Asian Journal of Chemistry; Vol. 29, No. 9 (2017), 2084-2090

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2017.20832

Synthesis and Characterization of Antitubercular Triazine-Chalcone Hybrid Molecules

AMAN RAWAT, ATINDER KAUR, SURJIT and HARPREET KAUR*

Department of Chemistry, Lovely Professional University, Phagwara-144 411, India

*Corresponding author: E-mail: harpreet2.kaur@lpu.co.in

Received: 20 April 2017; *Accepted*: 10 June 2017; *Published online*: 15 July 2017; AJC-18496

A new series of 1,3,5-triazine-chalcone hybrid molecules have been synthesized and evaluated *in vitro* for *Mycobacterium tuberculosis* H37Rv inhibitory potency using Alamar blue assay and the activity expressed as the minimum inhibitory concentration (MIC) in µg/mL. The antitubercular activity screening data revealed that the compounds **1** and **2B** demonstrated comparatively the most potent inhibitory activity, with MIC value 1.6 µg/mL. Most of the compounds displayed significantly promising activity.

Keywords: Heterocyclic chalcone, *Mycobacterium tuberculosis*, Antitubercular activity, Triazine-chalcone hybrid.

INTRODUCTION

The concept of "molecular hybridization" is in current demand as it can overcome the problem of drug resistance and enhance the existing libraries of anti-infective agents. The combination of two or more pharmacophores linked with each other results in new molecules with the hope that they would either give synergistic effect or additive pharmacological activities [1].

A number of heterocyclic compounds have been reported for their applications in the synthesis of biologically active and medicinally important molecules. The compounds which contain five membered rings having nitrogen and oxygen like isoxazole and pyrazoles are found to possess biological activities like antiviral, antitumor, anticonvulsant, analgesic, antioxidant, antidepressant, herbicidal, insecticidal, fungicidal, antibacterial, antimicrobial, cyclooxygenase inhibitory, antimicrobial, *etc*. [2]. A number of derivatives having *s*-triazine moiety have been reported, which can be used as polymers and drugs and also applicable as reactive dyes [3].

Mycobacterium tuberculosis (TB) is a serious public health problem and in today's world it is a leading infectious cause of death. World Health Organization (WHO) has launched a global project on antitubercular drug resistance in order to measure the effect of drug resistance. According to the data of global report on anti-tubercular drug resistance it has been shown that it is still present worldwide. However, a number of new chemical compounds having significant antitubercular activity have been recently discovered, but no new drug has entered the market from the last 55 years [4]. Tuberculosis is caused due to the several species of Mycobacteria that are aerobic pathogens of higher vertebrates which are actually intracellular, non-motile, Gram-positive and rod shaped obligates. In the current therapy to cure anti-TB, it will require almost nine months that is too long. Due to these factors the therapy for the patient have become very difficult. The known drawbacks of current existing drugs with the emergence of MDR strains and the increasing occurrence of death due to tuberculosis have arisen interest in the discovery and development of new anti-tubercular drugs with novel mode of action. So, there is an essential need to discover and develop the new anti-TB drugs in order to overcome the drug resistance problem [5].

There are basically two classes of antitubercular agents: The first line drugs which are given for six months are isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB), rifampin (RIF) and streptomycin. If due to bacterial drug resistance the treatment fails then second line drugs are used such as *p*-amino salicylic acid (PAS), kanamycin, fluoroquinolones, capreomycin, ethionamide and cycloserine are used. These drugs are mostly less effective and have serious side effects. Therefore, it is necessary to develop safe and cost-effective new antitubercular agents [6].

Chalcones are having wide range of biological activity and are found to be effective as antibacterial, antifungal, anti-inflammatory and anticancer *etc*. Chalcones are basically the α,β-unsaturated carbonyl compounds in which it contain ketoethylenic group. Chalcones have conjugated double bonds and a totally delocalized π-electron framework on both benzene rings.

The nitrogen containing heterocycles which are proved to be potent in the field of medicinal chemistry are the important class of compounds [7]. Triazine known from a long period of time as a class of six membered heterocyclic compound containing nitrogen. In 1,3,5-triazine all the nitrogen atoms are present symmetrically therefore it is also called as symmetrical triazine or *s*-triazine [4]. Recently it has been discovered that 2,4,6-trisubstituted-1,3,5-triazine scaffolds act as a potent inhibitor of *Mycobacterium tuberculosis* (Mtb) H37RV. The derivatives of *s*-triazine have also been found to possess a wide range of biological activities [8].

The pyrimidines and their derivatives have found to possess diverse pharmacological properties and a wide range of biological activities against unrelated DNA and RNA, viruses including polio herps viruses, diuretic, antitumor, anti HIV, cardiovascular and so on [7].

In the present work, the triazine-pyrimidine hybrids were synthesized by using different schemes and were evaluated for their *in vitro* antitubercular activity.

