An efficient synthesis of some new chalcone, acetyl pyrazoline and amino pyrimidine bearing 1,3,5- triazine nucleus as potential antimicrobial and antitubercular agent

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ABSTRACT

In an attempt to find a new class of antimicrobial and antitubercular agent, a new series of chalcone, acetyl pyrazoline and amino pyrimidine bearing 1,3,5- triazine nucleus were synthesized with appropriate chemical reagent. Chalcones (D1-D5) were synthesized by the classical Claisen-Schmidt condensation of substituted ketone (C) with variously substituted aldehydes via conventional method. Now treatment of chalcones with hydrazine hydrate/glacial acetic acid and guanidine hydrochloride/Alkali afforded the corresponding acetyl pyrazoline (E1-E5) and amino pyrimidine (F1-F5) derivatives respectively. The chemical structures of all newly synthesized compounds were established on the basis of their FTIR, 1H NMR, 13C NMR, LC-MS as well as elemental analysis. All the newly design compounds were assayed for their in vitro antimicrobial activity against selected pathogens by the Broth dilution method and in vitro antitubercular activity against Mycobacterium tuberculosis H37Rv using Lowenstein-Jensen MIC method. Most of the compounds showed appreciable antimicrobial activity against the all tested strains. Among the synthesized compounds D1, D3, E1, E3, E4, F3 and F4 exhibited excellent antimicrobial activity and said to be the most proficient members of the series. Compound D5 and F5 exhibited promising antitubercular activity.

Capsule Summary: 1,3,5-Triazine based some new chalcones, acetyl pyrazolines and aminopyrimidines were synthesized by conventional route which exerted good antimicrobial as well as antitubercular activity.

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INTRODUCTION

Tuberculosis (TB), an infection caused by Mycobacterium tuberculosis, remains a major global health problem in the world (Jaso et al., 2005). The most ordinary form of tuberculosis is pulmonary TB which is an extremely infectious and life-threatening infection. Moreover, the improved susceptibility to TB in human immunodeficiency virus (HIV)-infected populations is another serious health problem throughout the world (Aliyu et al., 2003). It is probable that between 2002 to 2020, approximately a billion people will be newly infected, more than 150 million people will get ill, and 36 million will die of TB (Mavitandela et al., 2006; Asif, 2015a,b,c). Often tuberculosis is accompanied by AIDS and survives as multidrug resistant tuberculosis (MDR-
TB) or as extensively/extremely resistant tuberculosis (XTR-TB), where neither standard antitubercular drug nor any of the regimens are potentially effective. The current chemotherapy is based on age-old drugs like Pyrazinamide, Isoniazid and Rifampicin for tuberculosis. The available treatment establishes a multidrug regime lasting a minimum of six months; although there is no guarantee that the complete sterilization of the infection will be obtained. Successful treatment of multidrug resistant (MDR)-TB and extensively drug resistant (XDR)-TB is even more challenging and requires even longer term treatment (Iseman. 1999). Furthermore, the increase in TB cases caused by MDR and XDR strains, and coinfection with HIV have pointed out the urgent need to develop new antitubercular drugs which will effectively kill MDR strains, less toxic and shortened duration of therapy. In pursuit of achieving this goal, our research efforts are focused on the development of novel structural moieties having antitubercular activity (Asif, 2016). Hence, for the purpose of obtaining new and more potent antitubercular compounds that can improve the current chemotherapeutic antituberculosis treatment, we have synthesized chalcones and convert them into its derivatives and tested for antimicrobial and antitubercular activity.

1,3,5-Triazine skeleton is one of the most appealing chemical core structure subjected to extensive study in recent years (Zhao et al., 2011). The triazine scaffold provides the basis for the design of biologically relevant molecules with widespread application as therapeutic (Chen et al., 2009; Baliani et al., 2005; Melato et al., 2008; Liu et al., 2015; Sunduru et al., 2010; Solankee. 2010). A generic terminology for the 1,3-diaryl-2-propen-1-one is chalcone. The privileged scaffold chalcone remained a fascination among researchers in the 21st Century because they have a unique structural feature of having a >C=O functional group in conjugation with >C=C<, ease of synthesis, diversity of substituents and wide range of biological properties. A classical method for synthesis of chalcone is Claisen-Schmidt condensation in which aldehyde react with acetoephone in the presence of aqueous alkaline bases (Ansari et al., 2005; Rao et al., 2004). Chalcones are also synthesised by using ultrasound irradiation (Calvino et al., 2006), Suzuki reaction (Eddarir et al., 2003) etc. Chalcones, either natural or synthetic, are known to exhibit a broad spectrum of various biological activities. The presence of α, β-unsaturated carbonyl moiety as well as of substituted aromatic rings renders the chalcones biologically active. Some substituted chalcones and their derivatives, including some of their heterocyclic analogues have been reported to possess a wide range of pharmacological activities such as cytotoxic (Salum et al., 2013), anti-retroviral (Rizvi et al., 2012), anti-malarial (Tomar et al., 2010), anti-platelet (Zhao et al., 2005), antitubercular (Gupta and Kaskhedikar. 2013), antimicrobial (Solankee et al., 2012) etc.... Cyclization of chalcone leading to benzodiazepine (Claramunt et al., 2006), pyrazoline (Montoya et al., 2014), pyrimidine (Agarwal et al., 2005), isoxazole (Solankee and Tailor. 2015), 1,4-diketone (Raghavan and Amuradha. 2002) etc.... derivatives have been a developing field within the realm of heterocyclic chemistry for the past several years. These observations led us to synthesize chalcone and convert into pyrazoline and pyrimidine derivatives exploring simple procedures.

