-oxa-1-azabicyclo[3.2.0]heptan-7-one was reported. In order to enhance the potency of this new class of inhibitors, a series of 3-substituted-4-oxa-1-azabicyclo[3.2.0]heptan-7-one have been synthesized

and their activities for inhibition of papain, cathepsin B, L, K and S have been evaluated.

Molecular modeling of 3-acetamido-4-oxa-1-azabicyclo[3.2.0]heptan-7-one with papain suggested that aromatic group substituted at C3 of the 4-oxa-1-azabicyclo[3.2.0]heptan-7-one ring may enhance binding with the hydrophobic S' site of the cysteine proteases. Compounds **4, 5** and **11** were predicted to be more active than the lead compound using the LUDI program[13]. The tricyclic anthraquinone ring in compound **7** appears to have a π–π interaction with the side chain of Trp_{177} of papain. Since it was reported[14] that 8-bromoacetyl-10-methylisoalloxazin can selectively react with the active site Cys_{25} of papain, the isoalloxazin derivatives **6** and **8** were designed. The triazole derivatives **13** and **14** were selected due to their structural similarity to our commercial drug Tazobactam **15**. The syntheses of compounds **2-8**, **11**, **13** and **14** are summarised in Scheme 1. (3RS, 5SR)-3-Bromomethyl-4-oxa-1-azabicyclo[3.2.0]heptan-7-one **1** was prepared according to a known method[15] from 4-acetoxy-2-azitidinone. Ester compounds **2-7** were synthesized by coupling **1** with acids in presence of Cs_2CO_3. Amination of **1** with the isoalloxazine derivative gave product **8**. The 3-azidomethyl-4-oxa-1-azabicyclo[3.2.0]heptan-7-one **9** and **12** were prepared from 4-acetoxy-2-azitidinone as described previously.[13] Catalytic hydrogenation of **9** gave an unstable 3-aminomethyl-4-oxa-1-azabicyclo[3.2.0]heptan-7-one. Thus the coupling reaction between **10** and 3-aminomethyl-4-oxa-1-azabicyclo[3.2.0]heptan-7-one was carried out in situ via the strong acylation agent, The acid chloride, which was used in the coupling reaction. Conversion of the carboxy of **10** to the acid chloride was carried out with thionyl chloride. Simultaneously, the azide **9** was reduced to 3-aminomethyl-4-oxa-1-azabicyclo[3.2.0]heptan-7-one. Without purification, mixing crude 3-aminomethyl-4-oxa-1-azabicyclo[3.2.0]heptan-7-one and the acid chloride gave the desired product **11**, which was purified by chromatography. The triazole derivatives **13**, **14** were prepared by cycloaddition of azide **9** or **12** with acetylene.

The inhibitory activities of these compounds against papain, cathepsin B, L, K, and S were determined according to the procedure described in the literature[16] by using Cbz-Pro-Phe-Arg-AMC as substrate for papain; 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 summarised in Table 1. All of these 3-substituted 4-oxa-1-azabicyclo[3.2.0]heptan-7-one compounds showed very selective profile. They only inhibit the papain type of cysteine proteases without inhibition of serine, metallo or aspartyl proteases. Some bicyclic or tricyclic substituents at the C3 of 4-oxa-1-azabicyclo[3.2.0]heptan-7-one can significantly increase the inhibition potency. Compound **7** is a very potent non-peptide inhibitor with IC_{50} values in the low nanomolar range. Molecular modeling of this compound with papain suggested that the tricylic anthraquinone moiety can interact optimally with Trp_{177} in the S1' subsite and with the hydrophobic pocket in the S2' subsite of the enzyme. The pure (3R, 5S)-isomer **14** is more potent than its enantiomeric mixture **13** which is in agreement with our previous finding that the (5S) stereochemistry at C5 of 4-oxa-1-azabicyclo[3.2.0]heptan-7-one ring is important for inhibition of cysteine proteases.


RCOOH
Cs_2CO_3
HMPA
55 °C, 4 hrs
1
2-7
2: R= -CH_3; 3: R= ; 4: R= ; 5: R= ;
6: R= ; 7: R= ;
(1) +
Cs_2CO_3
HMPA
55 °C, 4 hrs
8
H_2, Pd/C
EtOAc
9
10
$SOCl_2$
11
HC≡CH
acetone
13
12
14
15
COOH

Scheme 1

Table 1: In vitro inhibitory activity of oxapenam derivatives with cysteine proteases

Compd	IC_{50} (μM)				
	Papain	Cath B	Cath L	Cath K	Cath S
2	1.35	2.13	0.59	7.22	22.1
3	5.04	7.64	1.82	4.73	9.14
4	0.45	4.06	0.3	2.26	1.62
5	0.14	3.05	0.25	0.12	3.05
6	0.76	16.9	1.71	6.21	37.4
7	0.02	0.69	0.08	0.06	0.08
8	1.24	2.89	1.24	6.21	7.4
11	0.70	4.2	0.49	0.10	1.09
13	0.66	0.68	14.2	2.5	2.5
14	0.29	0.29	2.87	2.5	2.5

In summary, we have discovered that the non-peptide small molecular 3-substituted-4-oxa-1-azabicyclo[3.2.0]heptan-7-ones are very potent, selective inhibitors of the papain type of cysteine proteases. These results demonstrate that the potency of these inhibitors can be improved significantly by changing the substituents at C3 of the 4-oxa-1-azabicyclo[3.2.0]heptan-7-one ring to optimize the interactions with the S1' and S2' binding sites of the enzyme. Further modification at C3 of 4-oxa-1-azabicyclo[3.2.0]heptan-7-one could also be used to improve the pharmacokinetic properties of these inhibitors.

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