EXPERIMENTAL

Condenser, round bottom flask (250 mL), magnetic stirrer, beakers (500 mL), tripod stand, thermometer, guard tube, separating funnel, magnetic bead, measuring cylinder, test tubes, TLC plate, U.V. chamber.

p-Nitroacetophenone, Fe-powder, ethanol, methanol, ethylacetate, 2-chloro-4,6-dimethoxy-1,3,5-triazine, sodium hydroxide pellets, potassium hydroxide, conc. HCl, dry acetone, sodium carbonate, guanidine hydrochloride, 1,3-diphenyl propane-2-one, chloroform, ethyl acetate, butanol, hexane, distilled water, anisaldehyde, benzaldehyde, *p*-benzaloxy benzaldehyde, *p*-chloro benzaldehyde, *p*-cyano benzaldehyde. All the chemicals were purchased from Loba Chemical Ltd.

Synthesis of 4,6-diphenylpyrimidin-2-amine (2): 1,3-Diphenyl propen-2-one (0.01mol) (**1**) were taken in a 250 mL round bottom flask and dissolved in ethanol (25 mL). To this guanidine chloride (0.01 mol) and ethanolic KOH (5 mL of 40 %) were added. The reaction mixture was refluxed for 10 h. The mixture was then poured into water (ice-cold) and neutralized with dil. HCl. After neutralization, the solid was filtered, washed with excess of water, dried and recrystallized from ethanol from ethanol to give pure product.

Spectral data for compound 2: The IR of compound **2** exhibited ν(C=C) stretching = 1537-1494 cm^{-1}, ν(N-H) (1° amine) stretching = 3302 cm^{-1} and 3475 cm^{-1}, ν(C-N) stretching = 1220.98 cm^{-1}, ν(C-H)(S_{p2}) stretching = 3180.72 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.43 (s, 3H, -OCH_3), 5.1 (s, 2H, -NH_2), 7.0 to 8.0 (m, 20H, Ar-H and NH) [9]. The yield, melting point and R_f has been shown in Table-1.

Synthesis of compound 3: 4,6-Diphenylpyrimidin-2-amine (0.01 mol) and 2-chloro-4,6- dimethoxy-1,3,5-triazine (0.01 mol) were taken in round bottom flask and dissolved in dry acetone (50 mL). The reaction mixture was refluxed for 8 h. After checking the TLC, the reaction mixture was poured into ice-cold water. Neutralization of HCl was done by sodium carbonate solution (0.005 N, 10 mL water). The solid was filtered, washed and dried.

Spectral data for compound 3: The IR of compound **3** exhibited ν(N-H) = 3500-3300 cm^{-1} (N-H str. due to 2° amines), ν(C=C) =1633.76 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1222.91 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 802.41 cm^{-1}, ν(C-H)(s_{p2}) stretching = 3150-2950; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.33 (s, 6H, -2OCH_3), 6.5 (s, 1H (C-H), pyrimidine ring), 7.38-7.42 (m, 10H, Ar-H).

Synthesis of compound 7: 4-Aminoacetophenone (1.35 g, 0.01 mol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (1.75 g, 0.01 mol) were taken in round bottom flask and dissolved in dry acetone (50 mL). The reaction mixture was refluxed for 8 h. The reaction mixture was poured into ice water. After neutralization, the solid was filtered, washed and dried.

Synthesis of 1-[4-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)phenyl]-3-(3-methoxyphenyl)prop-2-en-1-one (1A-1E): Compound **7** (2.74 g, 0.01 mol) was dissolved in methanol (40 mL) in a 250 mL round bottom flask and to this 4-methoxy benzaldehyde (0.01 mol) was added. The solution was kept on stirring for 30 min at room temperature. To the reaction mixture sodium hydroxide solution (20 % w/v) was added. After checking the completion of the reaction by TLC, crushed ice was added in the reaction mixture and neutralization was done. The product was filtered and washed, dried and recrystallized from ethanol. Synthesis of **1B**, **1C**, **1D** and **1E** was done by same method.

Spectral data for compound 1A: The IR of compound **1A** exhibited ν(N-H) = 3330 cm^{-1} (N-H str. in 2° amine), ν(C-H) = 2843 cm^{-1} (C-H str. in aromatic ring), ν(C=O) stretching = 1640 cm^{-1}, ν(C=C) = 1632 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1220 cm^{-1} (C-O-C in ether); ν(C-N) stretching = 807 cm^{-1} (C-N- str. in *s*-triazine); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.33(s, 6H, -2OCH_3), 3.82 (s, 3H, P-OCH_3), 7.10-7.90 (m, 8H, Ar-H), 7.98 (d, 1H of –CO-CH=), 8.18 (d, 1H of Ar-CH=), 10.47(s, 1H, -NH) [10].

Spectral data for compound 1B: ν(N-H) = 3223.16 cm^{-1} (N-H str. in 2° amine), ν(C-H) stretching = 2887.53 cm^{-1} (C-H str. in aromatic ring), ν(C=O) stretching =1770.71 cm^{-1}, ν(C=C) = 1653-1618 cm^{-1} (-C=C- str. in aromatic ring),, ν(C=C) = 1548 cm^{-1} (-C=C- str. due to unsaturation with carbonyl), ν(C-O-C) stretching = 1271.13 cm^{-1} (C-O-C in ether); ν(C-N) stretching = 958 cm^{-1} (C-N- str. in *s*-triazine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.13-7.74 (m, 9H, Ar-H), 7.78 (d, 1H, HC=CH (H-α)), 8.01 (d, 1H, HC=CH (H-β)), 9.74 (s, 1H, NH), 3.33 (s, 6H, -2OCH_3) [5].

