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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTI-TUBERCULAR ACTIVITY OF 1, 3-THIAZINE DERIVATIVES

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ABSTRACT
Due to their potent biological activity, heterocyclic compounds have been used extensively in pharmacological research. Researchers' interest has been drawn to the heterocyclic molecule thiazine, which has four carbon atoms, one nitrogen atom, and one sulphur atom. It has interesting pharmacological properties. In the present work, a series of new thiazine derivatives were synthesized from substituted chalcone derivatives. Starting material was acetophenone derivatives. The structures of these compounds were confirmed by IR, NMR, Mass and elemental analysis. The synthesized compounds (IVa-IVe) were screened for anti-tuberculosis activity against *Mycobacterium tuberculosis* and the results show that some of these derivatives possess good activity against *Mycobacterium tuberculosis*.

Keywords: Thiazine, Anti-tubercular, NMR, Mass, Chalcone, Schiff bases.

1. INTRODUCTION
Due to the abundance of their structural components in many natural products including vitamins, hormones, antibiotics, etc., heterocyclic compounds have grown in importance. Thiazines are a class of heterocyclic compounds with an unsaturated six-membered ring that contains two carbon atoms, one nitrogen atom, and one sulphur atom. Several techniques have been developed for their synthesis [1].

Due to the abundance of their structural components in many natural products including vitamins, hormones, antibiotics, etc., heterocyclic compounds have grown in importance. It was discovered that 1, 3-thiazines, which have nitrogen and sulphur in their six-member heterocyclic ring system (N-C-S), are fairly stable pharmacophores. These findings were also true for other medicinally significant compounds, such as xylazine, an agonist at the 2-adrenergic receptor that is used to sedate, anaesthetize, relax muscles, and provide analgesia in animals [2] and chlormezanone (used as an anxiolytic and a muscle relaxant)[3].

Thiazines and their derivatives are among the most inventive sources of bioactive chemicals due to their special qualities, adaptability, and ease of availability [4, 5]. There are a few review publications [6-9] on thiazine compounds, however they are either incomplete or primarily concerned with 1, 3-thiazines.

TB is an airborne disease caused by the bacterium Mycobacterium tuberculosis (M. tuberculosis). Majority of TB cases are caused by M. tuberculosis, also called tubercle bacilli. M. tuberculosis spreads in airborne particles, which are 1-5 microns in diameter. Persons who have pulmonary or laryngeal TB disease may spread it through cough, sneeze, shout, or sing. Mycobacterium tuberculosis (Mtb) is the causative agent of the communicable disease tuberculosis (TB), which mostly affects the lungs and other body organs. In emerging nations like India, the number of persons with this condition has been rising, with an estimated 50 million people affected [10]. According to the WHO, there are 9 million more cases reported each year [11, 12]. Based on these data, scientists are concentrating more on creating new medication compounds to combat resistant Mycobacterium tuberculosis strains. This emphasises the requirement for on-going research and development of innovative and effective antimicrobial compounds to combat drug-resistant TB [13, 14].

Hence, with these observations we examine the feasibility and efficiency of an approach to synthesis

thiazine derivatives, which turns to exhibit significant antitubercular activities.

2. MATERIAL AND METHODS

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and 1H NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz. LC-MS of selected samples taken on LC-MSD-Trap-SL-01046. The purity of compounds was checked by thin layer chromatography on silica gel plate of 0.25 mm thickness using different solvent system. All the chemicals were laboratory grade and purchased from local market and thiazine derivatives were prepared by reported method.

2.1. Synthetic Protocol

2.1.1. Preparation of 2-hydroxy-3-nitro-5-chloro-acetophenone (II)

In glacial acetic acid, 3gm of 2-hydroxy-5-chloroacetophenone (I) was dissolved (3ml). This reaction mixture received drops of nitric acid while being constantly stirred. The reaction mixture's temperature was kept below zero degrees Celsius. One hour was given for the mixture to stand. It was stirred after being poured into ice-cold water. After that, crude product was filtered, dried, and recrystallized with ethanol produce a yellow solid.

