Research Article

Synthesis and Comparative Antimicrobial Study of Beta Lactam Derivatives

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ABSTRACT

This research work has been aimed at the synthesis of some novel heterocyclic analogues like Schiff bases and cyclization of Schiff bases to synthesize Beta lactam derivatives. The Schiff bases have been synthesized by refluxing equimolar (0.1 mole) quantities of a primary amine and aldehyde or ketone. The Beta lactam derivatives have been synthesized by refluxing equimolar (0.1 mole) quantities of Schiff base and chloroacetylchloride in presence of triethyl-amine. The synthesized Beta lactam derivatives were characterized by IR, NMR, Mass spectra followed by elemental analysis for carbon, hydrogen and nitrogen. Beta lactam derivatives are very important in medicinal and pharmaceutical fields because of their wide spectrum of biological activities. All the Beta lactam derivatives were screened for their in vitro antibacterial activity against two Gram +ve (*Staphylococcus aureus, Bacillus subtilis*) & two Gram –ve (*Escherichia coli, Pseudomonas aeruginosa*) bacterial strains by agar-well diffusion method. All Beta lactam derivatives exhibited varied activity against different bacteria. These studies may serve as a basis for the chemical modifications directed towards the development of a new class of antibacterial agents.

Keywords: Schiff bases, Beta lactam derivatives, Spectral study and Antimicrobial Study.

INTRODUCTION

Beta Lactam derivatives are very important in medicinal and pharmaceutical fields because of their wide spectrum of biological activities. From the literature survey up to date, Synthesis and biological activity of Beta lactam derivatives have also been reported by different authors[1-13]. The Beta lactams are 4-membered cyclic amides though the first member was synthesized by Staudinger in 1907, the Beta lactams as a class acquired importance since the discovery of penicillin which contains Beta lactam unit as an essential structural feature of its molecule, this interest continued unabated because of the therapeutic importance of Beta lactam antibiotics and recent findings of new naturally occurring Beta lactams. As a result of vigorous research, a vast literature has been accumulated over the years, and the chemistry of azetidinones continues to be blossoming field. The utility of azetidinones as synthons for various biologically active compounds, as well as their recognition as antibacterial, antifungal[14,15], anticancer[16], and cholesterol absorption inhibitors[17,18] has given impetus to these studies. The new synthesized novel Beta lactam derivatives were evaluated for antibacterial activity. Those compounds which showed significant antibacterial activity were selected for minimum inhibitory concentration studies.

MATERIAL AND METHODS

Experimental (analysis of synthesized compounds)

All chemicals used were of A.R. grade. Melting points were determined using open glass capillaries and are uncorrected. NMR spectra were recorded on NMR-Spectrometer (Bruker 300 M Hz). The ^{1}H-chemical shifts are expressed in ppm relative to tetramethylsilane (Me_4Si). IR spectra were recorded on FT-IR Spectrometer (Lambda Scientific). Micro analysis for C, H, and N were performed on Perkin Elmer CHN Analyzer. Mass spectra were obtained on Shimadzu GCMS-QP-2000 Mass Spectrometer.

General method for preparation of Schiff bases

0.1mole of amine (aniline, phenyl-hydrazine, 2,4-dinitrophenyl-hydrazine etc.) and 0.1 mole of carbonyl compound (antipyrine,

furfuraldehyde, p-hydroxy benzaldehyde etc.) were taken and mixed in glacial acetic acid (25 ml) the resultant mixture was refluxed for (1.5-10 Hrs.), cooled the precipitates and poured on to crush ice. The precipitates were filtered and washed with distilled water (2x10 ml). Recrystalization from ethanol afforded the purified Schiff base. Compounds were also purified by silica gel column chromatography (eluent: ethylacetate/hexane:: 1-3 : 9-7). The reaction was monitored by thin layer chromatography (TLC) and spots were visualized in iodine chamber.

General method for preparation of Beta lactam derivatives

0.1 mole of Schiff Base and 0.1mole of chloroacetylchloride were taken in 25ml ethanol/1,4-dioxane followed by 0.1 mole of triethylamine. The resultant mixture was refluxed for (1-9 Hrs.), cooled to room temperature and poured on to crush ice. Precipitates were filtered on suction pump and washed with distilled water (2x10 ml) and dried in air. Recrystalization from ethanol afforded the purified Beta lactam derivatives. Crude product was also purified by silica gel column chromatography (eluent: ethylacetate/hexane :: 1-3 : 9-7). The reaction was monitored by thin layer chromatography (TLC) and spots were visualized in iodine chamber.