Spectral data for compound 1C: ν(N-H) = 3495.13 cm^{-1} (N-H str. in 2° amine), ν(C-H) stretching = 2924.18 cm^{-1} (C-H str. in aromatic ring), ν(C=O) stretching =1678.13 cm^{-1}, ν(C=C) = 1601.12 cm^{-1} (-C=C- str. in aromatic ring),, ν(C=C) = 1556.61 cm^{-1} (-C=C- str. due to unsaturation with carbonyl), ν(C-O-C) stretching = 1269.2 cm^{-1} (C-O-C in ether); ν(C-N) stretching = 837.13 cm^{-1} (C-N- str. in *s*-triazine); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.18 (s, 2H, -OCH_2-Ar), 3.33 (s, 6H, -2OCH_3), 7.0-7.90 (m, 13H, Ar-H), 7.96 (d, 1H of –CO-CH=CH), 8.18 (d, 1H of Ar-CH=CH-), 10.12 (s, 1H, NH-) [10].

Spectral data for compound 1D: ν(N-H) = 3489 cm^{-1} (N-H str. in 2° amine), ν(C-H) stretching = 3057.27 cm^{-1} (C-H str. in aromatic ring), ν(C=O) stretching =1670-1620 cm^{-1}, ν(C=C) = 1552.75 cm^{-1} (-C=C- str. in aromatic ring), ν(C=C)

= 1446.66 cm^{-1} (-C=C- str. due to unsaturation with carbonyl), ν(C-O-C) stretching = 1269.2 cm^{-1} (C-O-C in ether); ν(C-N) stretching = 835.21 cm^{-1} (C-N- str. in *s*-triazine); ^{1}H NMR (400 MHz, DMSO *d*6, δ, ppm): 7.61 (d, *J* = 15.2 Hz, 1H, HC=CH (H-α)), 7.67-7.82 (m, 8H, Ar-H), 7.87 (d, *J* = 15.2 Hz, 1H, HC=CH (H-β)), 9.63 (s, 1H, NH), 3.33 (s, 6H, -2OCH_3) [5].

Spectral data for compound 1E: ν(N-H) = 3504.77 cm^{-1} (N-H str. in 2° amine), ν(C-H) stretching = 3032.20 cm^{-1} (C-H str. in aromatic ring), ν(C=O) stretching =1683.91 cm^{-1}, ν(C=C) = 1543.10 cm^{-1} (-C=C- str. in aromatic ring),, ν(C=C)=1456.30-1417.73 cm^{-1} (-C=C- str. due to unsaturation with carbonyl), ν(C-O-C) stretching = cm^{-1} (C-O-C in ether); ν(C-N) stretching = 837.13 cm^{-1} (C-N- str. in *s*-triazine).

Synthesis of N-[4-(2-amino-6-(4-methoxyphenyl) pyrimidin-4-yl) phenyl]-2,6-dimethoxypyrimidin-4-amine (2A-2E): Compound **1A** (0.01 mol) was dissolved in 25 mL ethanol in a 250 mL round bottom flask. To the solution guanidine hydrochloride (0.01 mol) and KOH (5 mL of 40 %) were added. Reflux the reaction mixture for 10 h. Completion of the reaction was confirmed with the help of TLC. After completion of reaction, the reaction mixture was cooled, poured into crushed ice, washed and dried to give the product.

The synthesis of **2B**, **2C**, **2D** and **2E** was carried out in the similar manner.

Spectral data for compound 2A: The IR of compound **2A** exhibited ν(N-H) = 3479.7 cm^{-1} (Broad N-H str. due to 1° and 2° amine), ν(C=C) = 1653.05 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1344.43 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 806.27; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.43 (s, 3H, -OCH_3), 5.1 (s, 2H, -NH_2), 7.0 to 8.0 (m, 20H, Ar-H and NH) [11]. The yield, melting point and R_f has been shown in Table-1.

Spectral data for compound 2B: The IR of compound **2B** exhibited ν(N-H) = 3238-3450 cm^{-1} (Broad N-H str. due to 1° and 2° amine), ν(C=C) = 1678-1618 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1089.82 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 956 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.43 (s, 3H, -OCH_3), 5.1 (s, 2H, -NH_2), 7.0 to 8.0 (m, 20H, Ar-H and NH) [11]. The yield, melting point and R_f has been shown in Table-1.

Spectral data for compound 2C: The IR of compound **2C** exhibited ν(N-H) = 3440-3500 cm^{-1} (Broad N-H str. due to 1° and 2° amine), ν(C=C) = 1640-1680 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1273.06 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 835.21 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.9 (s, 3H, -OCH_3), 5.1 (s, 2H, -NH_2), 7.0 to 8.0 (m, 20H, Ar-H and NH) [11]. The yield, melting point and R_f has been shown in Table-1.

Spectral data for compound 2D: The IR of compound **2D** exhibited ν(N-H) = 3450-3300 cm^{-1} (Broad N-H str. due to 1° and 2° amine), ν(C=C) = 1627.97 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1361.19 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 871.85 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.9 (s, 3H, -OCH_3), 5.1 (s, 2H, -NH_2), 7.0 to 8.0 (m, 20H, Ar-H and NH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.33 (s, 12H, -OCH_3), 6.72 (s, 1H (C-H), pyrimidine ring), 7.23 (m, 8H, Ar-H) [11].

