

“Synthesis of novel β -Lactam derivative and it's application.”

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Abstract: In this study, synthesis of novel β -Lactam derivative comprising of β -Lactam moiety and NEPA-NCA [(R)-ethyl 2-(((S)-4-methyl-2,5-dioxo oxazolidin-3-yl)methyl)-4-phenylbutanoate] is disclosed. The antibacterial activity of compound-X, was screened by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Compound-X showed significant activity in vitro against the three tested bacteria at concentrations of 1 mg/1 ml, 1 mg/5ml and 1 mg/10ml.

Key words: β -Lactam, NEPA-NCA, 7-ACCA

I. Introduction

Peptide research on drug design and drug discovery is one of the most promising fields in the development of new drugs. Peptides are synthesized by coupling the carboxyl group or C-terminus of one amino acid to the amino group or N-terminus of another. The possibility of unintended reaction is obvious; therefore protecting groups are usually necessary. Chemical peptide synthesis starts at the C-terminal end of peptide and ends at N-terminus. This is opposite to protein biosynthesis which starts at N-terminal end. The thing that captured the researcher's attention was the therapeutic effect of dipeptides.

NEPA-NCA (III) [(S)-ethyl 2-((S)-4-methyl-2,5-dioxooxazolidin-3-yl)-4-phenylbutanoate], which is hereby referred as NEPA-NCA, is an important side chain compound used in the synthesis of most cardiovascular drugs.

Using NEPA-NCA reacts with different amino acids in suitable condition reactions can give the different ACE inhibitors, for example; Ramipril, Trandolapril, Delapril, Imidapril, and Quinapril.HCl. Several compounds have been described where amino acids have been condensed with NEPA-NCA to get product of therapeutic use.

There is another class of compound known as β -Lactam compound. β -Lactam antibiotics (beta-Lactam antibiotics) are a broad class of antibiotics, consisting of all antibiotic agents that contain a β -Lactam ring in their molecular structures.

Most β -Lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics. Up until 2003, when measured by sales, more than half of all commercially available antibiotics in use were β -lactam compounds. β -Lactam antibiotics are indicated for the prophylaxis and treatment of bacterial infections caused by susceptible organisms.

7-ACCA (I) [(6R, 7R)-7-amino-3-chloro-8-oxo-5-thia-1-aza-bicyclo[4,2,0] oct-2-ene-2-carboxylic acid] is also a β -lactam compound which is used in preparation of cefaclor, a second generation antibiotic.

Accordingly the role of NEPA-NCA has been well demonstrated in literature justifying their potential as antihypertensive role. Similarly 7-ACCA has been also used as precursor of potential antibacterial drug. Accordingly it is planned to synthesize a compound using NEPA-NCA & 7-ACCA, that may yield into the preparation of a novel β -Lactam derivative and may possess significant biological activity.

Therefore it encouraged us to disclose a molecule comprising NEPA-NCA and β -Lactam compound like 7-ACCA.

So, the molecule (Compound-X) (M.F. $C_{22}H_{26}ClN_3O_6S$), chemical name: [(6R,7R)-3-chloro-7-((S)-2-((S)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino)propanamido)-8-oxo-5-thia-1-aza-bicyclo[4,2,0] oct-2-ene-2-carboxylic acid] has been synthesized by reaction of NEPA-NCA (III) (M.F. $C_{16}H_{19}NO_5$) with cephalosporin intermediate 7-ACCA (I) (M.F. $C_7H_7ClN_2O_3S$) in dichloromethane as solvent. (Scheme-1)

Experimentation

A suspension of (6R,7R)-7-amino-3-chloro-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylic acid (I) (25 g; 106.53 mili moles) and dichloromethane (300 ml) was heated to reflux with 107.91 milimoles of hexamethyldisilazane (HMDS) and 42.92 milimoles of trimethylchlorosilane (TMCS) for 4-6 hours. The solution containing (6R,7R)-trimethylsilyl-7-amino-3-chloro-8-oxo-5-thia-1-aza-bicyclo [4,2,0]oct-2-ene-2-carboxylate (II) i.e. silylated 7-ACCA was gradually cooled to room temperature (20-30°C) and subsequently added 122.5 milimoles of compound (III) i.e. NEPA-NCA. The above mixture was stirred for 2-3 hours then added water (250 ml) and tetrahydrofuran (125 ml) over a period of 10-20 minutes. The mixture was stirred at the same temperature for 30 -60 minutes to precipitate the product. Filtered the material and washed with

dichloromethane (50 ml) followed by water (50 ml) twice. Material was dried under vacuum at 40-45 °C for 5-6 hours (Yield 85-95 % molar).

