

## Synthesis of novel $\beta$ -Lactam derivative and its antibacterial activity

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**Abstract:** In this study, synthesis of novel  $\beta$ -Lactam derivative comprising of  $\beta$ -Lactam moiety 7-ACT i.e. (6R,7R)-7-amino-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylic acid and NEPA-NCA i.e. [(S)-ethyl 2-((S)-4-methyl-2,5-dioxoxazolidin-3-yl)-4-phenylbutanoate] is disclosed. The synthesis of intended compound has been characterized and confirmed by 1H-NMR, 13C-NMR and Mass. The antibacterial activity of this compound was screened by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. This compound showed significant activity in vitro against the three tested bacteria at concentrations of 1 mg/mL, 1 mg/5mL and 1 mg/10mL.

**Keywords:**  $\beta$ -Lactam, NEPA-NCA, 7-ACT

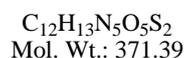
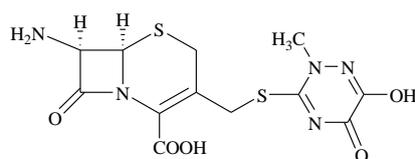
### I. Introduction

$\beta$ -Lactam antibiotics are specific class of antibiotics, consisting of all antibiotic agents that contain a  $\beta$ -Lactam ring in their chemical structures. Most of the therapeutically useful  $\beta$ -Lactam antibiotics possess the structurally modified side chains that enhance the activity of the drug. Large number of such analogs has been in use till date. But, in recent years, bacterial resistance to these antibiotics is found to be increasing at an alarming rate due to the resistance of bacteria to these drugs. As a result, successful treatment to bacterial infection is getting affected. In order to overcome this challenge, there is an ever-growing need to synthesize new antibiotics with enhanced activity. 7-ACT i.e. (6R,7R)-7-amino-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylic acid is a  $\beta$ -lactam compound which is used in synthesis of ceftriaxone.

Ceftriaxone sodium, which is prepared from 7-ACT, is a long acting third generation broad-spectrum cephalosporin injectable antibiotic with a broad spectrum of activity against Gram positive and gram negative bacteria. It is administered as disodium hemiheptahydrate salt. It finds applications in treatment of several infections. It is widely used in respiratory tract infections, urinary tract infections, including pyelonephritis and gonorrhea, septicemia, meningitis, burns, infections, postoperative infections, bone joints, soft tissue, skin and wound infections, abdominal infections (peritonitis, biliary and gastrointestinal infections) etc. and operative infection.

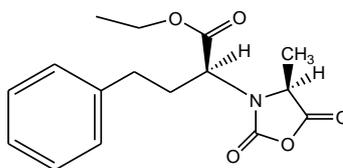
Ceftriaxone is often used (in combination, but not direct, with macrolide and/or aminoglycoside antibiotics) for the treatment of community acquired pneumonia. It is also a choice drug for treatment of bacterial meningitis caused by pneumococci, meningococci, *Haemophilus influenzae*, and susceptible enteric gram-negative rods, but not *Listeria monocytogenes*. It has also been used in the treatment of Lyme disease and gonorrhea.

The *syn*-configuration of the methoxyimino moiety confers stability of  $\beta$ -lactamase enzyme produced by many Gram negative bacteria. Such stability to  $\beta$ -lactamases increases the activity of ceftriaxone against otherwise resistant Gram negative bacteria. In place of the easily hydrolysed acetyl group of cefotaxime, ceftriaxone has a metabolically stable thiotriazinedione moiety. The 7-ACT mentioned above has the following details,



There is another class of compound known as NEPA-NCA i.e. [(S)-ethyl-2-((S)-4-methyl-2,5-dioxoxazolidin-3-yl)-4-phenylbutanoate]. It is a well-known chemical intermediate in pharmaceutical industry,

which plays an important role in synthesizing Angiotensin-I converting enzyme (ACE) inhibitors such as delapril, enalapril, imidapril, indolapril, moexipril, quinapril, ramipril and trandolapril. There are so many chemical substances available where different amino acids have been condensed with NEPA-NCA to yield product of medicinal use.

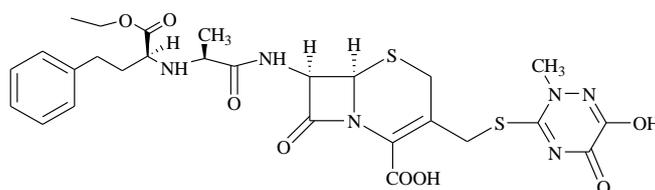


NEPA-NCA

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

The role of NEPA-NCA has been well reported in literature justifying their application as antihypertensive role. Similarly 7-ACT has been also used as precursor of potential antibacterial drug Ceftriaxone. Accordingly it is designed to synthesize a hybrid molecule of these two chemical compounds to generate functional molecule in which the characteristics of hybrid molecule are modulated, amplified or give rise to entirely new properties.

