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Antimicrobial studies of synthesized azetidinone derivatives from sulfamethoxazole moiety

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ABSTRACT

A series of novel Azetidinone derivatives have been synthesized from the intermediate schiff bases. Schiff bases are prepared from sulfamethoxazole moiety by reacting the hydrazide of the parent compound with different aromatic aldehydes. Cyclocondensation of schiff's bases with acetylchloride resulted in the formation of azetidinone derivatives. The products, characterized on the basis of satisfactory spectral data (IR, NMR, Mass spectroscopy), have shown moderate to good antimicrobial activity against some bacteria and fungi.

Keywords: Azetidinones, Schiff's bases, Antibacterial and Antifungal activities.

INTRODUCTION

One of the fundamental milestones in medicinal chemistry is represented by the knowledge acquired during the studies carried out on a simple four-member ring, β-lactam. 2-Azetidinones, commonly known as β-lactams, are well-known heterocyclic compounds [1]. The activity of the famous antibiotics such as penicillins, cephalosporins and carbapenams are attributed to the presence of 2-azetidinone ring in them. A large number of 3-chloromonocylic β-lactam possess powerful antibacterial [2,3], anti-inflammatory [4], antifungal [5,6,7], analgesic [8], anticonvulscant [9] and herbicidal [10] activities. The β-lactams also serve as synthons for many biologically important classes of organic compounds. Due to this, the investigation of chemistry and biology of these compounds continue to appeal the synthetic and medicinal organic chemists [11-13]. The present work is undertaken to explore more possibilities of finding a suitable derivatives, which would exceed its activity more than the already known drugs containing β-lactam ring.

By considering the above factors, it was thought to synthesize some substituted azetidinone derivatives derived from sulfamethoxazole moiety and screen them for biological activities.

EXPERIMENTAL SECTION

Melting points were determined by capillary method and were uncorrected. The IR spectra is recorded by using Shimadzu Perkin Ekmer 8201 PC IR Spectrometer using a thin film on potassium bromide pellets techniques and frequencies are expressed in cm^{-1}. The PMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer. All spectra were obtained in $CDCl_3$ and DMSO. Chemical shift values are reported as values in ppm relative to TMS (δ=0) as internal standard. The FAB mass spectra were recorded on JEOL SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6Kv, 10Ma) as the FAB gas.

Procedure for synthesis of schiff's bases:
Sulfadiazine moiety was converted into respective Schiff's bases in 3 steps

Step –I: Preparation of Sulphamethoxazole ethylacetate
A mixture of Sulphamethoxazole (0.1mol), ethylchloroacetate (0.1 mol) and anhydrous potassium carbonate (19.5gm, 0.15mol) in dry ethanol were refluxed on a water bath for 24 hours at 70°C . This reaction mixture of filtrate was then poured on to the ice-cold water and stirred well. The organic layer was extracted with ether and further the ether layer was washed with 5% HCl and dried over anhydrous sodium sulphate. Drying on water bath evaporated ether layer. Finally the resultant collected liquid was purified under reduced pressure to give pure Sulphonamide ethyl acetate.