Spectral data for compound 2E: The IR of compound **2D** exhibited ν(N-H) = 3504.77 cm^{-1} (Broad N-H str. due to 1° and 2° amine), ν(C=C) = 1683.91 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1273.06 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 837.13 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.32 (s, 3H, -OCH_3), 7.0 to 8.0 (m, 20H, Ar-H and NH) [11].

Synthesis of N-(4-(4-(2,6-dimethoxypyrimidin-4-ylamino)phenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-4,6-dimethoxy-1,3,5-triazin-2-amine (3A-3E): Compound **3A** (0.01 mol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (**6**) (1.75 g, 0.01 mol) were taken in a 250 mL round bottom flask and dissolved in dry acetone (50 mL). The reaction mixture was refluxed for 8 h. Completion of reaction was confirmed by TLC. The reaction mixture was poured into ice water. After neutralization, the solid was filtered, washed with excess of water and dried. Purification of compound was done by ethyl acetate and hexane.

Spectral data for compound 3A: The IR of compound **3A** exhibited ν(N-H) = 3608-3533 cm^{-1} (Broad N-H str. due to 2° amines), ν(C=C) = 1649.19 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1263.42 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 802.41 cm^{-1}, ν(C-H)(s_{p3}) stretching = 2966.62 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.37 (s, 15H, -OCH_3), 7.06 (d, 2H, Ar-H), 7.94 (s, 1H (C-H), pyrimidine ring), 7.84 (d, 2H, Ar-H). The yield, melting point and R_f has been shown in Table-1.

Spectral data for compound 3B: The IR of compound **3B** exhibited ν(N-H) = 3282.95 cm^{-1} (Broad N-H str. due to 2° amines), ν(C=C) = 1543.10 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1089.82 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 800.49 cm^{-1}, ν(C-H)(s_{p2}) stretching = 3026.69 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.32 (s, 12H, -OCH_3), 6.78 (s, 1H (C-H), pyrimidine ring), 7.85 (d, 2H, Ar-H), 8.22 (s, 4H, Ar-H), 7.91(d, 2H, Ar-H).

Spectral data for compound 3C: The IR of compound **3C** exhibited ν(N-H) = 3504.77 cm^{-1} (Broad N-H str. due to 2° amines), ν(C=C) = 1670.41 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1263.42 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 800.49 cm^{-1}, ν(C-H)(sp^2) stretching = 3059.20 cm^{-1}.

Spectral data for compound 3D: The IR of compound **3D** exhibited ν(N-H) = 3471.98 cm^{-1} (Broad N-H str. due to 2° amines), ν(C=C) = 1585.54 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1267.27 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 1055.1 cm^{-1}, ν(C-H)(s_{p2}) stretching = 2926.11 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.60 (s, 12H, -OCH_3), 6.78 (s, 1H (C-H), pyrimidine ring), 7.19 (d, 2H, Ar-H), 7.55 (m, 4H, Ar-H), 7.87(d, 2H, Ar-H).

Spectral data for compound 3E: ν(N-H) = 3354.77 cm^{-1} (Broad N-H str. due to 2° amines), ν(C=C) = 1678.13 cm^{-1} (-C=C- str. in aromatic ring), ν(C-O-C) stretching = 1269.20 cm^{-1} (C-O-C in ether), ν(C-N) stretching = 798.56 cm^{-1}, ν(C-H)$_{sp}$ stretching = 2893.92 cm^{-1}.

Anti-TB activity using alamar blue dye: The anti-tubercular activity of the synthesized compounds was checked against H_{37}RV *M. tuberculosis* using alamar blue assay. 200 μL of sterile water was added each well of 96 well plates. 100

TABLE-1
ANTITUBERCULAR ACTIVITY OF COMPOUNDS **1-3A-E**

Samples	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
TRZ	S	S	S	R	R	R	R	R
1	S	S	S	S	S	S	S	R
2	S	S	S	R	R	R	R	R
1-A	S	S	S	R	R	R	R	R
1-B	S	S	R	R	R	R	R	R
1-C	S	S	S	R	R	R	R	R
1- D	S	S	S	S	R	R	R	R
1-E	S	S	S	S	S	S	R	R
2-A	S	S	R	R	R	R	R	R
2-B	S	S	S	S	S	S	S	R
2-C	S	S	S	S	R	R	R	R
2-D	S	S	R	R	R	R	R	R
2-E	S	S	S	R	R	R	R	R
3-A	S	S	S	R	R	R	R	R
3-B	S	S	S	R	R	R	R	R
3-C	S	S	S	R	R	R	R	R
3-D	S	S	R	R	R	R	R	R
3-E	S	S	S	R	R	R	R	R

µL of the Middlebrook 7H9 broth was added in each well, serial dilutions were made directly. Drug concentration was checked for 100-0.2 µg/mL. Plates were incubated for five days at 37 °C. After 5 days 25 µL of 1:1 mixture of almar blue reagent and 10 % Tween 80 was added and the plates were further incubated for 24 h. A blue coloration shows bacterial growth inhibition and pink colour indicates bacterial cell growth. Pyrazinamide and streptomycin were used as standards for analysis [12].

