

# Schiff's Base Of Sulphonamide with Para Fluoro Benzaldehyde: Novel Potential Antimicrobial Agent

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## ABSTRACT

Antibacterial and antifungal diseases are very common all over the world. Currently used antimicrobial agents are not very useful due to the resistance developed by the microbes against them. In continuation to this, the present study was aimed at synthesizing Schiff's base of sulphonamide nucleus incorporated with para-substituted benzaldehyde showing good activity, with para-sulphonamido group playing a key role, and evaluating the potential of this agent as antimicrobial. The improvement achieved in potency of sulfonamide by introducing electron-withdrawing groups at the N<sup>1</sup>-position, which produced such highly potent drug as sulfadiazine, established the power of molecular modification in drug discovery. For the accomplishment of the proposed objective, the established method of synthesis with some modification was adopted, i.e. reacting sulphonamide and para substituted derivative of benzaldehyde resulting in formation of Schiff base or imine. Imine formation is acid catalysed, generally takes place fastest between pH 4-5 and is slow at very low or very high pH. In methodology a mixture of p-amido sulphonamide and p-fluoro benzaldehyde was refluxed in absolute ethanol for 12-14 hours in water bath in Dean Stark Apparatus, then Schiff's base was filtered, dried and recrystallised from absolute ethanol. The synthesized compound was subjected to physicochemical and spectral characterization. Antimicrobial activity of the compound was performed against microbes *E.coli* and *Aspergillus niger*. A concentration dependent increase in activity was observed which was due to molecular modification i.e. para fluoro benzaldehyde incorporated in sulphonamide nucleus forming Schiff's base or imine. From the results it is evident that this research resulted in producing novel potential Schiff's base of sulphonamide with enhanced antimicrobial activity.

**Keywords:** Antimicrobial Agent, Chemotherapy, Sulphonamides, Substituted benzaldehydes, Anti bacterial Agents, Anti fungal

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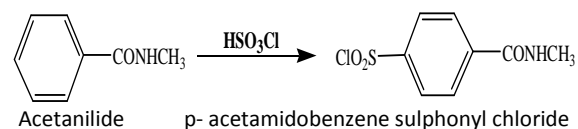
## INTRODUCTION

The modern era of antimicrobial chemotherapy dates back to 1936, with the introduction of sulfanilamide into clinical practice. Hundreds of antibiotics have been identified and developed to the stage where they are of value in therapy of infectious diseases.<sup>1</sup> Microbiologically produced drugs are known as antibiotics. The term antibiotic was introduced in 1942 by Waksman which mean substances produced by microorganism (microbial metabolic products) which suppress the growth of (static) or kill (cidal) the microorganism at very low concentrations. Sulpha drugs are a group of compounds used for eliminating a wide range of infections in human and other animal systems. Many therapeutically important sulpha drugs like sulphadiazine, sulphathiazole, sulphamerazine and so forth, posses  $\text{SO}_2\text{NH}_2$  moeity which is an important toxophoric function. The amide moiety is an important constituent of many biologically significant compounds. The importance of sulphonamides nucleus is well established in pharmaceutical chemistry. The discovery of mode of action of sulfonamides led to development of many new and effective antimetabolites. Because of structural similarity of sulphonamides with PABA, it competes with this substrate for bacterial enzyme Dihydropteroate synthase (DHPS). They, thus, inhibit the synthesis of bacterial folic acid and thereby the formation of essential cofactor forms. The improvement achieved in potency of sulfanilamide by introducing electron-withdrawing groups/heterocycles at the N1-position, which produced such highly potent drugs as sulfadiazine established the power of molecular modification in drug discovery.<sup>2</sup> (Fig.1)