Nitrogen containing heterocycles are perhaps by far the most explored heterocyclic compounds because of their occurrence in a numerous of natural products and biologically active compounds. For this reason, synthetic chemists continue to be interested in the construction and functionalization of these heterocycles. Pyrazolines are prominent two nitrogen-containing heterocyclic compounds and various procedures have been worked out for their synthesis. Pyrazolines have variety of methods for their synthesis but one of the popular methods is of Fischer and Knoevenagel i.e. the reaction of α, β- unsaturated ketones with hydrazine in acetic acid under refluxing condition (Fischer and Knoevenagel. 1887). However depending on the reactivity of molecules and need of the chemist, they had synthesized the pyrazolines under different solvent media and acidic or basic conditions (Powers et al., 1998; Amir et al., 2008; Voskienė and Mickevičius. 2009). The synthesis of pyrazolines remains of great interest due to the wide applications of such heterocycles in the pharmaceutical and agrochemical industry. Among the existing various pyrazoline type derivatives, 1-acetyl pyrazolines have been identified as one of the most promising scaffolds, which were found to display antioxidant (Jeong et al., 2004), cytotoxic (Ratkovic´ et al., 2010), anti-inflammatory (Barsou et al., 2006), immunosuppressive (Lombardino and Otterness. 1981), antitubercular (Yar et al., 2006), anticonvulsant (Archana et al., 2002), antimicrobial (Solankee et al., 2010) etc....activities due to the presence of C=N, N-N and other polar functional groups attached the pyrazoline moiety (Sasikala et al., 2012). Many class of chemotherapeutic agents containing pyrazoline nucleus are in clinical use such as orisul (bacterostatic), antipyrine (antipyrletic), butazolidine (anti-inflammatory). In view of these observations, we have report herein the synthesis of 1-acetyl pyrazoline derivatives from chalcone, which have been found to possess an interesting profile of antitubercular and antimicrobial activity. This gave a great impetus to the search for potential pharmacologically active drugs carrying pyrazoline substituents (Asif, 2016).

Among various heterocyclic systems, especially those containing pyridine and pyrimidine nucleus has been the subject of expanding research efforts in heteroaromatic and medicinal chemistry (Tu et al., 2008; Bagley et al., 2001; Ren et al., 2005). Pyrimidines are six membered heterocyclic ring compounds composed of nitrogen and carbon. They are present throughout nature in various forms and are the building blocks of numerous natural compounds from antibiotics to vitamins and liposaccharides. The most commonly recognized pyrimidines are the bases of RNA and DNA, the most abundant being cytosine, thymine or uracil. In medicinal chemistry, pyrimidine derivatives have been very well known for their therapeutic applications. Pyrimidine nucleus is present in barbituric acid and its several
derivatives which are used as hypnic drugs for the nervous system (Wang et al., 2004). In addition to this, pyrimidine nucleus is also found in alloxan, which is known for its diabeticogenic action in a number of animals (Lenzen and Panten, 1988). The pyrimidine moiety with some substitution shows promising antitumor activity as there are large numbers of pyrimidine based antimetabolites such as 5-Fluouracil (Blumenkranz et al., 1984), 5-Thiouracil (Al Safarjali et al., 2005) exhibits some useful antineoplastic activities. Apart from these activities, pyrimidines also possess antimicrobial (El-Essawy et al., 2010), antihypertensive (Pathak et al., 2006), antitubercular (Kumar et al., 2002) etc.... activities. In view of these findings and in continuation of our research work (Solankee et al., 2013; Solankee and Tailor, 2015), herein we reported the reaction of 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro-1', 4'-oxazine) - 6 - [4' - 3'' substitutedphenyl/2''furanyl/2''-proponen-1''-yl] phenylamino]-1,3,5-triazine (D1-D3) with different intermediates give subsequent conversion to products (E1-E5) and (F1-F5) possessing acetyl pyrazolines and amino pyrimidines components were investigated for in vitro antimicrobial and antitubercular activity.

MATERIAL AND METHODS

All the chemicals and solvents used for reaction were of analytical reagent (AR) grade. All the melting points were resolute in open capillary method and are uncorrected. IR spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using CDCl₃ as a solvent and TMS as an internal standard at 400 MHz operating frequency. Chemical shifts are reported in parts per million (ppm) and coupling constant (J) are reported in Hertz. Elemental analysis was carried out by Perkin-Elmer 2400 series-II elemental analyser (Perkin-Elmer, USA). Mass spectra were scanned on a Shimadzu LC-MS 2010 spectrometer (Shimadzu, Tokyo, Japan). Purity of the compounds were checked by thin layer chromatography using TLC aluminum sheets Silica Gel 60 F-254 (Merck) plates of 0.25 mm thickness and the spots were rendered visible by exposing to UV light or keeping the plates in iodine chamber. The compounds (D1-D5) were synthesized by the Claisen-Schmidt condensation reaction.

Preparation of 2-(3'-trifluromethylphenylamino)-4,6-dichloro-1, 3, 5-triazine (A)

Compound A was prepared by the condensation reaction of 3- trifluromethyl aniline (1.6 g, 0.01 mol) and cyanuric chloride (1.8 g, 0.01 mol) dissolved in acetone was constant stirring for 3 hours at 0 to 5 °C. Periodically, sodium carbonate solution (0.53 g, 0.005 mol) was added dropwise to neutralize HCl evolved during the reaction. The progress of the reaction was monitored by using TLC. Finally, the content of the mixture was poured into crushed ice. The solid separated out was filtered, washed with water, dried and recrystallized from ethanol to give 2 - (3' - trifluromethylphenylamino) - 4, 6 - dichloro - 1, 3,5- triazine (Zhou et al., 2006) (A).

