Project Report Summary

Novel Sulphonamide azodyes and their antimicrobial activities

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Minor Research Project

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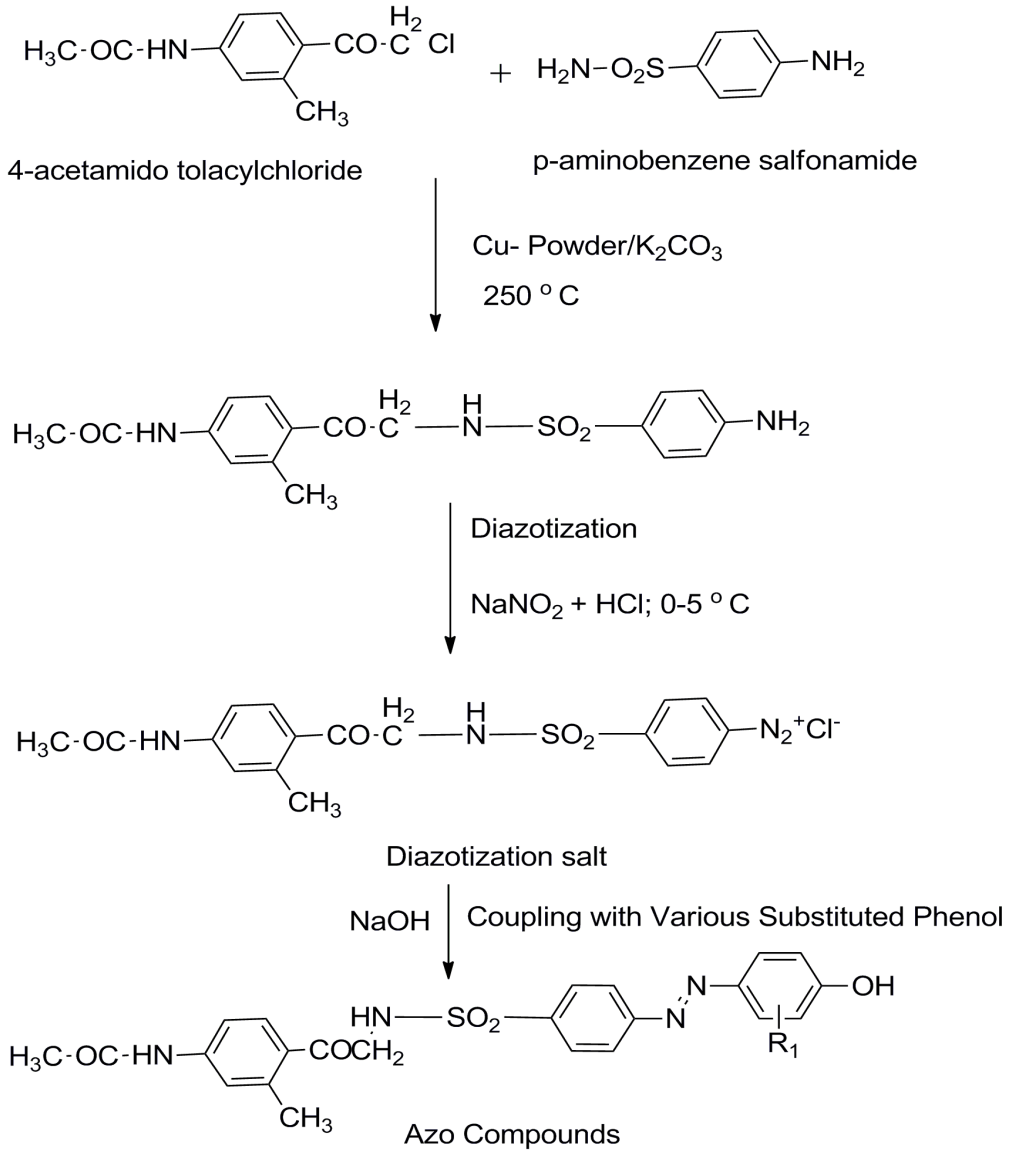
Objective:

The aim of the study leads towards synthesis of sulphonamide linked Azo compounds owing antibacterial activity. The enhancement of the activity will be studied as compared to standard sulphonamide drugs available. The new Azo compounds based on sulphonamide molecules will be designed and synthesised and further screened against various bacterial and fungal species.

Chemical Aspect:

All chemicals and solvents were obtained from commercial suppliers and were used without further purification. Melting points were determined on an electro thermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected.



