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STUDIES ON THE SYNTHESIS AND *IN-VITRO* ANTIMICROBIAL ACTIVITY EVALUATION OF SOME NOVEL β-LACTAMS CONTAINING SULFA DRUGS

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Keywords:

2-Azetidinones, β-Lactams, Antimicrobial activity screening, Sulfa drugs, Biological activity

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ABSTRACT: The present study aims to synthesize 2-azetidinone derivatives; 4-(3-chloro-1-(4-substituted-phenyl)-4-oxoazetidin-2-yl)-2-methoxyphenyl-acetate (c1-c6) and 2-allyl-4-(3-chloro-1-(4-Substituted-phenyl)-4-oxoazetidin-2-yl)-6-methoxyphenyl4-methylbenzenesulfonate (d1-d6) by electrocyclisation of imines with chloroacetylchloride in the presence of triethyle - amine and 1, 4-dioxane. The synthesized compounds were characterized by FT-IR, ^{1}H NMR spectra and elemental analysis and were screened *in-vitro* against three bacterial strains namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and two *fungal* strains namely *Aspergillus niger*, *Aspergillus flavus* by disc diffusion method. The compounds exhibited good antimicrobial activity.

INTRODUCTION: The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. β-lactam antibiotics such as penicillin have been the most widely used as antimicrobial drugs for many years. The development of multi-drug resistance has reduced the effectiveness of β-lactams and other antimicrobial drugs. The unique structural feature of β-lactams is the presence of four-membered β-lactam (2-azetidinone) ring which operate by forming a covalent adducts with membrane bound bacterial transpeptidases which are also known as penicillin binding protein and prevent the cell wall synthesis [1].

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The β-lactam ring system is found in a number of broad-spectrum β-lactam antibiotics like penicillins [2], cephalosporins [3], carbapenems, nocardicins and monobactams, which have been widely used as chemotherapeutics for treating microbial diseases [4]. β-lactams as heterocyclic compounds comprise the largest group of antibacterial agents and are preferred in antimicrobial therapy [5] due to their bactericidal properties and limited toxicity to humans. The importance of these heterocyclic compounds has been recognized and extensively studied in the field of synthetic organic chemistry due to their significant properties and applications. The interest continued unabated because of the therapeutic importance of β-lactam antibiotics and recent findings of new naturally occurring β-lactams. These also shows many other interesting biological properties, such as antibacterial [6, 7] antifungal [8], anti-inflammatory [9], anticancer [10 - 12], cholesterol absorption inhibitors [13, 14] and anti-hepatitis [15], human cytomegalovirus protease inhibitors [16], thrombin inhibitors [17], anti-hyperglycemic [18], anti-HIV [19], analgesic activities [20] and serine dependent enzyme inhibitors [21 - 22].

From medical point of view sulfonamides are used to treat bacterial type infections as antibiotics in animals as well as human beings [23 - 24]. The involvement of agriculture and pharma in enjoying various biological activities of sulfonamides is also producing both attention and attraction [25]. Sulfonamides are the main functional portion of many structures of drugs principally because of its stability as well as tolerance in humans [26 - 27]. Sulfa drugs are used for the treatment of gut infections, conjunctivitis, urinary tract infections, meningitis, eye lotions, bacillary dysentery and malaria [28 - 29].

Keeping this in mind the present effort is directed towards developing new antimicrobial agents with maximum efficacy in minimum concentrations. For this purpose we have synthesized two series of 2-azetidinones; 4-(3-chloro-1-(4-substituted-phenyl)-4-oxoazetidin-2-yl)-2-methoxyphenyl-acetate and 2- allyl- 4- (3- chloro- 1- (4- Substituted- phenyl)- 4- oxoazetidin- 2- yl) - 6- methoxyphenyl4-methyl benzenesulfonate and screened *in - vitro* against selected microbial pathogens.

MATERIALS AND METHODS:

Reagents and Instruments Used: All materials were of commercial reagents grade and purchased from Sigma-Aldrich. All reaction were monitored by Thin Layer Chromatography (TLC) on pre-coated sheets of 25 DS alufolin kieselgel 60 F_{254} silica gel 60 F_{254} (Merck) using UV-Vis fluorescence analysis chamber for detection. Melting points were measured in open glass capillary and are uncorrected. ^{1}H NMR spectra were recorded on a BRUKER AVANCE II-129 400 MHz FT-NMR spectrometer. FT-IR spectra were recorded on a Perkin Elmer FT-IR spectrophotometer. Elemental analysis was performed on Elementarvario MICRO cube CHN analyzer. All synthesized compound were purified by recrystellization in ethanol.

