Chemistry International 2(4) (2016) 189-200

International Scientific Organization
http://iscientific.org/
Chemistry International
www.bosaljournals.com/chemint/

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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A R T I C L E I N F O

Article type:
Research article
Article history:
Received October 2015
Accepted December 2015
October 2016 Issue
Keywords:
Chalcone
Acetyl pyrazoline
Amino pyrimidine
Antimicrobial activity
Antitubercular activity

A B S T R A C T

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 **(D_1-D_5)** 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 **(E_1-E_5)** and amino pyrimidine **(F_1-F_5)** derivatives respectively. The chemical structures of all newly synthesized compounds were established on the basis of their FTIR, ^{1}H NMR, ^{13}C 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* H_{37}Rv using Lowenstein-Jensen MIC method. Most of the compounds showed appreciable antimicrobial activity against the all tested strains. Among the synthesized compounds **D_1**, **D_2**, **D_3**, **E_1**, **E_3**, **E_4**, **F_3** and **F_4** exhibited excellent antimicrobial activity and said to be the most proficient members of the series. Compound **D_5** and **F_5** 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.

Cite This Article As: Anjani Solankee and Riki Tailor. 2016. An efficient synthesis of some new chalcone, acetyl pyrazoline and amino pyrimidine bearing 1,3,5-triazine nucleus as potential antimicrobial and antitubercular agent. Chemistry International 2(4) 189-200

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 (Mativandlela 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 acetophenone 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 Anuradha. 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 pyrmidine 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 Knovenagel. 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 (antipyretic), 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 hypnotic 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 diabetogenic 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-Fluorouracil (Blumenkranz et al., 1984), 5-Thiouracil (Al Safarjalani 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"-propenon-1"-yl} phenylamino]-1,3,5-triazine **(D_1-D_5)** with different intermidiates give subsequent conversion to products **(E_1-E_5)** and **(F_1-F_5)** 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. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using $CDCl_3$ 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 ^{o}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''-(3'''-methoxyphenyl)- 2''-propenon-1''-yl} phenylamino]-1,3,5-triazine (D_1)

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 temprature. 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 **(D_2-D_5)** were prepared by this given method.

Preparation of 2-(3'-trifluromethylphenylamino)-4-(tetrahydro- 1', 4'-oxazine)-6-[4'-{1''-acetyl 5''-(3'''-methoxyphenyl) 2''- pyrazolin 3''-yl} phenylamino]-1,3,5-triazine (E_1)

A 100 ml round bottomed flask, fitted with a reflux condenser, was charged with a mixture of an appropriate chalcone **(D_1)** (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 Na_2CO_3. The solid mass separated was collected by filtration, washed well with hot water and recrystallized from methanol to get product **(E_1)** in good yield with high purity. Similarly, the remaining compounds **(E_2-E_5)** were prepared by this same method.

Preparation of 2-(3'-trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[2''-amino-6''-(3'''-methoxyphenyl) pyrimidin-4''-yl} phenylamino]-1,3,5-triazine (F_1)

Compound **(D_1)** (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 **(F_1)** in good yield with high purity. In the same way, the remaining compounds **(F_2-F_5)** were prepared by this given method.

All the synthesized compounds **(D_1-D_5), (E_1-E_5)** and **(F_1-F_5)** were characterized by IR, ^{1}H NMR, and ^{13}C 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 **(D_1-D_5)**, **(E_1-E_5)** and **(F_1-F_5)** is outlined in Scheme 1.

SPECTRAL ANALYSIS DATA

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{3''-(3'''-methoxyphenyl)-2''-propenon-1''-yl} phenylamino]-1,3,5- triazine (D_1)

Yield 73%. Mp 122-124^{0}C. IR spectrum, v, cm^{-1}: 3336 (N-H), 3026 (=CH), 1656 (C=O), 1545 (C=C), 1431 (CH=CH), 1220 (C-O-C), 1045 (C-F), 806 (C=N), 689 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 3.6 (4H, t, C<u>H</u>$_2$), 3.7 (4H, t, C<u>H</u>$_2$), 3.8 (3H, s, 3-OC<u>H</u>$_3$), 6.7 (1H, d, *J* = 9.8, CO-C<u>H</u>=), 6.9 - 7.8 (12H, m, Ar-<u>H</u>), 8.1 (1H, d, *J* = 9.5, Ar-C<u>H</u>=), 8.2 (1H, s, N<u>H</u>). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 47.8 (CH_2), 53.6 (3-OCH_3), 65.3 (CH_2), 110.6 (CH), 112.5 (CH), 113.1 (CH), 115.8 (CH), 117.2 (CH), 122.0 (=CH), 122.8 (CH), 124.3 (CF_3), 125.2 (CH), 126.2 (C), 127.7 (CH), 131.1 (CH), 132.8 (CH), 135.3 (C), 141.3 (C), 142.3 (C), 143.5 (=CH), 146.9 (C), 158.3 (C), 164.5 (C=N), 166.5 (C=N), 169.3 (C=N), 175.5 (CO). Mass spectrum, m/z: 577 $(M+H)^+$. Found, %: C 62.52; H 4.74; N 14.55. $C_{30}H_{27}N_6F_3O_3$ Calculated, %: C 62.50; H 4.72; N 15.5.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{3''-(3'''-bromophenyl)-2''-propenon-1''-yl} phenylamino]-1,3,5- triazine (D_2)

