ANTIMYCOBACTERIAL ACTIVITY OF SULFONE DERIVATIVES BY LUCIFERASE REPORTER PHAGE (LRP) ASSAY

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Abstract: ***Schiff bases of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) obtained by the condensation of sulfone and ketones with 1:2 ratio have been synthesized and characterized by spectroscopic studies viz., Fourier-transform infrared, proton Nuclear Magnetic Resonance and Ultraviolet-visible. The synthesized compounds antimycobacterial activity has been against Mycobacterium tuberculosis $H_{37}Rv$ and clinical isolate of MTB Resistant to Isoniazid (H) and Rifampicin (R) and the results suggested that both derived compounds have significant antimycobacterial activity.***

Keywords: Antimycobacterial assay, Mycobacterium tuberculosis ($H_{37}Rv$), Isoniazid (H) and Rifampicin (R)

I. INTRODUCTION

One of the largest multisystemic single contagious diseases [1] is tuberculosis (TB). Robertkoch a German physician and Scientist was invented Tuberculosis in 1882. *Mycobacterium tuberculosis* (or TB), aerobic, a gram positive, rod shaped bacteria with size 1 to 4 micrometre [2] produce tuberculosis. More or less 60 % TB ill people are presented in China, India, Indonesia, Nigeria, Pakistan and South Africa [3]. 480,000 people with multidrug-resistant TB (MDR-TB) an additional 100,000 people with rifampicin-resistant TB (RR-TB). Some developing countries [4] 40 % of deaths in human immunodeficiency virus (HIV) co-infected individuals. TB communicate between people by cough and contact with fluid from the nose of diseased individual. About 85 % of TB develop in pulmonary tract and which is common site. TB can excellently cured with an amalgamation of antitubercular drugs. Active TB requires a minimum of six months of treatment with multiple drugs and without close administration many TB patients fails to complete a full course of medication, result in retreat and acquired drug resistance in the bacteria [5]. So, better TB treatment is need. Immunity against *Mycobacterium tuberculosis* infections is recognized to cellular immunity and TB patients usually have a disorder of immune functions.

Photonus pyralis, luciferase is one of the standard reporter molecules used in molecular biology and biochemistry. Luciferase can be used to monitor promoter response activity in bacteria, cultured cells and transgenic plants or animals. If it is performed under optimal conditions, could result in a direct relationship between the amount of light emitted from the sample and the transcriptional activity of the regulatory elements [6]. The oxidative carboxylation of luciferin a known bioluminescence reaction is catalysed by firefly luciferase. When luciferin is added to sample containing luciferase, there is an immediate light flash that reaches peak intensity at 0.3- 0.5 seconds that decays rapidly. This rapid exponential decay is caused by the reaction product, oxyluciferin which inhibits luciferase activity. The luciferase reporter *mycobacteriophage* technique has been described as an efficient system to decrease the time required for diagnosis and drug susceptibility testing of *Mycobacterium tuberculosis* and other mycobacteria. The first luciferase reporter phage (LRP), phAE40, was constructed from the *mycobacteriophage* TM4, a lytic phage able to infect mycobacteria of clinical importance including *M. tuberculosis*. These LRP assays offer an elegant means of detecting viable mycobacteria and provide a rapid tool for drug susceptibility screening [7].

II. EXPERIMENTAL PROCEDURE

2.1. Materials

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to standard procedures. Dapsone, benzophenone and acetone were obtained from Merck specialities Ltd., Mumbai, India.

2.2 Synthesis of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine)

Dapsone (0.5 mmol) quantity and benzophenone (1.0 mmol) was dissolved in 20 mL of ethanol and the solution was refluxed for 3 h under constant stirring. This condensation reaction was carried out by using acid catalyst (few drops of glacial acetic acid). The formed water was removed from the reaction mixture using sodium sulfate (dehydrating agent). After completion of the reaction, the mixture was reduced to half of its original volume using a water bath and kept aside at room temperature (**Scheme 1**). Pale greenish yellow crystals of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) was obtained from slow evaporation (Yield: 87%). The Schiff base is characterized by FTIR, UV- Vis and Proton NMR spectroscopy.

