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"Novel β-Lactam derivative: Synthesis and application"

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Abstract: *In this study, synthesis of novel β-Lactam derivative comprising of 7-ACA and NEPA-NCA [(R)-ethyl 2-(((S)-4-methyl-2,5-dioxo oxazolidin-3-yl)methyl)-4-phenylbutanoate] justifying advancement in their stability and more biological activity at comparatively lower concentration over earlier reported analogous material (IOSR Journal of Applied Chemistry (IOSR-JAC) e-ISSN: 2278-5736.Volume 7, Issue 7 Ver. I. (July. 2014), PP 16-20, is disclosed. The antibacterial activity of compound-Y was screened by assaying against Staphylococcus aureus, Escherichia coli and Bacillus subtilis. Compound-Y showed significant activity in vitro against the three tested bacteria at concentrations of 1 mg/ml, 1 mg/5ml and 1 mg/10ml.*
Key words: β-Lactam, NEPA-NCA, 7-ACA

I. Introduction

We have reported "Synthesis of novel β-Lactam derivative and it's application" in IOSR Journal of Applied Chemistry (IOSR-JAC) e-ISSN: 2278-5736.Volume 7, Issue 7 Ver. I. (July. 2014), PP 16-20, however this requires still improvement for justifying stability and greater biological activity even at lower concentration. This encouraged us to synthesize a novel β-Lactam derivative having longer stability even at room temperature and having more biological activity.

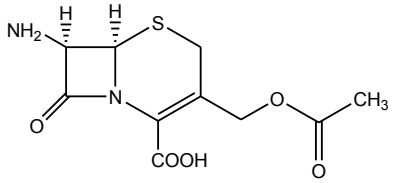
NEPA-NCA (III) [(S)-ethyl 2-((S)-4-methyl-2,5-dioxooxazolidin-3-yl)-4-phenylbutanoate], is a key side chain compound being used in the synthesis of most cardiovascular drugs like Ramipril, Trandolapril, Delapril, Imidapril, and Quinapril.HCl etc. These drugs are prepared by Using NEPA-NCA reacts with different amino acids in suitable condition reactions.

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

7-ACA (7-aminocephalosporanic acid) is the core chemical structure for the synthesis of cephalosporin antibiotics and intermediates. It can be obtained by chemoenzymatic hydrolysis of cephalosporin C.

7-ACA is used for the preparation of cefpodoxime proxetil, cefotaxime, ceftiofur, ceftriaxone, cefcapene hydrochloride, cefuroxime axetil and cefquinome sulfate.

Cefpodoxime proxetilis an oral third generation cephalosporin antibiotic. It is marketed as the prodrug cefpodoxime proxetil. It is active against most Gram positive and Gram negative organisms. Notable exceptions include Pseudomonas aeruginosa, Enterococcus, and Bacteroides fragilis. It is commonly used to treat acute otitis media, pharyngitis, sinusitis, and gonorrhea. It also finds use as oral continuation therapy when intravenous cephalosporins (such as ceftriaxone) are no longer necessary for continued treatment. Likewise cefotaxime, ceftiofur, ceftriaxone, cefcapene hydrochloride, cefuroxime axetil and cefquinome sulfate also having antibiotic properties and being used in the service of mankind.

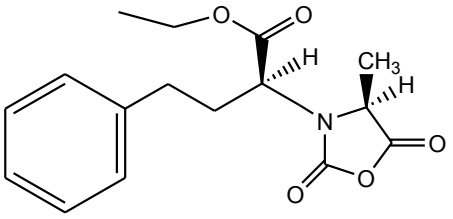


7-ACA

$C_{10}H_{12}N_2O_5S$
Mol. Wt.: 272.28

Substitution at 3-position of 7-ACA leads to the formation of different amino acid, which are also having antibiotic activity upon condensation with different chemical moieties. Following are the example of such substituted 7-ACA skeleton.