RESULTS AND DISCUSSION

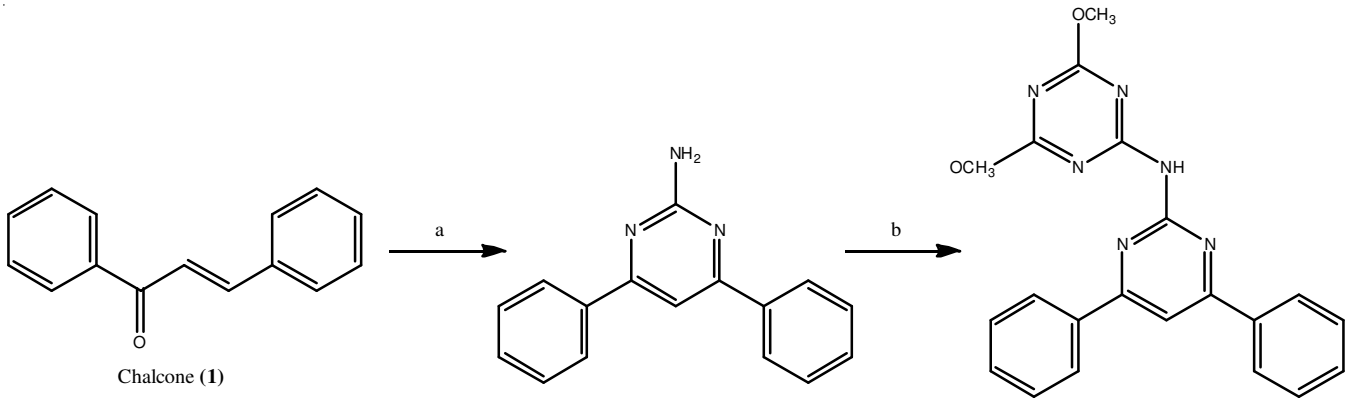
Two schemes were followed for the synthesis of compounds. Compounds **2** and **3** were synthesized using **Scheme-I** and compounds **1A-3E** were synthesized using **Scheme-II**.

Compound **2** was synthesized by reaction of un-substituted to form pyrimidine ring (**Scheme-I**). Compound **2** gave characteristic peaks at 3400-3200 cm^{-1} showing the presence of NH_2 group. The data was correlated with those present in literature [7].

Compound **3** was characterized by IR, ^1H NMR and ^{13}C NMR spectroscopy. It gave a single characteristic peaks at 3500-3300 cm^{-1} (2° amine) and the peaks of N-H stretching (1° amine) vanished. ^1H NMR done in DMSO-d_6 showing singlet at δ = 3.33 ppm for 6H (2-OCH_3) and at 6.5 ppm for 1H (C-H) of pyrimidine ring, multiplet at 7.38-7.42 ppm for 10H of aromatic ring were observed. The structure of compound **3** was confirmed to be N-(4,6-diphenylpyrimidin-2-yl)-4,6-dimethoxy-1,3,5-triazin-2-amine.

In **Scheme-II**, compound **5** was synthesized by the reaction of *p*-amino acetophenone with *s*-triazine in dry acetone. This was further reacted with substituted aromatic aldehyde to form compounds **1A-1E**. Compound **5** gave characteristic peak at 3300-3250 cm^{-1} for N-H stretching (2° amine) and for carbonyl stretching peak at 1670-1660 cm^{-1} was observed [5]. This confirmed the structure of compound **5** to be 1-[4-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)phenyl]ethanone.

Compounds **1A-1E** were synthesized by subsequent condensation of compound **5** with various aromatic aldehydes (**A-E**) in basic medium (40 % NaOH) using methanol as