2.3 Synthesis of N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)

Dapsone (0.5 mmol) quantity and acetone (1.0 mmol) was dissolved in 20 mL of ethanol and the solution was refluxed for 3 h under constant stirring. This condensation reaction was carried out by using acid catalyst (few drops of glacial acetic acid). The formed water was removed from the reaction mixture using sodium sulfate (dehydrating agent). After completion of the reaction, the mixture was reduced to half of its original volume using a water bath and kept aside at room temperature (**Scheme 1**). Pale yellow crystal was obtained from slow evaporation (Yield: 81%). The Schiff base is characterized by FTIR, UV- Vis and Proton NMR spectroscopy.

2.4 Antitubercular Assay

Anti-mycobacterial activity of the Schiff bases of derivatives of dapsone was evaluated by Luciferase reporter phage (LRP) assay against Standard strain of *M. tuberculosis* $H_{37}Rv$ and Clinical isolate of *M.tuberculosis* resistant to Streptomycin, Isoniazid, Rifampicin and Ethambutol (s,H,R & E) at two different concentrations (100 and 500 µg/mL). The Luciferase reporter phage assay methodology is rapid, inexpensive and less laborious for high throughput screening of compounds for their antimycobacterial activity compared to BATEC methodology which is costly, cumbersome and uses radioactive reagents. A compound is considered as an anti-tubercular agent if fifty percent reduction in relative lights units (RLU) is observed when compared to the control using Luminometer [8].

2.5 Microbial strain for anti-Mycobacterium tuberculosis Assays

Standard strain of *M.tuberculosis* $H_{37}Rv$ and clinical isolate of *M.tuberculosis* resistant to s,H,R & E maintained at National Institute for Research in Tuberculosis, Chennai were used for the antimycobacterial assay.

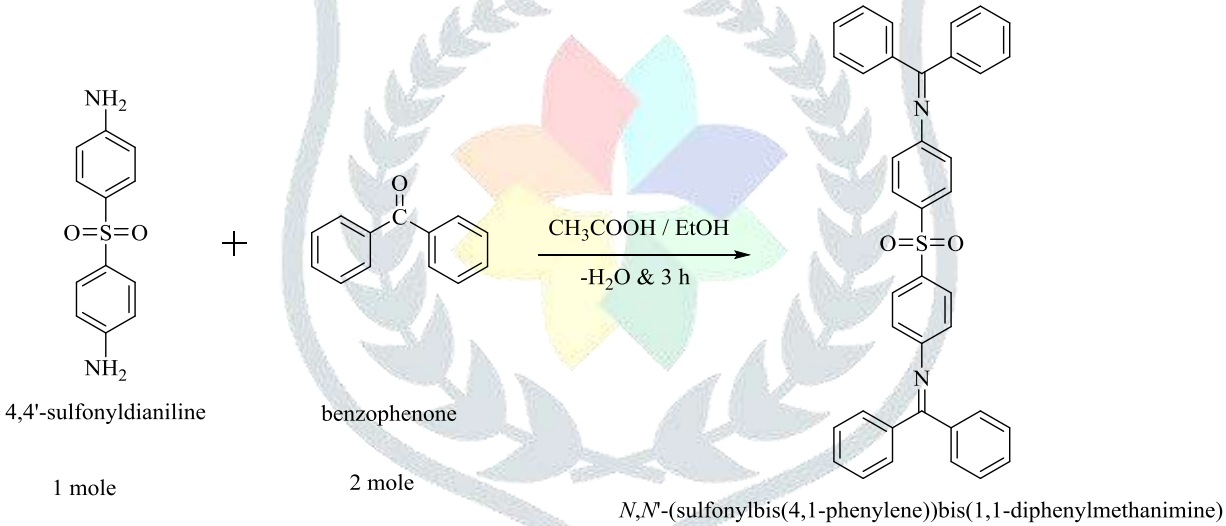
2.6 Luciferase reporter phage (LRP) assay

Standard strain $H_{37}Rv$ and a clinical isolate of *M.tuberculosis* resistant to s, H, R & E were grown in Middlebrook 7H9 complete medium 12 with and without solution of derivative of dapsone for 3 days at 37°C. Luciferase Reporter Phage Assay [9] was done using concentrations of 100 and 500 µg/ml of derivative of dapsone solution. Fifty-microliter bacterial suspension equivalent to MacFarlands No.2 standard was added to 400 µl of G7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this set up was incubated for 72 h at 37°C. After incubation, 50 µl of the high titer Luciferase reporter phage (phAE129) and 40 µl of 0.1 M $CaCl_2$ were added to all the vials and this setup was incubated at 37°C for 4 h. After incubation, 100 µl of the mixture was taken from each tube into a luminometer cuvette and an equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10s of integration in the Luminometer. Duplicate readings were recorded for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with control. The experiment was repeated when the mean RLU of the control was less than 1000 [10].