Preparation of 2-(3'-trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-chloro-1,3,5-triazine (B)

Tetrahydro - 1, 4 - oxazine (0.8 g, 0.01 mol) was added slowly to compound (A) (2.3 g, 0.01 mol) dissolved in acetone with constant stirring for 4 hours on a magnetic stirrer at room temperature. Periodically, sodium carbonate solution (0.5 g, 0.005 mol) was added dropwise to neutralize HCl evolved during the reaction. The progress of the reaction was monitored on TLC plate. After completion of the reaction, the content was poured into crushed ice. The solid separated out was filtered, washed with water, dried and recrystallized from ethanol to give 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - chloro - 1,3,5-triazine (B).

Preparation of 2-(3’-trifluromethylphenylamino)-4-(tetrahydro-1’, 4’-oxazine)-6-(4’-acetylphenylamino)-1,3,5-triazine (C)

Compound (B) (3.5 g, 0.01 mol) and 4-amino acetophenone (1.3 g, 0.01 mol) were dissolved in methanol mixed in 100 ml round- bottomed flask. Then the reaction mixture was heated under reflux temperature for 6 hours. During the reaction, sodium carbonate solution (0.5 g, 0.005 mol) was added to neutralize HCl evolved during the reaction. The progress of the reaction was monitored on TLC plate. After completion of the reaction, cool the mixture at room temperature and poured into crushed ice. Finally, the solid separated out was filtered, washed with water, dried and recrystallized from ethanol to give 2 - (3’ - trifluromethylphenylamino) - 4 - (tetrahydro - 1’, 4’ - oxazine) - 6 - (4’ - acetylphenylamino) - 1,3,5-triazine (C).

Preparation of 2-(3’-trifluromethylphenylamino)-4-(tetrahydro-1’, 4’-oxazine)-6-[4’-(3”-methoxyphenyl)-2”-proponen-1”-yl] phenylamino]-1,3,5-triazine (D₁)

Substituted acetophenone (C) (4.5 g, 0.01 mol) was dissolved in DMF and 3-methoxy benzaldehyde (1.3 g, 0.01 mol) was added to it in a 100 ml conical flask. Then the solution of 40% KOH (5 ml) was added in it to make alkaline. Then the reaction mixture was stirred for 24 hours on a magnetic stirrer at room temperature. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into crushed ice, neutralized with dilute hydrochloric acid and the mixture was agitated for 4 hours. The product was isolated by filtration and recrystallized from suitable solvent (ethanol) to give pure product. In the same way, the remaining compounds (D₂-D₅) were prepared by this given method.
Preparation of 2-(3′-trifluoromethylphenylamino)-4-(tetrahydro-1′, 4′-oxazine)-6-[4′-[1′-acetyl 5′-[3′′- methoxyphenyl]] 2′- pyrazolin 3′′-yl] phenylamino]-1,3,5-triazine (E1)

A 100 ml round bottomed flask, fitted with a reflux condenser, was charged with a mixture of an appropriate chalcone (D1) (5.7 g, 0.01 mol) dissolved in ethanol and hydrazine hydrate (0.75 g, 0.015 mol). To make the mixture acidic catalytic amount of glacial acetic acid (5 ml) was added. Then the reaction mixture was heated under reflux temperature for 5-6 hours. The progress of the reaction was monitored by TLC using toluene: methanol (12:6) eluent as mobile phase. After completion of the reaction, the mixture was cooled to room temperature then poured into crushed ice and neutralized with Na2CO3. The solid mass separated was collected by filtration, washed well with hot water and recrystallized from methanol to get product (E1) in good yield with high purity. Similarly, the remaining compounds (E2-E5) were prepared by this same method.

Preparation of 2-(3′-trifluoromethylphenylamino)-4-(tetrahydro-1′, 4′-oxazine)-6-[2′-amino-6′-[3′′-methoxyphenyl] pyrimidin-4′′-yl] phenylamino]-1,3,5-triazine (F1)

Compound (D1) (5.7 g, 0.01 mol) and guanidine hydrochloride (1.43 g, 0.015 mol) dissolved in ethanol was mixed in 100 ml round bottomed flask. To make this mixture alkaline 40% KOH (5 ml) was added to the reaction mixture and refluxed for 4-5 hours. The progress of the reaction was monitored by TLC using toluene: methanol (15:9 v/v) eluent as mobile phase. After completion of the reaction, the reaction mixture was poured into crushed ice and neutralized with dilute HCl. Finally, the product was filtered, washed with water, dried and recrystallized from methanol to afford the desired compound (F1) in good yield with high purity. In the same way, the remaining compounds (F2-F5) were prepared by this given method.

All the synthesized compounds (D1-D5), (E1-E5) and (F1-F5) were characterized by IR, 1H NMR, and 13C NMR, LCMS as well as elemental analysis. The characteristic data of the entire synthesized compounds are given in the spectral analysis data.

Reaction scheme

Methodical synthetic route for the target compounds (D1-D5), (E1-E5) and (F1-F5) is outlined in Scheme 1.