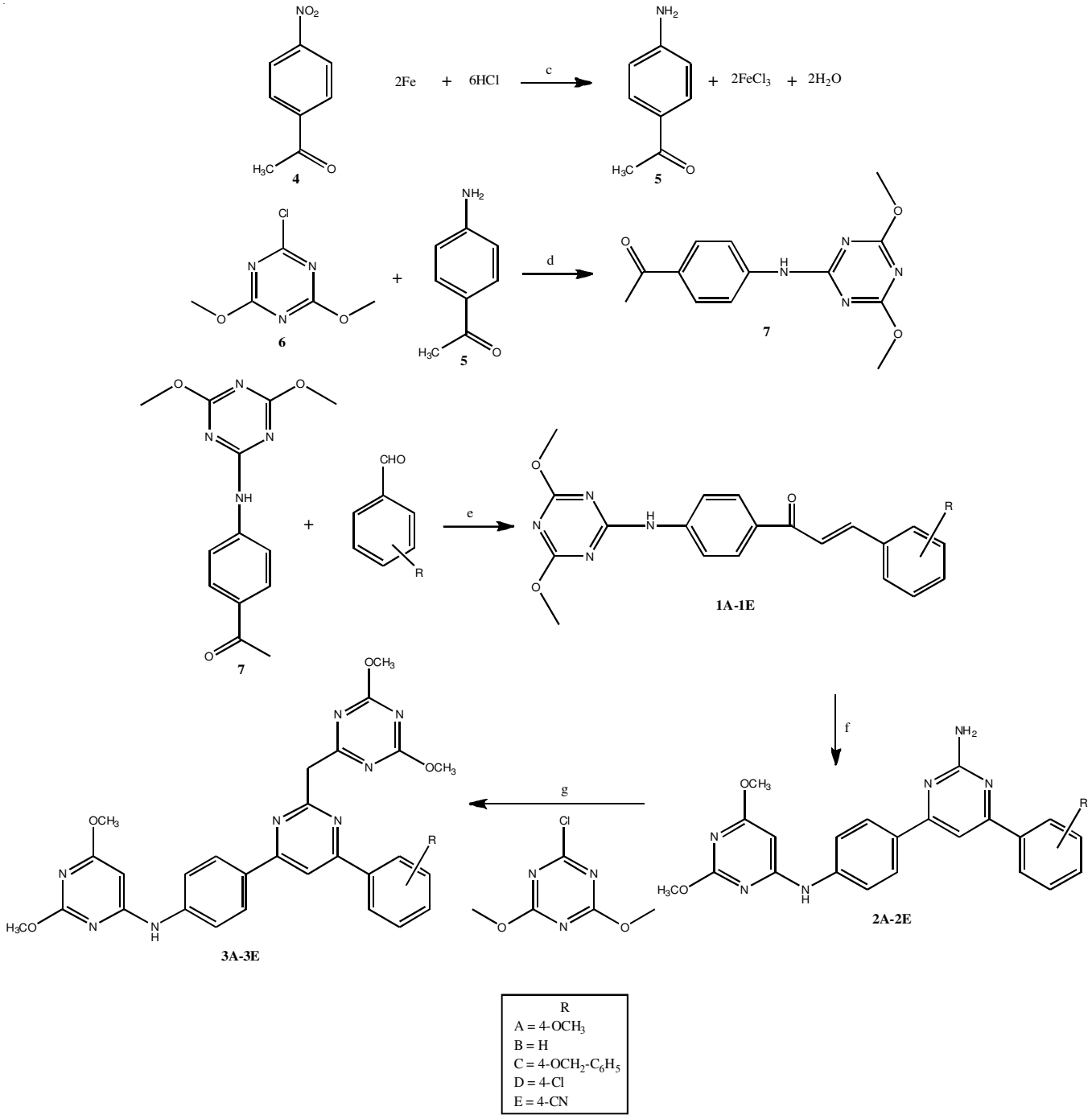
Chalcone **(1)**

4,6-Diphenylpyrimidin-2-amine **(2)**

N-(4,6-Diphenylpyrimidin-2-yl)-4,6-dimethoxy-1,3,5-triazin-2-amine **(3)**

Reagents and conditions: (a) Guanidine HCl, 40 % KOH, ethanol, reflux 10 h, (b) 2-chloro-4,6-dimethoxy-1,3,5-triazine, dry acetone, reflux 8 h

Scheme-I: Representation of synthesis of compound **1-3**



Reagents and conditions: (c) ethanol, reflux 6 h, 60 °C, (d) dry acetone, reflux 8 h, (3) methanol, 40 % NaOH, reflux 16 h, (f) guanidine HCl, 40 % KOH, ethanol, reflux 10 h, (g) dry acetone, reflux 8 h

Scheme-II: Representation of synthesis of compound **1A-3E**

solvent medium. Compound B gave characteristic peak at 3350-3200 cm^{-1} due to N-H stretching (2°amine) and at 1650-1600 cm^{-1} due to carbonyl stretching (C=O) group. The data was confirmed with those present in literature [5] and the structure of compound **1A** was confirmed to be 1-[4-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)phenyl]-3-(3-methoxyphenyl)prop-2-en-1-one.

Compound **1B** gave characteristic peak at 3250-3200 cm^{-1} due to N-H stretching (2° amine) and at 1680-1580 cm^{-1} due to carbonyl (C=O) group. The data was confirmed with those present in literature [5] and the structure of compound **1B** was confirmed to be 1-[4-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)-phenyl]-3-phenylprop-2-en-1-one.

Compound **1C** gave characteristic peak at 3500-3400 cm^{-1} due to N-H stretching (2°amine) and at 1700-1650 cm^{-1} due to carbonyl (C=O) group. The data was confirmed with those present in literature [5] and the structure of compound **1C** was confirmed to be 3-(3-(benzyloxy)phenyl)-1-[4-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)phenyl]prop-2-en-1-one.

Compound **1D** gave characteristic peak at 3500-3450 cm^{-1} due to N-H stretching (2°amine) and at 1700-1600 cm^{-1} due to carbonyl (C=O) group. The data was confirmed with those

present in literature [5] and the structure of compound **1D** was confirmed to be (E)-3-(4-chlorophenyl)-1-(4-(2,6-dimethoxypyrimidin-4-ylamino)phenyl)prop-2-en-1-one.

Compound **1E** gave characteristic peak at 3500-3550 cm^{-1} due to N-H stretching (2°amine) and at 1700-1600 cm^{-1} due to carbonyl (C=O) group. The structure of compound **1E** was confirmed to be 3-(3-(4-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)-phenyl)-3-oxoprop-1-enyl)benzonitrile.

Compounds **2A-2E** were synthesized by the reaction of compounds **1A-1E** with guanidine hydrochloride in basic medium (40 % KOH) to form pyrimidine ring. The structure of the synthesized compounds was assigned on the basis of IR, ^{1}H NMR. The proton NMR was done at 400 MHz in DMSO-d_6. The characteristic peaks at δ 3.5-4.0 ppm (singlet) for -OCH_3 group and at 5.0-5.3 ppm (singlet) for -NH_2 protons were observed. A multiplet at 7.0 to 7.5 ppm for aromatic proton were also observed.