## MATERIALS AND METHODS

### Procedure for step 1

A 500 ml two necked flask was equipped with a dropping funnel and a reflux condenser. Attached the top of the later a device for the absorption of hydrogen chloride. 20g (0.148 moles) of dry acetanilide was placed in the round bottom flask and 50ml (90g; 0.77 mol) of a good grade of chlorsulphonic acid in the dropping funnel and a calcium chloride guard tube was inserted into the later. Chlorsulphonic acid was added in small portions and flask was shaken from time to time to ensure thorough mixing. When the addition has been made, the reaction mixture was heated on a water bath for 1 hour in order to complete the reaction. Then it was allowed to cool and the oily mixture was poured in a thin stream with stirring into 300g of crushed ice (or ice water) contained in a 1- litre beaker. This operation was carried out carefully in the fume cupboard since excess of chlorsulphonic acid reacts vigorously with water. Flask was rinsed with a little and ice water and rinsing were added to the content of the beaker. Any lumps if formed were broken and mixture was stirred for several minutes in order to obtain a even suspension of granular white solid. P-acetamido benzene sulphonyl chloride was filtered off at the pump, washed with little cold water and drained well..

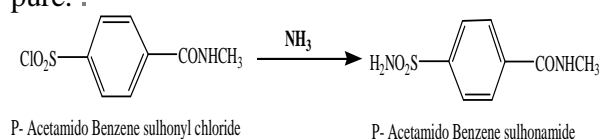


**Scheme -1**

### Procedure for Step 2

P-Acetamido benzene sulphonyl chloride was placed in the rinsed reaction flask, and a mixture of 70ml of concentrated ammonia solution and 70ml of water was added. The contents of the flask were

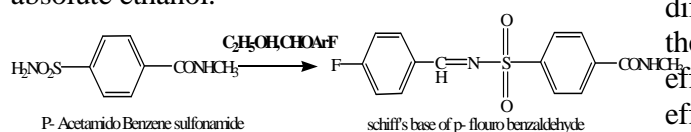
thoroughly mixed, and the mixture was heated with occasional swirling for 15 to 30 minutes. The sulphonyl chloride will be converted into pasty suspension of the corresponding sulphonamide. The suspension was cooled in ice, and then dilute sulphuric acid was added until the mixture is just acidic to litmus paper. Product was collected on Buchner funnel, washed with little cold water and drained as completely as possible. Product was dried at 100°C, and is sufficiently pure.<sup>3</sup>



Scheme -2

### Procedure for Step 3

A mixture of 2 (0.02 mole, 4.28g), p-flouro benzaldehyde (0.02 mole, 2.48 g) was taken in a Flat Bottom Flask fitted with  $\text{CaCl}_2$  guard tube. Then 45 ml of absolute ethanol was added to it. 1g of molecular sieves (4Å-5Å) and a pinch of  $\text{ZnCl}_2$  (anhydrous) were added for absorption of water generated in reaction, refluxed for 12-14 hours in water bath in Dean Stark Apparatus. After the completion of reaction, the solvent was removed by vacuum distillation and reaction mixture poured in crushed ice. Schiff's base was filtered, dried and recrystallised from absolute ethanol.<sup>4</sup>



Scheme -3

### Physicochemical and Spectral characterization

The synthesized compounds was subjected to physicochemical and spectral characterization. The compounds were purified by column chromatography by using

different grades of silica gel of column packing. Melting points reported are uncorrected. The Rf. values of synthesized compounds were determined. IR was done by applying 10 lbs sq. inch of pressure for about 2 minutes. The pellets were exposed to IR radiation using Shimadzu FTIR spectrophotometer to obtain the spectra. Mass spectra were recorded on Jeol/SX-102/DA-600 FAB mass spectrophotometer carried in SAIF department of CDRI, Lucknow. (Table 1)

### Pharmacological Evaluation

Different microbial species and strains have different degrees of susceptibility to different chemotherapeutic agents. Moreover, the susceptibility of a microorganism can change with time, even during therapy with a specific drug. Several tests have been used to indicate which chemotherapeutic agent is most likely to combat a specific pathogen.

### Principle Method involved: Disk Diffusion Method

Probably the most widely used, although not necessarily the best, method of testing is the disk diffusion method also known as Kirby-Bauer test. A Petri plate containing an agar medium is inoculated (seeded) amount of test organism. Next, filter paper disk impregnated with known concentrations of chemotherapeutic agents diffuse from the disk into the agar. The farther the agent diffuses from the disk, higher effective it is. If the chemotherapeutic agent is effective, a zone of inhibition forms around the disk after a standardized incubation.