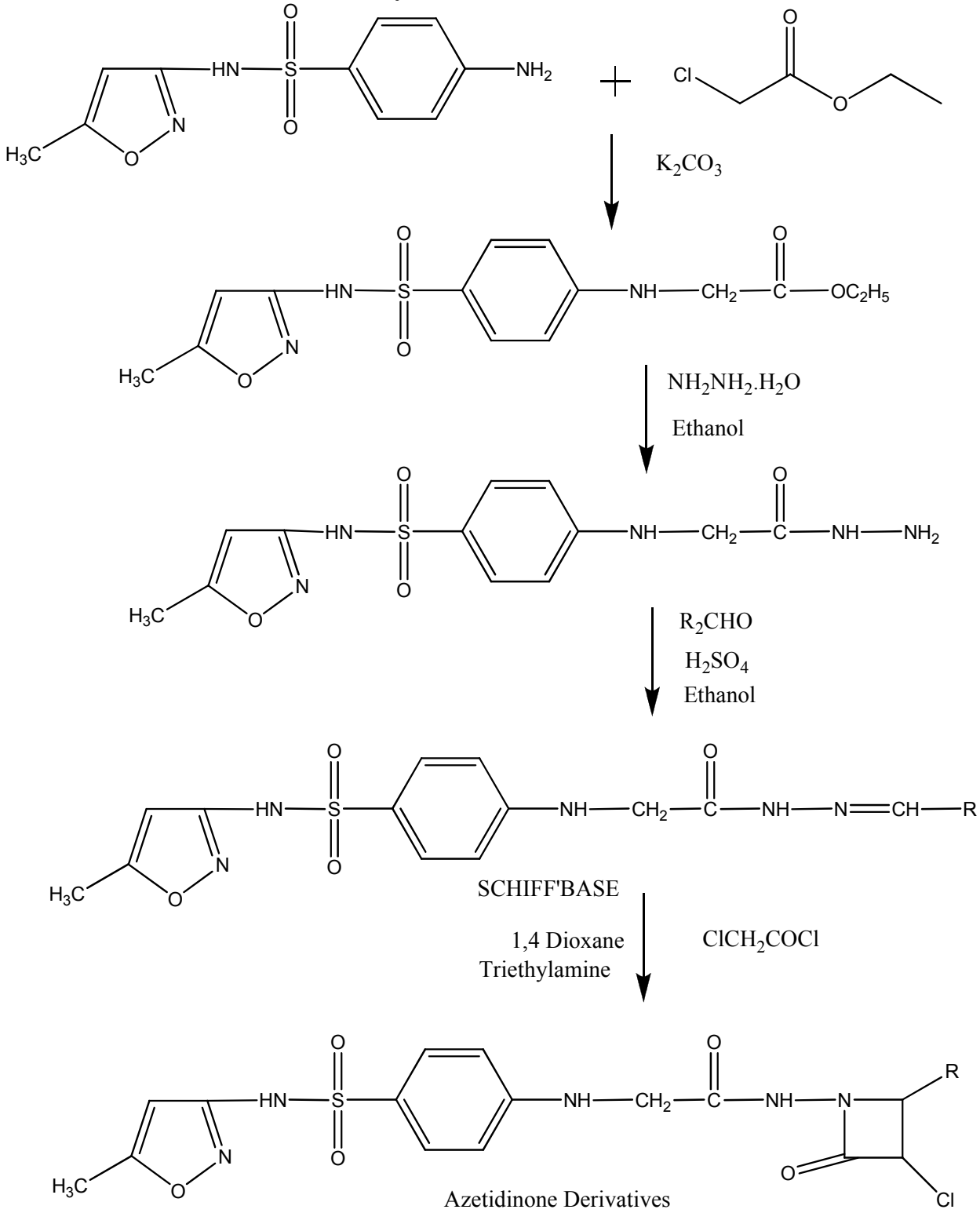
Step-II: Preparation of Sulphamethoxazole acetyl hydrazide
A mixture of Sulphamethoxazole ethylacetate (0.05mol), Hydrazine hydrate (99% 0.07 mol) in ethanol (100 ml) was refluxed for 6 hours. From the resultant mixture excess of ethanol was removed by distillation. On cooling, from the resultant mixture, white needle like crystals of Sulphonamide acetyl hydrazide began to separate. It was collected and then recrystallized from ethanol.

Step-III: Preparation of Schiff's bases of Sulphamethoxazole acetyl hydrazide
Mixture of Sulphamethoxazole acetyl hydrazide(0.01mol) dissolved in minimum quantity of ethanol and different aromatic or heterocyclic aldehydes (0.01 mol, dissolved in minimum quantity of ethanol) was refluxed together by employing sulphuric acid about 0.01 mol as catalyst in a round bottom flask on a water bath for 6 hours. The precipitate was filtered, washed with ice-cold water and recrystalized from ethanol.

Procedure for synthesis of Azetidinone analogs.
Chloroacetylchoride was added dropwise to Schiff's base (0.01mol) and triethylamine (0.02ml) in dioxane (25ml) at 5-10°C. The mixture was stirred for 20 hours and left at room temperature for three days. The contents were filtered, dried and recrystallized from ethanol.

N-[3-Chloro-4-(2-hydroxyphenyl)-2-oxo-azetine-1-yl]-2-(N'-5-methyl-3- isoxazolyl) sulfonamide
IR (KBr, cm^{-1}): 3394.59 (N-H) , 3021.5 (C-H) , 1734.72(C=O) of azetidinone ring, 1646.81 (CO-NH amidyl), 1566.0 (C-N), 750.67 (C-C aromatic), 683.41(C-Cl) , 1347.72(S $=O_2$) ,H^1 NMR (δ) in ppm: 5.47(1H,s, CH-Cl of azetidinone ring); 3.5(1H,s, azetidinone proton); 7.1 to 7.8 (12H,m,Ar-H); 8.53(1H,s,-CONH-); 4.76(1H,s, -NH SO2-); 3.3 to 3.0 (2H,s,-CH2-); 4.6 (1H,s,-NH-), MASS IN m/z: m/z 505.

Scheme 1: Synthesis of azetidinone derivatives



N - [3-Chloro-4-(4-methoxyphenyl)-2-oxo-azetine-1-yl]-2-(N'-5-methyl-3- isoxazolyl) sulfonamide

IR in cm^{-1}: 3609.84 (C-H), 1727.50 (C=O of azetidinone ring, 1641.5 (CO-NH amidyl), 1508.29 (C-N), 729.0 (C-C aromatic), 619.36 (C-Cl),1750.5 -OCH3 (aryl ester), 3015.5(C-H aromatic ring),H^1 NMR (δ) in ppm: 5.31 (1H,s, CH-Cl of azetidinone ring); 3.2 (1H,s, azetidinone proton); 6.9 to 7.6 (12H,m,Ar-H); 8.45 (1H,s,-CONH-); 4.03(1H,s, -NH SO_2-); 3.01 to 3.14 (2H,s,-CH_2-); 4.02 (1H,s,-NH-),6.44-6.61(3H,s,-OCH_3), MASS IN m/z: m/z 519.