Spectroscopic and elemental analysis data of Beta lactam derivatives is given below:

4-Chloro-1-[(2,4-dinitrophenyl)amino]-2-(furan-2-yl)-1,2-diazetidin-3-one

Yield 59%, dark brown powder; m.p.192-194 ^{0}C; IR (KBr) cm^{-1} : 3277 (N-H str.), 1740 (C=O str.), 1512 [(Ar-NO_2) asym. str.], 1326 [(Ar-NO_2) sym.str.], 1137 (C-NH str.) ^{1}H NMR (DMSO-d_6) :δ: 3.29 (s, 1H, Cl-CH-), 3.58 (s, 1H, Ar.-NH, exchangeable with D_2O), 6.61 (dd, 1H, Furyl C_4–H), 7.57 (d, 1H, Furyl C_3–H), 7.72 (d, 1H, Furyl C_5-H), 7.97-8.29 (m, 3H, 2,4-Dinitrophenyl). MS (m/z) 353.6 ($M^{\cdot+}$), 355.6, 352.5, 204.6, 197.0, 75.1. Anal. Calcd. for C_{12} H_8N_5 O_6Cl : C, 40.75 ; H, 2.28 ; N, 19.80 Found : C, 40.89 ; H, 2.30 ; N, 19.84.

4-Chloro-2-[4-(dimethylamino)phenyl]-1-[(2,4dinitrophenyl)amino]1,2diazetidin-3-one

Yield 50%, dark brown powder; m.p. 239-141 ^{0}C; IR (KBr) cm^{-1} : 3276 (N-H str.), 1743 (C=O str.), 1510 [(Ar-NO_2) asym. str.], 1325 [(Ar-NO_2) sym. str.]. ^{1}H NMR (DMSO-d_6) :δ: 3.07 [s, 6H, -N-$(CH_3)_2$], 3.32 (s, 1H, Cl-CH-), 3.59 (s, 1H, Ar.-NH-), 6.77 (d, 2H, J=9.0 Hz., H_b of -C_6H_4-N<), 7.81 (d, 2H, J=9.0 Hz., H_a of -C_6H_4-N<), 7.98-8.23 (m, 3H, 2,4-Dinitrophenyl). MS (m/z) 406.7 ($M^{\cdot+}$), 408.6, 405.7, 332.6, 330.6, 120.2, 119.2. Anal. Calcd. for $C_{16}H_{15}N_6O_5Cl$:C, 47.24; H, 3.72; N, 20.66; Found C, 47.48; H, 3.80; N, 20.27.

4-Chloro-1-[(2,4-dinitrophenyl)amino]-2-(4-hydroxyphenyl)1,2-diazetidin-3-one

Yield 50%, light maroon powder; m.p. 216-218 ^{0}C; IR (KBr) cm^{-1} : 3296 (O-H str.), 3269 (N-H str.), 1742 (C=O str.), 1515 [(Ar-NO_2) asy. str.], 1332 [(Ar-NO_2) sym. str.]. ^{1}H NMR (DMSO-d_6) :δ: 3.39 (s, 1H, Cl-CH<), 3.43 (s, 1H, -NH- exchangeable with D_2O), 5.51 (s, 1H, Ar.-OH exchangeable with D_2O), 6.69 (d, 2H, J=9.0 Hz., H_b of -C_6H_4-OH), 7.76 (d, 2H, J=9.0 Hz., H_a of -C_6H_4-OH), 8.03-8.34 (m, 3H, 2,4-dinitrophenyl). MS (m/z) 379.7 ($M^{\cdot+}$), 381.7, 378.6, 354.6, 353.6, 106.5, 104.5. Anal. Calcd. for $C_{14}H_{10}N_5O_6Cl$: C, 44.29; H, 2.66; N, 18.45; Found C, 44.41; H, 2.70; N, 18.64.

4-Chloro-2-[4-(dimethyl amino)phenyl]-1-(phenylamino)-1,2-diazetidin-3-one

Yield 60%, blackish brown powder; m.p. 64-66 ^{0}C; IR (KBr) cm^{-1}: 3270 (N-H str.), 1741 (C=O str.), 1136 (C-NH str.). ^{1}H NMR (DMSO-d_6) : δ: 3.05 [s, 6H, -N-$(CH_3)_2$], 3.34 (s, 1H, Cl-CH<), 3.57 (s, 1H, Ar.-NH, exchangeable with D_2O), 6.75 (d, 2H, J=9.0 Hz., H_b of -C_6H_4-N<), 7.17-7.62 (m, 5H, Ph-NH-), 7.89 (d, 2H, J=9.0 Hz., H_a of -C_6H_4-N<), MS (m/z) 316.8 ($M^{\cdot+}$), 316.6, 301.6, 239.6, 237.6, 224.7, 196.6. Anal. Calcd. for C_{16} H_{17} N_4 O Cl : C, 60.66; H, 5.41; N, 17.69. Found C, 60.85; H, 5.45; N, 17.73.