Spectral analysis data

2-(3′-Trifluoromethylphenylamino)-4-(tetrahydro-1′, 4′-oxazine)-6-[4′-[3′′-(3′′-methoxyphenyl)]-2′-proponen-1′′-yl] phenylamino]-1,3,5-triazine (D1): Yield 72%. Mp 128-130°C. IR spectrum, ν, cm⁻¹: 3321 (N-H), 3035 (ν=CH), 1640 (C=O), 1531 (C=C), 1487 (CH=CH), 1215 (C-O-C), 1065 (C-F), 802 (C=N), 745 (C-Cl), 696 (C-Chloro). 1H NMR spectrum (400 MHz, CDCl3), δ ppm (ν Hz): 3.4 (4H, t, CH2), 3.6 (4H, t, CH2), 6.8 (1H, d, J = 9.7, CO-CH3), 7.1 (1H, d, J = 9.8, Ar-CH=), 7.9 (12H, m, Ar-H), 8.1 (1H, s, NH). 13C NMR spectrum (400 MHz, CDCl3), δ ppm: 46.9 (CH2), 65.1 (CH3), 112.0 (CH), 113.2 (CH), 114.8 (CH), 116.6 (CH2), 117.2 (CH), 119.3 (=CH), 121.2 (CH), 123.7 (C=O), 124.5 (CF3), 126.7 (CH), 128.0 (C), 130.3 (CH), 131.5 (CH), 132.8 (CH), 143.2 (C), 144.3 (C), 145.8 (C), 164.9 (C=N), 168.1 (C=O), 171.5 (CO). Mass spectrum, m/z: 580 (M+H)+. Found, % C 59.91; H 4.18; N 14.48. C20H22F3O2Cl Calculated, % C 59.95; H 4.16; N 14.47.

2-(3′-Trifluoromethylphenylamino)-4-(tetrahydro-1′, 4′-oxazine)-6-[4′-[3′′-(2′′-chlorophenyl)-2′-proponen-1′′-yl] phenylamino]-1,3,5-triazine (D4): Yield 64%. Mp 136-138°C. IR spectrum, ν, cm⁻¹: 3321 (N-H), 3035 (ν=CH), 1640 (C=O), 1531 (C=C), 1487 (CH=CH), 1215 (C-O-C), 1065 (C-F), 804 (C=N), 756 & 656 (C-H). 1H NMR spectrum (400 MHz, CDCl3), δ ppm (ν Hz): 3.5 (4H, t, CH2), 3.6 (4H, t, CH3), 6.9 (1H, d, J = 9.3, CO-CH3), 7.2 - 7.8 (m, 11H, Ar-H), 8.1
Scheme 1: Synthetic route for the target compounds (D1-D3), (E1-E5) and (F1-F5)
(4H, concealed t, CH₂), 6.7 - 7.9 (12H, m, Ar-H), 8.1 (1H, s, NH). ¹³C NMR (400 MHz, CDCl₃), δ ppm: 31.2 (CH₃), 40.8 (CH₄), 44.5 (CH₂), 54.2 (3-OCH₃), 65.7 (CH-AR), 66.0 (CH₂), 110.5 (CH), 112.1 (CH), 113.8 (CH), 115.1 (CH), 117.5 (CH), 119.9 (CH), 121.5 (CH), 124.7 (CF₃), 126.0 (CH₂), 128.4 (CH), 133.4 (C), 134.2 (CH₂O), 142.9 (C), 144.8 (C), 147.0 (C), 150.3 (C), 152.4 (C-N), 164.5 (C-N), 165.2 (C-N), 167.0 (C-N), 169.2 (CO). Mass spectrum, m/z: 632.2 (M+H)+. Found, %: C 70.69; H 4.96; N 17.71.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-(1'-acetyl 5''-[3''-bromophenyl 2'-pyrazolin 3''-yl) phenylamino]-1,3,5-triazine (E₂): Yield 74%. Mp 158-160°C. IR spectrum, v, cm⁻¹: 3269 (NH), 3091 (=CH), 2935 (C-H), 1670 & 1570 (C-O & C=N), 1560 (-NH), 1499 (C=C), 1370 (CH₂), 1240 (C-O-C), 1073 (C-F), 808 (C=N), 690 (C-H), 590 (C-Br). ¹³C NMR spectrum (400 MHz, CDCl₃), δ ppm (J Hz): 1.9 (3H, s, COCH₃), 2.6 (1H, dd, J = 10.5 & 12.7, CH-CH), 3.2 (1H, dd, J = 10.5 & 12.7, CH-CH), 3.3 (4H, concealed t, CH₂), 3.6 (4H, concealed t, CH₂), 5.9 (1H, dd, J = 7.2 & 11.6, CH-CH₂-AR), 6.9 - 8.1 (12H, m, Ar-H), 8.3 (1H, s, NH). ¹³C NMR (400 MHz, CDCl₃), δ ppm: 25.6 (CH₃), 39.4 (CH₂), 42.3 (CH₃), 63.7 (CH-AR), 65.1 (CH₂), 111.7 (CH), 112.9 (CH), 114.0 (CH), 115.5 (CH), 116.2 (CH), 118.0 (CH), 120.3 (CH), 123.4 (C), 125.1 (C-F), 127.9 (CH₂), 129.1 (CH), 132.2 (C), 133.8 (CH), 136.5 (CH), 143.2 (C), 144.2 (C), 145.4 (C), 149.1 (C-N), 166.0 (C-N), 168.7 (C-N), 169.5 (C-N), 171.4 (CO). Mass spectrum, m/z: 681.4 (M+H)+. Found, %: C 78.55; H 5.38; N 10.05.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-(1'-acetyl 5''-[2''-chlorophenyl 2'-pyrazolin 3''-yl) phenylamino]-1,3,5-triazine (E₃): Yield 65%. Mp 136-138°C. IR spectrum, v, cm⁻¹: 3297 (-H), 3102 (C=O), 2889 (C-H), 1645 & 1590 (C=O & C=N), 1576 (NH), 1516 (C=C), 1356 (CH₂), 1256 (C-O-C), 1013 (C-F), 793 (C-N), 683 (C-Cl), 751 & 680 (C-H). ¹³C NMR spectrum (400 MHz, CDCl₃), δ ppm (J Hz): 2.9 (3H, s, COCH₃), 3.5 (4H, concealed t, CH₂), 3.6 (4H, concealed t, CH₂), 3.7 (1H, concealed dd, CH₂=CH₂), 4.6 (1H, concealed dd, CH₂=CH₂), 4.8 (1H, dd, J = 6.5 & 12.8, CH₂=CH₂-AR), 6.8 - 7.9 (12H, m, Ar-H), 8.0 (1H, s, NH). ¹³C NMR (400 MHz, CDCl₃), δ ppm: 22.4 (CH₂), 42.1 (CH₂), 43.8 (CH₂), 64.4 (CH-AR), 67.8 (CH₂), 109.2 (CH), 111.5 (CH), 113.2 (CH), 114.9 (CH), 115.1 (CH), 117.6 (CH), 119.4 (CH), 123.7 (CF₃), 125.0 (CH), 127.6 (CH), 130.4 (C), 131.9 (C), 132.3 (C), 134.0 (CH), 133.8 (C), 141.5 (C), 143.0 (C), 147.2 (C-N), 162.5 (C-N), 164.3 (C-N), 165.2 (C-N), 168.0 (CO). Mass spectrum, m/z: 636.9 (M+H)+. Found, %: C 58.49; H 4.41; N 17.57. C₃₃H₃₂F₃N₆O₆Cl: Calculated, %: C 58.45; H 4.43; N 17.59.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-(1'-acetyl 5''-[2''-furanyl 2'-pyrazolin 3''-yl) phenylamino]-1,3,5-triazine (E₄): Yield 72%. Mp 144-146°C. IR spectrum, v, cm⁻¹: 3307 (NH), 3064 (C=H), 2870 (C=H), 1651 & 1573 (C=O & C=N), 1569 (NH), 1559 (C=C), 1379 (CH₂), 1263 (C-O-C), 1103 (C-F), 799 (C=N), 748 & 699 (C-H). ¹³C NMR spectrum (400 MHz, CDCl₃), δ ppm (J Hz): 2.3 (3H, s, COCH₃), 2.8 (1H, dd, J = 10.8 & 12.9, CH=CH₂), 3.3 (4H, concealed t, CH₂), 3.5 (4H, concealed t, 4H, CH₃), 3.7 (1H, dd, J = 10.8 & 12.9, CH=CH₂), 5.2 (1H, dd, J = 8.3 & 12.8, CH=CH₂-AR), 7.1 - 8.2 (11H, m, Ar-H), 8.4 (1H, s, NH). ¹³C NMR (400 MHz, CDCl₃), δ ppm: 22.4 (CH₂), 42.1 (CH₂), 43.8 (CH₃), 64.4 (CH-AR), 67.8 (CH₂), 107.5 (CH), 109.4 (CH), 111.2 (CH), 113.5 (CH), 114.4 (CH), 116.0 (CH), 122.1 (CF₃), 126.7 (CH), 128.3 (CH), 132.8 (C), 133.4 (CH), 134.7 (CH), 140.4 (C), 142.9 (C), 152.5 (C), 153.7 (C-N), 164.3 (C-N), 165.1 (C-N), 167.0 (C-N), 172.2 (CO). Mass spectrum, m/z: 591.3 (M+H)+. Found, %: C 58.82; H 4.63; N 18.95. C₃₃H₃₂F₃N₆O₆Cl: Calculated, %: C 58.78; H 4.59; N 18.91.
Table 1: Antimicrobial activity data of the synthesized compounds (D1-D5), (E1-E3) and (F1-F5)

