Apr 5th, 8:00 AM - 12:00 PM

Synthesis of 2-Carbamoyl-4-Oxo-1,5-Diazabicyclo [3.2.1] Octane Derivatives as a Possible Inhibitors of Serine β-Lactamases

Haiyu Wang

Abbas Shilabin
East Tennessee State University

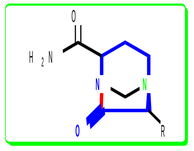
Synthesis of 2-Carbamoyl-4-oxo-1,5-diazabicyclo[3.2.1] octane Derivatives as Possible Inhibitors of Serine β-Lactamases

Haiyu Wang and Dr. Abbas G. Shilabin

Department of Chemistry, East Tennessee State University, Johnson City, TN 37614, U.S.A.

ABSTRACT

Antibiotic resistance is becoming ever more severe due in part to the increasing use of antibiotic drugs. One significant contributor to this problem is the production of β-lactamase enzymes that provide resistance to common β-lactam antibiotics such as penicillin. The scope of this research is to synthesize and study the β-lactamase inhibitors of 2-carbamoyl-4-oxo-1, 5-diazabicyclo [3.2.1] octane derivatives. β -lactamase inhibitors can inhibit the biological function of bacterial β-lactam and aid in the prevention of hydrolysis. Currently the research process is in the beginning stages of synthesizing three compounds: (R)-hexahydro-6-oxopyrimidine-4-carboxylic acid (**1a**), hexahydro-2,2-dimethyl-6-oxopyrimidine-4-carboxylic acid (**1b**) and hexahydro-6-oxo-2-phenylpyrimidine-4-carboxylic acid (**1c**). The future steps are to synthesize (R)-3-(methoxycarbonyl)-hexahydro-6-oxopyrimidine-4-carboxylic acid (**2a**), (R)-dimethyl tetrahydro-4-oxopyrimidine-1,6(2H)-dicarboxylate (**3a**) and (R)-methyl hexahydro-6-oxopyrimidine-4-carboxylate (**4a**).



INTRODUCTION

Based on molecular structures and amino acid sequences, β-lacamase can be divided into four classes: A, B, C, and D. Class A, C and D are serine enzymes acyl intermediate are produced at the active site during hydrolysis. In Class B, one or two zinc ions are required at the active site to maintain related biological functions.[1]

Class A (e.g. SHV, CTX-M, and SSHV) are broad-spectrum β-lactamases and mainly hydrolyze carbapenems such as cephalosporins, aztreonam, and penicillin. The general mechanism is Lys or Glu activation by water followed by nucleophilic attack on carbonyl carbon in lactam ring by serine.[2] As a result acyl-enzyme intermediate is produced. Clavulanate acid, sulbactam, and tazobactam can form stable covalent complexes and bind to enzyme irreversibly, which results in loss of the activity and function of class A lactamases.

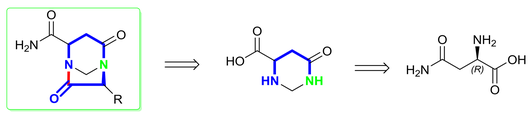
Class C lactamases (e.g. AmpC) which are encoded by bla genes on the chromosome are important enzymes in clinical diagnostics. AmpC lactamases are sensitive to oxacillin, aztreonam and cloxacillin, but are typically resistant to cephamycins, cephalosporins and penicillin. Tyr150 can activate Ser64 by attacking lactam ring in a hydrolysis process as general base.

Class D lactamases Ser67 in the chromosome sequences is activated for acylation by carboxylate Lys70 which serves as a general base during nucleophilic attack of lactam ring. Oxacillin, aztreonam and cloxacillin are typical antibiotics used to inhibit biological function and activity of Class C lactamases.[3] However, other most commonly used antibiotics such as cephamycins, cephalosporins and pencilicins show no effect in inhibiting activity of Class C enzymes. Some other inhibitors only can work when the enzymes are at low level, such as sulbactam, clavulanic acid and tazobactam.