4-Chloro-2-[4-(dimethyl amino)phenyl]-1-phenyl-1,2-diazetidin-3-one

Yield 64%, light brown powder; m.p. 96-98 ^{0}C; IR (KBr) cm^{-1} : 1738 (C=O str.). ^{1}H NMR ($CDCl_3$) : δ : 3.10 [s, 6H, $(CH_3)_2$-N-), 3.30 (s, 1H, Cl-CH<), 6.79 (d, 2H, J=9.0 Hz., H_b of -C_6H_4-N<), 7.33-7.78 (m, 5H, Ph-N<), 7.87 (d, 2H, J=9.0 Hz., H_a of -C_6H_4-N<). MS (m/z) 301.8 ($M^{\cdot+}$), 301.8, 226.5, 225.5, 196.5, 106.5, 104.5. Anal. Calcd. for $C_{16}H_{16}N_3OCl$: C, 63.68; H, 5.34; N, 13.93. Found C, 63.60; H, 5.28; N, 13.99.

4-Chloro-1-[(2,4dinitrophenyl)amino]-2-(2-hydroxyphenyl)-1,2-diazetidin-3-one

Yield 65%, red powder; m.p. 242-244 ^{0}C; IR (KBr) cm^{-1} : 3302 (O-H str.), 3270 (N-H str.), 1739 (C=O str.), 1513 [(Ar-NO_2) asym. str.], 1335 [(Ar-NO_2) sym. str.]. ^{1}H NMR (DMSO-d_6) : δ : 3.40 (s, 1H, Cl-CH<), 3.47 (s, 1H, Ar.-NH, exchangeable with D_2O), 5.53 (s, 1H, Ar.-OH), 6.93-7.24 (m, 4H, Ar. ring-A), 8.06-8.17 (m, 3H, Ar. ring-B). MS (m/z) 379.7 ($M^{\cdot+}$), 381.7, 378.6, 354.5, 353.5, 106.4, 104.4. Anal. Calcd.

for $C_{14}H_{10}N_5O_6Cl$: C, 44.29; H, 2.66; N, 18.45; Found C, 44.40; H, 2.68; N, 18.50.

4-Chloro-2-(2-hydroxyphenyl)-1-(phenylamino)-1,2-diazetidin-3-one

Yield 65%, light brown powder; m.p. 130-132 ^{0}C; IR (KBr) cm^{-1} : 3301 (O-H str.), 3275 (N-H str.), 1741 (C=O str.), 1135 (C-NH str.), ^{1}H NMR ($CDCl_3$: δ : 3.35 (s, 1H, Cl-CH), 3.61 (s, 1H, Ar.-NH-,exchangeable with D_2O), 5.50 (s, 1H, Ar.-OH, exchangeable with D_2O) 6.87-7.01 (m, 4H, Ar. ring-A), 7.13-7.32 (m, 5H, Ar. ring-B). MS (m/z) 289.7 (M^{+}), 290.5, 274.6, 261.7, 214.6, 212.6, 196.6. Anal. Calcd. for $C_{14}H_{12}N_3O_2Cl$: C, 58.04; H, 4.18; N, 14.51. Found C, 58.46; H, 4.25; N, 14.44.

1-(4-Amino-1,5-dimethyl-2-phenyl-2,3-dihydro-1*H*-pyrazol-3-yl)-4-chloro2(furan -2-yl)-1,2-diazetidin-3-one

Yield 35%, brownish-yellow powder; m.p.170-172 ^{0}C; IR (KBr) cm^{-1} : 3279(N-H str.), 1740 (C=O str.), ^{1}H NMR ($CDCl_3$) : δ : 1.29 (s, 3H, CH_3-C<), 2.53 (s, 3H, CH_3-N<), 2.62 (s, 1H, N-CH-), 3.17 (s, 1H, Cl-CH<), 3.57 (s, 2H, NH_2-C=C<,exchangeable with D_2O), 6.58 (dd, 1H, Furyl C_4-H), 6.68 (d, 1H, Furyl C_3-H), 6.89 (d, 1H, Furyl C_5-H), 6.98-7.36 (m, 5H, C_6H_5-N<). MS (m/z) 359.8(M^{+}), 315.7, 313.7, 203.6, 201.6, 106.5. Anal. Calcd. for $C_{17}H_{18}N_5O_2Cl$: C, 56.75; H, 5.04; N, 19.47. Found C, 56.60; H, 5.11; N, 19.35.