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<td>Chlo.</td>
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<tr>
<td>Cipr.</td>
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<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Gris.</td>
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<td>-</td>
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</tr>
<tr>
<td>Nyst.</td>
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</table>

Ampi: Ampicillin; Chlo: Chloramphenicol; Cipr: Ciprofloxacin; Gris: Gresofulvin; Nyst: Nystatin. *'-represent 'not tested'.

δ ppm: 47.6 (CH3), 64.5 (CH2), 99.7 (CH), 111.8 (CH), 112.0 (CH), 114.7 (CH), 116.9 (CH), 119.4 (CH), 122.0 (C), 123.7 (CF3), 125.4 (CH2), 127.4 (CH), 129.9 (CH), 131.1 (CH), 133.6 (C), 137.8 (C), 142.4 (C), 163.1, 163.5 & 165.6 (C), 167.1 (C=N), 168.2 (C=N), 170.7 (C=N). Mass spectrum, m/z: 633.7 (M+H)+. Found, %: C 54.25; H 3.75; N 18.96. C30H25N9F3OBr Calculated, %: C 54.23; H 3.79; N 18.97.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[2''-amino-6''-(2''-chlorophenyl) pyrimidin-4''-yl] phenylamin]-1,3,5- triazine (F1): Yield 70%. Mp 154-156°C. IR spectrum, ν, cm⁻¹: 3329 (NH2), 3056 (≡CH), 2961 (C-H), 1548 (C-NH), 1538 (C=C), 1671 (C=N), 1682 (C=N), 1707 (C=N). Mass spectrum, m/z: 633.7 (M+H)+. Found, %: C 54.25; H 3.75; N 18.96. C30H25N9F3OBr Calculated, %: C 54.23; H 3.79; N 18.97.