Synthesis of Precursors and Imines: Precursors and imines were synthesized according to literature[30].

General Procedure for the Synthesis of β-Lactams (c1-c6): Imine (a1-a6) (0.001mol) was added to a constantly stirred solution of 1, 4-dioxane (15ml), triethylamine (0.001mol) and chloroacetylchoride (0.001mol). The reaction mixture was stirred at 50 °C. The reaction vessel was then kept at room temperature for 30 min and refluxed for eight hours. On cooling the precipitate was obtained, which was filtered and thoroughly washed with water.

Synthesis of 4-(3-chloro-4-oxo-1-(4-(N-(thiazol-2- yl) sulfamoyl) phenyl) azetidin- 2- yl)- 2-methoxyphenyl-acetate (c6): Methyl-2-methoxy-4-[(*E*)- {[4-(1, 3- thiazol- 2- ylsulfamoyl) phenyl] imino}methyl]phenyl acetate (0.001mol) was added to a constantly stirred solution of 1,4-dioxane (15ml), triethylamine (0.001mol) and chloroacetylchoride (0.001mol). The reaction mixture was stirred at 50 °C. The reaction vessel was then kept at room temperature for 30 min and refluxed for eight hours. On cooling the precipitate was obtained, which was filtered and thoroughly washed with water.

Analytical Data: Molecular formula $C_{21}H_{18}ClN_3O_6S_2$; Yield; 51%, m.p.; 189 °C; Elemental analysis data Found (required %) C; 49.43 (49.6), H; 3.38 (3.57), N; 6.75 (6.98); FT-IR absorption frequencies in KBr (cm^{-1}) N-H (3378.67), C=C-(Ar) (1596), -OCH_3 (1471.42), C-H (Ar) (2846.4), C=O (β-lactam ring) (1783.08), C=O (ester) (1751.05);

^{1}H NMR Spectra (DMSO) δppm: CH_3 (ester) (3HS) 2.52, OCH_3 (3HS) 3.902, NH (1HS) 4.002, CH (β-lactam) (1HD) 5.156, CH-Cl (β-lactam) (1HD) 5.516, CH Ar (Ring A) (1HS) 7.116, Six doublets of Ar-rings (8HD) 6.526-8.813.

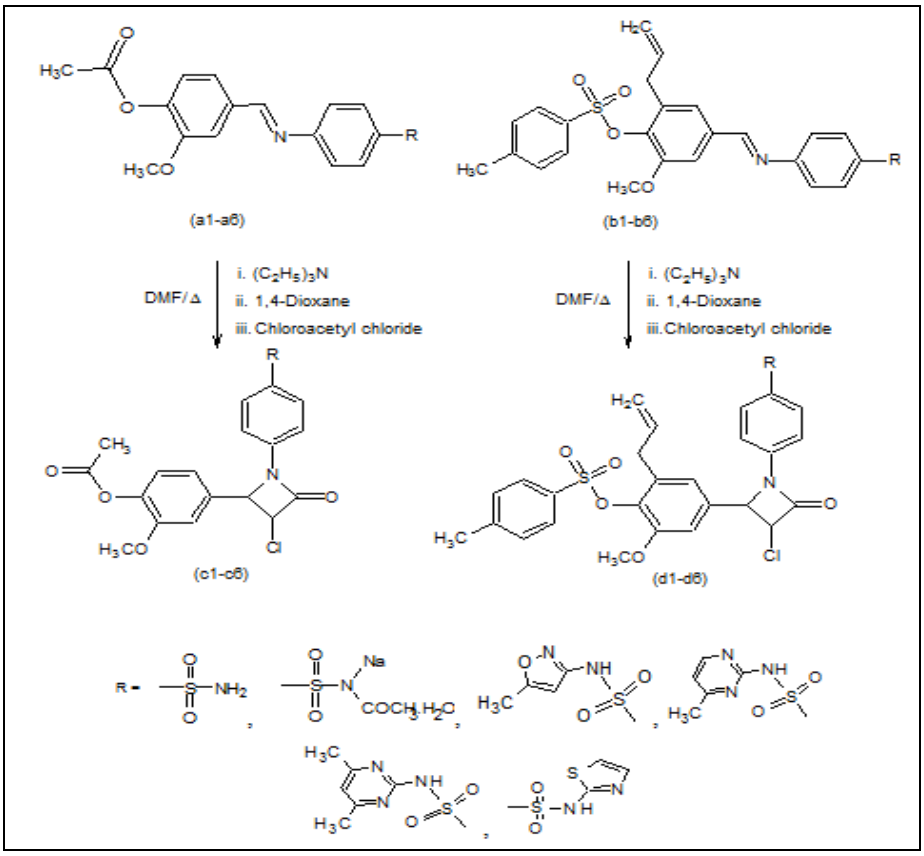
General Procedure for the Synthesis of β-Lactams (d1-d6): Imine (b1-b6) (0.001mol) was added to a constantly stirred solution of 1, 4-dioxane (15ml), triethylamine (0.001mol) and chloroacetylchoride (0.001mol). The reaction mixture was stirred at 50 °C. The reaction vessel was then kept at room temperature for 30 min and refluxed for 8 hours. On cooling the precipitate was obtained, which was filtered thoroughly, washed with water.