III. RESULTS AND DISCUSSION

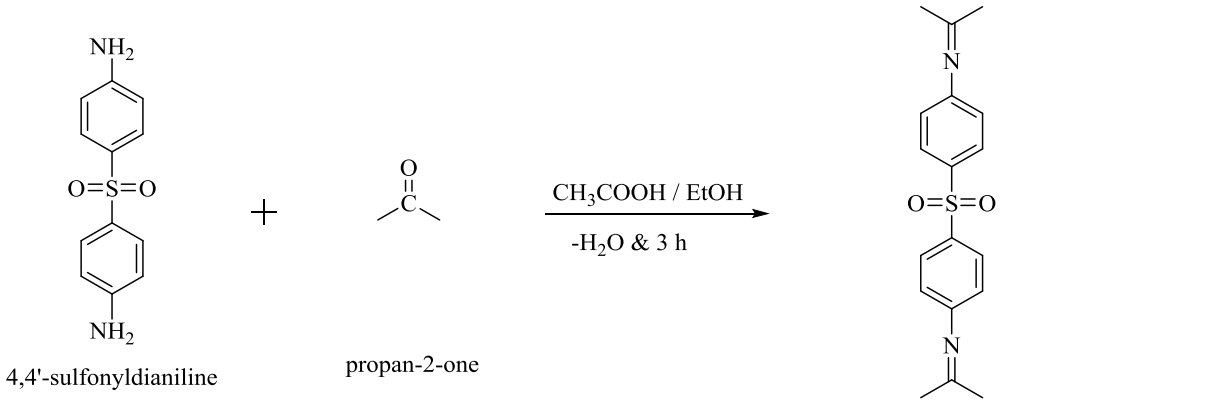
3.1 Reaction Synthesis of Ligands

Schiff base of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) prepare by the condensation of benzophenone and dapsone with 2:1 by **Scheme 1**



Scheme 1. Preparation of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine)

N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) prepare by 2:1 ratio of acetone and dapsone by **Scheme 2**.



4,4'-sulfonyldianiline propan-2-one *N,N'*-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)

1 mole 2 mole

Scheme 2. Preparation of N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)

3.2 UV-Visible spectra

The UV-visible spectra of Preparation of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) were recorded in aqueous solution (10^{-3} M) in the range of 200-1100 nm at room temperature. The electronic spectrum of derivatives of dapsone shows a broad band at 273.1 nm and 275.5 nm at the high energy 4.0000 (AU) of the compounds N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) absorptions in **Fig.1** and **3** respectively. These Schiff bases transition peaks at 273.1 and 275.6 nm of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) in **Fig. 2** and **4** shown n-Л* transition of the azomethine (-N=C<) chromospheres, 0.010 (% T) respectively. Therefore, the electronic spectrum of these Schiff bases have very less fluorescence property.

