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Synthesis and Spectral Characterization of 1, 4- Diazepines from 7-Aminocephalosporanic Acid and Their Biological Activity

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Abstract - Synthesis of biological active 1,4-diazepines are proceed by Diazotization of 3-(Acetyloxy-methyl) - 7 - amino - 8 - oxo - 5 - thia - 1-azabicyclo[4.2.0.] oct-2-ene-2-carboxylic acid (1) with Sodium nitrite and Hydrochloric acid, affords 3-(Acetyloxy-methyl)-7-[(E)-chlorodiazenyl]-8-oxo-5-thia-1-azabicyclo[4.2.0.] oct-2-ene-2-carboxylic acid (2). The compound (2) condense with various β-diketones (3a-c) to form new derivatives of various novel β-diketones (4a-c) of compound (2) and novel derivative of β-diketones (4a-c) are further reacted with Ethylenediamine (EDA) to form 1,4-diazepines (5a-c).

All structures of the newly synthesized compound were elucidates by elemental analysis and spectral studies. The newly compound (5a-c) was screened for its pharmacology as antibacterial and antifungal activity against various bacterial and fungal strains, showing excellent antibacterial and antifungal activity.

Keywords - 1,4-Diazepines, β-diketones/β-ketoester, Sodium nitrite, Ethylenediamine.

I. INTRODUCTION

7-Aminocephalosporanic acid, nucleus is a key intermediate in the production of many semi-synthetic antibiotics [1], can be synthesized by the chemical deacylation of cephalosporin C (CPC) employing iminoethers, nitrosyl chloride, and methanol. Since these steps involve toxic compounds causing environmental contamination, enzymatic methods of CPC deacylation are of great interest. Since cephalosporin acylase (CA) has very low activity toward CPC, most of the bioprocess reported methods involve a two-step conversion of CPC to 7-ACA. First, CPC is oxidized by D-amino acid oxidase, and the product, glutaryl-7-ACA (GL-7-ACA), is then converted to 7-ACA by GL-7-ACA acylase.

7-Aminocephalosporanic acid required for the production of most of the clinically used cephalosporins derivatives, i.e., semi synthetic cephalosporins. The repeated batch production of 7-ACA with entrapped cells of P. diminuta in different carriers were carried out for six cycles at optimal conditions. It was found that 33%, 38%, and 47% of activity was lost with chitosan, gelatin, and agar, respectively as immobilizing supports after the sixth cycle of operation [2]-[3].

The diazepine nucleus is a pharmacophoric scafforld and represent a class of heterocycles with a wide range of biological activities. Many of them are use as antiviral [4], anticonvulsant [5], psychotropics [6], antibacterial [7], herbicidal [8], anti-cancer [9]-[10]. Above biological activity of 1,4-Diazepines are of greater importance in pharmaceutical factor (PAF) antigonastic [11],1,4-diazepines and their derivatives possess anti HIV activities [12], serotoninergic S_3 antagonistic [13]-[14] activities.

1,4-diazepine nucleus has been proved as a versatile system in medicinal chemistry. A number of established drugs molecules like, Zometapine, Etizolam, Brotizolam, Clozapine and Dibenzepine are accessible starting from the corresponding 1, 4-diazepine [15].

In recent years the use of organic-inorganic hybrid immobilized solid support reagent have received great interest. These reagents not only simplify the purification process. But also provide help in

preventing release of reaction residue in to the environment. Many methods are reported in literature for the synthesis of 1, 4-diazepines [16].

In continuation of our ongoing research program to develop new reagent and synthetic procedure for the synthesis of heterocyclic compound [17]-[21], we report here a new convenient methods for the synthesis of diazonium salt containing 1,4-diazepine due to their importance in medicinal chemistry [22] to achieve this target we had synthesized β-diketones/β-ketoester (4a-c) which were condensed with EDA to obtain the corresponding substituted 1,4-diazepines (5a-c) with high yields.

II. EXPERIMENTAL WORK

A. General procedures

7-Aminocephalosporanic acid, sodium methoxide and β-Diketones were used as precursors for synthesis. Solvents were purified before use. Melting points were determined by open capillary method and are uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel. A mixture (7:2:1) of benzene: ethanol: ammonia was used as eluent to run the spot of the individual complexes solubilized in methanol. The observed movement of a single spot on the TLC plate confirmed that the complexes do not contain impurities.