Yield 76%. Mp 106-108^{0}C. IR spectrum, v, cm^{-1}: 3356 (N-H), 3090 (=CH), 1670 (C=O), 1523 (C=C), 1478 (CH=CH), 1245 (C-O-C), 1026 (C-F), 799 (C=N), 649 (C-H), 596 (C-Br). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 3.2 (4H, t, -C<u>H</u>$_2$), 3.4 (4H, t, -C<u>H</u>$_2$), 6.7 (1H, d, *J* = 9.6, CO-C<u>H</u>=), 7.9 (1H , d, *J* = 9.6, Ar-CH=), 7.2 - 8.0 (12H, m, Ar-<u>H</u>) 8.3 (1H, s, N<u>H</u>). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 46.8 (CH_2), 64.2 (CH_2), 111.1 (CH), 113.8 (CH), 114.2 (CH), 115.3 (CH), 117.8 (CH), 120.6 (=CH), 121.7 (CH), 122.4 (C), 124.9 (CF_3), 126.0 (CH), 127.8 (C), 129.1 (CH), 130.0 (CH), 133.4 (CH), 135.3 (C), 142.2 (C), 143.8 (C), 144.0 (=CH), 146.1 (C), 166.7 (C=N), 168.3 (C=N), 169.5 (C=N), 172.2 (CO). Mass spectrum, m/z: 625 $(M+H)^+$. Found, %: C 55.64; H 3.84; N 13.49. $C_{29}H_{24}N_6F_3O_2Br$ Calculated, %: C 55.59; H 3.86; N 13.44.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6- [4'-{3''-(2'''-chlorophenyl)-2''- propenon-1''-yl} phenylamino]-1,3,5- triazine (D_3)

Yield 72%. Mp 128-130^{0}C. IR spectrum, v, cm^{-1}: 3321 (N-H), 3035 (=CH), 1640 (C=O), 1531 (C=C), 1487 (CH=CH), 1215 (C-O-C), 1065 (C-F), 802 (C=N), 745 (C-H), 696 (C-Cl). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 3.4 (4H, t, C<u>H</u>$_2$), 3.6 (4H, t, -C<u>H</u>$_2$), 6.8 (1H, d, *J* = 9.7, CO-C<u>H</u>=), 7.8 (1H, d, *J* = 9.8, Ar-C<u>H</u>=), 6.8 - 7.9 (12H, m, Ar-<u>H</u>), 8.1 (1H, s, N<u>H</u>). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 46.9 (CH_2), 65.1 (CH_2), 112.0 (CH), 113.2 (CH), 114.8 (CH), 116.6 (CH), 117.2 (CH), 119.3 (=CH), 121.2 (CH), 123.7 (C), 124.5 (CF_3), 126.7 (CH), 128.0 (C), 130.3 (CH), 131.5 (CH), 132.8 (CH), 134.2 (C), 141.0 (C), 143.3 (C), 144.6 (=CH), 145.8 (C), 166.4 (C=N), 168.1 (C=N), 169.9 (C=N), 171.5 (CO). Mass spectrum, m/z: 580 $(M+H)^+$. Found, %: C 59.91; H 4.18; N 14.48. $C_{29}H_{24}N_6F_3O_2Cl$ Calculated, %: C 59.95; H 4.16; N 14.47.