1-{4-Amino-2-[(2,4dinitrophenyl)amino]-1,5-dimethyl-2,3-dihydro-1H-pyrazol-3-yl}-4-chloro-2-(furan-2-yl)-1,2-diazetidin-3-one

Yield 55%, yellow powder; m.p.195-197 ^{0}C; IR (KBr) cm^{-1}: 1738 (C=O str.), 1549 [(Ar-NO_2) asym. str.], 1348 [(Ar-NO_2) sym. str.]. ^{1}H NMR ($CDCl_3$) :δ: 1.20 (s, 3H, CH_3-C=C<), 2.59 (s, 1H, N-CH-C=), 2.70 (s, 3H, CH_3-N-N<), 3.21 (s, 1H, Cl-CH<), 3.61 (s, 2H, NH_2- C=C<, exchangeable with D_2O),), 4.19 (s, 1H, Ar.-NH-N<, exchangeable with D_2O),), 6.45 (dd, 1H, Furyl C_4-H), 6.95 (d, 1H,Furyl C_3-H), 7.07 (d, 1H, Furyl C_5-H), 7.19-8.38 (m, 3H, -C_6H_3-NO_2). MS (m/z) 464.8(M^{+}), 362.3, 337.7, 277.2, 201.3, 200.3. Anal. Calcd. for $C_{17}H_{17}N_8O_6Cl$: C, 43.93; H, 3.69; N, 24.12 Found C, 42.01; H, 3.75; N, 24.14.

RESULTS AND DISCUSSION

All the test compounds (Beta lactam derivatives) were screened for their in vitro antibacterial activity by agar-well diffusion method against Gram +ve (*Staphylococcus aureus, Bacillus subtilis*) & Gram –ve (*Escherichia coli, Pseudomonas aeruginosa*) microorganisms by preparing 200 µg/ml of test solution of each compound. Zone of inhibitions in mm were noted. The zone of inhibition of Beta lactam derivatives varied from 08 to 25 mm. The results have been shown in **Table No. 1**, those compounds which showed significant antibacterial activity were selected for minimum inhibitory concentration (MIC) studies by making dilutions of different concentrations varying from 1 to 50 µg/ml and the results of minimum inhibitory concentrations have been shown in **Table No. 2.** The activity of control dimethyl sulphoxide was also checked for its toxicity. Ampicillin was used as standard antibacterial agent for comparing the activity of test compounds.

The present investigations suggest that

All the test compounds (Beta lactam derivatives) have shown antibacterial activity against Gram +ve (*Staphylococcus aureus, Bacillus subtilis*) & Gram –ve (*Escherichia coli, Pseudomonas aeruginosa*) microorganisms. 1, 2, 4, 6 & 7 numbered compounds have shown significant activity. 3 numbered compound has shown moderate to significant activity. 5, 8 & 9 numbered compounds have shown weak to significant activity. It has been found that amongst all the test compounds taken for antibacterial evaluation, Comp. No. 2 & Comp. No. 7 have shown maximum activity against *Bacillus subtilis* & *Staphylococcus aureus,* respectively. The activity of control dimethyl sulphoxide was also checked for its toxicity and it has been found that it has no effect on the growth of any microorganisms taken.

CONCLUSION

All the Beta lactam derivatives exhibited varied activity against different bacteria. These studies may serve as a basis for the chemical modifications directed towards the development of a new class of antibacterial agents.

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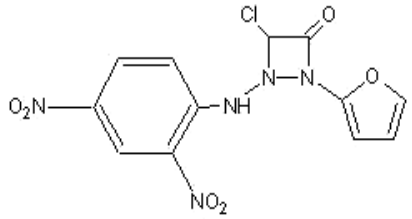
Table 1: Screening of Schiff bases and Beta-lactam derivatives For anti-bacterial activity (zone of inhibition in mm.)