### Experimental

#### Microbes

All microorganisms were obtained from MTCC (Microbial Type Culture Collection) Institute, Chandigarh.

### Standard preparations

A Standard preparation is an authentic sample of the appropriate antibiotic for which the potency has been precisely determined by reference to appropriate international standard. Ciprofloxacin and Griseofulvin were used as standard drugs. Standard sample preparations were made by dissolving in DMSO. DMSO below 10% v/v does not possess any antimicrobial activity.

### Test samples

Test samples of final compound were prepared in concentrations of 800µg/ml and 1000µg/ml using DMSO as solvent.

### Procedure

10 ml of the nutrient Broth suspension of test organisms were added to 100ml of sterile molten nutrient agar growth media (MTCC specified agar growth media for each organism) which has been cooled to 45°C, mixed well and poured into sterile Petri plates. The agar was allowed to solidify; it was then punched to make six wells/cups using a six mm sterile cork borer (separate borer for each organism) to ensure proper distribution of wells in periphery and one in centre. Agar plugs were removed and 50µl of test samples of each concentration of final compounds were poured in corresponding marked well by micropipettes after marking of concentration at the back of the wells. Triplicate plates of each organism were prepared. The plates were left at room temperature for 2 hrs to allow diffusion of samples and incubated face upward at corresponding temperature of each organism for 24 hrs. The diameter of zones of inhibition was measured to nearest millimeters (the cup size also being included).<sup>6,7</sup>

## RESULTS & DISCUSSION

The present study was aimed at synthesizing novel derivatives, having polycyclic structure with introduction of

sulphonamide nucleus incorporated with parafluoro substituted benzaldehydes showing good activity with para- sulphonamido group playing a key role. Schiff's Base was named after Hugo Schiff, is a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group but not hydrogen. Synthesis was done following a standard protocol<sup>8, 9, 10, 11</sup>. The synthesized compound was subjected to physicochemical and spectral characterization. Evaluation of these compounds as Antimicrobial agents of the was performed against microbes *E.coli* and *Aspergillus niger*. A concentration dependent increase in activity was observed which was due to molecular modification i.e. para fluoro benzaldehyde incorporated in sulphonamide nucleus forming Schiff's base or imine. From the results it is evident that this research resulted in producing novel potential schiff's base of sulphonamide with enhanced antimicrobial activity.

## CONCLUSION AND FUTURE PROSPECTIVE

The importance of sulphonamides is well established in pharmaceutical chemistry. A considerable number of sulphonamides are well known as antibacterial, carbonic anhydrase inhibitor, anticancerous, anti-inflammatory agents.<sup>12</sup> Sulfonamides are widely used in clinical practice even six decades after their discovery because of their wide pharmacologic and pharmacokinetic profile. In the current trend, this choice has declined due to gradual increase in resistance to them. Even though they are safe drugs but many adverse reactions appeared which suggested scientists to synthesize new derivatives, which are devoid of any adverse effects.<sup>13</sup>

Furthermore, accordingly in order to improve the previously observed activities it was decided to attach 4- amino sulphonyl phenyl group to some peculiar para

substituted benzaldehydes.<sup>14, 15</sup> The existing work shows that the sulphonamides nucleus acting as central and crucial scaffold with benzaldehydes derivatives having para fluoro substituents can lead to development of highly potent compounds having good to moderate level of various activities. After generating more and more new derivatives and assessing their biological activity data, one can go for exploration and study of the generated structures at molecular level and can easily correlate to their **Structure Activity Relationship** which will give a better model for the structure optimization. Thus, information obtained from this study can be utilized as a tool for further development of novel drug molecules.