2.1.2. Preparation of 2-hydroxy-3-nitro- 5-chloro-chalcones (III a-IIIe)[15]

In 50 ml of ethanol, 0.1mol of 2-Hydroxy-3-nitro-5-chloroacetophenone (II) was dissolved. Substituted benzaldehyde derivatives (0.1mol) (table-1) were then added, and the mixture was heated to boiling. Drop by drop, with continual stirring, 40 ml of aq. sodium hydroxide solution (40%) was added. The mixture was mechanically agitated at room temperature for approximately 30 minutes and then left overnight. Then, 50% hydrochloric acid solution was used to acidify it. The separated substance was filtered, and then washed with water and sodium bicarbonate (10%). The mixture of ethanol and acetic acid crystallised into the raw product.

2.1.3. Preparation of substituted thiazine's derivatives (IVa - IVe) [16]

Thiourea and 2-hydroxy-3-nitro-5-substituted chalcone were dissolved in ethanol at a concentration of 0.01mol each (25 ml). KOH solution (0.02M) was added to this aq (prepared from KOH in small amount of distilled water). After 2.5 hours of refluxing, the reaction mixture was cooled, diluted with water, and acidified with 1:1 HCl. From ethanol, the final product was filtered, dried, and crystallised (IVa - IVe). Other thiazine compounds were created using the same methodology. The Scheme shows how the reaction will proceed. (Figure-1)

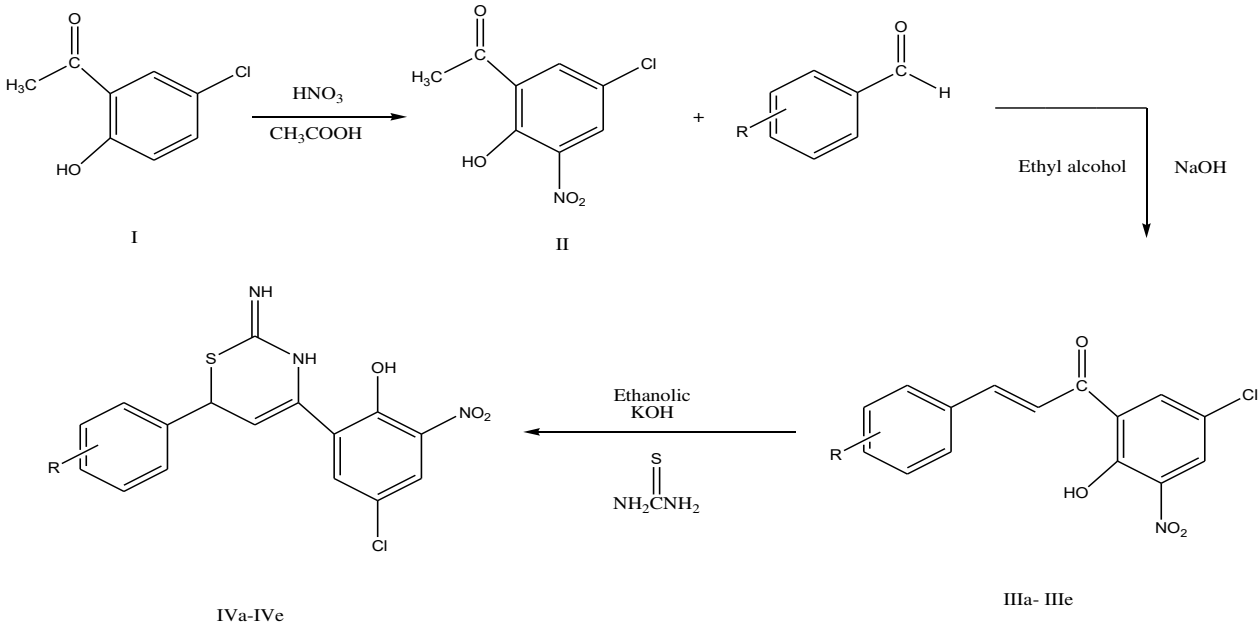
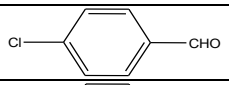
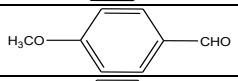
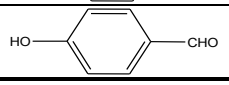