Compound **2A** gave two characteristic peaks at 3500-3300 cm^{-1} showing the presence of N-H stretching (1° amine) and the characteristic peak of α,β-unsaturation carbonyl group (1650-1600 cm^{-1}) was absent showing the condensation of guanidine hydrochloride with that group and a characteristic peak of C=N stretching was observed at 1530 cm^{-1}. The structure of the compound was confirmed to be N-[4-(2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl)phenyl]-2,6-dimethoxypyrimidin-4-amine.

Compound **2B** gave two characteristic peaks at 3450-3250 cm^{-1} showing the presence of N-H stretching (1° amine) and the characteristic peak of α,β-unsaturation carbonyl group (1680-1580 cm^{-1}) was absent showing the condensation of guanidine hydrochloride with that group and a characteristic peak of C=N stretching was observed at 1440 cm^{-1}. The structure of the compound was confirmed to be N-[4-(2-amino-6-phenylpyrimidin-4-yl)phenyl]-2,6-dimethoxypyrimidin-4-amine.

Compound **2C** gave two characteristic peaks at 3440-3500 cm^{-1} showing the presence of N-H stretching (1° amine) and the characteristic peak of α,β-unsaturation carbonyl group (1700-1650 cm^{-1}) was absent showing the condensation of guanidine hydrochloride with that group and a characteristic peak of C=N stretching was observed at 1540-1480 cm^{-1}. The structure of the compound was confirmed to be N-(4-(2-amino-6-(4-(benzyloxy)phenyl)pyrimidin-4-yl)phenyl)-2,6-dimethoxypyrimidin-4-amine.

Compound **2D** gave two characteristic peaks at 3450-3300 cm^{-1} showing the presence of N-H stretching (1° amine) and the characteristic peak of α,β-unsaturation carbonyl group (1700-1600 cm^{-1}) was absent showing the condensation of guanidine hydrochloride with that group and a characteristic peak of C=N stretching was observed at 1600-1450 cm^{-1}. The structure of the compound was confirmed to be N-(4-(2-amino-6-(4-chlorophenyl)pyrimidin-4-yl)phenyl)-2,6-dimethoxypyrimidin-4-amine.

Compound **2E** gave two characteristic peaks at 3500-3450 cm^{-1} showing the presence of N-H stretching (1° amine) and the characteristic peak of α,β-unsaturation carbonyl group (1700-1600 cm^{-1}) was absent showing the condensation of guanidine hydrochloride with that group and a characteristic peak of C=N stretching was observed at 1550-1450 cm^{-1}. The structure of the compound was confirmed to be 4-(2-amino-6-(4-(2,6-dimethoxypyrimidin-4-ylamino)phenyl)pyrimidin-4-yl)benzonitrile.

Compounds **3A-3E** were synthesized by further reaction of compound **2A-2E** with *s*-triazine in dry acetone at 60 °C. The structure of the compounds was assigned with the help of IR, ^{1}H NMR and ^{13}C NMR.

The ^{1}H NMR was done at 400 MHz in DMSO-d_6, showing characteristic peaks at δ =3.5-4.0 ppm for -OCH_3 proton and at 6.5-7.0 ppm for 1H (C-H) of pyrimidine ring. Two doublets at 7.10-7.60 ppm and a multiplet at 7.50-7.90 ppm for aromatic proton were also observed.

Compound **3A** gave a single characteristic peaks at 3600-3500 cm^{-1} of 2° amine and the peaks of N-H stretching (1° amine) was removed. The structure of compound **3A** was confirmed to be N-(4-(4-(2,6-dimethoxypyrimidin-4-ylamino)phenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-4,6-dimethoxy-1,3,5-triazin-2-amine.

Compound **3B** gave a single characteristic peaks at 3300-3250 cm^{-1} of 2° amine and the peaks of N-H stretching (1° amine) was removed. The structure of compound **3A** was confirmed to be N-(4-(4-(2,6-dimethoxypyrimidin-4-ylamino)phenyl)-6-phenylpyrimidin-2-yl)-4,6-dimethoxy-1,3,5-triazin-2-amine. Compound **3C** gave a single characteristic peaks at 3504 cm^{-1} of 2° amine and the peaks of N-H stretching (1° amine) was removed. The structure of compound **3A** was confirmed to be N-(4-(4-(benzyloxy)phenyl)-6-(4-(2,6-dimethoxypyrimidin-4-ylamino)phenyl)pyrimidin-2-yl)-4,6-dimethoxy-1,3,5-triazin-2-amine.

Compound **3D** gave a single characteristic peaks at 3500-3400 cm^{-1} of 2° amine and the peaks of N-H stretching (1° amine) was removed. The structure of compound **3A** was confirmed to be N-(4-(4-chlorophenyl)-6-(4-(2,6-dimethoxypyrimidin-4-ylamino)-phenyl)pyrimidin-2-yl)-4,6-dimethoxy-1,3,5-triazin-2-amine.

