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SYNTHESIS AND ANTIBACTERIAL SCREENING OF SOME AZETIDIN-2-ONES

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ABSTRACT

A new series of 4-[3-chloro-2- (4-hydroxy-3-methoxy benzyllidene)-4-oxoazetidin-1-yl] amino-N-(substituted) benzenesulfonamide, 4-{3-chloro-2- [5-methoxy- 2-nitro-4-(prop-2-en-1-yloxy) benzyllidene] -4-oxoazetidin -1-yl}] amino}-N-(substituted) benzenesulfonamide and 4-{3-chloro-2- [4-hydroxy- 3-methoxy- 5-(prop-2-en-1-yl) benzylidene] -4-oxoazetidin -1-yl} amino}-N-(substituted) benzenesulfonamide were synthesized using appropriate synthetic route. The chemical structures of all the synthesized compounds were deduced on the basis of elemental analysis and spectroscopic data. The antimicrobial activity of the synthesized compounds were screened against several microbes. Several of these molecules showed potent antimicrobial activity against *Escherichia coli*, *Staphyococcus aureus and Pseudomonas aeruginosa*, *Bacillus subtilis*, and significant structure-activity relationship (SAR) trends.

INTRODUCTION

Heterocycles constitute one of the most significant areas of research in the field of medicinal -lactam) heterocycles are considered as an chemistry[1].The azetidin-2-ones (important contribution of science to humanity[2], since they have been constituents of living organisms, natural products, drugs and many more substances useful to mankind and society in all walks of life. Their synthesis and evaluation has always drawn the attention of chemists& biologists over the years[3]. Since the discovery of penicillins[4], and cephalosporins as the most successful antibiotics, azetidin-2-ones have been the subject of regular discussion and investigation. The azetidin-2-ones antibiotics endowed with unique structure and potent antibacterial activity[5], include, penicillins, cephalosporins, carbapenems, nocardicins , clavulanic acid , sulbactams, and tazobactams .These molecules operate by forming a covalent adduct with membrane-bound bacterial transpeptidases, which are also known as penicillin binding proteins (PBPs), involved in the biosynthesis of cell walls.These mechanism-based inhibitors prevent the construction of cell wall and eventually -lactamaseinhibitory lead to cell lysis and death. Moreover, due to their action, azetidin-2-ones based heterocycles represent an attractive target of contemporary organic synthesis.Azetidin-2-onesand its derivatives are important compounds due to their broadrange of biological activities such as antimicrobial[6-16],antiubercular[17-19],anticancer[20-21],cholesterol absorption inhibition[22-23], antidiabetic[24], analgesic and anti-inflammatory[25], thrombin inhibition[26],antiparkinsonian[27], vasopressin via antagonist[28], anticonvulsant[29-30].

MATERIAL AND METHODS

All chemicals and solvents, reagents used in the present study were of analytical grade and solvents were used after distillation.All the melting points of the synthesized compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (MERCK) using n-hexane : ethyl acetate (8:2) solvent system. The developed chromatographic plates were visualized under UV at 254nm. IR spectra were recorded using KBr on Perkin Elmer spectrophotometer. ^{1}HNMR spectra in DMSO on a BRUKER FT-NMR instrument using TMS as internal standard and chemical shift values were expressed in ppm. Elemental analysis (CHN) was performed on Carlo Erba 1108. The present work is undertaken to explore more possibilities of finding a suitablederivatives, which would exceed its

