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Synthesis, Spectroscopic Characteristics and Antibacterial Study of Some Novel Sulphonamides

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Main purpose of this study was to produce some novel sulfonamides from already existing antibiotics having-NH_2 group in their structure by their simple and low-cost condensation reactions with *p*-toulene sulfonyl chloride. All reactions were carried out in aqueous media at controlled pH (8-10) with good yield. Characterization of synthesized compounds were done by using FTIR, ^{1}H NMR, ESI-MS and elemental analysis. Biological activity was evaluated against medically important Gram-positive and Gram-negative bacterial strains using MIC method after observing optical density value by spectrophotometer at 600 nm. All compounds were active against *E. coli* with MIC value less than 55 µg/mL, while N-((2S,5R,6R)-2-formyl-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptanes-6-yl)-N-tosylacetamide (**1**) showed pronounced results having MIC value less than 6 µg/mL for both strains.

Keywords: Sulfonamides, *p*-Toluene sulfonyl chloride, Antibiotics.

INTRODUCTION

The insist for new chemotherapeutic antibacterial leftovers attractive in branch of medicinal chemistry. The innovation of sulfonamides as antibacterial in near the beginning of 30 s was the opening of most charming period of chemotherapeutic agents [1-4].

Sulfonamide groups are not only antibacterial but are considered as a phramacophore which is present in a number of biologically active molecules, particularly in antimicrobial agents [5-9], additional to this many sulfonamides derivatives have been reported as carbonic anhydrase inhibitors [10-14], anticancer [15] and anti-inflammatory agents [16].

Some organism become resistant to all approved antibiotics and for their treatment there is need of potentially toxic drugs. There is overpowering need to build up highly effective and valuable antibacterial agents in order to treat infections which are caused by bacterial pathogens (resistant to existing antibiotics). Sulfonamides have broad spectrum of antibacterial activity because they behave as competitive inhibitors of enzymes dihydropteroate synthase (DHPS) and also having adverse effects especially in HIV patients [17]. As sulfonamides are inexpensive and highly active group so synthesis of novel sulfonamides with high activity is of great interest for pharmaceutical industry. The present study deals with synthesis of some new sulfonamides. The structure of synthesized compounds was recognized and confirmed by elemental analysis and from data obtained by spectral analysis *i.e.* IR, H NMR, ESI-MS and λ_{max}. Biological activity was studied using Gram-positive and Gram-negative strains of bacteria.

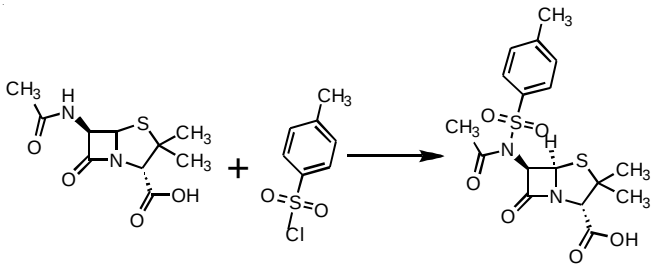
EXPERIMENTAL

p-Toluenesulphonyl chloride, penicillin, amoxicillin, cefixime, sodium carbonate and all solvents used were purchased from sigma Aldrich. Purification of synthesized compounds were done by precoated silica gel plates (Germany, Merck). Melting points were taken by using Gallen hamp apparatus. IR spectra for new compounds were obtained in range between 4000-600 cm^{-1} on Cary 630 Agilent FTIR spectrophotometer. PG-T90$^+$ UV-visible spectrophotometer for λ_{max} and flash HT plus elemental analyzer for analysing % of H, C, N and S, were used respectively. For mass determination of new molecules mass spectrometer having following specification was used Model: 6200 Series accurate-Mass, resolution: > 20,000 resolving power, ionization method: electrospry (ESI) Scanning speed: up to 40 spectra per second Mass range: 20 to 20,000 *m/z* Dynamic range: 10^5. ^{1}H NMR spectra in DMSO of synthesized

compounds were recorded on Bruker AC-iii 300 MHz instrument at 300 K, Chemical shifts were reported in δ (ppm) with respect to TMS as an internal standard; coupling constants were expressed in Hertz.

Synthesis of N-((2S,5R,6R)-2-formyl-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptanes-6-yl)-N-tosylacetamide (1): Penicillin (0.835 g, 0.1 mol) was taken in 25 mL water. It was stirred and pH was checked at regular intervals. After maintaining pH at 9 (0.264 g, 0.1 mol) *p*-toluene sulfonyl chloride was added. Stirring of this reaction mixture was continued and reaction completion was monitored by TLC. In order to precipitate the product 1 N HCl was added by maintaining the pH of reaction mixture at 2-3. Product was separated by filtration and recrystillized using ethanol (**Scheme-I**). Purification of product was confirmed by TLC using mobile phase of dichloromethane:ethanol (50:50).