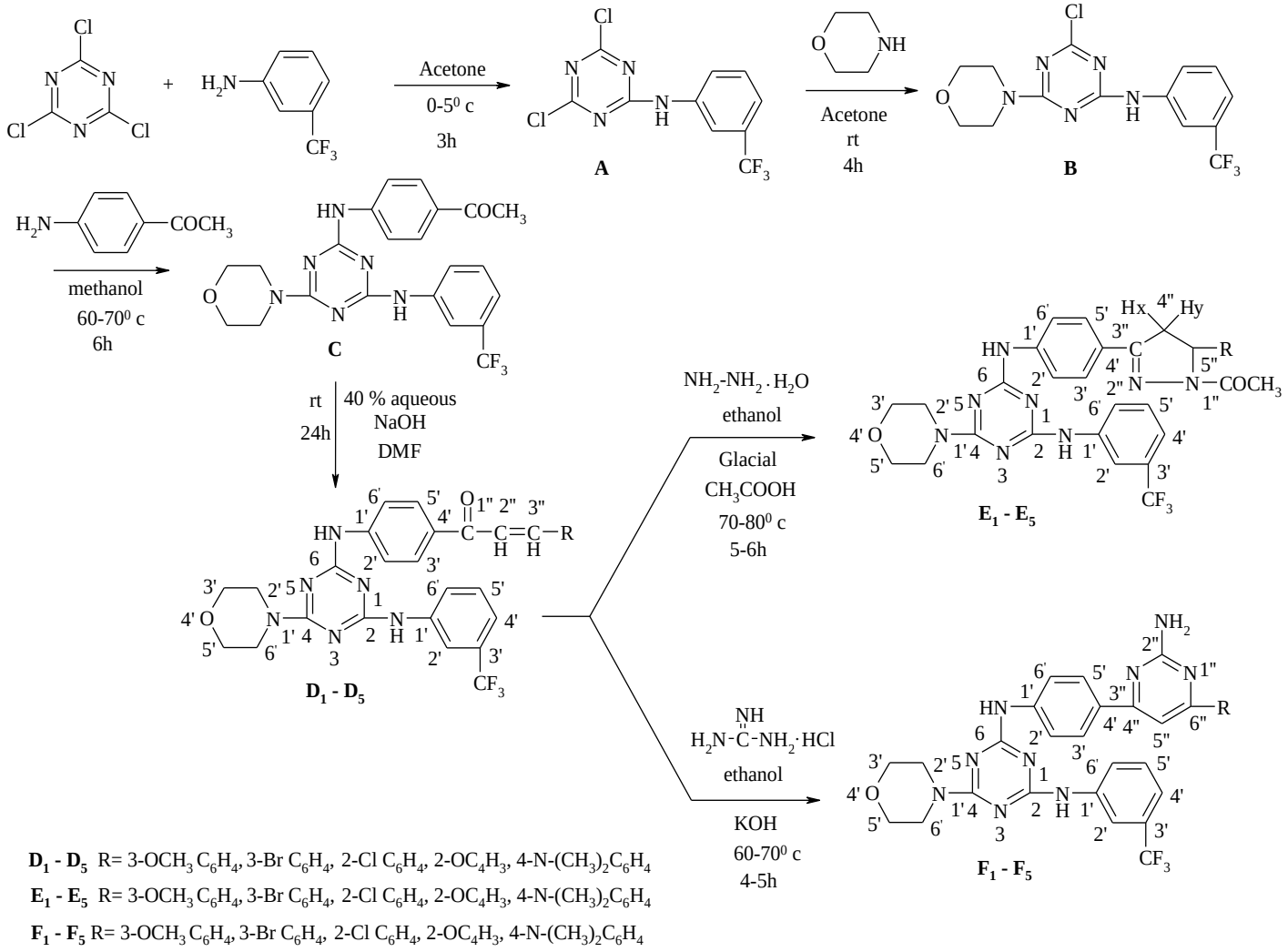
2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{3''-(2'''-furanyl)-2''-propenon-1''-yl} phenylamino]-1,3,5-triazine (D_4)

Yield 64%. Mp 136-138^{0}C. IR spectrum, v, cm^{-1}: 3321 (N-H), 3035 (=CH), 1640 (C=O), 1531 (C=C), 1487 (CH=CH), 1215 (C-O-C), 1036 (C-O-C), 1065 (C-F), 804 (C=N), 756 & 656 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 3.5 (4H, t, C<u>H</u>$_2$), 3.6 (4H, t, CH_2), 6.9 (1H, d, *J* = 9.3, CO-CH=), 7.2 - 7.8 (m, 11H, Ar-H), 8.1 (1H, d, *J* = 9.2, Ar-C<u>H</u>=), 8.4 (1H, s, N<u>H</u>). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 48.3 (CH_2), 68.6 (CH_2), 112.5 (CH), 113.7 (CH), 115.2 (CH), 116.7 (CH), 118.0 (=CH), 120.4 (CH), 122.2 (C), 123.7 (CF_3), 125.4 (CH), 127.2 (C), 129.2 (CH), 131.6 (CH), 132.4 (CH), 139.4 (C), 140.3 (CH), 143.9 (C), 145.3 (=CH), 146.7 (C), 151.5 (C), 164.3 (C=N), 167.3 (C=N), 168.2 (C=N), 172.6 (CO). Mass spectrum, m/z: 536 $(M+H)^+$. Found, %: C 60.40; H 4.33; N 15.70. $C_{27}H_{23}N_6F_3O_3$ Calculated, %: C 60.45; H 4.32; N 15.67.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6- [4'- {3''-(4'''-N, N-dimethylphenyl)-2''-propenon -1''- yl} phenylamino]-1,3,5- triazine (D_5)