	MICROBIAL SPECIES			
Compound No.	***E. coli***	***B. subtilis***	***P. aeruginosa***	***S. aureus***
MTCC Code	**1687**	**441**	**424**	**737**
1	18	21	17	22
2	21	25	21	23
3	15	17	15	16
4	21	23	17	21
5	15	08	18	14
6	22	24	18	24
7	17	22	18	25
8	15	17	08	08
9	14	18	08	15

00-09: Weak activity, 10-16: Moderate activity, 17-25: Significant activity

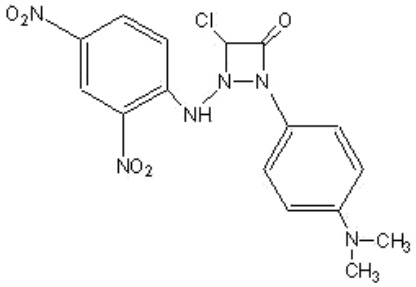
Table 2: Minimum inhibitory concentration (µg/ml.) of the Following compounds against selected bacteria

Compound No's.	1	2	4	6	7	Ampicillin
E. coli MTCC-1687	15	10	10	08	15	04
B. subtilis MTCC-441	10	07	08	07	10	03
p. aeruginosa MTCC-424	15	10	20	15	15	05
S. aureus MTCC-737	10	10	10	08	07	03



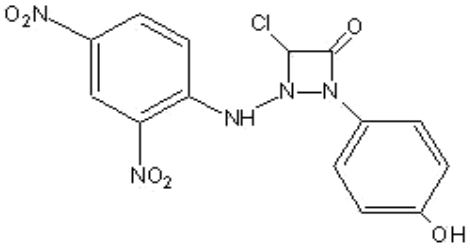
4-Chloro-1-[(2,4-dinitrophenyl)amino]-2-(furan-2-yl)-1,2-diazetidin-3-one

Comp. No. 1



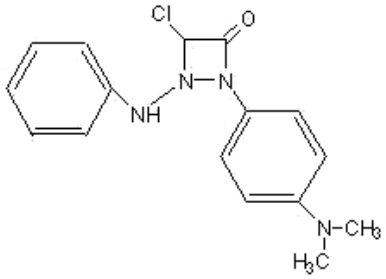
4-Chloro-2-[4-(dimethylamino)phenyl]-1-[(2,4-dinitro phenyl)amino]-1,2-diazetidin-3-one

Comp. No. 2



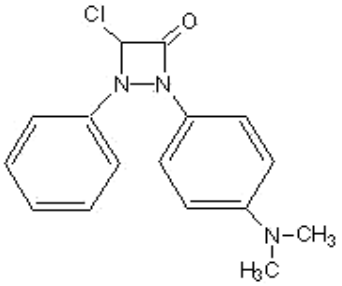
4-Chloro-1-[(2,4-dinitrophenyl)amino]-2-(4-hydroxy phenyl)-1,2-diazetidin-3-one

Comp. No. 3



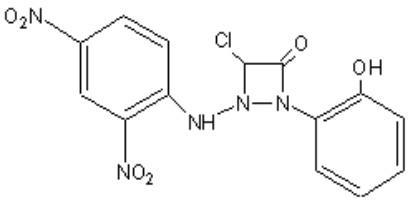
4-Chloro-2-[4-(dimethylamino)phenyl]-1-(phenyl amino)-1,2-diazetidin-3-one

Comp. No. 4



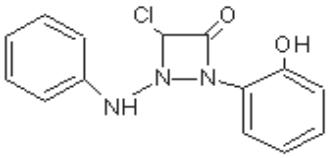
4-Chloro-2-[4-(dimethylamino)phenyl]-1-phenyl -1,2-diazetidin-3-one

Comp. No. 5



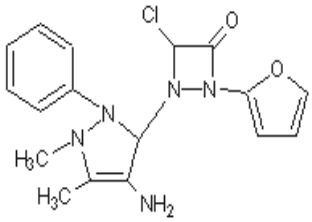
4-Chloro-1-[(2,4-dinitrophenyl)amino]-2-(2-hydroxyphenyl) -1,2-diazetidin-3-one

Comp. No. 6



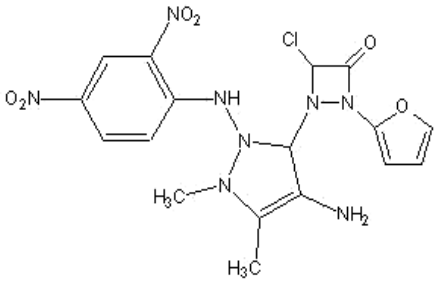
4-Chloro-2-(2-hydroxyphenyl)-1-(phenylamino) -1,2-diazetidin-3-one

Comp. No. 7



1-(4-Amino-1,5-dimethyl-2-phenyl-2,3-dihydro-1*H*-pyrazol -3-yl)-4-chloro-2-(furan-2-yl)-1,2-diazetidin-3-one

Comp. No. 8



1-(4-Amino-2-[(2,4-dinitrophenyl)amino]-1,5-dimethyl-2,3-dihydro-1*H*-pyrazol-3-yl)-4-chloro-2-(furan-2-yl)-1,2-diazetidin-3-one

Comp. No. 9

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