In vitro antimycobacterial and antimicrobial activity of some new pyrazoline, isoxazole and benzodiazepine derivatives containing 1,3,5-triazine nucleus via chalcone series

Anjani Solankee*, Riki Tailor & Kishor Kapadia

Department of Chemistry, B. K. M. Science College, Valsad 396 001, India
E-mail: dranjani_solankee@yahoo.com

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In the present study, three new combinatorial libraries of substituted phenyl pyrazoline 5a-e, isoxazole 6a-e and 1,5-benzodiazepine 7a-e derivatives have been synthesised via the reaction of chalcone 4a-e with phenylhydrazine hydrochloride, hydroxylamine hydrochloride and o-phenylenediamine respectively. The structures of all the newly synthesised compounds have been assigned on the basis of FTIR, 1H and 13C NMR, LC-MS, and elemental analysis. The title compounds have been screened for their preliminary in vitro antimicrobial activity against a panel of pathogenic strains S. aureus, S. pyogenus, E. coli, P. aeruginosa, C. albicans, A. niger and A. calavatus. Most of the compounds show appreciable antimicrobial activity against the tested strains. Compounds 8b, 8c, 9b, 9d, and 9f are considered as the best desired bioactive antimicrobial analogues of the series. In vitro antimycobacterial activity for all the synthesised compounds has been carried out against *Mycobacterium tuberculosis* H37 Rv. Compounds 5a, 6d and 7b display promising antimycobacterial activity.

Keywords: Phenyl pyrazoline, isoxazole, benzodiazepine, antimycobacterial activity, antimicrobial activity

The increasing cases of infectious diseases are influencing the world through their morbidity and mortality to the scientific community and have become a threat for human life worldwide. Tuberculosis is one of the major infectious diseases caused by *Mycobacterium tuberculosis*1,2. Tuberculosis is still the single largest infection having a high mortality rate and 0.1 to 0.3 percent of the population become infected every year in the developed countries. Especially, the rising prevalence of multidrug-resistant tuberculosis has significantly contributed to the increased difficulties in the control of tuberculosis. Often, tuberculosis is accompanied by AIDS and exists as multidrug resistant tuberculosis (MDR-TB) or as extensively drug resistant tuberculosis (XDR-TB), which generally affects the lungs, where neither standard antitubercular drugs nor any of the alternate treatments are effective3. So, the increasing rate of TB cases by MDR and XDR strains and the high rate of a co-infection with HIV have pointed out the urgent need to develop new antimycobacterial drugs which will effectively kill MDR and XDR strains, are less toxic, shortened duration of therapy and rapid mycobactericidal mechanism of action in the intracellular environment. Hence, the search for new and potent antitubercular agent is gaining interest.

1,3,5-Triazine nucleus is one of the most fascinating chemical core structures and subjected to broad study in recent years due to various biological activities, which have made it an indispensable anchor for the development of new therapeutic agents4,5. Chalcones are compounds of chalconoid group chemically known as 1,3-diarylprop-2-en-1-one and key precursors in the synthesis of a large array of biologically important heterocycles6-8. It is therefore, not surprising that many synthetic methods have been developed for the preparation of large number of heterocycles starting from chalcone precursors. Pyrazoline and isoxazole belong to the family of azoles i.e. pyrazoline constitutes a unique class of two nitrogen containing five membered heterocycle while isoxazole constitute a nitrogen and oxygen containing five membered heterocycle. During the past years considerable evidence has accumulated to demonstrate the importance of pyrazoline and isoxazole derivatives because of C=N, N−O, N−N and other polar functional groups presence in the ring. They are associated with a wide spectrum of biological activities such as anti-tumour9, apoptotic10, anti-inflammatory11, insect-antifeedent12, antiviral13, antimicrobial14, antiamoebic15, antitubercular16, etc. Benzodiazepines are an important class of nitrogen
containing seven membered heterocyclic compounds which find various applications in the field of synthetic as well as medicinal chemistry. Among all types of benzodiazepines (1,2-, 1,3-, 1,4-, 1,5-, 2,3- and 2,4-) only 1,4- and 1,5-benodiazepines have found a greater number of applications17. Along with these, they are also reported to have wide range of therapeutic activities such as anthelmintic18, anti-inflammatory19, anticancer20, antimicrobial21, antitubercular22, antiproliferative23, etc. Furthermore, benzodiazepines are valuable intermediates for the synthesis of fused ring compounds such as triazolo, oxadiazolo, oxazino and furanobenzodiazepines24-26. In view of the above mentioned facts and in continuation of our interest in the synthesis of nitrogen-containing heterocycles27,28 herein we report the synthesis, characterisation, antimycobacterial and antimicrobial screening of some new phenyl pyrazoline, isoxazole and 1,5-benzodiazepine derivatives containing 1,3,5-triazine core.

