

## Research Article

# Synthesis, Characterization and Pharmacological Screening of Substituted Sulphonamide Derivatives

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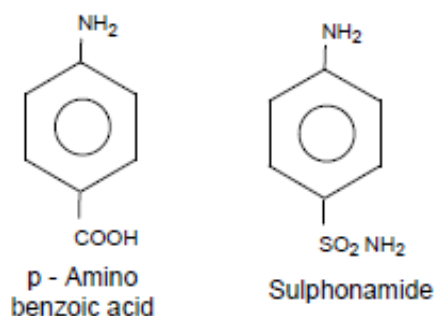
**Abstract:** Sulphonamides are an important class of heterocyclic compounds which possess wide spectrum of biological properties. The present work deals with the synthesis of some novel Sulphonamide derivatives using acetanilide, chlorsulphonic acid and various aromatic amines. All the synthesized compounds were characterized by IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR Spectroscopy. The compounds were evaluated for their antibacterial activity against Staphylococcus aureus 3TCC 3160 and Escherichia coli 3TCC 1652 using Streptomycin as reference standard and anti-inflammatory activity using Diclofenac as reference standard. Some of the analogues showed significant activity based on the substitutions on the aromatic ring.

**Keywords:** Sulphonamides, acetanilide, chlorsulphonic acid, anti-microbial, anti-inflammatory

### INTRODUCTION:

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating disease. Most of the activity is directed to new natural or synthetic organic compounds [1]. Heterocyclic nucleus imparts an important role in medicinal chemistry and serves as a key template for the development of various therapeutic agents. Significant number of compounds synthesized in industrial sector each year is heterocyclic in nature. Antibacterial sulfonamides are synthetic antimicrobial agents that contain the sulfonamide group. Sulphonamides (sulpha drugs) are one of a group of drugs derived from sulphanilamide that prevents the growth of bacteria. Sulphonamides compete with p-aminobenzoic acid (PABA) for the enzyme dihydropteroatesynthetase, which is important in the formation of folic acid that is required by the bacteria [2]. Folic acid is required for the synthesis of precursors of DNA and RNA both in bacteria and in mammals. Mammals obtain their folic acid in their diet but bacteria need to synthesize it. Sulphonamides inhibit the growth of bacteria but do not kill them i.e. their action is bacteriostatic. Many sulphonamides are rapidly excreted and very soluble in urine so they are used to treat infections of the urinary tract. Sulphonamides (sulpha drugs) are drugs that are derived from sulphanilamide, a sulphur-containing chemical. Most sulphonamides are antibiotics, but some are prescribed for treating ulcerative colitis [3]. Sulphonamide antibiotics work by disrupting the production of dihydrofolic acid, a form of folic acid that

bacteria and human cells use for producing proteins. In bacteria, antibacterial sulphonamides act as competitive inhibitors of the enzyme dihydropteroatesynthetase (DHPS), an enzyme involved in folate synthesis. Sulphonamides are therefore bacteriostatic and inhibit growth and multiplication of bacteria, but do not kill them. Humans, in contrast to bacteria, acquire folate (vitamin B9) through the diet [4].