## REFERENCES

1. Gilman, A.G.; Hardman, J.G.; Limbird, L.E. "Goodman and Gilman's the Pharmacological Basis of Therapeutics" 10th Ed, McGraw-Hill Companies Inc. 2001, 1143-1146.
2. Abraham, D.J. "Burger's Medicinal Chemistry & Drug Discovery" 6th Ed, John Wiley and Sons Inc. **2003**, 5, 539-543, 557-558. Kucukguzel, S.C.; Rollas, S.; Ozbek, A. *European Journal of Medicinal Chemistry* **2002**, 37, 197-206.
3. Furniss, B.S.; Hannaford, A.J.; Smith, P.W.G.; Tatchell, A.R. "Vogel's Textbook of Practical Organic Chemistry" 2nd Ed, Pearson Education **2007**, 882-885, 1284-1287.
4. Singh, S.P.; Parmar, S.S.; Raman, K.; Stenberg, V.I. *Chemical Review* **1981**, 81, 175-203.
5. Pavia, D.L.; Lampman, G.M.; Kriz, G.S. "Introduction to spectroscopy" 3rd Ed, Harcourt College Publishers **2001**, 13-101.
6. Tortora, G. J.; Funke, B.R.; Case, C.L. "Microbiology: An introduction" 8th Ed, Pearson education **2005**, 589-616.
7. Wiley, J.M.; Sherwood, L.M.; Woolverton, C.J.; "Prescot's, Harley and Klein's Microbiology" 7th Ed, Mc Graw Hill Companies Inc. **2008**, 57, 59, 835-885.
8. Cunico, W; Gomes, C.R.B.; Ferreira, M.L.G.; Capri, L.R.; Soares, M.; Wardell, S.M.S.V. *Tetrahedron Letters* **2007**, 48, 6217-6220.
9. International Union of Pure and Applied Chemistry. "Schiff base". Compendium of Chemical Terminology **Internet edition**.
10. Solomons, T.W.G.; Fryhle, C.B. "Organic Chemistry" 8th Ed, John Wiley and Sons Inc. **2004**, 972-975.
11. Carey, F.A. "Organic chemistry" 5th Ed, McGraw-Hill Companies Inc. **2004**, 724-729, 748, 934-935.
12. Joshi, S.; Khosla, N. *Bioorganic and Medicinal Chemistry Letters* **2003**, 13, 3747-3751.
13. Abraham, D.J. "Burger's Medicinal Chemistry & Drug Discovery" 6<sup>th</sup> Ed, John Wiley and Sons Inc. **2003**, 5, 539-543, 557-558.
14. Vazzana, I.; Terranova, E.; Mattioli, F.; Sparatore, F. *ARKIVOC*, **2004**, (V), 364-374.
15. Navidpour, L.; Shadnia, H.; Shafaroodi, H.; Amini, M.; Dehpour, A.R.; Shafee, A. *Bioorganic and Medicinal Chemistry* **2007**, 15, 1976-1982.

**Table 1. The Physical Properties of Synthesised Final Compound**

Sr.no	Compound	Mol. Formula	Melting range (°C)	% yield
1.	p-fluoro imine of p- amido sulphonamide	C <sub>15</sub> H <sub>13</sub> FN <sub>2</sub> O <sub>3</sub> S <sub>2</sub>	284-292	71%

**Table 2. Spectral Properties of the Synthesised Final Compound <sup>5,6</sup>**

Compound	Mol. Formula	Mol. wt.	IRcm <sup>1</sup> peaks	Interpretation of peaks	Mol. ion peak
p-fluoro imine of p- amido sulphonamide	C <sub>15</sub> H <sub>13</sub> FN <sub>2</sub> O <sub>3</sub> S <sub>2</sub>	352	2981.74	C-H Aromatic peak at 3250-3050	Mol. Ion peak at 353
			3409.91	NH (primary) peak at 3500-3100	
			2908.14	Aldehyde peak at 2900-2800	
			1731.96	C=O peak of aldehyde at 1740-1720	
			1157.21	S=O peak of sulphonamides at 1375-1140	
			1222.79	C-F peak at 1400-1000	



**Table 2. Antimicrobial Activity and Zone of Inhibition of Final Compounds**

Compound	Concentration ( $\mu\text{g/ml}$ )	Zone of inhibition (mm)	
		E.coli	A.niger
Novel			
Synthesized	800	16	12
compound	1000	18	14