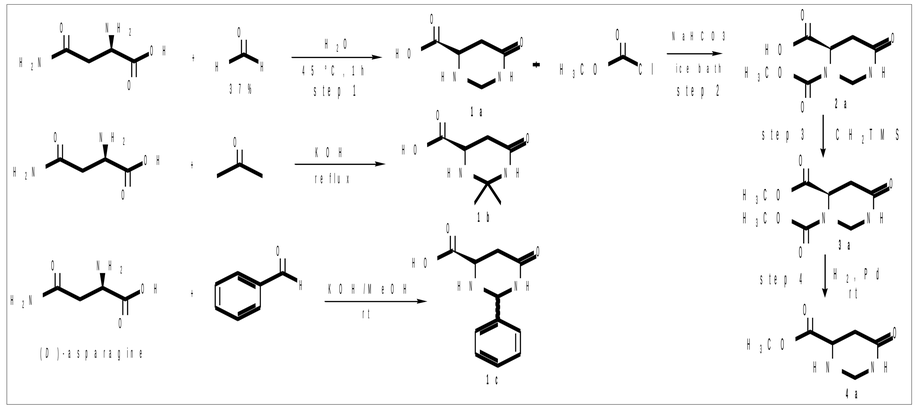
Class B lactamases are zinc ion dependent which is unique as compared with previous types of enzymes and their molecular structures are in a triangular shape. It can be divided into three subclasses based on zinc binding motifs, which are B1 (Zinc 1 ligands at His 116-His 118-His 119 and Zinc 2 ligands at Asp 120-Cys 221-His 263), B2 (Asn116-His118-His196 and Zinc 2 ligands at Asp 120-Cys221-His263), and B3 (Zinc1ligands at His116-His 118-196 and Zinc 2 ligands at Asp120-His121-His263).[4] Clinical research reveals that hydroxide ion located between zinc ions performs nucleophilic attack during hydrolysis and intermediate formed is stabilized by zinc ions. Protonation of nitrogen atom is the next step in hydrolysis. No effective antibiotics has been found to inhibit the activity of Class B since these type enzyme can catalyze hydrolysis of a wide range of lactams molecule.[5]

INHIBITOR DESIGN

Retrosynthetic analysis:



RESULTS



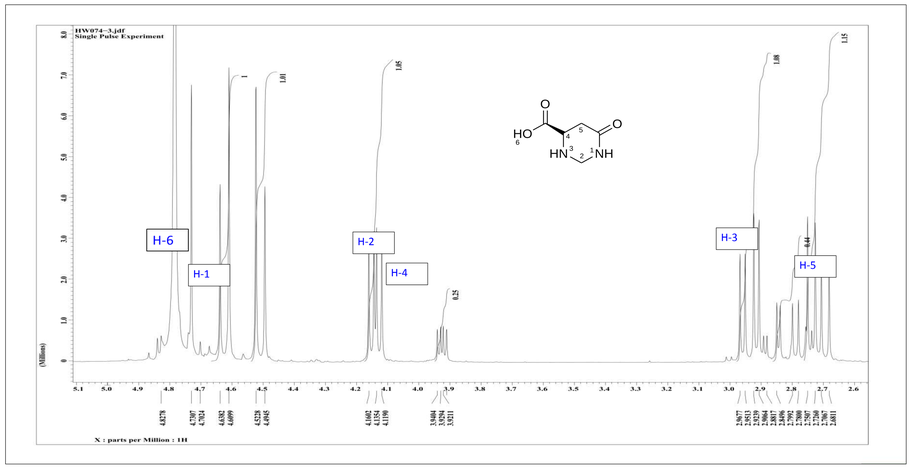
Scheme 1: Synthetic route of compound(1a),hexahydro-6-oxopyrimidine-4-carboxylic acid, compound(1b),hexahydro-2,2-dimethyl-6-oxopyrimidine-4-carboxylic acid and compound(1c),hexahydro-6-oxo-2-phenylpyrimidine-4-carboxylic acid.