Yield 68%. Mp 146-148^{0}C. IR spectrum, v, cm^{-1}: 3338 (N-H), 3041 (=CH), 1651 (C=O), 1547 (C=C), 1390 (CH_3), 1456 (CH=CH), 1228 (C-O-C), 1012 (C-F), 807 (C=N), 656 & 836 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 2.4 (3H, s, C<u>H</u>$_3$), 3.7 (4H, t, C<u>H</u>$_2$), 3.9 (4H, t, C<u>H</u>$_2$), 7.0 (1H, d, *J* = 10.2, CO-CH=), 7.8 (1H, d, *J* = 9.5, Ar-CH=), 6.8 - 8.0 (12H, m, Ar-<u>H</u>), 8.2 (1H, s, N<u>H</u>). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 41.3 (CH_3), 46.2 (CH_2), 62.4 (CH_2), 110.1 (CH), 112.3 (CH), 113.7 (CH), 114.5 (CH), 116.4 (=CH), 119.7 (CH), 122.4 (C), 125.7 (CF_3), 126.9 (CH), 128.6 (C), 130.1 (CH), 132.7 (CH), 133.4 (CH), 138.5 (C), 144.5 (C), 146.7 (=CH), 147.9 (C), 150.1 (C), 166.4 (C=N), 168.8 (C=N), 169.0 (C=N), 173.7 (CO). Mass spectrum, m/z: 589 $(M+H)^+$. Found, %: C 63.18; H 5.10; N 16.60%; $C_{31}H_{30}N_7F_3O_2$ Calculated, %: C 63.15; H 5.12; N 16.63.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{1''-acetyl 5''-(3'''- methoxyphenyl) 2''-pyrazolin 3''-yl} phenylamino]-1,3,5- triazine (E_1)



Scheme 1: Synthetic route for the target compounds **(D_1-D_5), (E_1-E_5)** and **(F_1-F_5)**

Yield 77%. Mp 111-113^{0}C. IR spectrum, v, cm^{-1}: 3360 (NH), 3009 (=CH), 2898 (C-H), 1663 & 1580 (C=O & C=N), 1542 (NH), 1508 (C=C), 1364 (CH_3), 1231 (C-O-C), 1032 (C-F), 801 (C=N), 682 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 2.4 (3H, s, COC$\underline{H}_3$), 3.0 (1H, dd, *J* = 11.3 & 13.2, C$\underline{H}^x$-CH), 3.6 (1H, dd, *J* = 11.3 & 13.4, C$\underline{H}^y$-CH), 5.5 (1H, dd, *J* = 5.9 & 12.8, C$\underline{H}$-CH_2-Ar), 3.9 (3H, s, 3-OC$\underline{H}_3$), 3.7 (4H, concealed t, C$\underline{H}_2$), 3.8 (4H, concealed t, C$\underline{H}_2$), 6.7 - 7.9 (12H, m, Ar-$\underline{H}$), 8.1 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 31.2 (CH_3), 40.8 (CH_2), 44.5 (CH_2), 54.2 (3-OCH_3), 65.7 (CH-Ar), 66.0 (CH_2), 110.5 (CH), 112.1 (CH), 113.8 (CH), 115.1 (CH), 117.5 (CH), 119.9 (CH), 121.5 (CH), 124.7 (CF_3), 126.0 (CH), 128.4 (CH), 133.4 (C), 134.2 (CH), 138.0 (CH), 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 60.79; H 4.96; N 17.69. $C_{32}H_{31}N_8F_3O_3$ Calculated, %: C 60.76; H 4.93; N 17.71.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{1''-acetyl 5''-(3'''- bromophenyl) 2''- pyrazolin 3''-yl} phenylamino]-1,3,5- triazine (E_2)

Yield 74%. Mp 158-160^{0}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 (CH_3), 1240 (C-O-C), 1073 (C-F), 808 (C=N), 690 (C-H), 590 (C-Br). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 1.9 (3H, s, COC$\underline{H}_3$), 2.6 (1H, dd, *J* = 10.5 & 12.7, C$\underline{H}^x$-CH), 3.2 (1H, dd, *J* = 10.5 & 12.7, C$\underline{H}^y$-CH), 3.3 (4H, concealed t, C$\underline{H}_2$), 3.6 (4H, concealed t, CH_2), 5.9 (1H, dd, *J* = 7.2 & 11.6, C$\underline{H}$-CH_2-Ar), 6.9 - 8.1 (12H, m, Ar-$\underline{H}$), 8.3 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 25.6 (CH_3), 39.4 (CH_2), 42.3 (CH_2), 63.7 (CH-Ar), 65.1 (CH_2), 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 (CF_3), 127.9 (CH), 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 54.61; H