activity more than the already known drugs containing azetidin-2-ones ring.With the alarming trends in microbial resistance to many azetidin-2-ones antibiotics it has become necessary to synthesize some novel azetidin-2-ones for bioassay of antimicrobial activity and the needfor drugs with more specific antimicrobial activity. Thereforeit was thought of interest to combineall the above-mentioned biolabile heterocyclic ringstogether in a molecular framework ofimines in order to enhance the additive effect towardthe biological activity. Keeping this in mind and in continuation of our earlier studies, we designed and synthesized 4-[3-chloro-2-(4-hydroxy-3-methoxybenzyllidene)-4-oxoazetidin-1-yl]amino-N-(substituted)benzenesulfonamide,4-{3-chloro-2-[5-methoxy-2-nitro-4-(prop-2-en-1-yloxy)benzyllidene]-4-oxoazetidin-1-yl}]amino}-N-(substituted) benzenesulfonamide and 4-{3-chloro-2-[4-hydroxy-3-methoxy-5-(prop-2-en-1-yl)benzylidene]-4-oxoazetidin-1-yl}amino}-N-(substituted)benzenesulfonamide with fascinating structural features. Moreover, in order to assess the antimicrobial potentiality of azetidin-2-ones nucleus and to investigate the structure-activity-relationship, the constructed molecules were screened for their antibacterial and antifungal activities. The synthetic pathway includes an electrocyclisation reaction of imine derivatives to give direct access to the desired4-[3-chloro-2-(4-hydroxy-3-methoxybenzyllidene)-4-oxoazetidin-1-yl]amino-N-(substituted)benzenesulfonamide,4-{3-chloro-2-[5-methoxy-2-nitro-4-(prop-2-en-1-yloxy)benzyllidene]-4-oxoazetidin-1-yl}] amino}-N-(substituted)benzenesulfonamide and 4-{3-chloro-2-[4-hydroxy-3-methoxy-5-(prop-2-en-1-yl)benzylidene]-4-oxoazetidin-1-yl} amino}-N-(substituted)benzenesulfonamide. The approach involves the initial preparation of precursors4-{[(E)-(4-hydroxy-3-methoxy benzyllidene] amino}-N-(substituted)benzenesulfonamide, 4-({((E)-[5 -methoxy- 2- nitro- 4-(prop -2 -en- 1 -yloxy) benzyllidene] amino}-N-(substituted)benzenesulfonamide and 4-{[(E)-(4-hydroxyl-3-methoxy-5-(prop-2-en-1-yl)benzylideneamino}-N-(substituted)benzenesulfonamide. The reactive imine derivatives were accessible via the reaction of anequimolar quantity of aromatic aldehyde containing allyl and allyloxy group with different sulpha drugs in ethanol at room temperature which resulted in the formation 4-{[(E)-(4-hydroxy-3-methoxy benzyllidene] amino}-N-(substituted)benzenesulfonamide, 4-({((E)-[5 -methoxy- 2- nitro- 4- (prop -2 -en- 1 -yloxy) benzyllidene] amino}-N-(substituted)benzenesulfonamide and 4-{[(E)-(4-hydroxyl-3-methoxy-5-(prop-2-en-1-yl)benzylideneamino}-N-(substituted)benzenesulfonamide [31]in good yield.

Further, the initially formed reactive imines underwent an electrocyclisation reaction of chloroacetyl chloride and triethyl amine in 1,4-dioxane afforded the target compounds4-[3-chloro-2-(4-hydroxy-3-methoxybenzyllidene)-4-oxoazetidin-1-yl]amino-N-(substituted) benzenesulfonamide,4-{3-chloro-2-[5-methoxy-2-nitro-4-(prop-2-en-1-yloxy) benzyllidene] -4-oxoazetidin-1-yl}]amino}-N-(substituted) benzenesulfonamide and 4-{3-chloro-2-[4-hydroxy-3-methoxy-5-(prop-2-en-1-yl)benzylidene]-4-oxoazetidin-1-yl}amino}-N-(substituted)benzene sulfonamide[32]in good yield. Typically, ketenes are generated thermallyfrom acid chlorides in presence of triethylamine, which on subsequent reaction with reactiveimines yield the desired azetidin-2-ones.