IR spectrum, ν, cm⁻¹: 3412 (NH2), 3089 (=CH), 2925 (C-H), 1640 (C=N), 1605 (≡NH), 1490 (C=C), 1323 (C-O-C), 1083 (C-F), 809 (C-N), 759 (C-H). 1H NMR spectrum (400 MHz, CDC13), δ ppm (J Hz): 4.2 (4H, concealed t, CH2), 4.4 (4H, concealed t, CH2), 5.6 (2H, s, NH2), 7.2 - 7.8 (11H, m, Ar-H), 8.0 (1H, s, NH). 13C NMR (400 MHz, CDCl3), δ ppm: 48.1 (CH2), 66.9 (CH2), 984 (CH), 1075 (CH), 1119.9 (C), 1125.8 (C), 1147.4 (CH), 116.9 (CH), 123.0 (CF3), 127.5 (CH), 129.3 (C), 132.2 (C), 134.9 (C), 136.1 (C), 139.5 (CH), 141.6 (C), 155.4 (C), 159.2, 162.6 & 164.0 (C), 165.2 (C=N), 167.9 (C=N), 169.2 (C=N). Mass spectrum, m/z: 576.2 (M+H)+. Found, %: C 58.42; H 4.15; N 21.87. Calculated, %: C 58.43; H 4.20; N 21.90.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[2''-amino-6''-(2''-N,N-dimethylaminophenylpyrimidin-4''-yl) phenylamin]-1,3,5-triazine (F1): Yield 70%. Mp 104-106°C. IR spectrum, ν, cm⁻¹: 3341 (NH2), 3049 (=CH), 2970 (C-H), 1652 (C-N), 1587 (NH), 1542 (C=C), 1361 (CH), 1250 (C-O-C), 1025 (C-F), 798 (C-N), 820 (C-H). 1H NMR spectrum (400 MHz, CDCl3), δ ppm (J Hz): 2.9 (3H, s, CH3), 3.8 (4H, concealed t, CH2), 4.0 (concealed t, 4H, -CH2O, oxazine ring), 4.5 (s, 2H-NH2), 6.9 to 8.1 (m, 13H, 12 Ar-H and 1-NH). 13C NMR (400 MHz, CDCl3), δ ppm: 42.5 (CH2), 45.3 (CH2), 65.4 (CH2), 102.3 (CH), 109.4 (CH), 110.8 (CH), 112.2 (CH), 114.7 (CH), 116.9 (CH), 124.3 (CF3), 126.7 (CH), 128.5 (CH), 131.2 (CH), 133.6 (CH), 135.0 (C), 137.2 (C), 141.5 (C), 156.3
(C), 164.6, 166.3 & 167.0 (C), 168.2 (C=N), 169.3 (C=N), 169.8 (C=N). Mass spectrum, m/z: 628.6 (M+H)+. Found, %: C 61.17; H 4.56; N 20.51. C_{22}H_{31}N_{10}F_{3}O Calculated, %: C 61.14; H 4.97; N 22.28.

**Methodology for in vitro antimicrobial screening (broth micro dilution method)**

Broth micro dilution method is one of the non-automated in vitro bacterial/fungal susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms which is carried out in tubes. The synthesized compounds were screened for antibacterial and antifungal activity against a panel of selected pathogens. DMSO was used as diluent to get desired concentration of drugs to test upon standard bacterial and fungal strains. The zone of inhibition produced by each compound was measured in μg/ml. Each synthesized compounds were diluted to 1000 μg/ml, 500 μg/ml and 250 μg/ml concentration for primary screen. The drugs found active in primary screening were similarly diluted to 200 μg/ml, 100 μg/ml, 50 μg/ml, and 25 μg/ml concentrations for secondary screen. The minimum inhibitory concentration (MIC) was determined and recorded at the lowest concentration inhibiting growth of the organism.

**Methodology for in vitro evaluation of antituberculosis activity (Lowenstein-Jensen method)**

The determination of antituberculosis activity of the synthesized compounds against *Mycobacterium tuberculosis* H37Rv were performed by Lowenstein-Jensen method with slight modification where 250 μg/ml dilution of each test compound were added liquid Lowenstein-Jensen Medium and then media were sterilized by inspissation method. A culture of *Mycobacterium tuberculosis* H37Rv growing on Lowenstein-Jensen medium was harvested in 0.85% saline in bijou bottles. All test compound make solution of 250 μg/ml concentration of compounds was prepared in DMSO. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 × 104 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which no development of colonies occurred or < 20 colonies was taken as MIC concentration of test compound.

**RESULTS AND DISCUSSION**

**Chemistry**

The synthetic route used to synthesize the unreported title compounds (D1-D3), (E1-E3) and (F1-F5) is illustrated in Scheme 1. The aim of the present study is to develop an efficient protocol with good to excellent yield in a short span of time. The formation of all these new heterocyclic derivatives were fully characterized by means of spectroscopic techniques such as FTIR, 1H NMR, 13C NMR and LC-MS that were in full agreement with their proposed structures. As an example, in the IR spectrum of compound D1, a strong absorption band is observed at 1431 and 1656 cm⁻¹ which corresponds to the stretching vibration of the CH = CH and C=O functionality of α, β unsaturated carbonyl group of chalcone moiety. The C-H bending vibrations for 1,3 disubstituted benzene ring, =CH and C=C functionality of aromatic ring were observed at 689, 1545 and 3026 cm⁻¹ respectively. The C=N stretching of 1,3,5-triazine core was observed at 806 cm⁻¹. The 1H NMR spectrum of compound D1 showed a doublet at δ 6.9 (J = 9.8 Hz) ppm for the -CO-CH= and at δ 8.1 (J = 9.5 Hz) ppm for the Ar-CH= of α, β unsaturated carbonyl group protons. The other remaining twelve aromatic protons appeared as a multiplet signal at δ 6.9-7.8 ppm. Finally, the 13C NMR spectra of the compound D1 was recorded in CDCl₃ and the spectral signals were in good agreement with the proposed structure. In the 13C NMR spectrum of compound D1, the most deshielded signal that appeared at δ 175.5 ppm was assigned to the carboxyl carbon of the chalcone moiety. The signal for CH = CH functionality of α, β- unsaturated carbonyl group was appeared at δ 122.0 and 143.5 ppm. The signals for aromatic carbons appeared between at δ 110.6-146.9 ppm in the 13C signal.

