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SYNTHESIS AND EVALUATION OF ANTI-MICROBIAL ACTIVITY OF SOME SUBSTITUTED NOVEL AZETIDINONE DERIVATIVES

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ABSTRACT

A series of Azetidinone analogues were synthesized characterized and screened for the presence of anti-microbial activity. The final test compounds were characterized by UV, IR and NMR. The purity of the compounds was ascertained by TLC technique. The test compounds were tested on four bacterial species i.e., E.coli, S.aureus, P.aeruginosa, B.subtilis. Amoxicillin was used as a standard drug for comparing the test results.

Keywords: Azetidinone analogues, Characterization, Anti-microbial activity.

INTRODUCTION

Diseases caused by bacteria are widespread worldwide. Many natural and synthetic products containing Azetidinone moiety as Penicillins, Cephalosporins were reported to possess various Anti-bacterial property. Many of these Antimicrobial activities were attributed to the presence of chiral centres present at 5th and 6th position of beta lactam ring. Azetidinone analogues have been extensively explored for developing pharmaceutical antibiotics like Penicillins, Cephalosporins, Carbapenas, Monobactams etc. A large number of 3-chloro monocyclic beta lactams having substitutions at position 1 and 4 possess powerful anti-microbial, anti-convulsant and anti-tubercular activity[1]. The simplest beta lactam contains 2-azetidinone moiety.

The treatment of many infections is mainly based on the use of Antibiotics. In recent years, a number of Antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistant genes[2]. In addition this problem, Antibiotics are often associated with mild to severe adverse effects[3]. Azetidinone analogues are particularly used to possess various microbial activities like anti-fungal, anti-viral, anti-bacterial etc[4]. Therefore, there is a need to develop alternatives or novel antibacterial molecules for the treatment of infectious diseases. Hence the objective of the present study is to synthesize a novel azetidinone analogue and evaluate its antimicrobial property.

EXPERIMENTAL

Synthetic chemistry

The present study was undertaken to synthesize a series of Phenyl hydrazinamides, azetidinone analogues and to screen for anti-microbial activity. The target compounds were obtained in four steps.

Step-I

1.446gms of phenyl hydrazine was dissolved in ethyl acetate and stirred continuously. 0.951ml of ethyl chloro formate was dissolved in ethyl acetate. Both the solutions were mixed and few drops of triethylamine were added. Then this mixture was kept for stirring in the presence of ice cubes for 6 hours. After 6 hours the substance was filtered and dried.

Step-II
The first step intermediate ethyl ester of phenyl hydrazine was weighed and dissolved in 2ml of hydrazine hydrate in ethanol and was refluxed for 6 hours. From the resultant mixture excess of ethanol was removed by distillation on cooling and then yellow crystals that were formed are collected and dried.

STEP-III
The second intermediate hydrazide was weighed and dissolved in minimum quantity of ethanol and 2ml of benzaldehyde (0.01mole) was also dissolved in minimum quantity of ethanol. After mixing both the solutions, it was refluxed together employing sulphuric acid about 0.01mole as catalyst in a round bottomed flask on a water bath for 6hrs. In the similar way, the second step intermediate is also treated with various substituted benzaldehydes i.e., p-hydroxy benzaldehyde, p-methoxy benzaldehyde, p-methyl benzaldehyde, p-dimethyl amino benzaldehyde to get the corresponding substituted Schiff's bases.

STEP-IV
The third step intermediates i.e. substituted Schiff's bases were weighed and chloroacetyl chloride was added drop wise to Schiff's bases (0.0083 moles) followed with triethyl amine (0.0166 mole) in dioxane at 5-10^{o}C. The mixtures were stirred for 20 hours and left at room temperature for 3 days. Then the mixtures were filtered, dried and recrystallized from ethanol. The % yield, Rf values and melting points of the synthesized derivatives are tabulated in Table-1.

Table-1: Physical data of Azetidinone derivatives (1a – 1e)