IR (film, ν_{max}, cm^{-1}): 1150.35 (N-*S*=*O* str.), 1055.12 (C-N str), 1641.45 (-N-*C*=*O*), 815.32 (C-S str), 930.16 (S-N str), 3388.12 (-OH). δ_H (300 MHz; DMSO): 9.28 (1H, s, -OH), 7.22-7.47 (4H, m, Ar*H*), 5.14-5.27 (1H, d, *J* = 6.9, -COC*H*N-), 4.10 (1H, s, -C*H*COOH), 4.00-4.03 (1H, d, *J* = 6.9, -C*H*S (CH_3), 2.49 (3H, s, -TsCH_3), 2.32 (3H, s, -COCH_3), 1.88 (6H, s, -C(CH_3)$_2$); *m/z* (ESI) 412; HRMS (ESI) calcd. for $C_{17}H_{20}N_2O_6S_2$; $C_{17}H_{20}N_2O_6S_2Na^+$ 435.13. Mass analysis for mass 435.1381000 and found to be 435.1392490. Anal. calcd. for $C_{17}H_{20}N_2O_6S_2$ (m.w. 412): C, 49.50; H, 4.89; N, 6.79; S,15.55 %. Found: C, 49.48; H, 4.87; N, 6.77; S, 15.53 %.



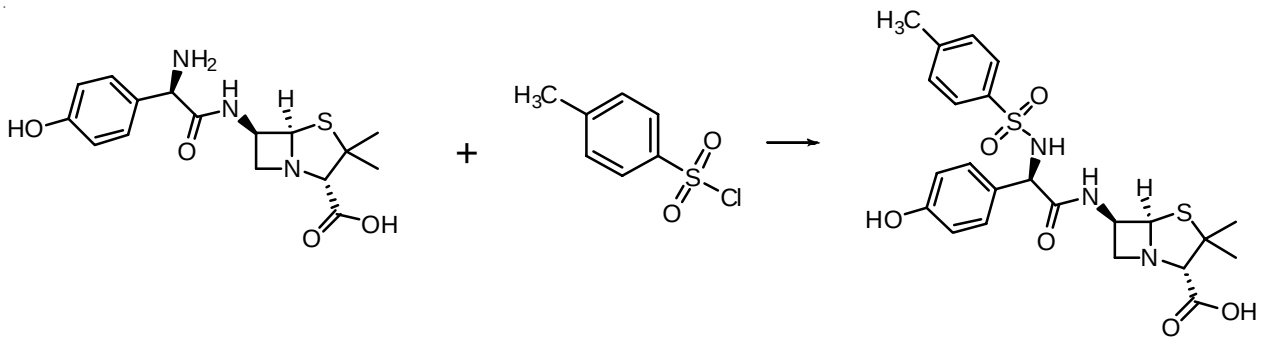
Scheme-I

Synthesis of (2S,5R,6R)-6-((R)-2-(4-hydroxyphenyl)-2-(4-methylphenylsulfonamido)acetamido)-3,3-dimethyl-4-thia-1-azabicyclo[3,2,0]heptanes-2-carboxylic acid (2): (0.9125 g, 0.1 mol) amoxicillin was dissolved in 25 mL water in a round bottom flask and stirred for some time. pH was checked and maintained at 8-9. Then (0.264 g, 0.1 mol) *p*-toluene sulfonyl chloride was added to the above reaction mixture and pH was strictly maintained at 8-9. Reaction mixture was stirred for 2-3 h at room temperature and reaction completion was monitored by TLC. After reaction completion 1 N HCl was added and product was precipitated out at pH 2-3. After filtration purification was done by recrystallization from ethanol (**Scheme-II**). Product purification was confirmed by TLC using mobile phase of acetic acid:butanol:water (10:70:20).

