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RESEARCH ARTICLE

Comparitive Antitubercular Activity of Sulfadrug Substituted 1, 4-Thiazines and 1, 3- Thiazines

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ABSTRACT

Tuberculosis has an on-going impact on global public health in the 21st century. Although many active antitubercular agents have since been developed, a disturbing co-occurrence with the use of present drugs as single agent has developed drug resistance. This has made use to investigate for new thiazine derivatives for finding more effective antitubercular agents. The present study was designed to synthesise some novel sulpha drug substituted 1, 4- thiazines by using O-amino thiophenol, maleic anhydride, ethanol and formaldehyde and also 1, 3-thiazine was prepared from chalcones obtained by Claisen Schimidt condensation reaction. Then the synthesised compounds were screened for their antimycobacterial activity by using Micro plate Alamar Blue Assay (MABA). The results indicate 1, 4-thiazine derivatives and 1, 3- thiazine derivative have considerable antimycobacterial activity. Out of the 5 derivatives, E4 (Sulfamethoxazole substituted 1, 4- thiazine) is most effective and showing activity at 25µg/ml. The study suggests that 1, 4-Thiazine derivatives shows potent antimycobacterial activity than 1, 3-thiazine derivative.

KEYWORDS

Tuberculosis, 1, 4-Thiazines, 1, 3-Thiazine, MABA method

INTRODUCTION

Tuberculosis is the second most common cause of death from infectious disease after those due to HIV/AIDS. Roughly one-third of the world's population has been infected with *M. tuberculosis*, with new infections occurring in about 1% of the population each year. However, most infections with *Mycobacterium tuberculosis* do not cause TB disease, and 90–95% of infections remain asymptomatic. Pulmonary tuberculosis is the most important form of tuberculosis.

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TB primarily affects the lungs, but it can also affect organs in the central nervous system, lymphatic system, and circulatory system among others. It is caused by the organism *Mycobacterium tuberculosis*[1,2,3,4]. The spread of multidrug resistance TB (MDR TB) and extensively drug-resistance TB (XDR-TB) causes the new challenges for the prevention, treatment and control of this deadly disease[5].

Thiazine derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis[6]. Literature survey reveals that Thiazine derivatives, has been great interest due to its wide range of biological activities such as anti-diabetic, anti-histaminic, antibacterial, antifungal, phagocytic activity of human

neutrophils, antagonistic potassium channel-opening agents, antioxidant, analgesic, antiinflammatory, anti-tuberculosis, antitumor, antihelminthic, insecticidal, nitric oxide synthase inhibitor, smooth muscle relaxants, urokinase inhibitors. The derivatives of thiazine act as myocardial calcium channel modulators. Synthesis of novel thiazine derivatives as antimycobacterial agents helps in the battle against pathogenic *Mycobacterium tuberculosis*[7,8,9,10].

In continuation of synthetic work on biologically active compounds, it is interesting to note that the Cephalosporin contain a 1, 3-thiazine ring, which is active core of Cephalosporin β- lactam antibiotics. It has been observed that there is no Cephalosporin with a 1, 4- thiazine nucleus. In the light of above fact we have synthesised some new sulfa drug substituted1,4-thiazine derivatives by changing sulphur to the fourth position from the third position by mannich reaction, which consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. The final product is a β-amino-carbonyl compound also known as a Mannich base. In the developmental phase, Mannich bases of thiazines are biologically active heterocyclic compounds and important potential new drugs. Then these synthesised 1, 4-thiazine derivatives compared with 1, 3 thiazine derivative to see whether they have same activity like 1, 3-thiazine moiety[11,12,13,14].

In the context to the above principle, the present research work was aimed at to synthesize sulfa drug substituted 1, 4 thiazines and 1, 3-thiazine derivative and to further compare their antimycobacterial activities.

MATERIALS AND METHOD

All the chemicals and reagents are collected from Chemco and Nice pharmaceuticals. Melting points were determined in an open capillary method and are uncorrected. Infrared spectra were recorded on SHIMADZU IR Affinity- 1 spectrometer by using KBr pellet technique.

General Procedure for the Synthesis of Mannich Base Derivatives [14]

Scheme 1

Step 1: Synthesis of (3-oxo-3, 4-dihydro-2H-1, 4-Benzothiazin-2-yl) Acetic Acid (ST_1)

To a solution of maleic anhydride (0.05mol) in diethyl ether (20ml) a solution of O- amino thiophenol (0.05mol) or in diethyl ether (20ml) was added. The reaction mixture was stirred at room temperature for 2 hours. The precipitate was filtered and washed with ether and recrystallized from ethanol to get pure (ST_1). Yield: 95%. Melting point: 201°.