The elemental analysis (C, H, N) of compound was performed on Carlo Erba-1108 elemental analyzer, their result were found to be in good agreement with the calculated value. The IR spectra were recorded on a Nicolet-Magna-FT-IR-550 spectrometer in using KBr pellets. ^{1}H NMR spectra were run on model DRX 300 at 300.13 and 75 MHz respectively.

B. Synthesis of 3-[(acetyloxy) methyl]-7-[(E)-chlorodiazenyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (2)

Pure 7-Aminocephalosporanic acid (2.72g., 0.01 M) HCl (3.64 ml, 0.1M) (1) and 3ml. water was taken in a round bottomed flask and stirred it 10 minute on a magnetic stirrer at temperature of 0-5º C. Then added $NaNO_2$ (0.759 g, 0.01 M) and 3ml water solution, temperature of the reaction mixture was maintained between 0-5º C. Thus diazonium salt remained in the solution.

C. Synthesis of 3-[(acetyloxy) methyl]-7-[(E)-(2, 4-dioxopentan-3-yl/1, 3-dioxo-1-phenylbutan-2-yl/1, 3-dioxo-1,3-diphenylpropan-2-yl) diazenyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (4a-c)

Placed sodium methoxide (1.0g., 0.02 M) and β-diketones/β-ketoester (3) (0.01mole) in a dry round bottomed flask fitted with a guard tube and stirred it for one hour on a magnetic stirrer at a temperature of 50°C, until a creamy mass was obtained. The compound (2) (1.60g, 0.005M) was then added in small portions and dry toluene (5 ml) was added as solvent to affect proper stirring of the reaction mixture. The reaction mixture was refluxed at 100°C for about twenty hours. The completion of the reaction was monitored through TLC. When the reaction was completed, the reaction mixture was cooled and toluene was removed under reduced pressure. The reaction mixture was extracted with chloroform and then chloroform layer was washed thrice with water. The chloroform layer was then dried over sodium sulphate and filtered. The chloroform was distilled off and the viscous mass was precipitated by using a mixture of ethylacetate and petroleum ether (2:8). The crude product was recrystallized from absolute alcohol. Purity of diketone was checked through TLC using benzene: ethanol: ammonia (7: 2: 1) upper layer as mobile phase.

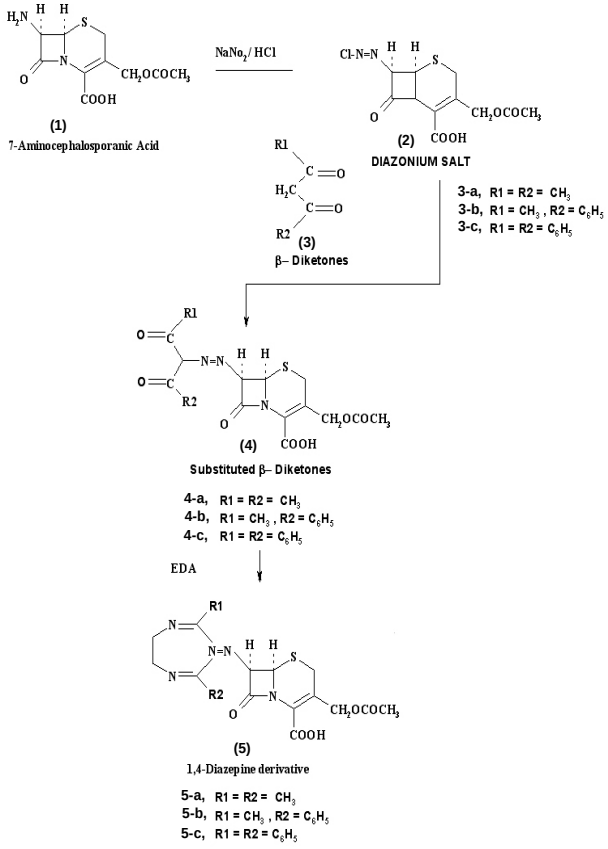
D. Synthesis of 1,4-diazepines derivatives (5a-c)

β-Diketone (4a-c) (2g., 0.005M) was placed in a two necked round bottomed flask along with dry ethanol (10ml.). To the reaction mixture, added glacial acetic acid (2.5ml.) followed by dropwise addition of ethylenediamine (1.2 ml, 0.02M) using a dropping funnel. The progress of the reaction was checked through TLC. After the completion of the reaction, the reaction mixture was refluxed for a period of about eight hours and cooled to room temperature. The solvent from the reaction mixture was removed under reduced pressure. The viscous mass so obtained was thoroughly washed with dry ether to remove

unreacted β-diketone, and finally a dry powdered mass was obtained. The crude product was crystallized from acetone. Purity of the compound was checked through TLC using upper layer of benzene: ethanol: ammonia (7: 2: 1) as the mobile phase. Physical and analytical data of compound 4a-c are shown in Table I.