The IR spectrum of compound E1 exhibited the appearance of a strong absorption band at 1656 cm⁻¹ corresponding to the stretching vibration of the C=O functionality of acetyl group attached at N₁ position in pyrazoline ring. A broad stretching band for the C=N functionality of pyrazoline unit and C=C functionality of aromatic ring were observed at 1580 and 1508 cm⁻¹ respectively. The presence of the CH₃ group and C=N=H stretching of pyrazoline ring were appeared at 1364 and 2898 cm⁻¹ respectively. The aromatic C-H bending vibrations for 1,3 disubstituted benzene ring and C=N stretching of 1,3,5-triazine nucleus were observed at 682 and 801 cm⁻¹ respectively. The 1H NMR spectrum of compound E1 showed a singlet at δ 2.4 ppm for the COCH₃ proton. The pro-chiral methylene protons C₄=H of pyrazoline appeared as two distinct doublets of a doublet at δ 3.0 ppm (J = 11.3 and 13.2 Hz) and at δ 3.6 ppm (J = 11.3 and 13.4 Hz) for the CH=CH and CH=CH protons, thereby indicating that both the protons are magnetically non-equivalent and diastereotopic while the chiral C₅=CH proton of pyrazoline appeared as a doublet of a doublet at δ 5.5 ppm (J = 5.9 and 12.8 Hz) due to CH=CH₂Ar proton. The other remaining eleven aromatic protons appeared as a multiplet signal at δ 6.7-8.1 ppm. Finally, the 13C NMR spectra of the cyclized product E1 was recorded in CDCl₃ and the spectral signals were in good agreement with the proposed structures. In the 13C NMR spectrum of compound E1, the shielded signal at δ 31.2 and 40.8 ppm were assigned to the methyl and methylene carbon of pyrazoline ring. The most deshielded signal that appeared at
The signals for aromatic carbons appeared between at δ 110.6-158.7 ppm in the $^{13}$C spectrum. There are no absorptions in the region of 1600-1700 cm$^{-1}$ in IR spectra of compound F. The absence of a C=O group of chalcone moiety in these structures and further confirmation of cyclization of chalcone into a pyrimidine ring. The other remaining twelve aromatic protons resonate as a multiplet signal at δ 6.8-8.1 ppm. $^{13}$C NMR spectrum of compound F showed a signal at 101.3 ppm due to the -CH carbon of pyrimidine ring and signal at δ 163.1 and 164.7 ppm assigned to the C=N carbon of pyrimidine ring assigned the pyrimidine unit. The signals for aromatic carbons appeared between at δ 110.6-158.7 ppm in the $^{13}$C spectrum. There are no absorptions in the region of 1600-1700 cm$^{-1}$ in IR spectra of compound F. The obtained elemental analysis values are in good agreement with theoretical data. Further, mass spectra of all the title compounds showed molecular ion peak $M^+$ corresponding to their exact mass which is in agreement with its proposed structure.

**Antimicrobial activity**

All the synthesised compounds were evaluated for their antibacterial activity against two Gram-positive bacteria (Staphylococcus aureus MTCC 96 and Streptococcus pyogenes MTCC 442) and two Gram-negative bacteria (Escherichia coli MTCC 443 and Pseudomonas aeruginosa MTCC 441) by using ampicillin, chloramphenicol and ciprofloxacin as the standard antibacterial drugs. Antifungal activity was screened against three fungal species (Candida albicans MTCC 227, Aspergillus niger MTCC 282 and Aspergillus clavatus MTCC 1323) by using griseofulvin and nystatin as the standard antifungal drugs. The minimal inhibitory concentration (MIC) values of all the synthesised compounds were determined in terms of μg/ml by the Broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS, 2000). The results are summarised in Table 1. The antibacterial screening of chalcone (D1-D5), 1-acetyl pyrazoline (E1-E5) and 2-amino pyrimidine derivatives (F1-F5) pointed out that in Gram-positive bacteria, compounds E4 (MIC = 50 μg/ml), E2 (MIC = 62.5 μg/ml) showed an outstanding inhibitory effect against Staphylococcus aureus as compared to ampicillin (MIC = 250 μg/ml) and equipotent to chloramphenicol and ciprofloxacin (MIC = 50 μg/ml) while compound F2 (MIC = 100 μg/ml) and D1 (MIC = 125 μg/ml) also showed appreciable activity to ampicillin (MIC = 250 μg/ml) and modest to chloramphenicol and ciprofloxacin (MIC = 50 μg/ml) against Staphylococcus aureus organism. In the case of inhibiting Streptococcus pyogenes, compounds D4, D5 and F2 (MIC = 62.5 μg/ml) exhibited excellent inhibitory effect compare to ampicillin (MIC = 100 μg/ml) and equivalent to chloramphenicol and ciprofloxacin (MIC = 50 μg/ml) whereas compounds D2, D3, E1, E4 and F3 (MIC = 100 μg/ml), D1, E2, E3 and F4 (MIC = 125 μg/ml) exerted equally potential to ampicillin (MIC = 100 μg/ml) and less potential to chloramphenicol and ciprofloxacin (MIC = 50 μg/ml) against Streptococcus pyogenes.