Step 2: Synthesis of Mannich Bases

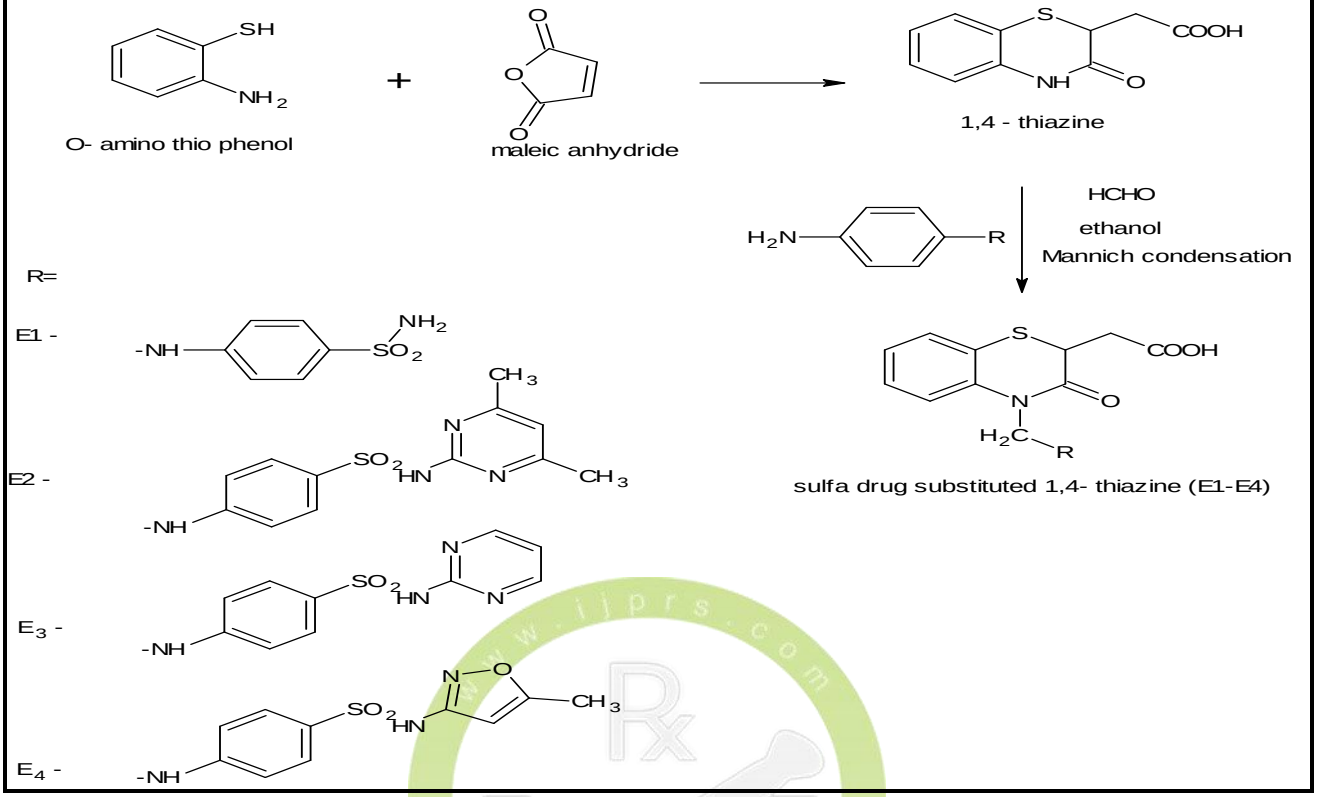
A mixture of compound (ST_1) (0.01 mol) was dissolved in ethanol (15 ml) followed by addition of sulfa drugs (0.01 mol) and formaldehyde (0.02 mol) to undergo Mannich reaction. The reactants were refluxed for 2-10 hours with continuous stirring at 70-75^{0}c.The completion of reaction was checked by TLC. After completion of reaction the mixture was poured into ice water and kept in a refrigerator overnight. The product precipitated out and was filtered, dried and recrystallized with ethanol to give solid compounds.

Scheme 2[15]