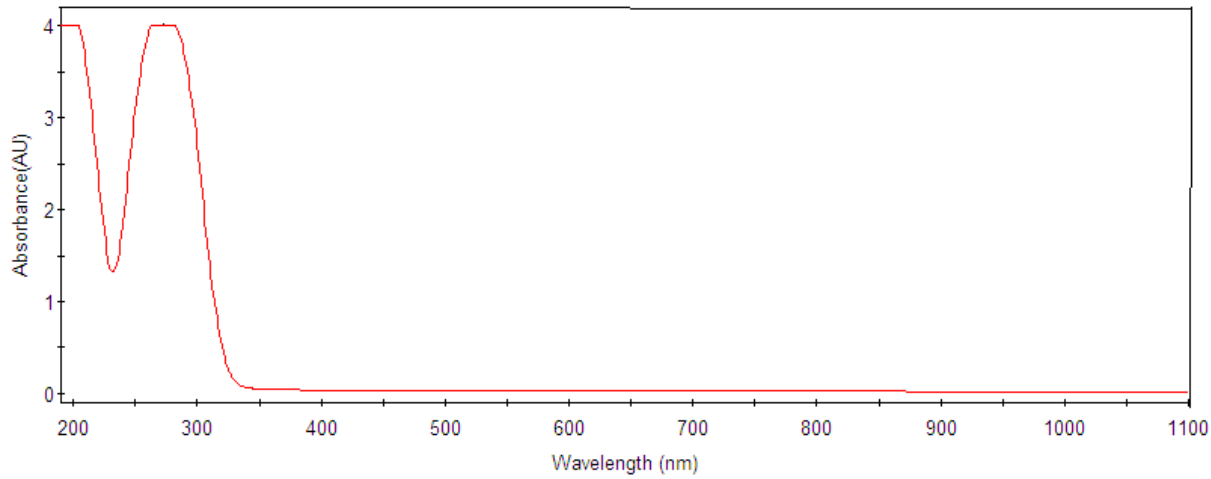


Fig.1 UV Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine)

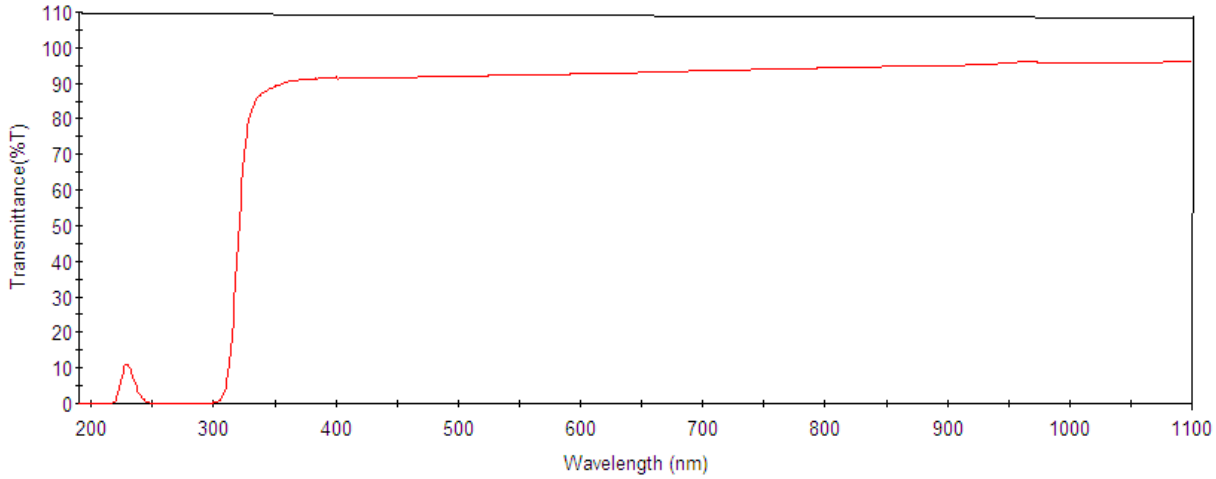


Fig.2 Visible Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine)