Antibacterial activity of compound-X was tested by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Synthetic compound-X (1 mg) were immersed in 1 ml, 5 ml and 10 ml of 1% aq. Sodium bicarbonate solution and left at room temperature for one hour so that it get dissolved completely. The bacteria *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were inoculated into separate nutrient broths and incubated at 27°C for 24 h. Broth culture of the test bacterium (0.2 ml) was evenly spread on a nutrient agar plate under sterile conditions with the help of sterile glass spreader. Sterile filter paper discs (What man No. 1; 7 mm) were soaked in test of compound-X, allowed to dry it for a few minutes in a sterile Petri dish. Each disc was placed at the center of a nutrient agar plate, which were earlier inoculated with the respected bacterium. Filter paper discs having 50 μ m of distilled water and cephalosporin were used separately as controls. All experiments were maintained in triplicate. The Petri dishes were incubated at 27 °C. After 24 h, the diameter of any clear inhibition zone around the discs was measured.

II. Result & Discussion

Spectral analysis was performed to characterize the compound. The characterization of spectral data confirms the structure of product compound-X. ¹H-NMR, ¹³C-NMR, Mass and FTIR exhibited distinct characteristics and confirm the formation of desired product, Compound-X. Also found that the compound have good stability on storage under dry condition at low temperature (<8°C). However the product molecule is sensitive to moisture and direct sun light. ¹H-NMR, ¹³C-NMR, IR and MASS spectral data of compound (X) are in Table-1, 2, 3 and 4 respectively. Antibacterial activity of compound-X was analyzed by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Compound-X showed significant activity in vitro against the three tested bacteria at concentrations of 1mg/1ml, 1mg/5ml and 1mg/10ml. To determine the antibacterial sensitivity of compound, it was compared with cephalosporin under the same conditions. The degree of antibacterial activity of Compound- X was calculated. The compound-X showed almost similar activity as the cephalosporin at 1mg/1ml (fig.5) and 1mg/5ml but activity at 1mg/10ml was less in compare to cephalosporin at the 1mg/10 ml. The inhibitory zone diameters of compound-X against *Staphylococcus aureus*, *E. coli* and *Bacillus subtilis* were 10.13 , 9.25 and 10.80mm at 1mg/1ml concentration (fig.5a) while 6.6, 6.0 and 5.8 mm at 1ml/5ml concentration (fig.5b). The concentration 1mg/10ml exhibited very less inhibition for all tested organisms i.e. 1.8, 0.9 and 1.1mm respectively (fig.5c). (Table-5)

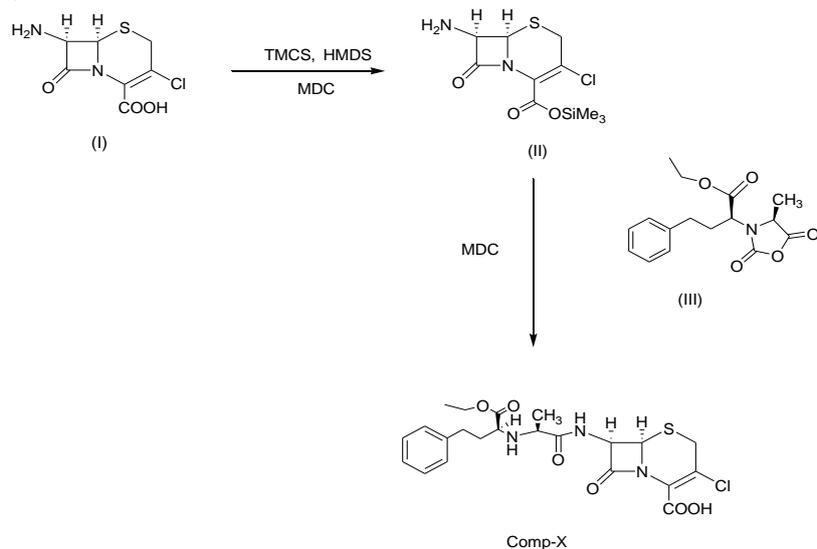
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Scheme-1:



Chemical Structure:

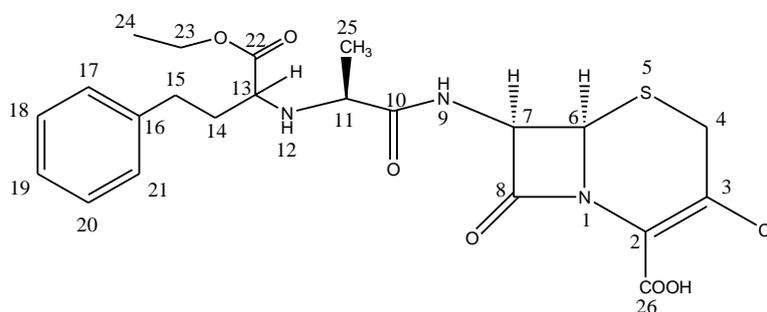


Table-1: ^1H NMR: In D_2O

Chemical shift (δ ppm)	Assignments	
1.979-2.007	(d, 3H)	H-25
2.172 -2.210	(t, 3H)	H-24
2.214-2.312	(t, 1H)	H-12
2.321-2.402	(q,2H)	H-14
2.542-2.624	(q, 2H)	H-23
3.431-3.534	(d, 1H)	H-4
3.692-3.737	(d, 1H)	
3.824-3.912	(q, 1H)	H-13
4.021-4.124	(d, 1H)	H-11
4.221-4.324	(t, 2H)	H-15
4.846 – 5.111	(d, 1H)	H-6
5.123-5.147	(2d, 1H)	H-7
5.214 - 5.224	(t, 1H)	H-19
5.862-5.941	(d, 2H)	H-17, H-21
6.212 - 6.252	(d, 2H)	H-18, H-20