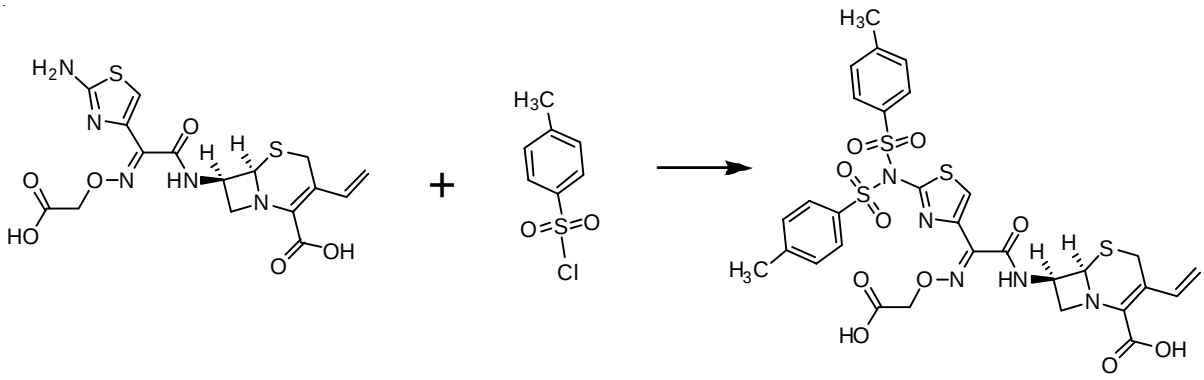
IR (film, ν_{max}, cm^{-1}): 1148.67 (N-*S*=*O* str), 1050.4 (C-N str), 1641.88 (-N-*C*=*O*), 814.49 (C-S str), 932.6 (S-N str,), 3239.36 (OH). δ_H (300 MHz; DMSO): 9.48 (1H, s,-COO*H*), 8.45-8.54 (1H, d, *J* = 6.5 -N*H*CO-), 8.14-8.27 (1H, d, *J* = 6.9-N*H*SO_2-), 7.03-7.73 (8H, m, Ar*H*), 6.75 (1H, s, Ar-O*H*), 6.48-6.51 (1H, d, *J* = 6.7,-C*H*NHSO_2), 6.03-6.31 (1H, q, *J* = 6.6, -C*H*NHCO), 5.73-5.89 (1H, d, *J* = 6.6-C*H*S (CH_3)$_2$), 5.53 (1H, s,-C*H*COOH), 4.89-5.27 (1H, dd, *J* = 6.5, 1.2, -C*H*HN), 4.20-4.60 (1H, dd, *J* = 6.4, 1.2, -CH*H*N), 3.32 (3H, s, ArCH_3), 2.31 (6H, s, -C (CH_3)$_2$); *m/z* (ESI) 505.13; HRMS (ESI) calcd for $C_{23}H_{27}N_3O_6S_2$; $C_{23}H_{27}N_3O_6S_2Na^+$ 528.23 Mass analysis for mass 528.2301450 and found to be 528.2390850. Anal. calcd. for $C_{23}H_{27}N_3O_6S_2$ (m.w. 505.61): C, 54.64; H, 5.38; N, 8.31; S, 12.68 %. Found: C, 54.62; H, 5.36; N, 8.29; S, 12.66 %.

Synthesis of (6R,7R)-7-((E)-2-((carboxymethoxy)imino)-2-(2-(4-methyl-N-tosylphenylsulfonamido)thiazol-4-yl)-acetamido)-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (3): (1.33 g, 0.1 mol) cefixime was dissolved in 25 mL water, when pH of solution became 9 then (0.264 g, 0.1 mol) *p*-toluene sulfonyl chloride was added. This reaction mixture was stirred at room temperature; under controlled pH. Monitoring of preceding reaction was done by use of TLC. At the end of reaction 1 N HCl was added, product was precipitated out at pH 2-3, which was separated out by filtration (**Scheme-III**). Purification of product was performed by recrystillization using methanol. TLC of purified product was done using mobile phase dichloromethane:ethanol (60:40).

IR (film, ν_{max}, cm^{-1}): 1159.48 (N-*S*=*O* str), 1086 (C-N), 1652.80 (-N-*C*=*O*), 819.62 (C-S), 958.92 (S-N). δ_H (300 MHz; $CDCl_3$): 11.07 (1H, s,-COO*H*), 10.56 (1H, s,-COO*H*), 8.48-8.52 (1H, d, *J* = 6.3 -N*H*CO-), 7.94-8.25 (4H, m, Ar*H*), 7.79 (1H, s, -C=C*H*S-), 7.54-7.64 (4H, m, Ar*H*), 6.64-6.78 (1H, dd, *J* = 6.3, 1.3, -C*H*=CH_2), 5.83-5.88 (1H, d, *J* = 6.5,-C*H*N (S)), 5.42-5.50 (1H, dd, *J* = 6.4, 1.4, -CH=C*H*H), 5.21-5.32 (1H, dd, *J* = 7.1, 1.3, -CH=CH*H*), 5.08 (1H, s, -OCH_2COOH), 4.86-4.97 (1H, q, *J* = 6.5, -C*H*NHCO), 3.48-3.66 (1H, dd, *J* = 6.2, 1.2, -C*H*HN), 3.23-3.35 (1H, dd, *J* = 6.3, 1.2, -CH*H*N), 3.95-3.03 (2H, dd, *J* = 4.3, 1.4,-C*H*HS), 2.34 (6H, s, -Ar (CH_3)$_2$); *m/z* (ESI) 747.84; HRMS (ESI) calcd for $C_{30}H_{29}N_5O_{10}S_4$;