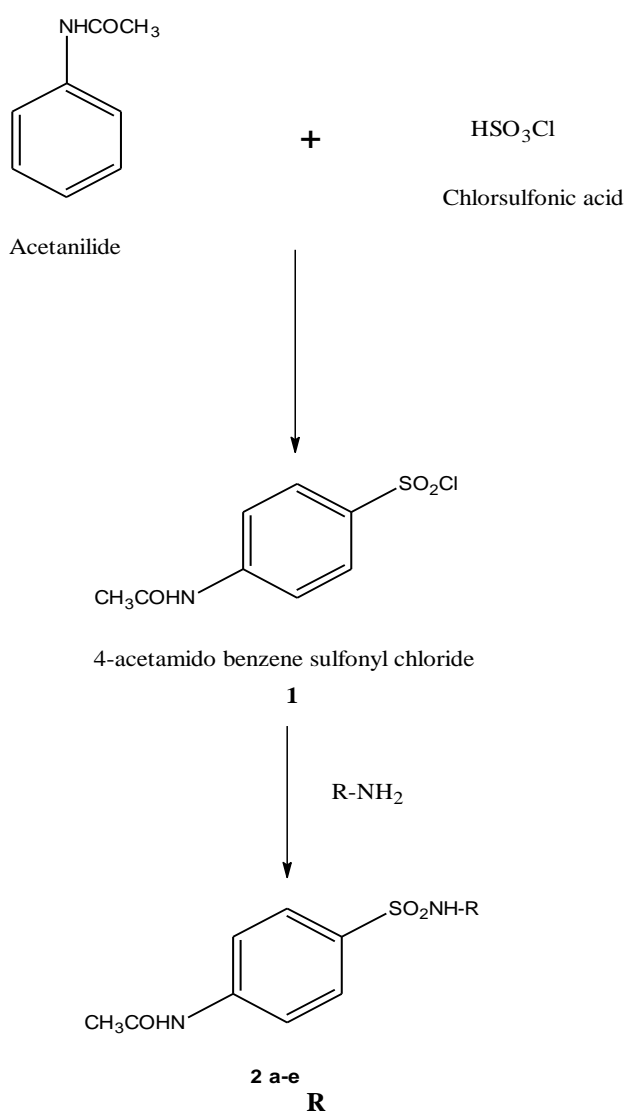


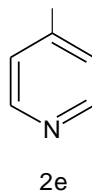
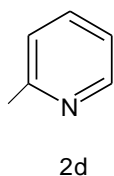
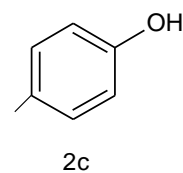
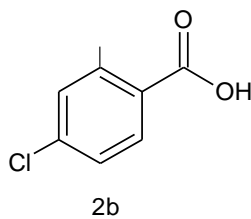
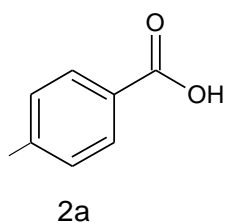
The sulphonamide chemical moiety is also present in other medications that are not antimicrobials, including thiazide diuretics (including hydrochlorothiazide, metolazone, and indapamide), loop diuretics (including furosemide, bumetanide, and torsemide), sulfonylureas (including glipizide, glyburide, among others), and some COX-2 inhibitors (e.g., celecoxib), and acetazolamide. Sulfasalazine, in addition to its use as an antibiotic, is also used in the treatment of inflammatory bowel disease [5].

Sulfonamides have the potential to cause a variety of untoward reactions, including urinary tract disorders, haemopoietic disorders, porphyria, and hypersensitivity reactions. When used in large doses, they may cause a strong allergic reaction. Two of the most serious are Stevens–Johnson syndrome and toxic epidermal necrolysis (also known as Lyell syndrome). Approximately 3% of the general population have adverse reactions when treated with sulphonamide antimicrobials. Of note is the observation that patients with HIV have a much higher prevalence, at about 60% [6]. Hypersensitivity reactions are less common in nonantibioticsulfonamides, and, though controversial, the available evidence suggests those with hypersensitivity to sulphonamide antibiotics do not

have an increased risk of hypersensitivity reaction to the nonantibiotic agents. A key component to the allergic response to sulphonamide antibiotics is the arylamine group at N4, found in sulfamethoxazole, sulfasalazine, sulfadiazine, and the anti-retroviral amprenavir and fosamprenavir. Other sulphonamide drugs do not contain this arylamine group; available evidence suggests that patients who are allergic to arylaminesulfonamides do not cross-react to sulfonamides that lack the arylamine group, and may therefore safely take non-arylamine sulfonamides. It has therefore been argued that the terms 'sulfonamide allergy' or 'sulfa allergy' are misleading, and should be replaced by a reference to a specific drug (e.g. 'cotrimoxazole allergy') [7].

**Synthetic Scheme:**





## MATERIALS AND METHODS

### Experimental work:

#### General procedure for the synthesis of different sulphonamides:

##### Step I: Synthesis of 4-acetamidobenzenesulphonylchloride:

Take 25g (0.185 mol) of acetanilide in a 500 ml round bottom flask and add 63ml (1 mol) of chlorosulphonic acid in small portions with continuous shaking. After complete addition reflux the reaction mixture for one hour on a water bath. Cool and pour the reaction mixture into 300g of crushed ice. Filter and wash with water and dry the product. The obtained product can be used as such in the next step.