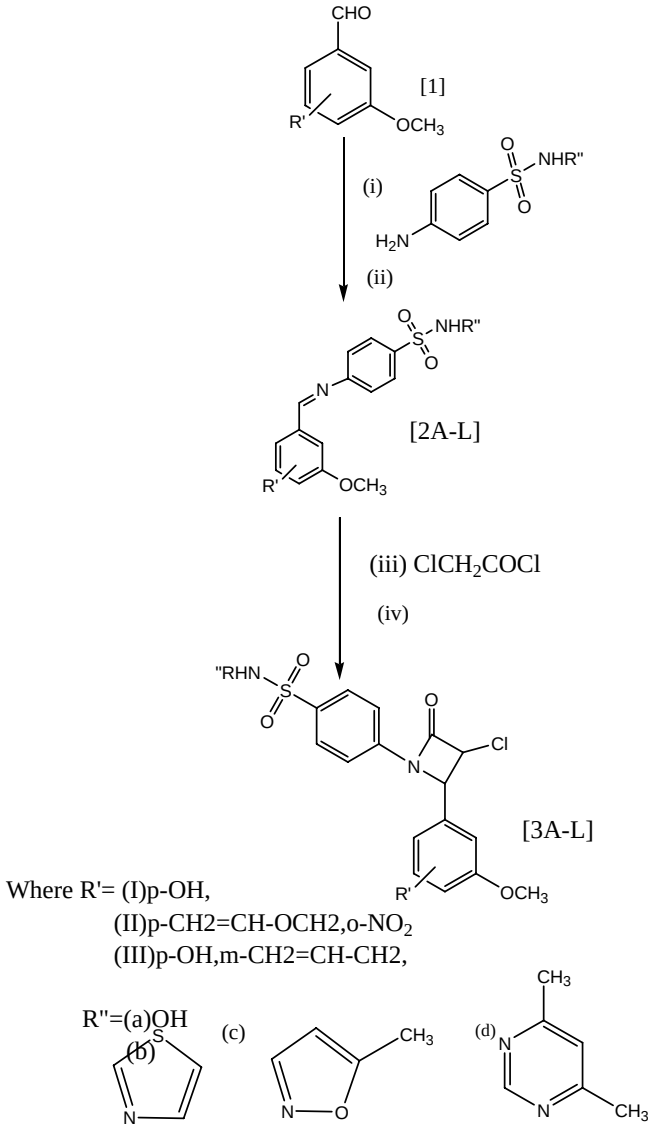
General procedure for the synthesis of azetidinones:

Imine (0.001mole) was added to a constantly stirred solution of 1,4 dioxane (15ml), triethylamine (0.001 mole) and chloroacetyl chloride (0.001mole). The reaction mixture was stirred at 50^{o}C. The reaction vessel was then kept at room temperature for 30min and refluxed for 8hr. On cooling the precipitate was obtained, which was filtered, thoroughly washed with water.

RESULTS AND DISCUSSION

Structures of newly obtained compounds have been ascertained on the basis of their consistent IR, ^{1}H NMR and mass spectral assignments [33]. All the newly synthesizedazetidin-2-ones 3(A-L) were assayed *in vitro* for their growth inhibitory activity against pathogenic micro-organisms. The antibacterial activity were tested by the disc-diffusion[34-35]. method using selected Gram positive and Gram negative strains of *Escherichia coli, Staphyococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis* and compared to reference drug Streptomycin and Penicillin-G. The experimental results of antibacterial activity indicated a variable degree of efficacy of the compounds against different strains of bacteria. It has been observed that *E.coli* show moderate to good inhibition activity while *P.aeruginosa* show less to moderate activity. *S.aureus* show good to maximum inhibition nearly in all compounds while *B. subtilis* show moderate to good inhibition activity.[Table-I] In table the 3A,3G,3K show most excellent potent activity, while other derivatives show mild to moderate activity.