Table I
Physical and Analytical Data of Compound

Compound	R1	R2	Melting point (^{0}C)	Yields%	Molecular formula
4a	CH_3	CH_3	175	45	$C_{15}H_{17}N_3O_7S$
4b	CH_3	C_6H_5	180	60	$C_{20}H_{19}N_3O_7S$
4c	C_6H_5	C_6H_5	185	50	$C_{25}H_{21}N_3O_7S$



Scheme – 1

E. Spectral Data

3-[(acetyloxy) methyl -7-[(E)-(5,7-dimethyl-3,6-dihydro-2H-1,4-diazepine-6-yl)diazenyl]-8-oxo-5thia-1-azabicyclo [4.2.0.] oct-2-ene-2-carboxylic acid (**5a)**

^{1}H-NMR: 1.951(6H,s, CH_3,), 3.623(4H,m,N-CH_2), 2.056 (3H,s,CH_3(C=O), 4.504 (2H,s, CH_2(C=O), 5.362 (1H,s,-CH=), 3.388 (2H,dd, S-CH_2), 4.996 (1H,d,CH), 5.726 (1H,d,CH).

3-[(acetyloxy) methyl – 7-[(E)-(5-phenyl-7-methyl-2,3-dihydro-6H-1,4-diazepine-6-yl)diazenyl]-8-oxo-5thia-1-azabicyclo [4.2.0.] oct-2-ene-2-carboxylic acid (**5b)**

^{1}H-NMR: 7.373-7.747(5H,m, Ar-H,), 1.982(6H,s,CH_3), 3.543-3.774 (4H,m,N-CH_2), 2.056 (3H,s, CH_3(C=O), 4.503 (2H,s,-CH_2(C=O), 5.413 (1H,s, CH=), 3.388 (2H,dd,S-CH_2), 5.009 (1H,d,CH), 5.718(1H,d,CH)

3-[(acetyloxy) methyl-7-[(E)-(5,7-diphenyl-2,3-dihydro-6H-1,4-diazepine-6-yl)diazenyl]-8-oxo-5thia-1-azabicyclo [4.2.0.] oct-2-ene-2-carboxylic acid (**5c)**

^{1}H-NMR : 7.495-7.785(10H,m, Ar-H), 3.611-3.727(4H,m,N-CH_2), 2.056 (3H,s,CH_3(C=O),4.503 (2H,s,-CH_2(C=O), 5.413 (1H,s, -CH=), 3.388 (2H,dd,S-CH_2), 5.009 (1H,d,CH), 5.718(1H,d,CH)

III. BIOLOGICAL ACTIVITY

A. Antibacterial Activity

The antibacterial activity of newly synthesized complex was tested in vitro against two Gram-positive bacteria Staphylococcus aureus (MSSA 22) and Bacillus subtilis (ATCC 6051) and two Gram-negative bacteria Escherichia Coli (K 12) and Pseudomonas aeruginosa (MTCC 2488) strains using disc diffusion method. The discs measuring 5 mm in diameter were prepared from Whatmann No. 1 filter paper sterilized by dry heat at 140 ºC for 1 h. The sterile discs previously soaked in a concentration of the test complex were placed in a nutrient agar medium. The plates were invested and kept in an incubator for 24 h at 37 ºC. The inhibition zone thus

formed was measured (in mm) after 24 h. The screening was performed for 100 lg/ml concentration of test 5a,5b and 5c compound and antibiotic disc, Tetracycline (30 lg/disc, Hi-Media) was used as control. The nutrient broth which, logarithmic serially two fold diluted amount of test CT complex and controls, was inoculated within the range 10^{-4}–10^{-5}cfu/ml. The cultures were incubated for 24 h at 37 ºC and growth was monitored visually and spectro-photometrically like. The lowest concentration (highest dilution) required to arrest the growth of bacteria is regarded as minimum inhibitory concentration (MBC). To obtain the diameter of zone, 0.1 ml volume was taken each and spread on agar plates. The number of colony forming units (cfu) was counted after 24 h of incubation at 35 ºC.