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

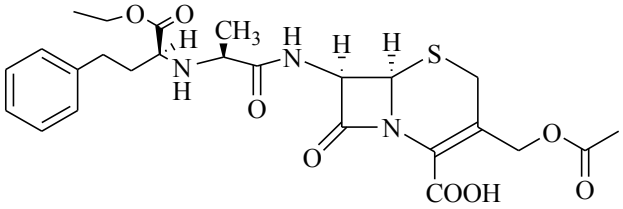


NEPA-NCA

$C_{17}H_{21}NO_5$
Mol. Wt.: 319.35

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

In the present work, the molecule (Compound-Y) (M.F. $C_{25}H_{31}N_3O_8S$), chemical name: [(6R,7R)-3-(acetoxymethyl)-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-ACA (I) (M.F. $C_{10}H_{12}N_2O_5S$) in dichloromethane as solvent. (Scheme-1)



Comp-Y

$C_{25}H_{31}N_3O_8S$
Mol. Wt.: 533.59

II. Experimentation

A suspension of (6R,7R)-3-(acetoxymethyl)-7-amino-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylic acid (I) (20 g; 73.45 mili moles) and dichloromethane (200 ml) was heated to reflux with 107.91 milimoles of hexamethyldisilazane (HMDS) and 42.92 milimoles of trimethylchlorosilane (TMCS) for 3-4 hours. The solution containing (6R,7R)-trimethylsilyl-3-(acetoxymethyl)-7-amino-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylate (II) i.e. silylated 7-ACA was gradually cooled to room temperature (20-30°C) and subsequently added 84.56 milimoles of compound (III) i.e. NEPA-NCA. The above mixture was stirred for 3-4 hours then added water (200 ml) and acetone (50 ml) over a period of 20-30 minutes. The mixture was stirred at the same temperature for 45 -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 4-6 hours (Yield 85-90 % molar).

Antibacterial activity of compound-Y was analyzed by assaying against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. Synthetic compound-Y (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-Y, 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 µm 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.

III. Result & Discussion

Compound-Y was characterized by spectral analysis like NMR, Mass and IR. The characterization of spectral data confirms the structure of product compound-Y. 1H-NMR, 13C-NMR, Mass and FTIR exhibited distinct characteristics and conform the formation of desired product, Compound-Y. It was also observed that the compound-Y shows substantial stability on storage under dry condition at room temperature (approx. 25°C).

1H-NMR, 13C-NMR, IR and MASS spectral data of compound (Y) are tabulated Table-1, 2, 3 and 4 respectively. Antibacterial activity of compound-Y was analyzed by assaying against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. Compound-Y showed significant activity in-vitro against the three tested bacteria at concentrations of 1mg/ml, 1mg/5ml and 1mg/10ml. To determine the antibacterial sensitivity of compound, it was compared with standard cephalosporin under the same conditions. The degree of antibacterial activity of Compound- Y was calculated.

The biological activity of compound-Y is comparable with standard cephalosporin at 1mg/ml (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-Y against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were 9.5, 8.0 and 8.6 mm at 1mg/ml concentration (fig.5a) while 7.2, 6.5 and 6.2 mm at 1ml/5ml concentration (fig.5b). The concentration 1mg/10ml also exhibits good inhibition for all tested organisms i.e. 4.4, 5.0 and 5.1mm respectively (fig.5c). (Table-5)

Acknowledgements

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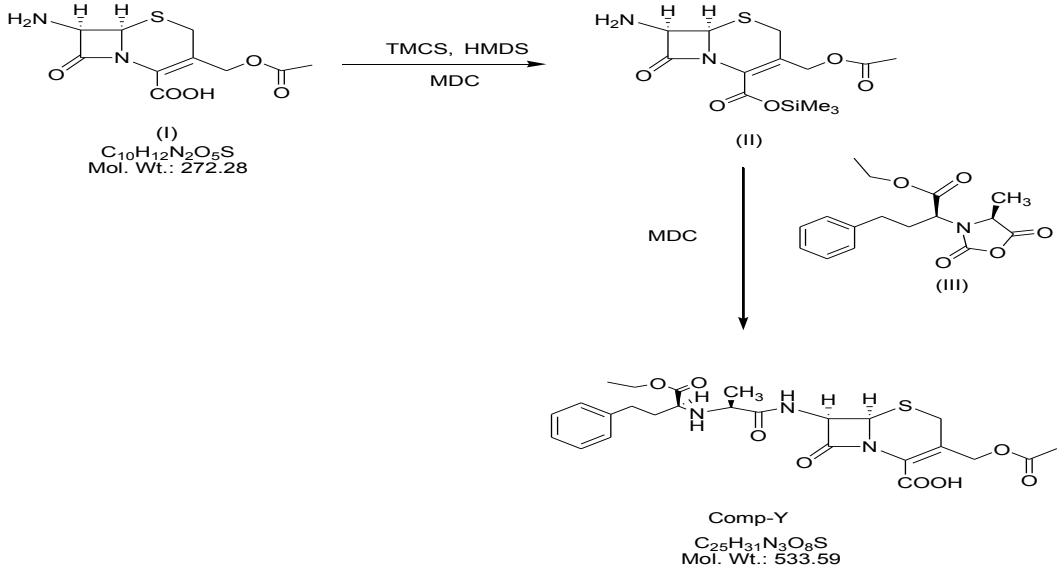
Scheme-1:



Chemical Structure:

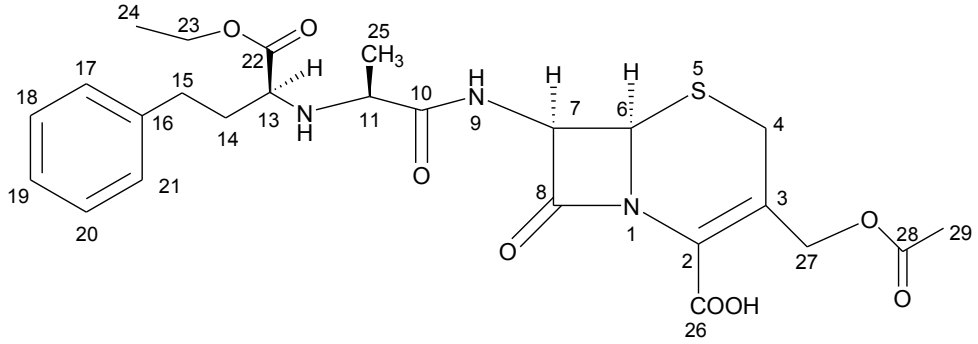


Table-1: 1H NMR: In D_2O

Chemical shift (δ ppm)	Assignments	
1.958-1.990	(d, 3H)	H-25
2.026- 2.116	(s, 3H)	H-29
2.195 -2.212	(t, 3H)	H-24
2.225-2.319	(t, 1H)	H-12
2.331-2.421	(q,2H)	H-14
2.546-2.664	(q, 2H)	H-23
3.531-3.644	(d, 1H	H-4
3.712-3.739	(d, 1H)	
3.841-3.982	(q, 1H)	H-13
4.061-4.146	(d, 1H)	H-11
4.261-4.372	(t, 2H)	H-15
4.721-4.795	(s, 2H)	H-27
4.852 – 5.143	(d, 1H)	H-6
5.128-5.151	(2d, 1H)	H-7
5.221 - 5.242	(t, 1H)	H-19
5.871-5.968	(d, 2H)	H-17, H-21
6.231 - 6.264	(d, 2H)	H-18, H-20

Table-2: 13C NMR:

Chemical shift (δ ppm)	Carbon assignment
20.31	C-24
20.52	C-29
25.27	C-25
57.29	C-27
58.14	C-15
58.68	C-4
61.24	C-14
61.96	C-11
62.12	C-13
62.26	C-6
64.38	C-7
115.91	C-2
116.32	C-3
126.39	C-19
128.83	C-17,C-21
131.36	C-18,C-20
161.54	C-16
162.82	C-8
167.78	C-26
168.52	C-22
169.15	C-28
174.18	C-10
174.18	C-8

Table-3: IR: Frequency Assignment:

Functional groups	Wave number (cm-1)
N-H stretch of amide	3255
C-H stretching	3047, 2975, 2965
C=O stretching	1794, 1755, 1723
C-H bending	1482, 1457
C-O stretching	1143, 1102

Table-4: Mass: ESI mode, m/z $C_{25}H_{31}N_3O_8S$: calculated: 533.59 Found $(M+H)^+$: 534.6