Fig. 1: Synthetic route of targeted compounds

Table 1: List of various aromatic amines

S. No.	Compounds Code	Substituted Aromatic Amine (R)	Structure of Aromatic Amine (R)
1	IVa	p-chlorobenzaldeyde	
2	IVb	p-bromobenzaldeyde	
3	IVc	p-methoxybenzaldeyde	
4	IVd	p-nitro benzaldeyde	
5	IVe	p-hydroxybenzaldeyde	

2.2. Biological Activity

The *in-vitro* antitubercular activity of the synthesised compounds was tested against the *Mycobacterium Tuberculosis*. The screening result were compared with Moxifloxacin (Zone of inhibition Z.I. = 24-26 mm) as a reference drug. The screening culture medium was nutrients agar (Bacteriological Grade, Qualigen Fine Chem. Mumbai, India) and antitubercular screening was performed by employing by filter paper disc method. The solvent used was 10% of DMSO in methanol and biological screening result was mentioned in mm (millimeter), showing a diameter of inhibition zone and these are categoried as 6 mm for mild, 7-13 mm for moderate, 14-26 mm for efficacy, respectively.

3. RESULTS AND DISCUSSION

3.1. Chemistry

All the novel thiazine derivatives were synthesized, purified and separated by using column chromatography or recrystallization method. Synthesized compounds were characterized by using Elemental analysis, FT-IR, ^{1}HNMR and Mass spectrometric studies. The integration curves fully support the orientation of protons in the analyzed compounds. Furthermore, all the compounds demonstrated the characteristic chemical shifts for the thiazine nucleus. Additionally, synthesized compounds were analyzed by mass spectra and indicated no difference in the fragmentation pattern among the set of synthesized series.

3.1.1. IV_a: 4-chloro-2-(6-(4-chloro-phenyl)-3,6-dihydro-2-imino-2H-1,3-thiazin-4-yl)-6-nitro-phenol

Yellowish Grey solid, Molecular Formula: $C_{16}H_{11}Cl_2N_3O_3S$, Molecular weight: 396.25, Yield: 67.38%, M.P.: 189-191°C, R_f value: 0.86, FT-IR (KBr, cm^{-1}): 3434.67(O-HStr.), 3117.92(=C-H Str.), 1629.41 (C=C Str.), 1254.04 (C-N Bend.), 710.77 (Ar C-H Bend.),743.35 (C-Cl Bend.), ^{1}H-NMR (400 MHz, DMSO, δ ppm):2.00 (s, 1H, NH), 4.5 (d, 1H, CH), 5.00 (s, 1H, OH), 6.9-7.91 (m, 8H, Ar-H). Mass Spectra: M^+ 245.20, M^{+2} 398.26. Elemental Analysis, % found (% required): C, 48.46 (48.5); H, 2.78 (2.80); N, 10.56 (10.6); O, 12.09 (12.11), Cl, 17.82 (17.89); 8.11(8.09).

3.1.2. IV_b: 2-(6-(4-bromophenyl)-3,6-dihydro-2-imino-2H-1,3-thiazin-4-yl)-4-chloro-6-nitro-phenol

Greyish brown colored solid,Molecular Formula: $C_{16}H_{11}BrClN_3O_3S$, Molecular weight: 440.70,Yield: 63.33%, M.P.: 202-204°C, R_f value: 0.66, FT-IR (KBr, cm^{-1}):3434.65(O-H Str.), 3068.65(=C-H Str.), 1657.18(C=C Str.), 1257.53 (C-N Bend.), 676.84 (Ar C-H Bend.), 730.43(C-Cl Bend.),667.01 (C-Br Bend.), ^{1}H-NMR (400 MHz, DMSO, δ ppm): 2.00 (s, 1H, NH), 4.5 (d, 1H, CH), 5.00 (s, 1H, OH), 6.9-7.87 (m, 8H, Ar-H). Mass Spectra: M^+398.26, M^{+2} 442.07. Elemental Analysis, % found (% required): C, 43.58 (43.61); H, 2.56 (2.52); N, 9.56 (9.54); O, 10.83 (10.89), Cl, 8.10 (8.04); S, 7.23 (7.27); Br, 18.09 (18.13).