Therefore a protocol was designed to synthesize the intended molecule from NEPA-NCA and  $\beta$ -Lactam compound like 7-ACT. The synthetic protocol involves the multi-step synthesis of a  $\beta$ -Lactam derivative (Compound-A) (M.F.  $C_{27}H_{32}N_6O_8S_2$ ), chemical name: [(6R,7R)-7-((S)-2-((S)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino)propanamido)-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylic acid] by reaction of NEPA-NCA (M.F.  $C_{16}H_{19}NO_5$ ) with cephalosporin intermediate 7-ACT (M.F.  $C_{12}H_{13}N_5O_5S_2$ ). (Scheme-1) The process involves silylation of 7-ACT i.e. (6R,7R)-7-amino-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylic acid using Hexamethyldisilazane (HMDS) and Trimethylchlorosilane (TMCS) in dichloro methane as solvent. The next step involves the condensation of silylated 7-ACT with NEPA-NCA followed by desilylation and work-up step to afford the desired product compound-A i.e. : (6R,7R)-7-((S)-2-((S)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino)propanamido)-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4,2,0]oct-2-ene-2-carboxylic acid. This 7-ACT upon condensation with NEPA-NCA will convert into the compound-A of following formula;



Compound-A

$C_{27}H_{32}N_6O_8S_2$   
Mol. Wt.: 632.71

## II. Experimentation

A suspension of (6R,7R)-7-amino-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4,2,0] oct-2-ene-2-carboxylic acid (I) (25 g; 67.31 mili moles) and MDC (dichloromethane) (300 mL) was heated to reflux with 68.18 milimoles of hexamethyldisilazane (HMDS) and 27.12 milimoles trimethylchlorosilane (TMCS) for 4-6 hours. The solution containing (N, O)-bistrialkylsilyl (6R,7R)-7-amino-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid (II) was slowly cooled to room temperature (20-30°C) and subsequently added 77.40 milimoles of compound (III). The above mixture was stirred 2-3 hours then added water (400 mL) and tetrahydrofuran (100 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 water 50 mL twice. Material was dried under vacuum at 40-45°C for 5-6 hours (Yield 85-95%).

Antibacterial activity of compound-A was analyzed by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Synthetic compound 1mg was immersed in 1mL, 5mL and 10mL of 1%

Sodium bicarbonate solution and left at room temperature for one hour so that it dissolved completely. Antibacterial test of compound-A was done *in-vitro*. The bacteria *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were inoculated into separate nutrient broths and incubated at 27°C for 24 hours. 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 compound-A, 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 $\mu$ 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 hours, the diameter of any clear inhibition zone around the discs was measured.

### III. Result & Discussion

The Compound-A, prepared by utilizing the 7-ACT and NEPA-NCA was analysed by the spectral studies. Compound-A was characterized by spectral analysis like 1H-NMR (400 MHz), 13C-NMR (300 MHz), and Mass. The characterization of spectral data confirms the structure of product compound-A. 1H-NMR, 13C-NMR and Mass exhibits their distinct characteristics and confirms the formation of desired product i.e. Compound-A.

It was also observed that the compound-A shows substantial stability on storage under dry condition at low temperature (approx. 2-8°C). 1H NMR, 13C NMR and MASS spectral data of compound-A are tabulated in Table-1, 2 and 3 respectively.

Antibacterial activity of compound-A was tested by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Compound-A showed biological activity *in-vitro* against the three tested bacteria at concentrations of 1g/1mL, 1g/5mL and 1g/10mL. To determine the antibacterial sensitivity of compound-A, it was compared with standard cephalosporin under the same conditions. The degree of antibacterial activity of compound-A was calculated.