The biological activities of the synthesized CT complex have been studied for its antibacterial and antifungal activities using disc diffusion methodin vitro against two Gram-positive bacteria Staphylococcus auras (MSSA 22) and B. subtilis (ATCC 6051) and two Gram-negative bacteria E. Coli (K 12) and P. aeruginosa at concentration of 100 lg/ml. Tetracycline was used as standard drug for the comparison of bacterial results and screening data are given in table. The newly synthesized CT complex have exerted significant inhibitory activity against the growth of the tested bacterial strains and data reveal that complex have significant influence on the antibacterial profile of S. aureus and B. subtilis. The complex exhibited good inhibitory results against E. coli and P. aeruginosa as reported in Table II.

Table II
Antibacterial Activity of Synthesized Compound

Compound	Staphylococcus Aureus	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa
5a	32.5(±1.89)	31.0 (±1.09)	29.0 (±0.70)	27.0 (±1.33)
5b	27.0 (±0.87)	28.0 (±0.82)	25.0 (±1.02)	24.0 (±0.72)
5c	24.10 (±1.20)	23.05 (±0.71)	22.0 (±1.67)	21.0 (± 0.68)
Tetracycline (stand.)	35.5 (±0.82)	30.0 (± 0.82)	28.0 (±1.40)	24.0 (± 1.05)

B. Antifungal Activity

The newly synthesized 5a,5b and 5c complex was also screened for its antifungal property against Aspergillus niger (Laboratory isolate), Candida albicans(IQA-109) and Penicilliumsp (Laboratory isolate) in DMSO using standard agar disc diffusion method . The synthesized xxx complex was dissolved in DMSO. All cultures were routinely maintained on Sabouraud's dextrose agar (SDA, Hi-media, Mumbai) and incubated at 28 ºC. Spore of fungal stain lawning was formed from 7 days old culture on sterile normal solution and diluted to obtain approximately105 cfu/ml. The culture was centrifuged at 1000 rpm, pellets was resuspended and diluted in sterile NSS to obtain a viable count 105 cfu/ml. The inoculum of non-sporing fungi C. albicanswas performed by growing the culture in SD broth at 37 ºC overnight. With the help of spreader, 0.1 ml volume of the approximately diluted fungal culture suspension was taken and spread on agar plates. The fungal activity of CT complex was compared with Nystatin (30 lg/disc Hi-Media) as standard drug. The cultures were incubated for 48 h at 37 ºC and the growth was monitored. Antifungal activity was determined by measuring the diameters of the zone (mm) in triplicates sets.

The synthesized 5a, 5b and 5c complex was also examined for its antifungal activity and Nystatin was used as standard drug for comparison of antifungal results. The test CT complex exhibited excellent inhibitory results for A. niger (Laboratory isolate), C. albicans (IQA- 109) and Penicilliumsp (Laboratory isolate) as given in Table III. The data revealed that the CT complex has produced the marked enhancement in the potency as antifungal agent.

Table III
Antifungal Activity of Synthesized Compound

Compound	Aspergillus niger	Candida albicans	Penicillium sp.
5a	39.20(±0.72)	32.0(±0.76)	44.0 (±0.75)
5b	32.0 (±0.82)	34.0(±0.84)	38.0 (±0.84)
5c	27.10 (0.88)	28.0(±0.88)	32.0 (±0.82)
Nystatin (stand.)	26.0 (±0.82)	35.3(±1.02)	25.0 (±0.88)

V. CONCLUSION

The new method for the synthesis of 1,4-diazepines were found clean and had operational simplicity. This simple and reproducible method affords various 1,4-diazepines with short reaction times, excellent yields and without the formation of undesirable by products. The synthesized compound evaluated (5a-c) shows antifungal, antimicrobial and anthelmintic activities. More extensive study is needed to confirm the preliminary results and mode of action studied are required to be able to optimize the effectiveness of this series of compound (5a-c).

7-aminocephalosporanic acid (7-ACA) (1) was diazotized with sodium nitrite and Hydrochloric acid and water to obtain Diazonium salt.(2). The condensation reaction of compound (2) with various β-diketones/ β-ketoesters(3a-c) in the presence of sodium methoxide yields corresponding substituted β-diketones/ β-ketoesters (4a-c). The compound (4a-c) were characterized by elemental analysis and spectral studied.(Table 1,2) compound (4a-c) on the reaction with EDA to affords substituted 1,4-diazepine (5a-c). Scheme-1

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VII. REFERENCES

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