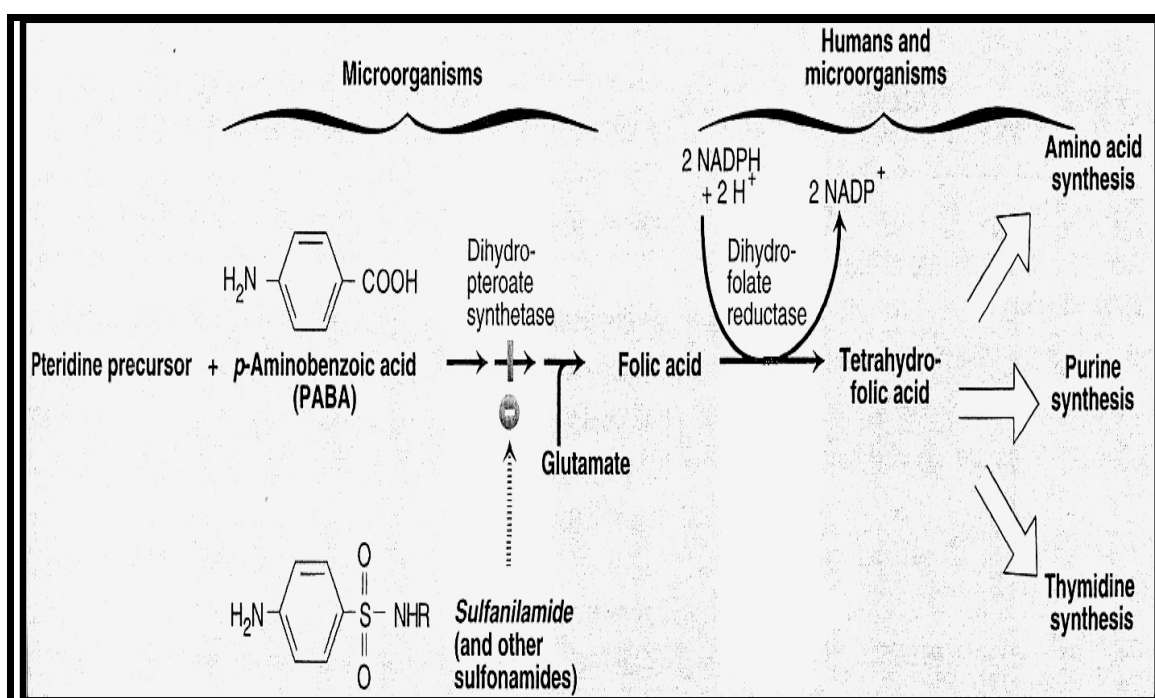


Figure.1. Mode of Action of Sulfonamides.

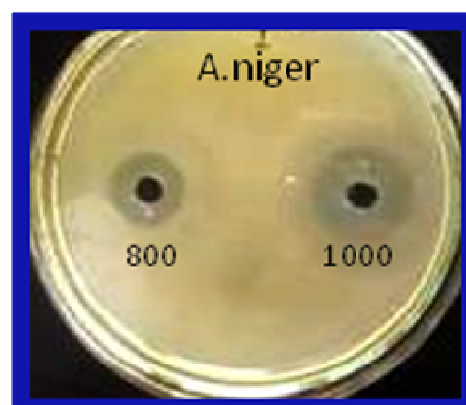
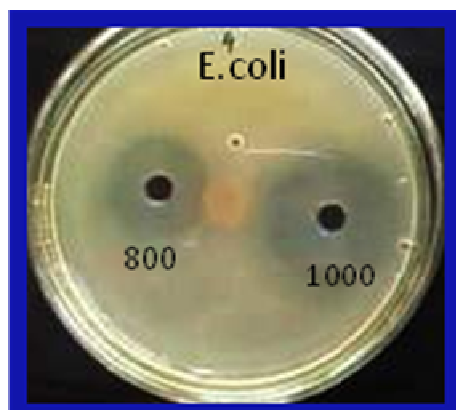


Figure.2. Zone of Inhibition.