Synthesis of 2-allyl-4-(3-chloro-4-oxo-1-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)azetidin- 2-yl)- 6-methoxyphenyl-4-methylbenzenesulfonate (d6): (Z)- 2-allyl- 6-methoxy- 4- (((4-(N-(thiazol- 2-yl) sulfamoyl) phenyl) imino) methyl) phenyl-4-methyl-benzenesulfonate (0.001 mol) was added to a constantly stirred solution of 1, 4-dioxane (15ml)

triethylamine (0.001 mol) and chloroacetylchoride (0.001 mol). The reaction mixture was stirred at 50 °C. The reaction vessel was then kept at room temperature for 30 min and refluxed for 8 h. On cooling the precipitate was obtained, which was filtered and thoroughly washed with water.

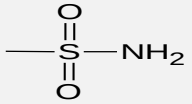
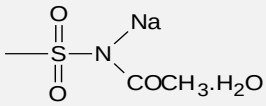
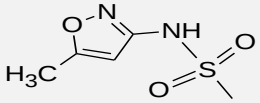
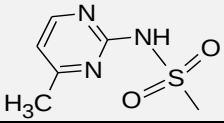
Analytical Data: Molecular formula $C_{29}H_{26}ClN_3O_7S_3$; Yield; 64% m.p.; 186 °C; Elemental analysis data Found (requires %) C 53.43 (52.76), H 3.58 (3.97), N 5.95 (6.36); FT-IR absorption frequencies in KBr (cm^{-1}) N-H (3300.67), C=C- (Ar) (1550), -OCH_3 (1425.42), C-H (Ar) (2900.4), C=O (β-lactam ring) (1779.08), C=O (ester) (1740.05).

^{1}H NMR Spectra (DMSO) δppm: CH_3 (3HS) 2.28, CH_2 (2HS allyl group) 3.22 OCH_3 (3HS) 3.52, NH (1HS) 4.04, CH_2 (2HS) 5.00, CH (β-lactam) (1HD) 5.156, CH-Cl (β-lactam) (1HD) 5.516, CH Ar (Ring A) (1HS) 7.116, Six doublets of Ar-rings (8HD) 6.526-8.813.



SCHEME 1: ROUTE FOR THE SYNTHESIS OF β-LACTAMS

TABLE 1: PHYSICAL DATA OF β-LACTAMS DERIVED FROM METHYL-2-METHOXY-4-[(*E*)-{[4-(1, 3-THIAZOL-2-YLSULFAMOYL)PHENYL]IMINO}METHYL]PHENYLACETATE

S. no	Compound	R	M. P. (°C)	Yields %	Molecular formula
1.	c1		186	54	$C_{18}H_{17}ClN_2O_6S$
2.	c2		203	52	$C_{20}H_{20}ClN_2NaO_8S$
3.	c3		179	45	$C_{22}H_{20}ClN_3O_7S$
4.	c4		182	58	$C_{23}H_{21}ClN_4O_6S$

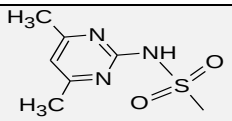
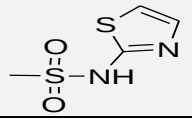
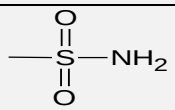
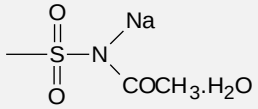
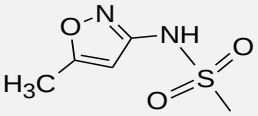
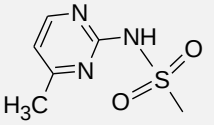
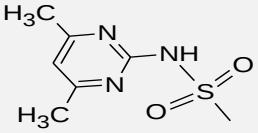
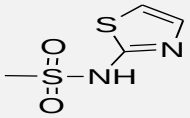
5.	c5		194	47	$C_{24}H_{23}ClN_4O_6S$
6.	c6		189	51	$C_{21}H_{18}ClN_3O_6S_2$