3.1.3. IV_c: 4-chloro-2-(3,6-dihydro-2-imino-6-(4-methoxy phenyl)-2H-1,3-thiazin-4-yl)-6-nitro -phenol

Pale brown colored solid, Molecular formula: $C_{17}H_{14}ClN_3O_4S$, Molecular weight: 391.83, Yield: 68.16%, M.P.:231-233°C, R_f value: 0.72,FT-IR (KBr, cm^{-1}): 3375.68(O-H Str.), 3146.93 (=C-H Str.), 1602.38 (C=C Str.), 1278.82 (C-N Bend.), 761.10 (Ar C-H Bend.), 753.59 (C-Cl Bend.). ^{1}H-NMR (400 MHz, DMSO, δ ppm): 2.00 (s, 1H, NH), 3.73 (s, 3H,

OCH_3), 4.5 (d, 1H, CH), 5.00 (s, 1H, OH), 6.65-7.92 (m, 8H, Ar-H). Mass Spectra: M^+236.99, M^{+2} 393.21. Elemental Analysis, % found (% required): C, 52.07 (52.11); H, 3.59 (3.6); N, 10.69 (10.72); O, 16.30 (16.33), Cl, 9.12 (9.05); S, 8.17 (8.18).

3.1.4. IV_d: 4-chloro-2-(3,6-dihydro-2-imino-6-(4-nitro phenyl)-2H-1,3-thiazin-4-yl)-6-nitro-phenol

Creamiest browncolored solid, Molecular Formula: $C_{16}H_{12}ClN_3O_4S$, Molecular weight: 315.71, Yield: 73.44%, M.P.:215-217°C, R_f value: 0.73, FT-IR (KBr, cm^{-1}): 3369.95(O-H Str.), 3117.92 (=C-H Str.), 1629.41 (C=C Str.), 1292.84 (C-N Bend.), 710.77(Ar C-H Bend.), 753.66 (C-Cl Bend.). ^{1}H-NMR (400 MHz, DMSO, δ ppm): 2.00 (s, 1H, NH), 4.5 (d, 1H, CH), 5.00 (s, 1H, OH), 6.9-8.07 (m, 9H, Ar-H). Mass Spectra: M^+ 212.09, M^{+2} 315.58. Elemental Analysis, % found (% required): C, 50.86 (50.87); H, 3.19 (3.20); N, 11.10 (11.12); O, 16.93 (16.94); Cl, 9.36 (9.38); S, 8.52 (8.49).

3.1.5. IV_e: 4-chloro-2-(3,6-dihydro-2-imino-6-(4-hydroxy phenyl)-2H-1,3-thiazin-4-yl)-6-nitrophenol

Pale reddish orange colored solid, Molecular Formula: $C_{16}H_{13}ClN_3O_5S$, Molecular weight: 377.81, Yield: 70.06%, M.P.: 207-209°C, R_f value: 0.79, FT-IR (KBr, cm^{-1}): 3407.09(O-H Str.), 3108.02 (=C-H Str.), 1609.91 (C=C Str.), 1296.02 (C-N Bend.), 734.56 (Ar C-H Bend.), 781.07 (C-Cl Bend.). ^{1}H-NMR (400 MHz, DMSO, δ ppm): 2.00 (s, 1H, NH), 4.5 (d, 1H, CH), 5.00 (s, 2H, OH), 6.9-8.07(m, 9H, Ar-H). Mass Spectra: M^+ 233.54, M^{+2} 379.45. Elemental Analysis, % found (% required): C, 48.77 (48.80); H, 3.09 (3.07); N, 10.65 (10.67); O, 20.33 (20.31); Cl, 9.06 (9.00); S, 8.17 (8.14).