4.11; N 16.49. $C_{31}H_{28}N_8F_3O_2Br$ Calculated, %: C 54.64; H 4.14; N 16.44.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{1''-acetyl 5''-(2'''- chlorophenyl) 2''-pyrazolin 3''-yl} phenylamino]-1,3,5-triazine (E_3)

Yield 65%. Mp 136-138^{0}C. IR spectrum, v, cm^{-1}: 3297 (-H), 3102 (=CH), 2889 (C-H), 1645 & 1590 (C=O & C=N), 1576 (NH), 1516 (C=C), 1356 (CH_3), 1256 (C-O-C), 1013 (C-F), 793 (C=N), 683 (C-Cl), 751 & 680 (C-H); ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 2.9 (3H, s, COC$\underline{H}_3$), 3.5 (4H, concealed t, CH_2), 3.6 (4H, concealed t, CH_2), 3.7 (1H, concealed dd, C$\underline{H}^x$-CH), 4.6 (1H, concealed dd, C$\underline{H}^y$-CH), 4.8 (1H, dd, *J* = 6.5 & 12.8, C$\underline{H}$-CH_2-Ar), 6.8 - 7.9 (12H, m, Ar-$\underline{H}$), 8.0 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 22.4 (CH_3), 42.1 (CH_2), 43.8 (CH_2), 64.4 (CH-Ar), 67.8 (CH_2), 109.2 (CH), 111.5 (CH), 113.2 (CH), 114.9 (CH), 115.1 (CH), 117.6 (CH), 119.4 (CH), 123.7 (CF_3), 125.0 (CH), 127.6 (CH), 130.4 (C), 131.9 (C), 132.3 (CH), 134.0 (CH), 138.6 (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. $C_{31}H_{28}N_8F_3O_2Cl$: Calculated, %: C 58.45; H 4.43; N 17.59.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{1''-acetyl 5''-(2'''- furanyl) 2''-pyrazolin 3''-yl} phenylamino]-1,3,5- triazine (E_4)

Yield 72%. Mp 144-146^{0}C. IR spectrum, v, cm^{-1}: 3307 (NH), 3064 (=CH), 2870 (C-H), 1651 & 1573 (C=O & C=N), 1569 (NH), 1559 (C=C), 1379 (CH_3), 1263 (C-O-C), 1103 (C-F), 799 (C=N), 748 & 699 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 2.3 (3H, s, COC$\underline{H}_3$), 2.8 (1H, dd, *J* = 10.8 & 12.9, C$\underline{H}^x$-CH), 3.3 (4H, concealed t, CH_2), 3.5 (4H, concealed t, 4H, CH_2), 3.7 (1H, dd, *J* = 10.8 & 12.9, C$\underline{H}^y$-CH), 5.2 (1H, dd, *J* = 8.3 & 12.8, C$\underline{H}$-CH_2-Ar), 7.1 - 8.2 (11H, m, Ar-$\underline{H}$), 8.4 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 22.4 (CH_3), 42.1 (CH_2), 43.8 (CH_2), 64.4 (CH-Ar), 67.8 (CH_2), 107.5 (CH), 109.4 (CH), 111.2 (CH), 113.5 (CH), 114.4 (CH), 116.0 (CH), 122.1 (CF_3), 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. $C_{29}H_{27}N_8F_3O_3$: Calculated, %: C 58.78; H 4.59; N 18.91.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[4'-{1''-acetyl 5''-(4'''-N, N-dimethylphenyl) 2''-pyrazolin 3''-yl} phenylamino]-1,3,5- triazine (E_5)

Yield 68%. Mp 154-156^{0}C; IR spectrum, v, cm^{-1}: 3368 (NH), 3106 (=CH), 2903 (C-H), 1640 & 1591 (C=O & C=N), 1545 (-NH), 1526 (C=C), 1380 (CH_3), 1219 (C-O-C), 1086 (C-F), 803 (C=N), 823 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 3.2 (3H, s, COC$\underline{H}_3$), 2.0 (3H, s, CH_3), 2.6 (1H, concealed dd, C$\underline{H}^x$-CH), 3.5 (1H, concealed dd, C$\underline{H}^y$-CH), 3.9 (4H, concealed t, CH_2), 4.1 (4H, concealed t, CH_2), 4.3 (1H, concealed dd, C$\underline{H}$-CH_2-Ar), 7.0 - 7.8 (12H, m, Ar-$\underline{H}$), 8.0 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 21.2 (CH_3), 40.3 (CH_3), 42.8 (CH_2), 44.5 (CH_2), 63.6 (CH-Ar), 65.1 (CH_2), 111.3 (CH), 112.8 (CH), 113.4 (CH), 115.1 (CH), 117.3 (CH), 118.2 (CH), 120.1 (CH), 124.7 (CF_3), 125.8 (CH), 126.0 (CH), 129.3 (C), 131.8 (CH), 133.2 (CH), 134.3 (C), 142.0 (C), 144.5 (C), 145.0 (C=N), 148.3 (C), 165.0 (C=N), 167.6 (C=N), 168.7 (C=N), 170.5 (CO). Mass spectrum, m/z: 646.1 (M+H)$^+$. Found, %: C 61.43; H 5.32; N 19.50. $C_{33}H_{34}N_9F_3O_2$ Calculated, %: C 61.39; H 5.30; N 19.52%.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[2''-amino-6''-(3'''-methoxyphenyl) pyrimidin- 4''-yl} phenylamino]-1,3,5-triazine (F_1)