TABLE 2: PHYSICAL DATA OF β-LACTAMS DERIVED FROM (Z)-2-ALLYL-6-METHOXY-4-(((4-(N-(THIAZOL-2-YL)SULFAMOYL)PHENYL)IMINO)METHYL)PHENYL-4-METHYLBENZENESULFONATE

S. no	Compound	R	M.P. (°C)	Yield %	Molecular formula
1.	d1		181	72	$C_{26}H_{25}ClN_2O_7S_2$
2.	d2		198	55	$C_{28}H_{28}ClN_2NaO_9S_2$
3.	d3		174	63	$C_{30}H_{28}ClN_3O_8S_2$
4.	d4		176	69	$C_{31}H_{29}ClN_4O_7S_2$
5.	d5		192	58	$C_{32}H_{31}ClN_4O_7S_2$
6.	d6		186	64	$C_{29}H_{26}ClN_3O_7S_3$

Antimicrobial Activity: All the synthesized β-lactams (c1-c6) and (d1-d6) were screened *in-vitro* for their antibacterial activity against *E. coli*, *P. aeruginosa*, *B. subtilis* and antifungal activity against *A. niger*, *A. flavus* in order to determine their structure activity relationship. Chloremphenicol and Fluconazole have been taken as reference drugs for activity screening.

TABLE 3: RESULTS OF *IN-VITRO* ANTIMICROBIAL ACTIVITY OF β-LACTAMS (C1-C6)

		Zone of inhibition (mm)				
S. no	Compound	*E. coli*	*P. aeruginosa*	*B. subtilis*	*A. niger*	*A. flavus*
1	c1	18	12	18	14	14
2	c2	14	16	20	18	16
3	c3	14	20	24	16	16
4	c4	14	14	20	16	20
5	c5	16	16	18	20	16
6	c6	20	18	20	16	22
7.	Control	28	24	32	24	36

Concentration = 1000µg/ml.
Control for bacterial/fungal strain: Chloremphenicol / Fluconazole.

TABLE 4: RESULTS OF *IN-VITRO* ANTIMICROBIAL ACTIVITY OF β-LACTAMS (D1-D6)

		Zone of inhibition (mm)				
S. no	Compound	*E. coli*	*P. aeruginosa*	*B. subtilis*	*A. niger*	*A. flavus*
1.	d1	16	16	20	16	16
2.	d2	14	20	16	16	16
3.	d3	12	24	30	20	20

4.	d4	14	22	20	16	24
5.	d5	16	14	20	14	24
6.	d6	20	16	28	20	28
7.	Control	28	24	32	24	36

Concentration = 1000µg/ml.
Control for bacterial/fungal strain: Chloremphenicol / Fluconazole.

RESULTS AND DISCUSSION: All the β-lactams (c1-c6 and d1-d6) were screened *in-vitro* for their antibacterial activity against *E. coli, P. aeruginosa, B. subtilis* and for antifungal activity against *A. niger, A. flavus*. The Reference antibacterial drug was Chloremphenicol for bacterial and Fluconazole for fungal strains.

Observations from **Table 3** and **Table 4** are as follows:

- β-lactam (c3) with acetyloxy and sulphamethoxazole group exhibit better antibacterial activity against *Bacillus subtilis*.
- β-lactam (c6) with acetyloxy and sulphathiazole moiety exhibit better antifungal activity against *A. flavus.*
- β-lactams (d3 and d6) with allyl, sulphonyloxy and sulphamethoxazole/sulphathiazole groups exhibits better antibacterial activity against *Bacillus subtilis*.
- β-lactam (d6) with allyl, sulphonyloxy and sulphathiazole moiety exhibits better antifungal activity against *A. flavus.*

CONCLUSION: A series of substituted β-lactams have been successfully synthesized by electrocyclisation of imines with chloroacetyl chloride in the presence of triethylamine and 1, 4-dioxane. The results showed that the synthesized compounds possess good antimicrobial activities against the tested microorganisms with compounds (c3), (c6), (d3) and (d6) displaying good activity.

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CONFLICT OF INTEREST: Nil.

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