Table-2: ^{13}C NMR:

Chemical shift (δ ppm)	Carbon assignment
20.40	C-24
25.17	C-25
58.34	C-15

58.75	C-4
61.10	C-14
61.84	C-11
62.03	C-13
62.31	C-6
64.30	C-7
115.98	C-2
116.25	C-3
126.34	C-19
128.74	C-17,C-21
131.53	C-18,C-20
161.49	C-16
162.96	C-8
167.67	C-26
168.84	C-22
174.16	C-10
174.16	C-8

Table-3: IR: Frequency Assignment:

Functional groups	Wave number (cm-1)
N-H stretch of amide	3229
C-H stretching	3028, 2981, 2936
C=O stretching	1787, 1740, 1701
C-H bending	1496, 1460
C-O stretching	1122, 1099

Table-4: Mass:

Name of Bacteria	Compound X			Standard Cephalosporin		
	Inhibition activity in mm at 1mg/1ml conc.	Inhibition activity in mm at 1mg/5ml conc.	Inhibition activity in mm at 1mg/10ml conc.	Inhibition activity in mm at 1mg/1ml conc.	Inhibition activity in mm at 1mg/5ml conc.	Inhibition activity in mm at 1mg/10ml conc.
<i>Staphylococcus aureus</i>	10.13	6.6	1.80	11.0	7.5	7.0
<i>Escherichia coli</i>	9.25	6.0	0.9	9.5	7.0	6.3
<i>Bacillus subtilis</i>	10.80	5.8	1.1	11.0	7.0	6.5

ESI mode, m/z C₂₂H₂₆ClN₃O₆S: calculated: 495.12 Found (M+H)⁺: 496.1

Table-5: Biological Activity:

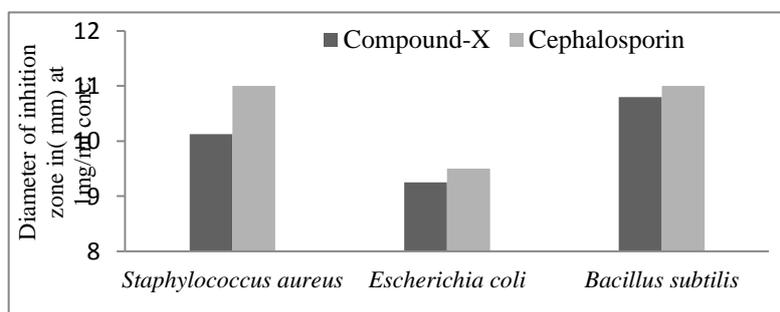


Fig. 5a. Antibacterial activity of compound X and Cephalosporin at 1mg/1ml.

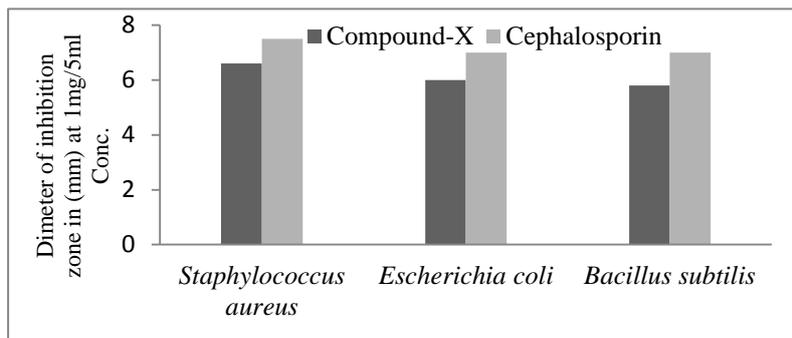


Fig. 5b. Antibacterial activity of compound X and Cephalosporin at 1mg/5ml

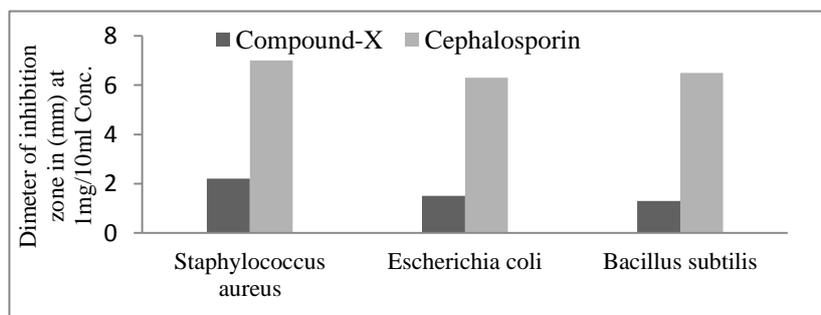


Fig. 5c. Antibacterial activity of compound X and Cephalosporin at 1mg/10ml