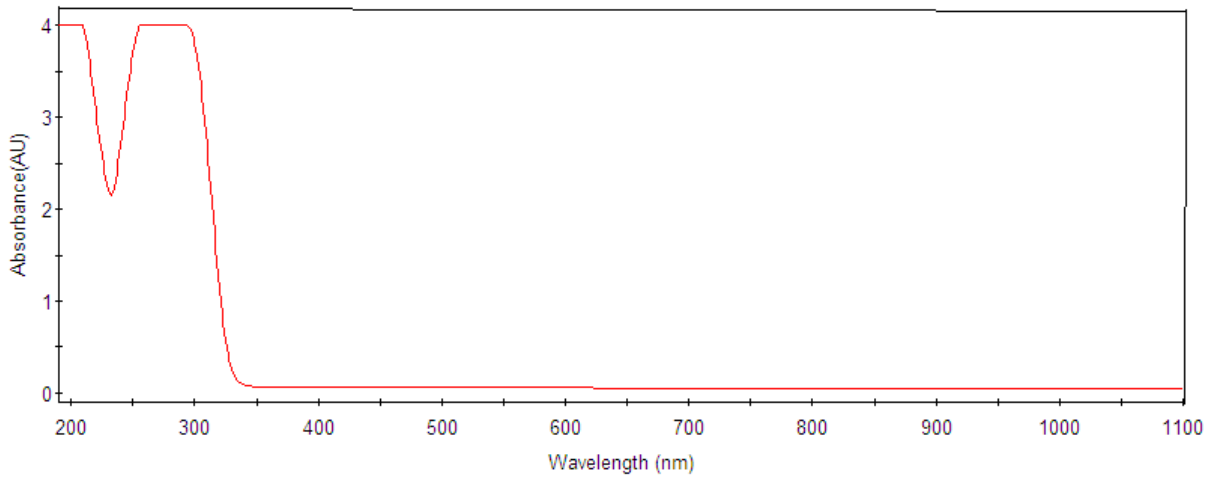


Fig. 3 UV Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)

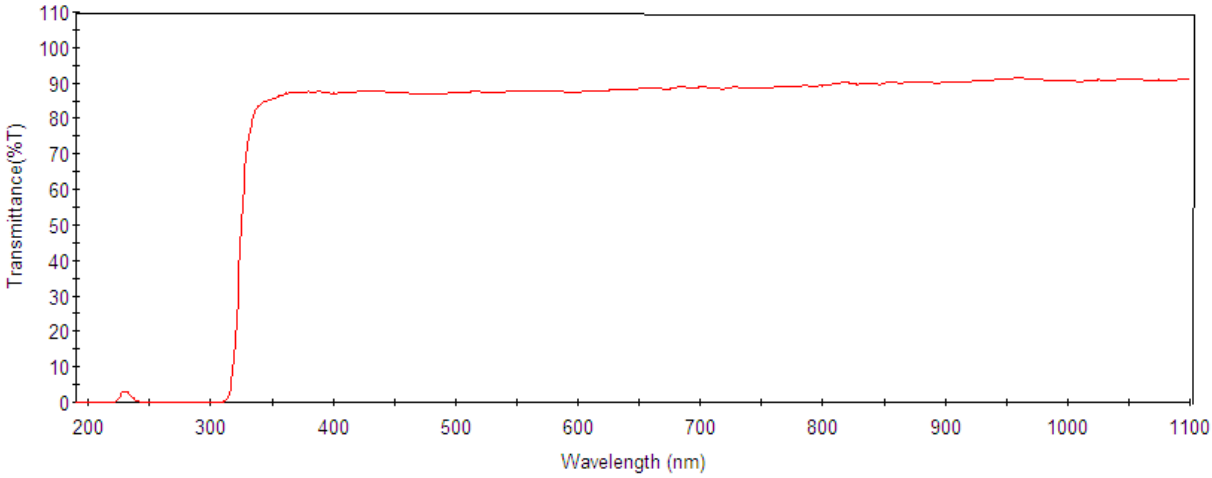


Fig.4 Visible Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)

3.3 IR Spectra

The vibrational spectra is valuable information regarding the nature of functional group of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine). The IR spectra of the N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) data showed in **Fig.5** and **Fig.6**, a sharp with strong peak at 1592.84 cm^{-1} and 1591.73 cm^{-1} indicate the azomethine (-N=CRR') group of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) respectively. A sharp and with strong peak at 898.16 cm-1 and sharp with medium peak at 695.07 cm-1 indicate phenylaromatic carbon with sulphur (ArC-S) single bond of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) respectively. The sulphur double bonded with oxygen in sulfone a sharp with strong peak appear at 1145 cm-1 and weak sharp band at 1336 cm-1 for N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and a sharp with strong peak at 1144 cm-1 and a weak sharp peak at 1336 cm-1 for N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine).