Table-5: Biological Activity:

	Compound -Y			Standard cephalosporin		
Name of Bacteria	Inhibition activity in mm at 1mg/ml conc.	Inhibition activity in mm at 1mg/5ml conc.	Inhibition activity in mm at 1mg/10ml conc.	Inhibition activity in mm at 1mg/ml conc.	Inhibition activity in mm at 1mg/5ml conc.	Inhibition activity in mm at 1mg/10ml conc.
Staphylococcus aureus	9.5	7.2	4.4	11.1	7.4	6.9
Escherichia coli	8.0	6.5	5.0	9.3	6.0	6.2
Bacillus subtilis	8.6	6.2	5.1	10.9	7.1	6.4

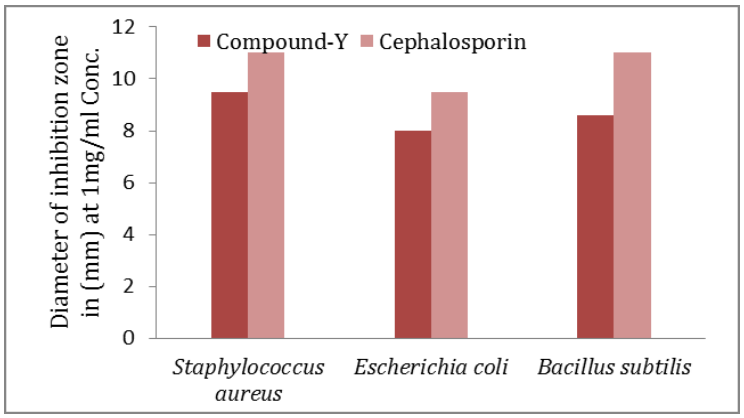


Fig.5a. Antibacterial activity of compound Y and Cephalosporin at 1mg/ml

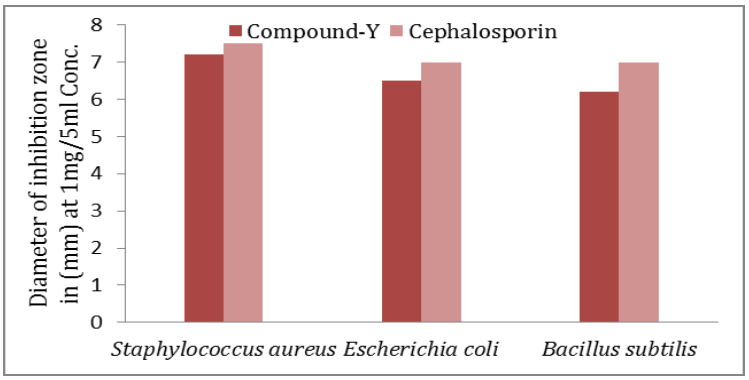


Fig.5b. Antibacterial activity of compound Y and Cephalosporin at 1mg/5ml

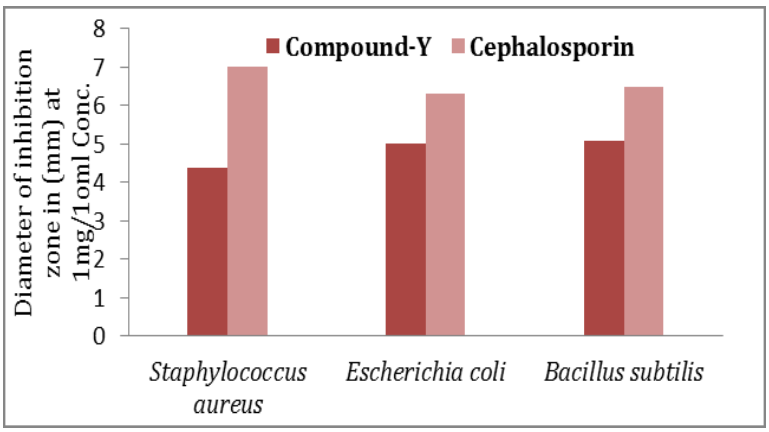


Fig.5c. Antibacterial activity of compound Y and Cephalosporin at 1mg/10ml