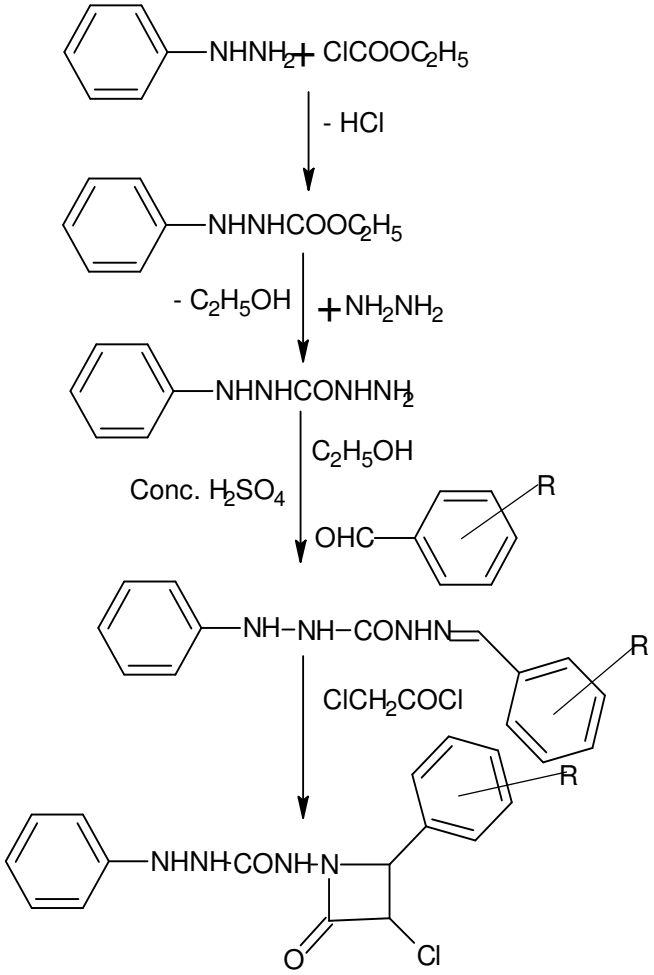
Compound No.	R	Melting point (^{o}C)	R_f value	Yield (%)
1a	-H	122-129	0.53	76.5
1b	-OH	130-136	0.51	71.24
1c	$-OCH_3$	139-147	0.48	68.32
1d	$-CH_3$	125-133	0.63	66.54
1e	$-N(CH_3)_2$	150-152	0.84	61.12

Characterization of the synthesized compounds

1. N-[3-chloro-2-(phenyl)-4-oxaazetidin-1-yl]Phenyl hydrazinamide (1a)
Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapours. The final compound displayed a Rf value of 0.53. The final compound was also characterized by UV and IR. The UV absorption peak of the compound was observed at 235nm. The IR spectra of the final compound displayed bands at C=O of beta lactam 1735cm^{-1}, C=O stretch of amide 1680cm^{-1}, C-H stretch of aromatic ring 3100 cm^{-1}, N-H stretching displayed bands at 1495- 1520 cm^{-1}, C=O stretching of acyl at 1660 cm^{-1}, bands at 789.88 cm^{-1} due to C-Cl stretching of the beta lactam ring. HNMR (DMSO-d_6) - Δ6.33-6.35 (beta lactam-phenyl), 2.119 (-NH 2^{o}amine), 7.00 – 7.48 (5H, Ar-H).

2. N-[3-chloro-2-(4^{1}-hydroxy phenyl)-4-oxaazetidin-1-yl]Phenyl hydrazinamide (1b)
Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapours. The final compound displayed a Rf value of 0.51. The final compound was also characterized by UV and IR. The UV absorption peak of the compound was observed at 241nm. The IR spectra of the final compound displayed bands at C=O of beta lactam 1735cm^{-1}, C=O stretch of amide 1680cm^{-1}, C-H stretch of aromatic ring 3100 cm^{-1}, N-H stretching displayed bands at 1495-1520 cm^{-1}, C=O stretching of acyl at 1660 cm^{-1}, bands at 789.88 cm^{-1} due to C-Cl stretching of the beta lactam ring, bands at O-H stretch broad peak observed in 3400 cm^{-1}. HNMR (DMSO-d_6) - Δ6.33-6.35 (beta

lactam-phenyl), 2.119 (-NH 2°amine), 7.00 – 7.48 (5H, Ar-H), Δ6.94-7.39 (substituted hydroxy benzaldehyde).



Where R=H, - OH, $-CH_3$, $-OCH_3$, $-N(CH_3)_2$

Scheme-1: Schematic representation of the synthesis

3. N-[3-chloro-2-(4[1]-methoxy phenyl)-4-oxaazetidin-1-yl]Phenyl hydrazinamide (1c)

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapours. The final compound displayed a Rf value of 0.48. The final compound was also characterized by UV and IR. The UV absorption peak of the compound was observed at 243nm. The IR spectra of the final compound displayed bands at C=O of beta lactam 1735cm^{-1}, C=O stretch of amide 1680cm^{-1}, C-H stretch of aromatic ring 3100 cm^{-1}, N-H stretching displayed bands at 1495-1520 cm^{-1}, C=O stretching of acyl at 1660 cm^{-1}, bands at 789.88 cm^{-1} due to C-Cl stretching of the beta lactam ring, bands at 1744 cm^{-1} was observed due to ester stretching. HNMR (DMSO-d_6) - Δ6.33-6.35 (beta lactam-phenyl), 2.119 (-NH 2°amine), 7.00 – 7.48 (5H, Ar-H), Δ6.20-6.23 (substituted methoxy benzaldehyde).

4. N-[3-chloro-2-(4[1]-methyl phenyl)-4-oxaazetidin-1-yl]Phenyl hydrazinamide (1d)

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots

were observed in iodine vapours. The final compound displayed a Rf value of 0.63. The final compound was also characterized by UV and IR. The UV absorption peak of the compound was observed at 240nm. The IR spectra of the final compound displayed bands at C=O of beta lactam 1735cm^{-1}, C=O stretch of amide 1680cm^{-1}, C-H stretch of aromatic ring 3100 cm^{-1}, N-H stretching displayed bands at 1495-1520 cm^{-1}, C=O stretching of acyl at 1660 cm^{-1}, bands at 789.88 cm^{-1} due to C-Cl stretching of the beta lactam ring. HNMR (DMSO-d_6) - Δ6.33-6.35 (beta lactam-phenyl), 2.119 (-NH 2^{o}amine), 7.00 – 7.48 (5H, Ar-H), Δ6.95-7.40 (substituted methyl benzaldehyde).