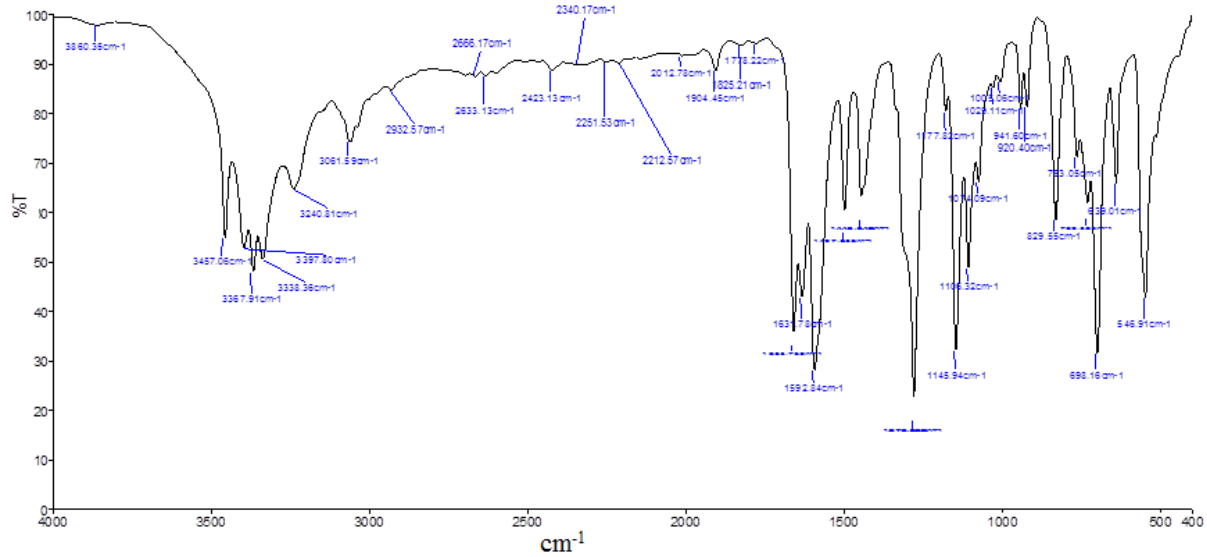


Fig.5 FTIR Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine)

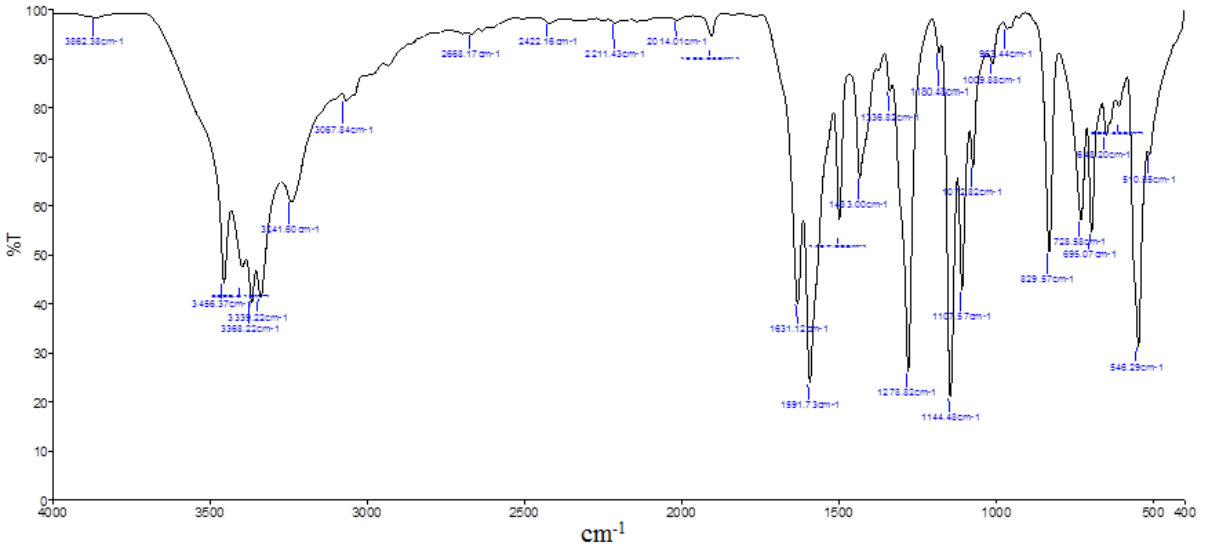


Fig.6 FTIR Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)

3.4 1H NMR Spectra

The ^{1}H NMR results show N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) in **Fig.7** and **Fig.8.** In N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) a strong with sharp doublet peak appear at 7.7 ppm (d, 8H) (J=9 Hz), a doublet at 7.6 ppm (d, 8H) (J=7.2 Hz) & a triplet at 7.5 ppm (t, 4H) (J=7.5 Hz) indicate benzylidene ring protons shifts and two sharp doublets appear at 7.4 ppm (d, 4H) (J=8.4 Hz) & 6.5 ppm (d, 4H) (J=8.4 Hz) indicate sulfone proton shifts. There are two sharp with medium doublet peaks appear at 7.4 ppm (d, 4H) (J=8.4 Hz) & 6.5 ppm (d, 4H) (J=8.7 Hz) of sulfone protons shifts and a sharp with strong singlet appear at 2.5 ppm (s, 12H) indicate methyl protons shift.