In the case of inhibiting Gram-negative bacteria, compounds D2, E5 and F4 (MIC = 62.5 μg/ml) demonstrated excellent activity compared to ampicillin (MIC = 100 μg/ml) whereas compounds D3, E3 and F3 (MIC = 100 μg/ml) showed equipotential to ampicillin (MIC = 100 μg/ml) and less potential to chloramphenicol (MIC = 50 μg/ml) and ciprofloxacin (MIC = 25 μg/ml) against Escherichia coli. Compounds D1, D4, E1 (MIC = 50 μg/ml) exhibited an outstanding inhibitory effect against Pseudomonas aeruginosa as compared to ampicillin (MIC = 100 μg/ml) and comparable to chloramphenicol (MIC = 50 μg/ml) and modest ciprofloxacin (MIC = 25 μg/ml) whereas compounds D2, E2, E6, F3 and F4 (MIC = 100 μg/ml) exerted equipotent to ampicillin (MIC = 100 μg/ml) and mild to chloramphenicol (MIC = 50 μg/ml) and modest ciprofloxacin (MIC = 25 μg/ml) against Pseudomonas aeruginosa. Compounds D2, E5, F1 and F3 (MIC = 200 μg/ml) better to ampicillin (MIC = 250 μg/ml) whereas compounds D4, D5, E3, F2 (MIC = 250 μg/ml) comparable to ampicillin (MIC = 250 μg/ml) against Staphylococcus aureus. The remaining compounds showed moderate to good activity to inhibit the growth of bacterial pathogens and were found less effective than the employed standard drugs. The antibacterial results revealed that most of the prepared compounds showed improved activity against the Gram-negative bacteria rather than Gram-positive bacteria.

From in vitro antifungal activity data, it is found that compounds D2, E3, F2, F3 (MIC = 100 μg/ml) and E1, E2 (MIC = 200 μg/ml) displayed highest antifungal activity against Candida albicans as compared to griseofulvin (MIC = 500 μg/ml) and modest to nystatin (MIC = 100 μg/ml) while compounds D1, D3, D4, D5, E5, F1, F4, F5 (MIC = 500 μg/ml) showed the same potency as griseofulvin (MIC = 500 μg/ml) against Candida albicans. Compounds D4, D5, E4 and F3 (MIC = 100 μg/ml) depicted equipotent to griseofulvin (MIC = 100 μg/ml) and nystatin (MIC = 100 μg/ml) against Aspergillus niger. Compounds D1, D3, E5 and F5 (MIC = 100 μg/ml) found equipotent to griseofulvin (MIC = 100 μg/ml) and nystatin (MIC = 100 μg/ml) against Aspergillus clavatus.
Antitubercular activity

The encouraging results of the antimicrobial screening prompted us to screen the title compounds for their in vitro antitubercular activity. The in vitro antitubercular activity of all the newly synthesized compounds were determined by using Lowenstein-Jensen medium (conventional method) against Mycobacterial tuberculosis H37Rv strain (Rattan 2000). The observed results are presented in Table 2 in the form of inhibition (%), relative to that of standard antitubercular drugs isoniazid and rifampicin. Compounds demonstrating more than 90% inhibition in the primary screening were retested at lower concentration (MIC) in a Lowenstein-Jensen medium and evaluated for their MIC values. Among the compounds screened for antitubercular activity, compounds D$_5$ (MIC = 62.5 µg/ml) and E$_5$ (MIC = 62.5 µg/ml) were found to possess the greatest potency against Mycobacterium tuberculosis with 92 and 91% inhibition respectively (Table 3). Other derivatives showed moderate to poor antitubercular activity.

CONCLUSIONS

In conclusion, the present study reports the successful synthesis of a new series of 1-acetyl pyrazoline and 2-amino pyrimidine derivatives from chalcone bearing 1,3,5-triazine nucleus with the aim of discovering innovative structure leads serving as potent antimicrobial and antitubercular agents. The screening results revealed that all the compounds exhibited moderate to excellent activities against all the pathogenic strains. Upon varying the substitution on aryl ring appended to the chalcone, pyrazoline and pyridine ring, the activities changed drastically. Among the fifteen newly synthesised compounds, analogues D$_1$, D$_2$, D$_3$, E$_5$, E$_4$, E$_3$, F$_3$ and F$_1$ possessing electron withdrawing atom/group such as methoxy, chloro and nitro at the meta or para position were identified as the most potent antibacterial agents and compound E$_3$ and F$_3$ were found to be the most effective antifungal agent with relatively low cytotoxicity. The results described here merit further investigations in our laboratory using a forward chemical genetic approach in finding lead molecules as antimicrobial agents. Compounds D$_5$ and E$_5$ displayed excellent antituberculosis activity. Consequently, the compounds proved to be worthy for further modifications to obtain more efficacious antibacterial and antitubercular compounds.

ACKNOWLEDGEMENTS

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REFERENCES


Amir, M., Kumar, H., Khan, S.A., 2008. Synthesis and pharmacological evaluation of pyrazoline derivatives as

### Table 2: In vitro antitubercular activity (% inhibition) of the synthesized compounds (D$_1$-D$_5$), (E$_1$-E$_5$) and (F$_1$-F$_5$) at concentration 250 µg/mL

<table>
<thead>
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<th>Sample</th>
<th>Inhibition (%)</th>
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<tbody>
<tr>
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<tr>
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### Table 3: In vitro antitubercular activity of compounds exhibiting greater inhibition

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