Scheme: Synthetic strategies leading to Azo Compounds

Table 1: Substitutional pattern of Azo compounds

Compounds	**R_1 (Substituted Phenols)**	**% Yield**	**Melting Point (°C)**
D1	-H	80	212
D2	2-Cl	74	209
D3	3-Cl	75	206
D4	4Cl	72	203
D5	2-Br	81	215
D6	3-Br	78	217
D7	4-Br	69	222
D8	2-NO_2	70	212
D9	3-NO_2	73	216
D10	2-CH_3	66	209
D11	3-CH_3	74	206
D12	4-CH_3	69	208
D13	2-OCH_3	70	212
D14	3-OCH_3	76	219
D15	4-OCH_3	74	220
D16	3-OH	79	202
D17	1-Naphthol	66	199
D18	2-Naphthol	63	198

Biological Aspect:

Antimicrobial activity:

So far as microorganisms concerned with the study of biological activity of complexes, parental ligands and metal salts, the Bacterial organisms used in present study were:

Micro organisms:

Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger and Aspergillus clavatus.

Materials:

- Sterile graduated pipettes of 10ml, 5ml, 2ml and 1ml sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles
- Pasteur pipettes
- Overnight broth culture of test and control organisms (same as for disc diffusion tests)
- Required micro organism.
- Required solvent for the micro organism.
- Sterile distilled water - 500ml and suitable nutrient broth medium
- A suitable rack to hold 22 tubes in two rows.

Stock solution preparation:

Stock solution can be prepared using the formula

$$\frac{1000}{P} \times V \times C = W$$

Where,

P = Potency given by the manufacturer in relation to the base

V = Volume in ml required

C = Final concentration of solution (multiples of 1000)

W = Weight of the antimicrobial to be dissolved in the volume V

Method:

All the ATCC culture was collected from institute of microbial technology, Bangalore. 2% Luria broth solution was prepared in distilled water while, pH of the solution was adjusted to 7.4±0.2 at room temperature and sterilized by autoclaving at 15 lb pressure for 25 min. The tested bacterial and fungal strains were prepared in the luria broth and incubated at 37 °C and 200 rpm in an orbital incubator for overnight. Sample solutions were prepared in DMSO for concentration 200, 150, 100, 50, 25, 12 and 6µg/mL. The standard drug solution of Streptomycin (antibacterial drug) and Nystatin (antifungal drug) were prepared in DMSO. Serial broth micro dilution was adopted as a reference method. 10 µl solution of test compound was inoculated in 5 mL luria broth for each concentration respectively and additionally one test tubes was kept as control. Each of the test tubes was inoculated with a

suspension of standard microorganism to be tested and incubated at 35 °C for 24 h. At the end of the incubation period, the tubes were examined for the turbidity. Turbidity in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration. The antimicrobial activity tests were run in triplicate.

Table 2: Antimicrobial, results of compounds (**D1-D18**)

Compounds D1-D18	**Minimal Inhibition Concentration [a] of microorganisms (µg/mL)**						
	Bacteria				**Fungi**		
	SA	*BS*	*EC*	*PA*	*CA*	*AN*	*AC*
D1	>200	200	200	200	200	200	200
D2	150	150	200	150	200	150	>150
D3	150	100	200	150	>150	100	200
D4	100	150	100	50	150	150	100
D5	150	100	150	150	150	>150	150
D6	200	>150	200	200	>200	200	200
D7	100	50	100	100	150	200	200
D8	100	50	150	150	100	150	150
D9	100	200	150	>100	200	150	100
D10	150	50	100	200	100	>50	150
D11	150	150	200	150	200	150	>150
D12	100	50	100	100	150	200	200
D13	100	50	100	100	150	200	200
D14	100	50	100	100	150	200	200
D15	150	150	200	150	200	150	>150
D16	100	50	100	100	150	200	200
D17	100	50	100	100	150	200	200
D18	150	150	200	150	200	150	>150
CQ	12	12	>6	12	>12	12	12
Streptomycin	12	6	6	12	NT	NT	NT
Nystatin	NT	NT	NT	NT	6	12	12

Conclusion:

Hereby, I report the synthesis of diazotized sulphonamide based compounds by divergent step by step manner. The synthesized compounds were screened against bacterial species and fungal species. The synthesized compounds rendered moderate activity against the microbial species and thus further optimization and modification could be applied leading to increase in activity of the azotized compounds.