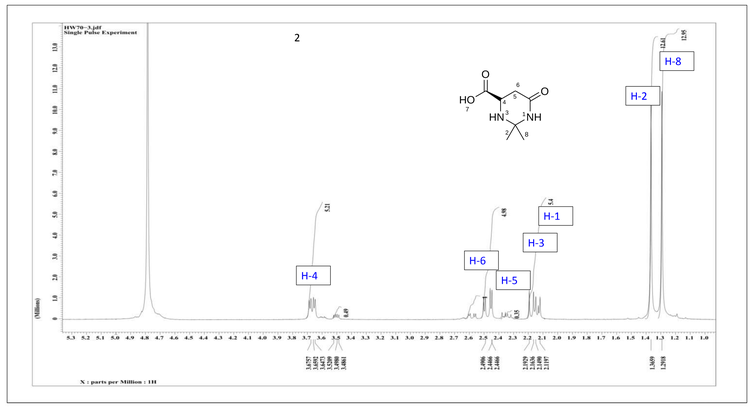
EXPERIMENTAL PROCEDURE

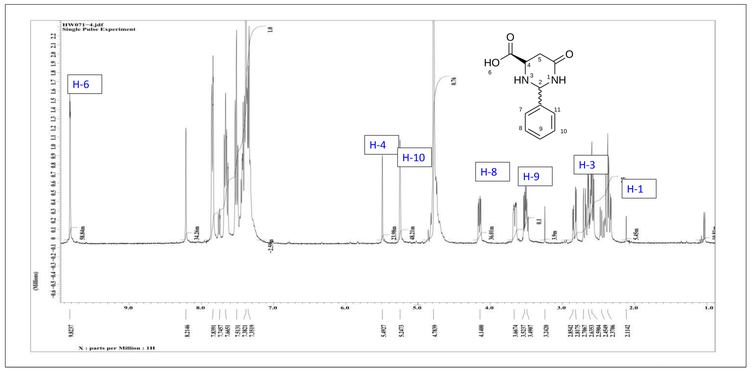
Compound 1a: To a solution of D-asparagine(30.1526 g) in 400 mL water at 45 °C, add 16.25 mL of 37% formaldehyde. After stirring for 10 hours at 45°C the solution was cooled down to -5°C to give a white slurry. The slurry was allowed to warm up 0°C, then the precipitate collected by vacuum filtration. The compound then put into oven to dry for over night.

Compound 1b: To a mixture of D-asparagine(1.2 g, 8 mmol) in HPLC grade acetone(12 mL), KOH solid (0.55 g, 8 mmol) was added into. The reaction set at oil bath at 120 °C, then do reflux for 10 hours, magnetic stirring rate set at 400 rpm.

Compound 1c: Obtain 1.5 g (10 mmol) D-asparagine monohydrate, dissolve in 30 mL methanol and input 0.68 g (20 mmol) potassium hydroxide, then add 1.43 mL benzyaldehyde dropwise, and the reaction mixture was kept at 25 °C for 12 hours. Then add 10 mL ether to the reaction flaks to wash and filtrate to obtain white sold.







CONCLUSION

We have synthesized several compounds (**1 a-c**) and we are currently in the process of producing **1a** derivatives (**2a**, **3a**, and **4a**). All chemical structures were characterized based on ^{1}H- and ^{13}C-NMR spectra. We will continue our multistep synthetic pathway to make the target compound. The biological activity of all compounds will be tested in our lab or in collaboration with other research groups. The focus of our bioassay will be on antibacterial potency or/and inhibitory activity against class A and C serine β-lactamases.

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Chemistry and The School of Graduate Studies at ETSU. We are also grateful for the financial support of the ETSU Office of Research and Sponsored Programs Administration (ORSPA).

REFERENCES

1. Watkins, R., Papp-Wallace, K., Drawz, S., & Bonomo, R. Novel beta-lactamase inhibitors: A therapeutic hope against the scourge of multidrug resistance. *Frontiers In Microb.,* 2013, Dec 24, Vol.4.
2. Miller, M.; Kale, M.; Reddy, K.; Tentarelli, S.; Zambrowski, M.; Zhang, M.; Verheijen, J. Alkylidene Oxapenem beta-Lactamase Inhibitors Revisited: Potent Broad Spectrum Activity but New Stability Challenges. *ACS Med. Chem. Lett.*, 2014, 5, 915-920.
3. Shrestha, B., Bhuyan, N., & Sinha, B. Analysis of Two Cephalosporin Drugs Ceftriaxone and Cefepime Alone and in Presence of Two [beta]-Lactamase Inhibitors Sulbactam and Tazobactam. *Asian J. Chem.*, 2011, 23, 4751-4756.
4. Xia, Y., Cao, K., Zhou, Y., Alley, M., Rock, F., Mohan, M., .Plattner, J. Synthesis and SAR of novel benzoxaboroles as a new class of β-lactamase inhibitors. *Bioorg. Med. Chem. Lett.*, 2011, 21, 2533-6.
5. Olsen, Lars, Jost, Sandra, Adolph, Hans-Werner, Pettersson, Ingrid, Hemmingsen, Lars, & JA [cedilla]rgensen, Flemming S. New leads of metallo-[beta]-lactamase inhibitors from structure-based pharmacophore design. *(Report). Bioorg. Med. Chem.,* 2006, *14, 2627.*