##### Step II: Synthesis of different Sulphonamides (2a-e):

Amines (0.02 mol) were suspended in 100 ml water and the pH 9-10 was maintained by adding basic aqueous solution of sodium carbonate (10%). Then 4-acetamidobenzenesulphonylchloride (0.02 mol; 1) was added in the reaction mass slowly over 10-15 min. After complete addition of compound 1, the reaction mixture was stirred and monitored with TLC (n-hexane: Ethyl acetate; 7:3) for the completion of the reaction. Then conc.HCl was added slowly to adjust the pH to 2.0. The reaction mixture was reserved at room temperature for 15 min; white solid was filtered, washed with distilled water and dried to obtain the corresponding compound (2a-e).

## ANTI-MICROBIAL ACTIVITY

### Diffusion assays:

Diffusion assays are carried out on a solid medium usually an agar medium, which is suitable for the growth of the test organism. The compound to be assayed is allowed to diffuse through the medium in a radial fashion from a cup. So the adjacent growth of the test organism is either depressed, as with an antibacterial which depresses the growth, or stimulated as with a growth factor. The diameter of this area reflects the concentration of the compound being assayed and it is compared with similar zones produced various known concentrations of standard or reference

compound. There are two types of diffusion assays, although somewhat similar, each has its own particular advantages. Those methods are paper disc method and cup plate method [8-12].

In paper disc method paper discs are applied with 0.1ml of testing substance. A standardized amount of agar medium, perhaps 10ml, is placed in petri plates and allowed to solidify. As soon as this base layer is solidified, a standardized amount of the same or a different agar medium inoculated with a test organism is added above the base layer and allowed to solidify to form the seeded agar layer. Then paper discs are placed on solidified agar medium. The number of discs used for plate depends on expected sizes of the zones, since the zones should not overlap.

In cup plate method, instead of using discs, cups or cylinders are made on the solidified and seeded agar medium. These cylinders are filled with the appropriate dilutions of the solutions to be assayed or with solutions containing known concentrations of the reference compound and the plates are incubated for a specific period of time kept at constant temperature. The diameters of the zones are measured in millimetres and the concentrations in the solutions under assays are determined by comparison with standard[9].

### Experimental work:

In the present experiment the antimicrobial activity was tested by cup plate assay method. The antimicrobial activity of Sulphonamides was tested and compared with the standard streptomycin solution at two different concentrations i.e., 500µg and 1mg. DMSO is used as a solvent.

### Test organisms:

Staphylococcus aureus 3TCC 3160  
Escherichia coli 3TCC 1652

### Procedure:

Medium was inoculated at 1% level with 18hrs old cultures of the above mentioned test organisms and

were transferred into sterile Petri dishes. The medium in the plates was allowed to set at room temperature for about 10min and they were set to solidify in a refrigerator for 30min. After that cylinders were made in the medium. The test solutions which were prepared in DMSO along with the standard solution of streptomycin were placed in their respective cylinders. The plates thus prepared were left to stand in a refrigerator for about 1hr to allow the test solution for diffusion. Then incubation of the above plates was done for 24hrs at 37°C. The plates were examined for zones of inhibition and the inhibition zone diameters were recorded in the table.

#### IN-VITRO ANTI-INFLAMMATORY ACTIVITY:

##### Procedure:

The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity [13-14]. Blood will be collected from healthy volunteer. The collected blood mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood will be centrifuged at 3000 rpm and packed cells will be washed with isotonic saline (0.85%, pH 7.2) and a 10% (v/v) suspension will be made with isotonic saline. The assay mixture contains the drug (1000µg/ml), 1ml of phosphate buffer (0.15M, pH 7.4) and 2ml of hypotonic saline (0.36%) and 0.5ml of HRBC suspension. Diclofenac will be used as reference drug. Instead of hypotonic saline 2ml of distilled water will be used in the control. All the assay mixtures will be incubated at 37°C for 30 minutes and centrifuge at 3000 rpm. The absorbance of the supernatants will be estimated using UV spectrophotometer 560 nm.