Scheme-II



Scheme-III

TABLE-1
PHYSICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

Compd.	m.f.	Colour of compounds	R_f value	λ_{max} [a]	m.p. (°C)	Yield (%)
1	$C_{17}H_{20}N_2O_6S_2$	Green	1.20	270	240	56
2	$C_{23}H_{27}N_3O_6S_2$	Light green	0.44	255	210	55
3	$C_{30}H_{29}N_5O_{10}S_4$	Light brown	0.33	289	180	77

[a]Solvent used for λ_{max} was ethanol for all compounds.

$C_{30}H_{29}N_5O_{10}S_4Na^+$ 770.92 Mass analysis for mass 747.9210341 and found to be 747.9223402. Anal. calcd. for $C_{30}H_{29}N_5O_{10}S_4$ (m.w. 747.84): C, 48.18; H, 3.91; N, 9.36; S, 17.15 %. Found: C, 48.16; H, 3.89; N, 9.34; S, 17.13 %.

Antibacterial assay: Luria-Bertani broth was used as a growth medium due to it efficient results in bacterial growth [17]. LB broth was prepared by taking 4 g of tryptone, 2 g of yeast extract and 4 g of sodium chloride in 400 mL of distilled water, using NaOH (1 N) pH was maintained at 7. This prepared medium was autoclaved at 125 °C for 0.5 h. Sample solutions were prepared in ethanol and ethanol was also used as control. Using micropipette different volumes of samples were taken in appendroff tubes. All this process was done in Laminar flow of BSL-2 in order to keep contamination away.

Each sample was screened against two bacterial strains, in 5-50 µg concentration range. For each bacterial strain 3 test tubes were labelled. 2 mL of LB broth and 20 µL of bacterial strains [Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*)] were added in these tubes. After that stocks of 5 µL, 10 µL, 20 µL containing 5 µg, 12.5 µg and 50 µg were added. These tubes were incubated at 37 °C for complete 24 h. After this optical density of each medium and control medium was taken at 600 nm.

RESULTS AND DISCUSSION

Synthesized sulphonamides are soluble in common organic solvents. Physical properties of synthesized sulphonamides are given in Table-1.

In compound **1**, ^{1}H in -COC*H*N- is coupled with the neighbouring proton [-C*H*S (CH_3)] present in the four member ring to give a doublet at 5.14-5.27 ppm and *vice versa*. It was observed that 1st doublet appeared at up field side than the 2nd one which might be due to the high deshielding effect of nitrogen than sulphur. A broad singlet was noticed at 1.88 ppm whose integration indicated the presence of 6 proton of two methyl groups.

In compound **2**, there is a diasterocentre *i.e.* -CH^{a}H^b present in the four member ring. Both protons in -CH_2 are different from each other, coupled with each other to give a doublet and that doublet is further coupled with neighbouring ^{1}H to give a doublet of doublet. Two doublets of doublet were found at 4.89-5.27 and 4.20-4.60 ppm, respectively.

In compound **3**, chemical shift values were calculated for a diasterocentre at 3.48-3.66 and 3.23-3.35 ppm respectively. In this molecule, there is vinyl group (-CH=CH_2-) in which all three protons are chemically non-identical and every one gave a doublet of a doublet. Their activities against both bacterial strains are shown in Table-2.

TABLE-2
IC_{50} DETERMINATION OF DIFFERENT SYNTHETIC PRODUCTS AGAINST DIFFERENT STRAINS OF BACTERIA

Compound No.	*S. aureus*	*E. coli*
1	5 µg	5 µg
2	–	5 µg
3	12.5 µg	50 µg

Note: The amount of compound at which 50 % bacterial growth is inhibited with reference to control is called IC_{50} value.

Minimum inhibition concentration values for all synthesized sulphonamides shows a discrepancy between 5 to 50 µg. It is clear that best MIC values for both strains were obtained by new sulphonamide molecule compound **1** *i.e.* 5 µg, however compound **2** showed good result for only one strain that is *S. aureus* having IC_{50} value at concentration of 5 µg and compound **3** proved more resistant to *E. coli* than *S. aureus* having MIC value 12.5 µg.

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