Yield 70%. Mp 139-141^{0}C. IR spectrum, v, cm^{-1}: 3306 (NH_2), 3064 (=CH), 2968 (C-H), 1670 (C=N), 1580 (NH), 1509 (C=C), 1245 (C-O-C), 1070 (C-F), 800 (C=N), 675 & 842 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 3.6 (4H, concealed t, CH_2), 3.7 (concealed 4H, t, CH_2), 3.8 (3H, s, 3-OC$\underline{H}_3$), 5.1 (2H, s, NH_2), 6.8 - 8.2 (12H, m, Ar-$\underline{H}$), 8.5 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 46.5 (CH_2), 55.6 (3-OCH_3), 67.1 (CH_2), 101.3 (CH), 110.6 (CH), 112.4 (CH), 114.5 (CH), 117.3 (CH), 120.8 (CH), 124.0 (CF_3), 126.3 (CH), 127.8 (CH), 129.2 (CH), 130.4 (CH), 131.6 (C), 135.9 (C), 141.0 (C), 158.7 (C), 163.1, 164.7 & 166.8 (C), 165.4 (C=N), 167.5 (C=N), 169.1 (C=N). Mass spectrum, m/z: 615.8 (M+H)$^+$. Found, %: C 60.45; H 4.56; N 20.51. $C_{31}H_{28}N_9F_3O_2$: Calculated, %: C 60.48; H 4.58; N 20.48.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[2''-amino-6''-(3'''-bromophenyl) pyrimidin-4''-yl} phenylamino]-1,3,5- triazine (F_2)

Yield 72%. Mp 120-122^{0}C; IR spectrum, v, cm^{-1}: 3321 (NH_2), 3031 (=CH), 2945 (C-H), 1650 (C=N), 1568 (NH), 1545 (C=C), 1226 (C-O-C), 1045 (C-F), 805 (C=N), 696 (C-H), 578 (C-Br); ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 3.7 (4H, concealed t, CH_2), 3.9 (4H, concealed t, CH_2), 5.3 (2H, s, NH_2), 7.0 - 8.2 (13H, m, 12 Ar-$\underline{H}$), 8.4 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 47.6 (CH_2), 64.5 (CH_2), 99.7 (CH), 111.8 (CH), 112.0 (CH), 114.7 (CH), 116.9 (CH), 119.4 (CH), 122.0 (C), 123.7 (CF_3), 125.4 (CH), 127.4 (CH), 129.9 (CH), 131.1 (CH), 133.6 (C), 137.8 (C), 142.4 (C), 161.3, 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. $C_{30}H_{25}N_9F_3OBr$ Calculated, %: C 54.23; H 3.79; N 18.97.

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6- [2''-amino-6''-(2'''-chlorophenyl) pyrimidin-4''-yl} phenylamino]-1,3,5- triazine (F_3)

Yield 67%. Mp 154-156^{0}C. IR spectrum, v, cm^{-1}: 3329 (NH_2), 3056 (=CH), 2961 (C-H), 1648 (C=N), 1574 (NH), 1538 (C=C), 1220 (C-O-C), 1064 (C-F), 802 (C=N), 768 (C-H), 690 (C-Cl). ^{1}H NMR spectrum (400M Hz, $CDCl_3$), δ ppm (*J* Hz): 3.3 (4H, concealed t, CH_2), 3.5 (4H, concealed t, CH_2), 4.9 (2H, s, NH_2), 6.8 - 8.0 (12H, m, Ar-$\underline{H}$), 8.1 (1H, s, N$\underline{H}$). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 48.1 (CH_2), 66.9 (CH_2), 98.4 (CH), 110.5 (CH), 111.4 (CH), 113.8 (CH), 115.0 (CH), 117.6 (CH), 122.8 (CF_3), 126.9 (CH), 127.7 (CH), 130.1 (CH), 132.5 (CH), 133.7 (C), 134.2 (C), 136.3 (C), 140.0 (C), 163.6, 165.1 & 166.9 (C), 167.6 (C=N), 169.5 (C=N), 170.4 (C=N). Mass spectrum, m/z: 620.5 (M+H)$^+$. Found, %: C 58.15; H 4.04; N 20.30. $C_{30}H_{25}N_9F_3OCl$ Calculated, %: C 58.12; H 4.06; N 20.33.