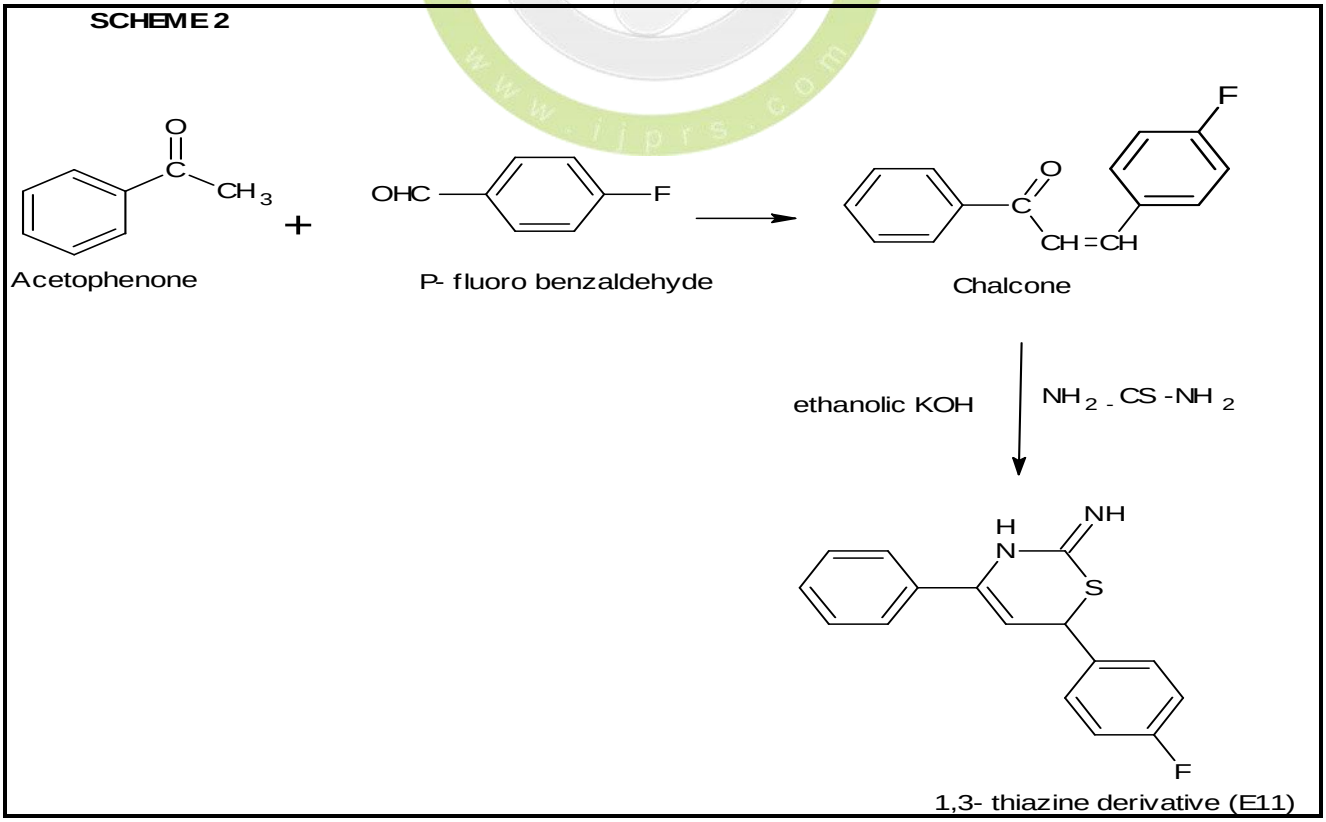
Step 1: Preparation of Chalcone by Claisen Schimidt Condensation Reaction

A solution of 22g of Sodium hydroxide in 200 ml of water and 122.5 ml of rectified spirit were placed in a 500 ml flask and stirred. The flask was immersed in a bath of crushed ice and poured 0.01 mol P-fluro benzaldehyde and 0.01 mol Acetophenone, alternatively and stirred continuously and keep the temperature at about 25°c and stir vigorously until the mixture was thick that stirring is no longer effective. The stirrer was removed and the reaction mixture was placed in an ice chest refrigerator overnight. The product was filtered with Buchner funnel, washed with cold water until the washings are neutral to litmus and then with 20 ml of ice cold rectified spirit. Crude chalcone after drying in

SCHEME 1



SCHEME 2



the air and recrystallized from ethanol. The yield: 70%, MP: 85-88°c, pale yellow solid.

Step 2: Preparation of 1, 3-thiazine Derivative

0.01mol chalcone and 0.01mol thiourea were dissolved in ethanol (25ml). To this aq.KOH solution (0.02mol) was added (prepared from KOH in small amount of distilled water). The reaction mixtures refluxed for 2.5 hours, cooled, diluted with water and acidifies with 1:1 HCl. The product was filtered, dried and recrystallized from ethanol.

Anti-Tubercular Activity[16]

The anti tubercular activities of the compounds were assessed against *Mycobacterium tuberculosis* using Microplate Alamar Blue Assay (MABA) method.

Antitubercular screening was carried out by Middle brook 7H9 Broth Base (M198) medium against H37RV Strain. [Middle brook 7H9 Broth Base (M198) + Middle Brook ADC growth supplement] containing standard drug as well as control Middle brook 7H9 Broth Base (M198) was also inoculated with *Mycobacterium tuberculosis* of H37RV Strain.

200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 well plates to minimized evaporation of medium in the test wells during incubation. The 96 well plates received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml.

Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULTS AND DISCUSSION

In the present work, totally five compounds were synthesised. Scheme 1 involves the formation of some 1, 4- thiazines by using O-amino thiophenol and maleic anhydride. Further 4 sulpha drug substituted mannich bases (E1-E4) were synthesised from (3-oxo-3, 4-dihydro-2H-1, 4-benzothiazin-2-yl) acetic acid with ethanol and formaldehyde. Scheme 2 involves the synthesis of chalcone by Claisen-Schmidt condensation of P-fluoro benzaldehyde and acetophenone. The synthesized chalcones were cyclised to their corresponding 1, 3 thiazine derivatives by condensation with thiourea and ethanolic sodium hydroxide. Purity of the all synthesised compounds was checked by thin layer chromatographic method. Melting points were determined in an open capillary method and are uncorrected.

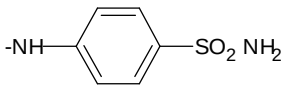
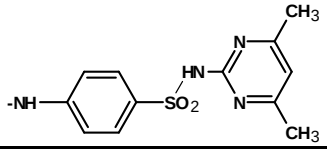
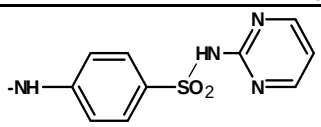
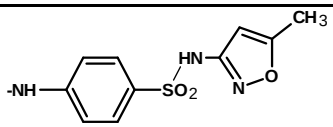
IR spectrum gave an idea about the functional groups present in the compounds. The IR region ranges between 4000–650 cm^{-1} were recorded on SHIMADZU IR Affinity- 1 spectrometer which showed different vibration levels of molecules by using KBr pellet technique.

Then the synthesised compounds were screened for their antimycobacterial activity by using Alamar Blue assay (MABA). The compounds were screened for antitubercular activity at the concentration of 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100µg/ml by using standard drugs such as Pyrazinamide, Streptomycin and Ciprofloxcin. The result was found that compound E4 (Sulfamethoxazole substituted 1, 4- thiazine) is most effective showing activity at 25µg/ml. Then all other compounds were effective at 50µg/ml levels.

CONCLUSION

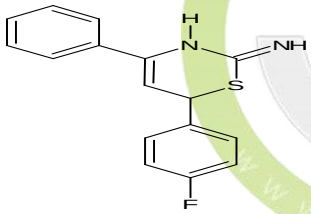
The present study concluded that sulfa substituted 1, 4-thiazine derivatives show more potent antimycobacterial activity than 1, 3-thiazine derivative. Let us be optimistic that these synthesized compounds could be useful as specific antimycobacterial agents against Tuberculosis.

Table 1: Physical data of newly synthesized sulfa drug substituted 1, 4- thiazines

Compound	R	Mole. formula	Mol. wt	m.p (°c)	% yield	Rf value
E1		$C_{17}H_{17}O_5S_2N_3$	407.20	233-235	75	0.73
E2		$C_{23}H_{23}O_5S_2N_5$	513.33	188-190	70	0.69
E3		$C_{21}H_{19}O_5S_2N_5$	485.0	215-217	68	0.70
E4		$C_{21}H_{20}N_4O_6S_2$	488.27	186-188	74	0.74

*solvent system: Acetone: Methanol: Chloroform (2:1:1)

Table 2: Physical data of newly synthesized 1, 3- thiazine

Compound	R	Mole. formula	Mol. wt	m.p (°c)	% yield	Rf value
E11		$C_{16}H_{13}N_2SF$	283	60 – 62	70	0.6164

*solvent system: Acetone: Methanol: Chloroform (2:1:1)

Table 3: IR Spectral Data (KBr Pellet Method)

Types of Vibrations	Wave number (cm^{-1})				
	E1	E2	E3	E4	E11
NH stretching	3204.87	3375.43	3377.36	3383.29	3178.69
Aromatic CH stretching	2990.76	3072.60	3037.89	2989.79	3064.89
OH stretching	2919.39	2916.37	2924.09	2920.35	
C=O stretching	1588.45	1672.28	1710.86	1588.45	
SO_2 stretching	1398.45	1311.59	1327	1396.52	
C-N stretching	1157.34	1149.57	1149	1087.90	1678.07
C-S stretching	670.29	663.51	675.09	673.19	692.44

Table 4: Antitubercular Activity of Synthesized Compounds

Sample No	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
E1	S	S	R	R	R	R	R	R
E2	S	S	R	R	R	R	R	R
E3	S	S	R	R	R	R	R	R
E4	S	S	S	R	R	R	R	R
E11	S	S	R	R	R	R	R	R
Standards	Pyrazinamide					3.125µg/ml		
	Streptomycin					6.25µg/ml		
	Ciprofloxacin					3.125µg/ml		

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