5. N-[3-chloro-2-(4^1-dimethyl amino phenyl)-4-oxaazetidin-1-yl]Phenyl hydrazinamide (1e)

Melting point was determined in an open capillary tube using an electro thermal digital melting point apparatus and is uncorrected. Compound was checked for its purity by TLC on silica gel plates and spots were observed in iodine vapours. The final compound displayed a Rf value of 0.84. The final compound was also characterized by UV and IR. The UV absorption peak of the compound was observed at 253nm. The IR spectra of the final compound displayed bands at C=O of beta lactam 1735cm^{-1}, C=O stretch of amide 1680cm^{-1}, C-H stretch of aromatic ring 3100 cm^{-1}, N-H stretching displayed bands at 1495-1520 cm^{-1}, C=O stretching of acyl at 1660 cm^{-1}, bands at 789.88 cm^{-1} due to C-Cl stretching of the beta lactam ring, C-N displayed bands at 1250 cm^{-1}. HNMR (DMSO-d_6) - Δ6.33-6.35 (beta lactam-phenyl), 2.119 (-NH 2^{o}amine), 7.00 – 7.48 (5H, Ar-H), Δ5.99-6.00 (substituted dimethyl amino benzaldehyde).

Preparation of the test compound

The synthesized compounds were insoluble in water. So, an organic solvent like 10% v/v Dimethyl sulphoxide (DMSO) in distilled water was prepared. The test compounds were prepared in the concentration of 50µg/ml using DMSO as the solvent.

Standard drug

Amoxicillin was taken as the standard drug so as to compare the anti-microbial activity of the test compounds. Amoxicillin is also prepared in the concentration of 50µg/ml in DMSO.10% of DMSO in distilled water is used as control.

Microorganisms used

Staphylococcus aureus, Bacillus subtilis, E.coli and Pseudomonas aeruginosa pure cultures were chosen for the study.

Anti-microbial activity

In-vitro anti-microbial study was carried on Muller Hinton agar plates which were maintained at 37^{o}C for 24hrs by agar diffusion cup plate method. All the test compounds synthesized were screened for anti-microbial activity at 50µg/ml concentration against the following four bacterial strains i.e., Staphylococcus aureus, Bacillus subtilis, E.coli and Pseudomonas aeruginosa. Staphylococcus aureus, Bacillus subtilis, E.coli and Pseudomonas. Amoxicillin was used as the standard drug to compare the test results under similar conditions. 10% Dimethyl Sulphoxide was used as solvent control for anti-bacterial activity. The results of the test compound were expressed in terms of Zone of Inhibition (mm). The zone of inhibition of the test and standard are presented in Table-2.

Table-2: Statistical Data of Anti-microbial activity

Compound No.	Anti-microbial activity at 50µg/ml Zone of inhibition (mm)			
	Staphylococcus aureus	*Bacillus subtilis*	*E.coli*	*Pseudomonas aeruginosa*
1a	13.62 ± 0.51	10.35 ± 0.54	13.65 ± 0.24	15.69 ± 0.48
1b	10.15 ± 0.28	11.88 ± 0.47	20.94 ± 0.28	18.35 ± 0.35

1c	15.89 ± 0.45	14.96 ± 0.51	17.36 ± 0.06	14.26 ± 0.23
1d	14.69 ± 0.32	13.58 ± 0.17	16.68 ± 0.42	16.37 ± 0.18
1e	11.98 ± 0.45	10.26 ± 0.58	13.22 ± 0.49	15.86 ± 0.23
Standard	19.25 ± 0.56	15.87 ± 0.39	21.43 ± 0.36	22.67 ± 0.47

All values are mean ± SEM and analyzed by one way ANOVA followed by Dunnet test

RESULTS AND DISCUSSION

The azetidinone analogues were synthesized with the starting material phenyl hydrazine and the final compounds (1a – 1e) were synthesized with two intermediate compounds formed in between. The final compounds were checked for their purity by thin layer chromatography technique. The compounds were also characterized by UV, IR and NMR. As beta lactam antibiotics are very popular anti-microbial agents worldwide, the synthesized azetidinone analogues were screened for anti-microbial activity. The compounds showed mild to moderate level of Anti-microbial activity when tested against four species of bacteria. Azetidinone derivatives 1a, 1e were active against Pseudomonas aeruginosa and derivatives 1b, 1c were active against E.coli. Derivative 1d was found to be active against E.coli and Pseudomonas aeruginosa. The active anti-microbial activity of the 1d azetidinone derivative might be attributed to the electron releasing nature of the $–CH_3$ group. The test compounds can be further tested on various species of fungus and moulds to ascertain its anti-microbial activity on a broader scale which is our future plan of research work.

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