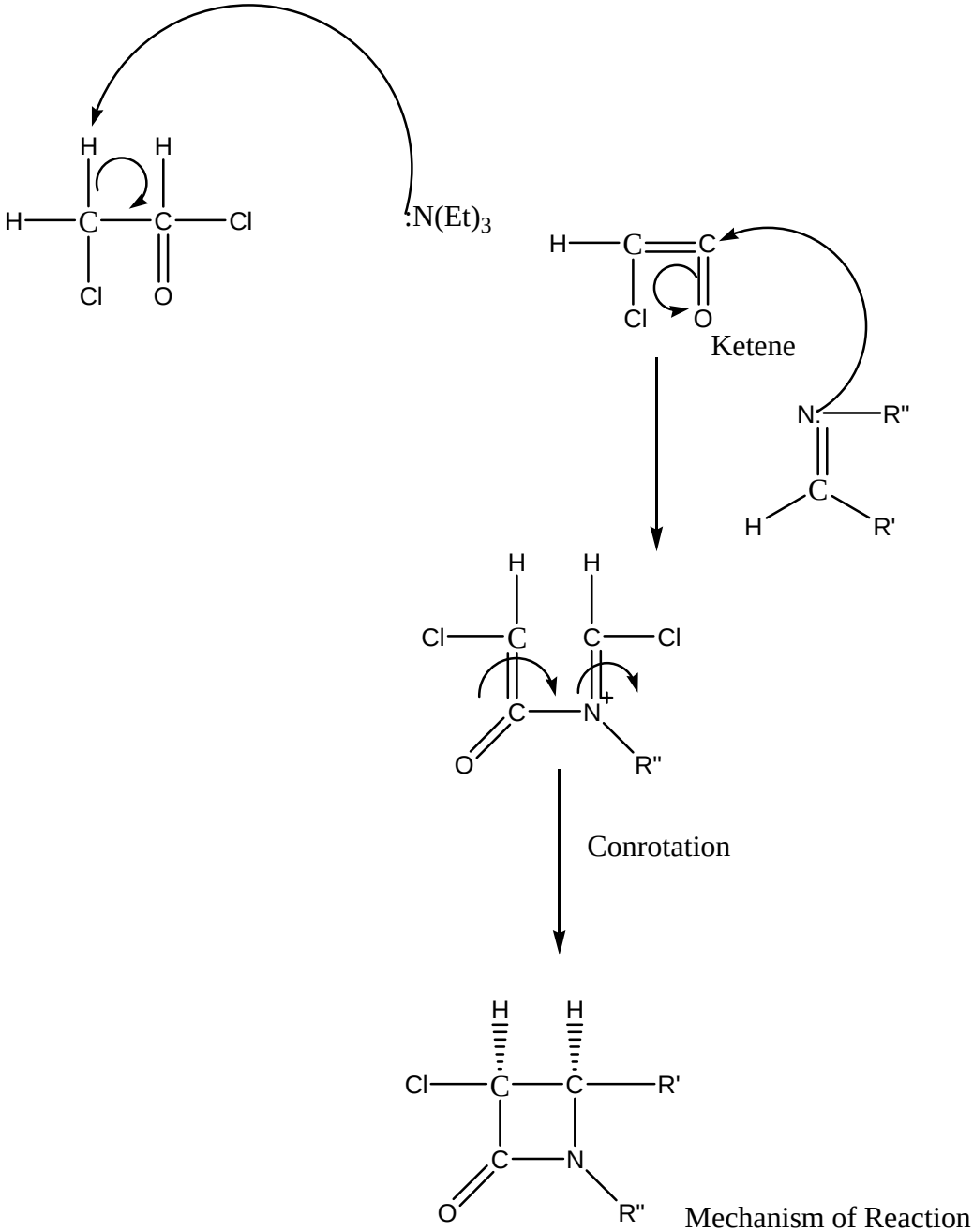
Scheme of Reaction



Where R'= (I)p-OH,
(II)p-CH2=CH-OCH2,o-NO_2
(III)p-OH,m-CH2=CH-CH2,

R"=(a)OH (b) (c) (d)

Reagents and conditions:(ii)EtOH, reflux,2h; (iv)$(C_2H_5)_3N$, 1,4-dioxane,stirred1h,reflux,8h



Mechanism of Reaction

CONCLUSION

The results of antimicrobial screening clearly indicate that the nature of substituent and their position on azetidin-2-ones nucleus affected the in vitro activity significantly. The presence of electron-withdrawing groups on the aromatic ring in general increases the antibacterial activity of tested compounds. All the four sulphamethoxazole, sulphanilimide, sulphathiazole and sulphadimidine are classical bioisosteres so obey steric and electronic definition of classical

bioisosteres. Sulphamethoxazole substituent frequently appears in many drugs and it follows the trend here also as sulphamethoxazole substituted compounds seem to be more potent than other sulpha substituents. Antimicrobial activity of sulphanilimide and sulphathiazole is average. Here we are able to identify some interesting structure-activity relationship on azetidinone ring. Our results encourage the synthesis of azetidin-2-ones analogs to give new class of antibacterial agents.

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31. Experimental: All melting points were measured in open capillary and are uncorrected.The chemicals and reagents used were of AR grade. The product mixtures were analyzed by thin-layer chromatography (TLC) on silica gel sheets. IR spectra (in cm^{-1}) were recorded on PerkinElmer 337 spectrometer. 1H NMR was recorded on a Varian EM-390 MHz NMR spectrometer in DMSO-d_6, chemical shifts are given in d ppm using TMS as an internal standard. Mass analysis was performed on Jeol SX-102 spectrometer using FAB technique.
32. General procedure for the synthesis of imines [2A-L]: An equimolar mixture (0.005 M) of aldehyde and appropriate amine in ethanol was refluxed for 3 h. The reaction mixture was cooled in an ice bath and a drop of sulfuric acid was added to it. The product obtained was filtered, washed and recrystallised by EtOH as shiny yellow crystals.
33. General procedure for the synthesis of b-lactam [3A-L] : In a closed vessel containingcompound (0.001 M) in 20 mL of 1,4-dioxan,0.095 mL of chloroacetyl chloride and 0.16 mL of triethylaminewere added and the reaction mixture was stirredat 50 _C for 1 h.