Table 1: Antimicrobial activity data of the synthesized compounds (D_1-D_5), (E_1-E_5) and (F_1-F_5)

Sample	Minimum Inhibitory Concentration (µg/mL)						
	Gram-positive bacteria		Gram-negative bacteria		Fungi		
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. clavatus*
D_1	250	125	125	50	500	250	100
D_2	200	100	62.5	100	100	250	>1000
D_3	250	100	100	125	500	250	100
D_4	125	62.5	125	50	500	100	250
D_5	250	62.5	100	125	500	100	1000
E_1	62.5	100	125	50	200	>1000	1000
E_2	62.5	125	200	100	200	500	500
E_3	250	125	100	125	100	250	100
E_4	50	100	100	200	250	100	1000
E_5	200	250	62.5	100	500	>1000	100
F_1	200	250	250	250	500	500	500
F_2	250	62.5	125	200	100	500	500
F_3	200	100	100	100	100	100	200
F_4	100	125	62.5	100	500	250	>1000
F_5	250	200	62.5	200	500	200	100
Ampi.	250	100	100	100	-	-	-
Chlo.	50	50	50	50	-	-	-
Cipr.	50	50	25	25	-	-	-
Gris.	-	-	-	-	500	100	100
Nyst.	-	-	-	-	100	100	100

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

2-(3'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[2''-amino-6''-(2'''-furanyl) pyrimidin-4''-yl} phenylamino]-1,3,5- triazine (F_4)

Yield 70%. Mp 118-120^{0}C. IR spectrum, v, cm^{-1}: 3412 (NH_2), 3089 (=CH), 2925 (C-H), 1640 (C=N), 1605 (-NH), 1490 (C=C), 1232 (C-O-C), 1083 (C-F), 809 (C=N), 759 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 4.2 (4H, concealed t, CH_2), 4.4 (4H, concealed t, CH_2), 5.6 (2H, s, NH_2), 7.2 - 7.8 (11H, m, Ar-H), 8.0 (1H, s, NH). ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 48.1 (CH_2), 66.9 (CH_2), 98.4 (CH), 107.5 (CH), 111.9 (CH), 112.5 (CH), 114.7 (CH), 116.9 (CH), 123.0 (CF_3), 127.5 (CH), 129.3 (CH), 132.2 (CH), 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'-Trifluromethylphenylamino)-4-(tetrahydro-1', 4'-oxazine)-6-[2''-amino-6''-(4'''-N, N-dimethylphenyl) pyrimidin-4''-yl} phenylamino]-1,3,5-triazine (F_5)

Yield 70%. Mp 104-106^{0}C. IR spectrum, v, cm^{-1}: 3341 (NH_2), 3049 (=CH), 2970 (C-H), 1652 (C=N), 1587 (NH), 1542 (C=C), 1361 (CH_3), 1250 (C-O-C), 1025 (C-F), 798 (C=N), 820 (C-H). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ ppm (*J* Hz): 2.9 (3H, s, CH_3), 3.8 (4H, concealed t, CH_2), 4.0 (concealed t, 4H, -CH_2, oxazine ring), 4.5 (s, 2H,-NH_2), 6.9 to 8.1 (m, 13H, 12 Ar-H and 1-NH); ^{13}C NMR (400 MHz, $CDCl_3$), δ ppm: 42.5 (CH_3), 45.3 (CH_2), 65.4 (CH_2), 102.3 (CH), 109.4 (CH), 110.8 (CH), 112.2 (CH), 114.7 (CH), 116.9 (CH), 124.3 (CF_3), 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. $C_{32}H_{31}N_{10}F_3O$ 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 inspissations 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 **(D_1-D_5)**, **(E_1-E_5)** and **(F_1-F_5)** 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, ^{1}H NMR, ^{13}C NMR and LC-MS that were in full agreement with their proposed structures. As an example, in the IR spectrum of compound **D_1**, 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 ^{1}H NMR spectrum of compound **D_1** 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 ^{13}C NMR spectra of the compound **D_1** was recorded in $CDCl_3$ and the spectral signals were in good agreement with the proposed structure. In the ^{13}C NMR spectrum of compound **D_1**, the most deshielded signal that appeared at δ 175.5 ppm was assigned to the carbonyl 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 ^{13}C spectrum.