3.2. Biological Activity [17]

The novel synthesized compounds have shown moderate to strong activity against *M. tuberculosis* compared to standard drug. The compound having chloro substituent on phenyl ring (Iva) was found to be most active and show good antitubercular activity against *M. tuberculosis* with zone of inhibition 20mm. Upon further observation, it was analyzed that minimum 08 mg weight of IVa is required to inhibit the growth of *Mycobacterium tuberculosis* (MIC-value) and 15 mg weight of compound is required to inhibit the 50% growth of microorganism (MIC_{50}).

Compound IV_b show moderate antitubercular activity against *M. tuberculosis* with zone of inhibition 17mm. Upon further observation, it was analyzed that minimum 10 mg weight of IV_b is required to inhibit the growth of *Mycobacterium tuberculosis* (MIC value) and 17 mg weight of compound is required to inhibit the 50% growth of microorganism (MIC_{50} value).

Compound IV_c show remarkable antitubercular activity against *M. Tuberculosis* with zone of inhibition 21 mm and minimum 07 mg weight is required to inhibit the growth of *Mycobacterium tuberculosis* (MIC value) and 25 mg weight of the compound required to inhibit the 50% growth of microorganism.

Table 2: Zone of Inhibition (mm) & MIC Value of synthesized thiazine Compounds

Compound Code	Z.I.(mm)	MIC	MIC_{50}
IV_a	14	12	17
IV_b	20	10	15
IV_c	21	05	10
IV_d	12	17	30
IV_e	15	14	1
Moxifloxacin	27	02	04

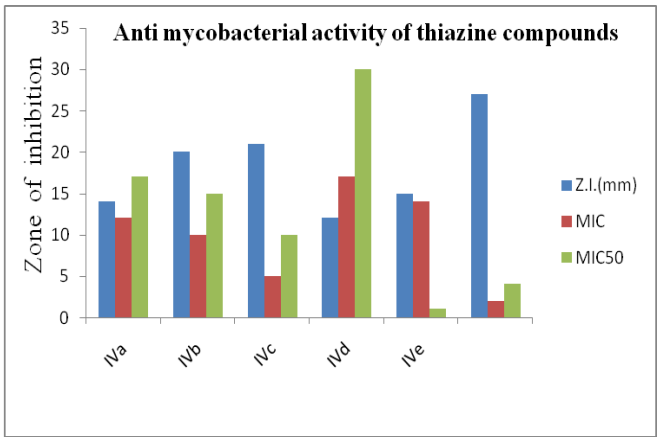


Fig. 2: Anti-mycobacterial activity of synthesized thiazine derivatives

Compound IV_d show moderate antitubercular activity against *M. Tuberculosis* with zone of inhibition 14 mm. Upon further observation, it is analyzed that minimum 12 mg weight of IV_d is required to inhibit the growth of *Mycobacterium tuberculosis* (MIC value) and 18 mg weight of compound is required to inhibit the 50% growth of microorganism (MIC_{50} value).

Compound IV_e show good antitubercular activity against *M. tuberculosis* with zone of inhibition 20 mm and

minimum 10 mg weight is required to inhibit the growth of *Mycobacterium tuberculosis* (MIC value) while 15 mg weight of compound is required to inhibit the 50% growth of microorganism (MIC_{50} value) (Table 2, Fig. 2).

4. CONCLUSION

A series of thiazine derivatives had been synthesized and characterized by IR, NMR, mass and elemental analysis. The final compounds were screened for antitubercular activity against *M. tuberculosis* strains. *In-vitro* anti tubercular activities of all synthesized compounds against *M. Tuberculosis* by taking the Moxifloxacin (Z.I. = 24-26 mm) as standard drug and MIC and MIC_{50} value of each synthesized compounds were evaluated. Such test results indicate that compound IV_a (Z.I. = 20 mm, MIC = 05 mg), IV_b (Z.I. = 20 mm, MIC = 10 mg), IV_c (Z.I. = 21 mm, MIC = 5 mg), IV_d (Z.I. = 20 mm, MIC = 10 mg) and IV_e (Z.I. = 21 mm MIC = 5 mg) are potential anti-tubercular agents and capable to inhibit grow. Results were significant to standard drug Moxifloxacin.

5. ACKNOWLEDGMENT

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Conflict of Interest

None declared

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