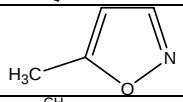
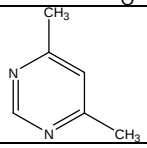
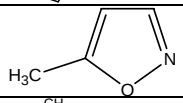
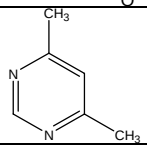
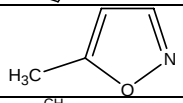
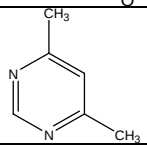
The reaction mixture was then kept at room temperature for 30 min and further refluxed for 8 h.The filtrate was concentrated under reduced pressure and poured into ice-cold water. The product so obtained was recrystallised from methanol as light brown crystals.

34. Analytical data of compound (3A): mp (_C) 200, yield 35%: IR KBr (m cm_1) 3512 (O–H), 1760 (C=O, fourmemberedlactam), 1578 (N=N), 1498 (N=O, asym), 1349(N=O, sym), 1602, 1599, 1464 (C–C, ring str), 1H NMR(d) ppm 4.2 (s, 1H, OH), 5.1 (d, 1H, J3,4 = 4.4 Hz, C4–H),5.8 (d, 1H, J3,4 = 4.4 Hz, C3–H), 7.1–7.4 (m, 12H, ArH),:Elemental analysis, found (calcd) (%) C, 61.23(61.99): H, 3.18 (3.69); N, 13.41 (13.77).
35. In disc-diffusion assay, few colonies of organisms were inoculated in 2–5 mL nutrient broth and grown for 2.5 h. The agar plates were dried and inoculated by spreading the bacterial suspension evenly over it. The sterile paper discs (6 mm) impregnated with fixed dose viz., 400 lg/mL of compound were placed on the preinoculated surface. The disc-bearing plates were incubated at 37 _C and examined at 48 h for zone of inhibition, if any, around the disc. Chloromycetin was used in assay as a standard control drug. An additional negative control disc without any sample but impregnated with equivalent amount of solvent (DMF) was also used in the assay. The diameter of inhibition zone is directly proportional to the degree of sensitivity of bacterial strain and the concentration of compound under test.

Physical characteristics of the synthesized azetidin-2-ones:

S.no	Comp.	M.p(^{0}c)	Yield	Colour
1	3A	200^0	35%	Black colour
2	3B	190^0	40%	Yellowish colour
3	3C	210^0	40%	Black colour
4	3D	180^0	45%	Reddish brown colour
5	3E	140^0	40%	Pale yellow colour
6	3F	130^0	35%	Brown colour
7	3G	120^0	40%	Black colour
8	3H	135^0	40%	Black colour
9	3I	120^0	45%	Black colour
10	3J	130^0	35%	Black colour
11	3K	110^0	40%	Brown colour
12	3L	110^0	42%	Black-brown colour

Table-I : Results of *in-vitro* antibacterial & Antifungal activity observed for the synthesizedazetidin-2-ones compounds through disc diffusion assay.

	R	R"	Inhibition zone diameters[a]			
			E. coli	***P. aeruginosa***	***S.aureus***	***Bacillus species***
3A	p-OH	H	10	10	32	12
3B	p-OH		11	14	22	-
3C	p-OH		16	-	26	14
3D	p-OH		10	14	26	14
3E	p- CH_2=CH-OCH_2' o-NO_2	H	14	-	22	-
3F	p- CH_2=CH-OCH_2' o-NO_2		16	-	28	16
3G	p- CH_2=CH-OCH_2' o-NO_2		16	-	32	24
3H	p- CH_2=CH-OCH_2' o-NO_2		11	-	28	-
3I	p-OH,m-CH_2=CH-CH_2	H	-	-	-	-
3J	p-OH,m-CH_2=CH-CH_2		-	-	-	-
3K	p-OH,m-CH_2=CH-CH_2		10	10	-	10
3L	p-OH,m-CH_2=CH-CH_2		-	12	-	-
Standard		Streptomycin	28	24		
Standard		Penicillin-G			38	32
Standard		Terbiforce				

Antibacterial and antifungal susceptibility of compounds was measured in terms of zone of growth inhibitions.

(-) means no activity.

[a& b] Inhibition zone diameters in millimeters at 1000 µg/mL concentration of compounds.