The IR spectrum of compound **E_1** 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 N_1 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 CH_3 group and C_4"-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 ^{1}H NMR spectrum of compound **E_1** showed a singlet at δ 2.4 ppm for the $COCH_3$ proton. The pro-chiral methylene protons C_4"-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 CHx-CH and CHy-CH protons, thereby indicating that both the protons are magnetically non-equivalent and diastereotopic while the chiral C_5"-H proton of pyrazoline appeared as a doublets of a doublet at δ 5.5 ppm (J = 5.9 and 12.8 Hz) due to CH-CH_2-Ar proton. The other remaining eleven aromatic protons appeared as a multiplet signal at δ 6.7-8.1 ppm. Finally, the ^{13}C NMR spectra of the cyclized product **E_1** was recorded in $CDCl_3$ and the spectral signals were in good agreement with the proposed structures. In the ^{13}C NMR spectrum of compound **E_1**, 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 δ 169.2 ppm was assigned to the carbonyl carbon of the acetyl group attached with the pyrazoline unit. The signals for aromatic carbons appeared between at δ 110.5-150.3 ppm in the ^{13}C spectrum.

The IR spectrum of compound F_1 showed a strong characteristic band at 1650 cm^{-1} and 3306 cm^{-1} due to the C=N and NH_2 group of pyrimidine ring. The C_5"-H stretching of pyrimidine ring was observed at 2968 cm^{-1}. The aromatic C=C stretching, C-H bending vibrations for 1,3 disubstituted benzene ring were appeared at 1509 and 675 cm^{-1} respectively. The C=N stretching of 1,3,5-triazine nucleus was observed at 800 cm^{-1}. The ^{1}H NMR spectrum of compound **F_1** showed a sharp singlet at δ 5.1 ppm due to the NH_2 protons and it also showed a sharp singlet at δ 7.5 ppm due to HC=C which confirmed the cyclization of the 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_1** showed a signal at 101.3 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 which 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_1** which indicating the absence of a C=O group of chalcone moiety in these structures and further confirmed the cyclization of chalcone in to amino pyrimidine. Moreover, distinctive singlet around at δ 3.7-3.9 ppm stands for methoxy group of aryl ring attached to chalcone, pyrazoline and pyrimidine unit, singlet around at δ 8.0-8.5 ppm stands for secondary amine attached with 1,3,5-triazine which confirmed the presence of 1,3,5-triazine nucleus. 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.

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

Sample	Inhibition (%)
D_1	81
D_2	58
D_3	46
D_4	32
D_5	92
E_1	50
E_2	32
E_3	65
E_4	87
E_5	91
F_1	56
F_2	85
F_3	62
F_4	50
F_5	88
Isoniazid	99
Rifampicin	98

Table 3: *In vitro* antitubercular activity of compounds exhibiting greater inhibition

Sample	Inhibition (%)	MIC (µg/mL)
D_5	92	62.5
E_5	91	62.5
Isoniazid	99	0.2
Rifampicin	98	40

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 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 **(D_1-D_5)**, 1-acetyl pyrazoline **(E_1-E_5)** and 2-amino pyrimidine derivatives **(F_1-F_5)** pointed out that in Gram-positive bacteria, compounds E_4 (MIC = 50 µg/ml), E_1 and E_2 (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 F_4 (MIC = 100 µg/ml) and D_4 (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 D_4, D_5 and F_2 (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 D_2, D_3, E_1, E_4 and F_3 (MIC = 100 µg/ml), D_1, E_2, E_3 and F_4 (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 D_2, E_5, F_4 and F_5 (MIC = 62.5 µg/ml) demonstrated excellent activity compared to ampicillin (MIC = 100 µg/ml) while compounds D_3, D_5, E_3, E_4 and F_3 (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 D_1, D_4, E_1 (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 D_2, E_2, E_5, F_3 and F_4 (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 D_2, E_5, F_1 and F_3 (MIC = 200 µg/ml) better to ampicillin (MIC = 250 µg/ml) while compounds D_3, D_5, E_3, F_2 (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 D_2, E_3, F_2, F_3 (MIC = 100 µg/ml) and E_1, E_2 (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 D_1, D_3, D_4, D_5, E_5, F_1, F_4, F_5 (MIC = 500 µg/mL) showed the same potency as griseofulvin (MIC = 500 µg/ml) against *Candida albicans*. Compounds D_4, D_5, E_4 and F_3 (MIC = 100 µg/ml) depicted equipotent to griseofulvin (MIC = 100 µg/ml) and nystatin (MIC = 100 µg/ml) against *Aspergillus niger*. Compounds D_1, D_3, E_3, E_5 and F_5 (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_1**, **E_3**, **E_4**, **F_3** and **F_4** 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 antitubercular activity. Consequently, the compounds proved to be worthy for further modifications to obtain more efficacious antibacterial and antitubercular compounds.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal, B. K. M. Science College, Valsad for providing research amenities, Atul Ltd. (Atul) for the FTIR analysis, RSIC Punjab University for the ^{1}H NMR, ^{13}C NMR, mass spectral analysis as well as elemental analysis and Microcare Laboratory, Surat, for antitubercular and antimicrobial activity.

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