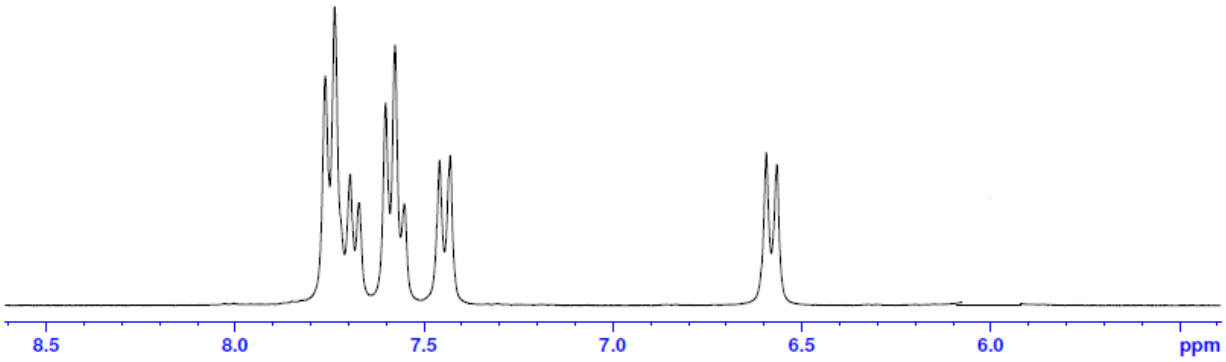


Fig.7 ^{1}HNMR Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine)

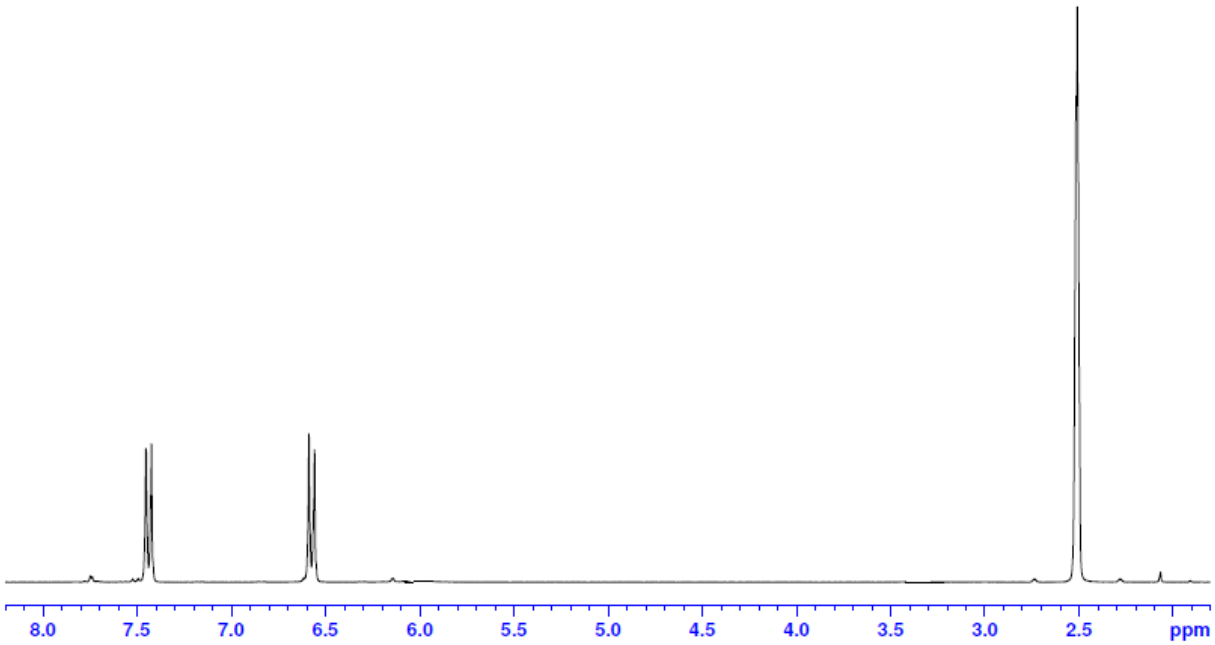


Fig.8 ^{1}NMR Spectrum of N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)