The biological activity of compound-A was compared as against standard cephalosporin at 1 mg/ 1mL, 1 mg/ 5mL and at 1 mg/ 10mL. The inhibitory zone diameters of compound-A against *Staphylococcus aureus*, *E. coli* and *Bacillus subtilis* were 5.5, 5.1 and 6.3 mm at 1mg/1mL concentration (fig.4a) while it was 4.2, 4.0 and 4.8 mm at 1mg/5mL concentration (fig.4b) and it was found 1.4, 1.0 and 1.1mm at 1mg/10 mL concentration respectively (fig.4c). (Table-4)

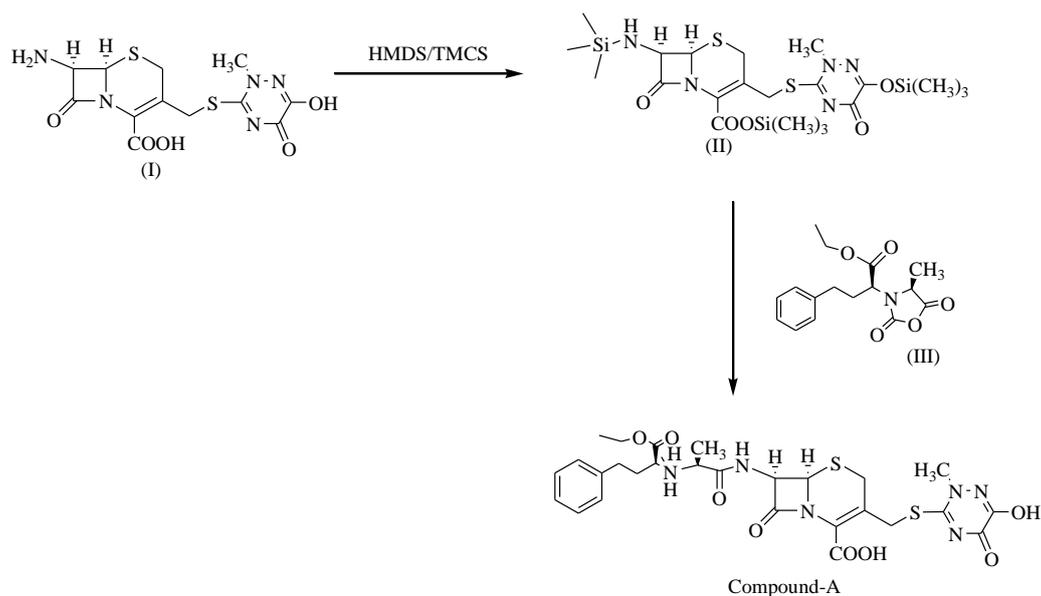
### Acknowledgements

We are thankful to Central Drug Research Institute (CDRI), Lucknow for giving the spectral data and analysis.

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Scheme-1:



Chemical Structure:

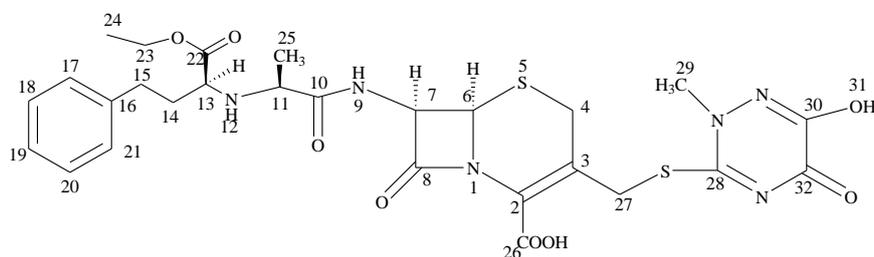


Table-1:  $^1\text{H}$  NMR: In  $\text{D}_2\text{O}$

Chemical shift ( $\delta$ ppm)	Proton assignments	
1.879-1.976	(d, 3H)	H-25
2.062 -2.180	(t, 3H)	H-24
2.281-2.302	(m, 2H)	H-14
2.411-2.488	(s, 3H)	H-29
2.557 – 2.572	(t, 2H)	H-15
3.341-3.434	(d, 1H)	H-4
3.572-3.597	(d, 1H)	
3.651-3.724	(t, 1H)	H-13
3.811-3.846	(s, 2H)	H-27
3.916-3.988	(q, 1H)	H-11
4.221-4.324	(q, 2H)	H-23
4.775 – 4.928	(d, 1H)	H-6
5.243-5.357	(d, 1H)	H-7
6.731 - 6.945	(m, 5H)	H-17, H-18, H-19, H-20, H-21

Table-2:  $^{13}\text{C}$  NMR:

Chemical shift ( $\delta$ ppm)	Carbon assignment
14.41	C-24
18.78	C-25
21.03	C-27
26.85	C-4
31.13	C-15
33.22	C-14
37.45	C-29
57.23	C-11
58.14	C-6
58.45	C-13
60.17	C-7
62.10	C-23