$$\text{Percent stabilization} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

#### RESULTS AND DISCUSSION:

The melting point of organic compound was determined by Thiel's melting point tube (Capillary tube method). The IR spectra of the compounds were carried out in FT-IR Bruker  $\alpha$ -T model and only characteristics peaks were reported. The <sup>1</sup>H NMR and <sup>13</sup>C NMR of the compounds were carried out in Bruker AMX 400 MHz NMR with TMs as internal standard. The solvent used was Deuterated Dimethyl sulfoxide. All the reactions were monitored over silica

gel-G TLC plates and spots were visualized by iodine vapors or by irradiation with ultraviolet light (254 nm).

#### 4-([4-(acetyl amino) phenyl]sulfonyl)amino benzoic acid (2a):

Molecular formula: C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>5</sub>S; Melting Point: 205-208 °C; IR (KBr Pellet,  $\nu$  in cm<sup>-1</sup>): 3030.37 (Ar C-H Str), 2945.68 (CH<sub>3</sub> Str), 3245.68 (N-H Str), 1667.59 (Amide C=O Str), 1784.97 (Acid C=O Str), 3245.68 (Acid O-H Str), 1398.66 & 1158.92 (S=O Str).

#### 2-([4-(acetylamino) phenyl] sulfonyl)amino)-4-chlorobenzoic acid (2b):

Molecular formula: C<sub>15</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>5</sub>S; Melting Point: 247-250 °C; IR (KBr Pellet,  $\nu$  in cm<sup>-1</sup>): 3030.17 (Ar C-H Str), 2859.02 (CH<sub>3</sub> Str), 3386.80 (N-H Str), 1666.01 (Amide C=O Str), 1766.24 (Acid C=O Str), 3386.80 (Acid O-H Str), 1314.69 & 1169.04 (S=O Str), 760.56 (C-Cl Str). HNMR: 2.04 (s, CH<sub>3</sub>), 2.51 (s, N-H), 12.57 (s, -CO-OH), 6.8 (s, N-H), 6.86-10.27 (s, Ar-H); <sup>13</sup>CNMR: 168 (C=O), 153 (CO-OH), for benzene 112 (C<sub>2</sub>&C<sub>6</sub>), 113 (C<sub>3</sub>&C<sub>5</sub>), 118 (C<sub>1</sub>), 118 (C<sub>6</sub>), chloro benzene, 126 (C<sub>5</sub>), 127 (C<sub>6</sub>), 132 (C<sub>3</sub>), 135 (C<sub>4</sub>), 137 (C<sub>1</sub>), 144 (C<sub>2</sub>), 40 (-CH<sub>3</sub>).

#### N-[4-(4-hydroxyphenyl) sulfamoyl]phenyl]acetamide (2c):

Molecular formula: C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S; Melting Point: 234-237 °C; IR (KBr Pellet,  $\nu$  in cm<sup>-1</sup>): 3033.48 (Ar C-H Str), 2840.64 (CH<sub>3</sub> Str), 1641.14 (Amide C=O Str), 3613.27 (Phenol O-H Str); 1161.20 (S=O Str).

#### N-[4-(pyridin-2-ylsulfamoyl)phenyl] acetamide (2d):

Molecular formula: C<sub>13</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S; Melting Point: 192-195 °C; IR (KBr Pellet,  $\nu$  in cm<sup>-1</sup>): 3117.25 (Ar C-H Str), 2976.45 (CH<sub>3</sub> Str), 3409.70 (N-H Str), 1664.75 (Amide C=O Str), 1382.96 & 1156.30 (S=O Str).

#### N-[4-(pyridin-4-ylsulfamoyl) phenyl] acetamide (2e):

Molecular formula: C<sub>13</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S; Melting Point: 216-218 °C; IR (KBr Pellet,  $\nu$  in cm<sup>-1</sup>): 3100.79 (Ar C-H Str), 2945.72 (CH<sub>3</sub> Str), 3259.11 (N-H Str), 1678.82 (Amide C=O Str), 1369.46 & 1164.55 (S=O Str); HNMR: 1.9 (s, -CH<sub>3</sub>), 2.50 (s, N-H), 4.06 (s, N-H), 6.8-8.08 (s, Ar-H); <sup>13</sup>CNMR: 40 (-CH<sub>3</sub>), 131 (C=O), 108 (benzene C<sub>1</sub>-C<sub>6</sub>), 122 (pyridine C<sub>1</sub>, C<sub>2</sub>, C<sub>4</sub>&C<sub>5</sub>), 126 (pyridine C<sub>3</sub>).