3.5 Antimycobacterial Activity by Luciferase Reporter Phage (LRP) Assay

The antimycobacterial activity of dapsone derived Schiff bases N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) were tested against *Mycobacterium tuberculosis* H_{37}Rv and Clinical isolate of MTB Resistant to Ioniazid (H) and Rifampicin (R) by Luciferase Reporter Phage (LRP) assay with percentage of Relative Light Units (RLU) by two different concentrations were found in 50 µg/ml and 100 µg/ml.

Antimycobacterial activity from the **Table 1**, N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) in 37.72 and 48.13 percentage of reduction in relative light units in 50 µg/ml and 100 µg/ml against *Mycobacterium tuberculosis* H_{37}Rv respectively. N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) have 53.90 and 62.39 percentage of reduction in relative light units in 50 µg/ml and 100 µg/ml against *Mycobacterium tuberculosis* H_{37}Rv respectively. Where, Rifampicin as standard and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) have more activity than N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine).

From the **Table 1**, antimycobacterial activity against clinical isolate of MTB Resistant to Isoniazid (H) and Rifampicin (R) Schiff bases of N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) have 49.13 and 63.54 percentage of reduction in relative light units in 50 µg/ml and 100 µg/ml against *Mycobacterium tuberculosis* H_{37}Rv respectively. For, N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) have 60.90 and 68.19 percentage of reduction in relative light units in 50 µg/ml and 100 µg/ml against *Mycobacterium tuberculosis* H_{37}Rv respectively against clinical isolate of MTB Resistant to Isoniazid (H) and Rifampicin (R). Where the standard have only 21.76 percentage of reduction of relative light units in the concentration of 2 µg/ml but N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) have 2.2570 and 2.9210 times greater than standard in the concentrations of 50 µg/ml and 100 µg/ml respectively and N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) have 2.7980 and 3.1498 times greater than standard in the concentrations of 50 µg/ml and 100 µg/ml respectively.

Table 1. Results of Screening assay for Antimycobacterial Activity

Compounds Name	% of Reduction in RLU			
	Mycobacterium Tuberculosis $H_{37}Rv$		Clinical isolate of MTB (Resistant to Isoniazid (H) and Rifampicin (R))	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
A	37.72	48.13	49.13	63.54
B	53.97	62.39	60.90	68.19
Rifampicin (2 µg/ml)	82.70		21.76	

A – N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine)
B - N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine)
Criteria: Antimycobacterial activity indicated by fifty per cent reduction in relative light units (RLU) in the presence of compound in comparison with compound free control.

IV. CONCLUSIONS

In the present research studies, our efforts were to synthesis and characterize of sulfone derived Schiff bases by condensation method in 1:2 ratio of dapsone with two different ketones and . These synthesized compounds were characterized by various physicochemical and spectral analyses. From the Results of Screening assay for Antimycobacterial Activity of these Schiff bases were good inhibition of antimycobacterial assay. N,N'-(sulfonylbis(4,1-phenylene))bis(propan-2-imine) have more active than N,N'-(sulfonylbis(4,1-phenylene))bis(1,1-diphenylmethanimine) for antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and Clinical isolate of MTB Resistant to Ioniazid (H) and Rifampicin (R) by Luciferase Reporter Phage (LRP) assay with percentage of Relative Light Units (RLU) by two different concentrations were found in 50 µg/ml and 100 µg/ml. Its criteria was given under the **Table 1** for, reference of activity.

V. ACKNOWLEDGEMENTS

The authors thanks to **Dr. Prabhu Seenivasan**, Consultant Microbioligist, Department of Bacteriology, **National Institute of for Research in Tuberculosis** (Indian Council of Medical Research) Mayour V.R.Ramanathan Road, Chetput, Chennai-600031 for, Antimycobacterial activity work done and one of the authors Dr.G.Vijayakumar express thanks to UGC, New Delhi, India (**MRP-6282/15/UGC-SERO**) for the financial support to carried out this research work.

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