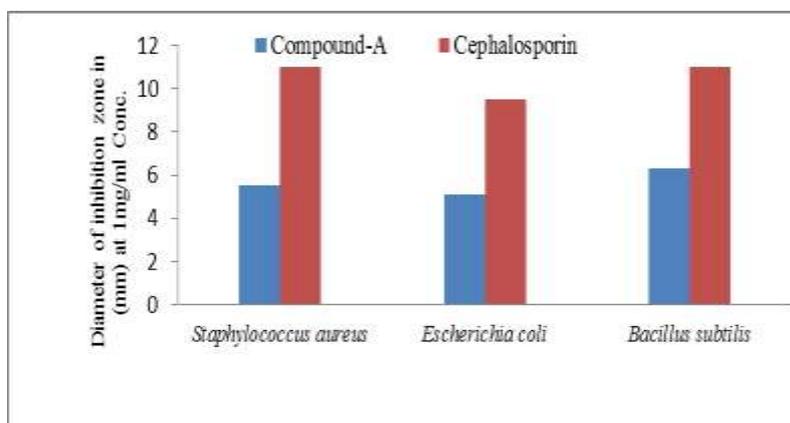
122.23	C-2
127.21	C-3
128.32	C-19
129.14	C-17, C-21
131.05	C-18, C-20
139.56	C-16
156.24	C-30
165.91	C-28
166.75	C-8
169.24	C-26
170.13	C-22
173.02	C-10
189.16	C-32

**Table-3: Mass:**  
ESI mode, m/z  $C_{27}H_{32}N_6O_8S_2$ : calculated: 632.71

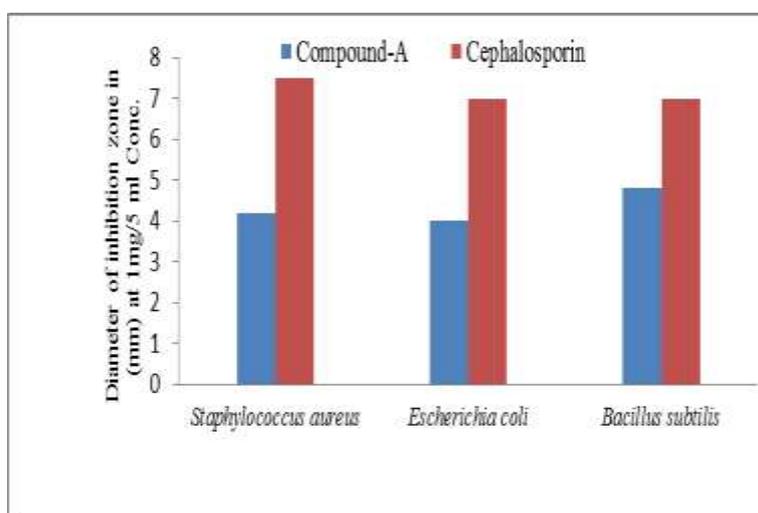
m/z (amu)	Assignment
633.6	[M+H] <sup>+</sup>

**Table-4: Biological Activity:**

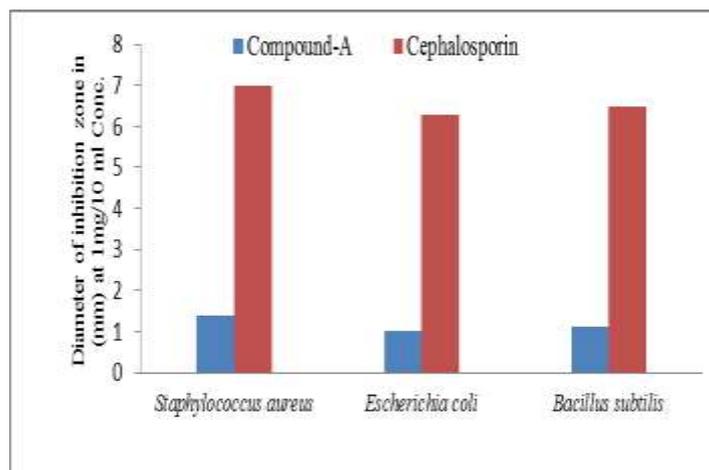
Name of Bacteria	Compound-A			Standard cephalosporin		
	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc
<i>Staphylococcus aureus</i>	5.5	4.2	1.4	11.0	7.5	7.0
<i>Escherichia coli</i>	5.1	4.0	1.0	9.5	7.0	6.3
<i>Bacillus subtilis</i>	6.3	4.8	1.1	11.0	7.0	6.5



**Fig.4a.** Antibacterial activity of compound-A and Cephalosporin at 1mg/mL



**Fig.4b.** Antibacterial activity of compound-A and Cephalosporin at 1mg/5mL



**Fig.4c.** Antibacterial activity of compound-A and Cephalosporin at 1mg/10mL