Table-1: Characterization data of Synthesised Compounds

CompoundCode (Sulphonamides)	Mol. Formula	Mol.wt. (g/mol)	M.P (°C)	Yield (%)	R <sub>f</sub> * Value
2a	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub> S	334.3	205-208	80.42	0.89
2b	C <sub>15</sub> H <sub>13</sub> ClN <sub>2</sub> O <sub>5</sub> S	368.8	247-250	82.25	0.98
2c	C <sub>14</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub> S	306.3	234-237	76.56	0.53
2d	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S	291.3	192-195	81.26	0.54
2e	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S	291.3	216-218	78.92	0.40

\*Mobile phase-n-hexane: Ethyl acetate = 7:3

### Anti - microbial Activity of the Synthesized Compounds:

In the present study we used different species to measure inhibitory potential of chalcones. Results of anti-microbial screening of the product have suggested an anti-microbial activity on the species like *Escherichia coli*, *Staphylococcus aureus*.

Comparing the effect of the sulphonamides on microbial growth allowed concluding on structural elements responsible for effective zone of inhibition.

The order of zone of inhibition of chalcones is as follows:



All the 5 were evaluated for anti-microbial activity by cup plate method at the concentration of 250µg/ml and 500µg/ml using *Streptococcus aureus* and *Escherichia coli* bacteria. Results are represented in tabular column.

**Table-2: Anti-microbial screening of the Synthesized Compounds**

S.No:	CompoundCode (Sulphonamides)	Zone of inhibition(mm)			
		Gm <sup>+Ve</sup>		Gm <sup>-Ve</sup>	
		<i>S.aureus</i>		<i>E.Coli</i>	
		250µg/ml	500µg/ml	250µg/ml	500µg/ml
1	2a	8	8	10	12
2	2b	8	16*	8	8
3	2c	14	14	10	13
4	2d	8	9	8	9
5	2e	9	10	8	9
Control	DMSO	8		8	
Standard	streptomycin	18*		20*	

(\*) Significant zone of inhibition bore size-8mm

### In-vitro Anti-inflammatory screening:

In the present study in-vitro anti-inflammatory activity was checked for the synthesized compounds.

The absorbance of the supernatants will be estimated using UV spectrophotometer 560 nm.

**Tab.3: In-vitro anti-inflammatory screening of Synthesized compounds**

S.No	COMPOUND CODE	ABSORBANCE	PERCENTAGE INHIBITION
1	2a	0.518	66.3
2	2b	0.432	71.9
3	2c	0.400	70.1
4	2d	0.732	52.4
5	2e	0.586	61.8
9	Diclofenac	0.306	80.1
10	Control	1.538	-----

Diclofenac is used as reference drug. Compound 2b has shown highest percentage of inhibition compared to other derivatives. Compounds 2c, 2a, 2e, 2d has shown moderate percentage of inhibition.

### CONCLUSION

By using acetanilide, chlorosulphonic acid and various aromatic amines novel sulphonamide derivatives were synthesized. All the 5 synthesized derivatives of sulphonamides were evaluated with physical, analytical characterization and biological screening such as anti-microbial and *In-vitro* anti-inflammatory.

From the above results it is evident that synthesized sulphonamide derivatives 2a-2e showed significant broad spectrum antibacterial activity at

500µg/ml & 1mg/ml concentration levels when compared with standard drug Streptomycin. In particular, compounds containing the withdrawing groups (halogens) show the maximal activity. Compounds containing the chloro, phenolic substituents attached to aromatic ring shows the better activity compared with other substituents attached to the aromatic nucleus.

A future aspect of the Synthesised derivatives of sulphonamides is to carry out in-vivo studies to bring Potential effects.

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