Compound **3E** gave a single characteristic peaks at 3450-3300 cm^{-1} of 2° amine and the peaks of 1° NH_2 were vanished. The structure of compound **3A** was confirmed to be 4-(2-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)-6-(4-(2,6-dimethoxypyrimidin-4-ylamino)phenyl) pyrimidin-4-yl)-benzonitrile.

Furthermore, the compounds **1-3** (**A-E**) were evaluated for their *in vitro* antitubercular activity using H_{37}RV *Mycobacterium tuberculosis* by alamar blue assay (Fig. 1). The MIC (minimum inhibitory concentration) is expressed in minimum in μg/mL. From the results, it is revealed that the compounds **1** and **2B** are showing the maximum inhibition of bacterium, with MIC value of 1.6 μg/mL. The compound **1E** showed promising activity, with MIC values 3.12 μg/mL. Some of compounds like **1D** and **2C** showed activity at 12.5 μg/mL (Table-1) [12].

Conclusions

In order to explore the novel concept of developing hybrid molecules for tuberculosis and in view of the excellent anti-TB activity exhibited by triazine, chalcone and pyrimidine moieties. A series of eighteen triazine-chalcone and triazine-chalcone-pyrimidine hybrids were synthesized and evaluated for their ant-TB activity.

In the present study, it is found that Compound **1** and **2B** are very potent against tuberculosis at a concentration of 1.6

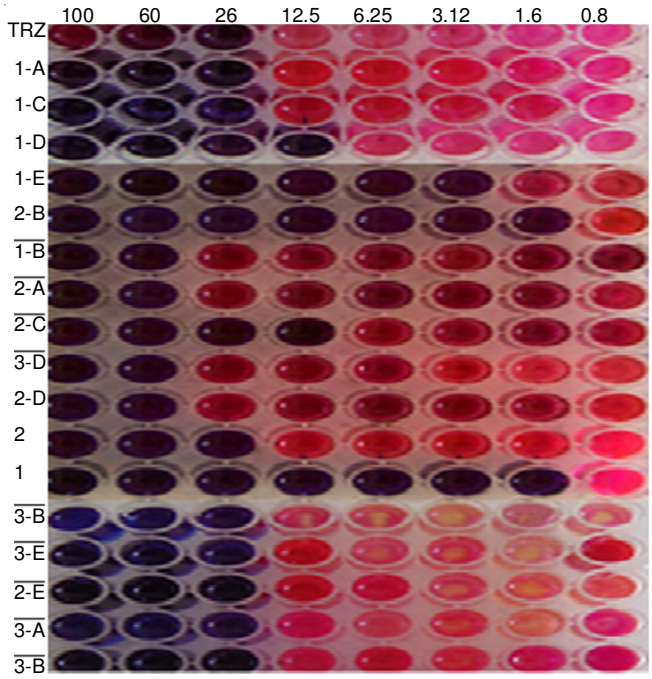


Fig. 3. Anti-TB results of the synthesized compounds

µg/mL, whereas compound **1E** is efficacious at a concentration of 3.12 µg/mL. Compounds like **1D** and **2C** are effective at 12.5 µg/mL whereas all others are sensitive at a concentration of 25 µg/mL.

A number of lead compounds have been synthesized. Further work could be undertaken to develop quite potent drug against one of the World's most deadly disease.

The third series containing triazine-chalcone-pyrimidine is reported for the first time. Further work could be on the third series to find out its medicinal importance.

REFERENCES

1. V.R. Avupati and R.P. Yejella, *World J. Pharm Pharm. Sci.*, **3**, 278 (2014).
2. R. Ghosh and A. Das, *World J. Pharm. Pharm. Sci.*, **3**, 578 (2014).
3. A. Solanki and R. Patel, *Indian J. Chem.*, **53B**, 1448 (2014).
4. M.R. Gajula and Y.V.R. Reddy, *Eur. J. Chem.*, **5**, 374 (2014); https://doi.org/10.5155/eurjchem.5.2.374-379.1027.
5. S.R. Dwarampudi, G.S. Dannana, V.R. Avupati and V.S.M. Bendi, *Eur. J. Chem.*, **5**, 570 (2014); https://doi.org/10.5155/eurjchem.5.4.570-576.1098.
6. R. Saha, M.M. Alam and M. Akhter, *RSC Adv.*, **5**, 12807 (2015); https://doi.org/10.1039/C4RA14440F.
7. V. Sharma and K.V. Sharma, *Rasayan J. Chem.*, **4**, 17 (2011).
8. F.G. Khan, M.V. Yadav, S.R. Khapate and A.D. Sagar, *Indo-Am. J. Pharm. Res.*, **5**, 1447 (2015).
9. S. Syam, S.I. Abdelwahab, M.A. Al-Mamary and S. Mohan, *Molecules*, **17**, 6179 (2012); https://doi.org/10.3390/molecules17066179.
10. A. Solankee, K. Patel and R. Patel, *E-J. Chem.*, **9**, 1897 (2012); https://doi.org/10.1155/2012/638452.
11. A. Solankee and Y. Prajapati, *Rasayan J. Chem.*, **2**, 9 (2009).
12. M.C.S. Lourenço, M.V.N. de Souza, A.C. Pinheiro, M. de L. Ferreira, R.S.B. Gonçalves, T.C.M. Nogueira and M.A. Peralta, *Arkivoc*, 181 (2007); https://doi.org/10.3998/ark.5550190.0008.f18.