N-[3-Chloro-4-(4-dimethylaminophenyl)-2-oxo-azetine-1-yl]-2- (N'-5-methyl-3-iso azolyl) sulfonamide
IR in cm^{-1}: 3438.87(N-H), 3217.84 (C-H),1770.43 (C=O) of azetidinone ring, 1694.94 (CO-NH amidyl),1443.96(C-N),813.43 (C-C aromatic), 646.43(C-Cl), H^1 NMR (δ) in ppm: 5.46 (1H,s, CH-Cl of azetidinone ring); 3.6 (1H,s, azetidinone proton); 7.3 to 7.90 (12H,m,Ar-H); 8.55 (1H,s,-CONH-); 3.90 (1H,s, -NH SO_2-); 3.01 to 3.14 (2H,s,-CH_2-); 4.79 (1H,s,-NH-), MASS IN m/z: m/z 526

Table 1: Physical Data of Schiff's Bases

S. No.	COMP. No.	PHYSICAL STATE	M.P.(˚C)	% YIELD
1	SB1	Yellow crystals	195-198˚C	72.0%
2	SB2	Brown crystals	210-212˚C	75.5%
3	SB3	Reddish brown crystals	220-222˚C	78.5%
4	SB4	Pale yellow crystals	235-238˚C	65.5%
5	SB5	Yellow crystals	215-218˚C	68.5%
6	SB6	Orange yellow crystals	230-233˚C	65.5%

Table 2: Physical Data of newly synthesized Azetidinone Derivatives

S. No.	COMP. No.	PHYSICAL DATA	M.P.(˚C)	%YIELD
1	SBz1	Yellow crystals	225-228	69.5
2	SBz2	Brown crystals	230-235	60.5
3	SBz3	Reddish brown crystals	215-218	55.0
4	SBz4	Brown crystals	218-220	70.5
5	SBz5	Yellow brown crystals	240-242	78.5
6	SBz6	Yellow orange crystals	195-198	69.5

Determination of Antimicrobial Activity:

Modified Kirby-Bauer method was used for the evaluation of antimicrobial activity of the synthesized compounds. Circular paper discs of 6mm diameter were impregnated with the specific amount of the test sample and were placed on a Mueller Hinton agar medium in a petri plate, which was inoculated on its surface with one of the test organisms. After incubation, the plates were observed for the growth inhibition zones around paper disc. The diameter of the zone of inhibition is proportional to the antimicrobial activity of the substance. The diameters of the zones of inhibition were compared with that produced by the standard antibiotics.

Table3 : Data of antimicrobial activity of azetidinone derivative derived from Sulfamethoxazole

S. No.	*COMP. No.*	*Diameter of zone of inhibition (mm)*				
		B.subtilis	***S.sureus***	***E. Coli***	***P.aeruginosa***	***C.albicans***
1	SBz1	14	18	15	16	7
2	SBz2	14	16	12	15	6
3	SBz3	16	19	17	18	8
4	SBz4	15	18	14	17	8
5	SBz5	15	16	15	18	10
6	SBz6	14	16	14	15	6
Sulfamethoxazole		14	15	16	17	8
Ampicillin		16	20	18	20	-
Griseofulvin		-	-	-	-	10

All the test compounds were tested at 50µg level. To obtain this, solutions containing 10 mg/ml of the test compounds were prepared in sterile dimethyl formamide (DMF) and 5µl each of the solutions were added into each disc using a micropipette. Sulfadiazine(10µg), sulfamethoxazole

(10µg), Ampicillin (10µg) and griseofulvin (25µg) were used as standard antibiotics. All the solutions were prepared in aseptic conditions.

Four different bacterial cultures viz., a *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* and one fungal culture, *Candida albicans* were used as test organisms for evaluation of antibacterial and antifungal activity.

RESULTS AND DISCUSSION

Synthesis of azetidinone derivatives by the earlier described method resulted in products with good yield. Purity of the intermediate and final product was determined by their melting point using Thomas Hoover apparatus (open capillary method) and also scuretinsed with TLC method. The final products were purified by the recrystallization techniques with dioxan and ethanol. Analysis of spectral data of the synthesized compounds revealed the useful information about their structures.

All the synthesized azetidinone derivatives derived with sulfamethoxazole have shown moderate to good antibacterial and antifungal activity in comparison to that of the standard drugs ampicillin, sulfadiazine and griseofulvin.Compound SB_z3, SB_z4, SB_z5, have shown significant antibacterial activity. Compound SB_z1, SB_z3, SB_z4 SB_z5 have shown good antifungal activity.

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