Results and Discussion
Chemistry
The synthetic route used to synthesise the unreported title compounds 5a-e, 6a-e and 7a-e is illustrated in Scheme I. The aim of the present study is to develop an efficient protocol with good to excellent yield in a short span of time. All these new heterocyclic derivatives were fully characterised by means of spectroscopic techniques such as FTIR, 1H and 13C NMR, LC-MS as well as elemental analysis. As an example, in the IR spectrum of compound 5a, a broad stretching band for the C=N functionality of pyrazoline unit and the C_d"-H stretching of pyrazoline ring were observed at 1610 cm\(^{-1}\) and 2965 cm\(^{-1}\) respectively. The aromatic C-H bending vibrations for 1,3-disubstituted benzene ring and C=C functionality of aromatic ring is observed at 686 and 1521 cm\(^{-1}\) respectively. The C=N stretching of 1,3,5-triazine core was observed at 802 cm\(^{-1}\). In the 1H NMR spectrum of compound 5a, the pro-chiral methylene protons C_d"-H of pyrazoline appeared as two distinct doublet of a doublet at \(\delta \) 2.0 (\(J = 11.5 \) and 13.0 Hz) and at \(\delta \) 3.1 (\(J = 11.5 \) and 13.2 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_d"-H proton of pyrazoline appeared as a doublet of a doublet at \(\delta \) 5.2 (\(J = 4.6 \) and 11.8 Hz) due to CH-CH2-Ar proton. The other remaining seventeen aromatic protons

![Scheme I — Synthetic route for the target compounds 5a-e, 6a-e and 7a-e](image-url)
appeared as a multiplet signal at δ 6.9-8.0. Finally, the $^{13}$C NMR spectrum of the cyclised product 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 5a, the shielded signal at δ 37.5 was assigned to the methylene carbon of pyrazoline ring. The most deshielded signal that appeared at δ 149.7 was assigned to the C=N carbon of the pyrazoline unit. The signals for aromatic carbons appeared between δ 111.3 and 147.9 in the $^{13}$C spectrum. The IR spectrum of compound 6a exhibited the disappearance of absorption at 1660 cm$^{-1}$ corresponding to >C=O group of chalcone and a strong band at 1595 cm$^{-1}$ for 1,3-disubstituted benzene ring. In the C=O groups of chalcone moiety in these structure and further confirmed the cyclisation of chalcone into its derivative. In addition, distinctive singlet was observed around at δ 3.7-4.0 for methoxy group of aryl ring attached to pyrazoline, isoxazole and diazepine unit and singlet around δ 8.1-8.4 stands for secondary amine attached with 1,3,5-triazine. The obtained elemental analysis values are in good agreement with theoretical data.

**Antimicrobial activity**

The antimicrobial activity of newly synthesised compounds 5a-e, 6a-e and 7a-e was carried out by micro broth dilution method$^{29}$ according to National Committee for Clinical Laboratory Standards (NCCLS, 2002). The synthesised compounds were screened for antibacterial activity against a panel of selected pathogens Gram positive (S. aureus MTCC 96 and S. pyogenus MTCC 442), Gram negative (E. coli MTCC 443 and P. aeruginosa MTCC 441) bacterial species and for antifungal activity, a panel of selected pathogens of fungal (C. albicans MTCC 227, A. niger MTCC 282 and A. calavatus MTCC 1323) species were used. Ampicillin, Chloramphenicol and Ciprofloxacin were used as standard antibiotic drugs for antibacterial activity while Greseofulvin and Nystatin were used as standard drug for antifungal activity. 2% DMSO solution 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 synthesised 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. The results are summarised in Table I.

Upon reviewing antimicrobial data (Table I), it has been observed that in Gram positive bacterial strains, compounds 6b and 6e exerted an outstanding inhibitory effect i.e. MIC = 50 µg/mL and 62.5 µg/mL respectively against Staphylococcus aureus compared to Ampicillin (MIC = 250 µg/mL) and similar inhibitory effect to Chloramphenicol and Ciprofloxacin (MIC = 50 µg/mL) whereas compounds 5b and 7d (MIC = 125 µg/mL), 5e and 7e (MIC = 200 µg/mL) were found to
possesses significant activity compared to Ampicillin (MIC = 250 µg/mL) and compounds 5a, 5c, 5d, 6a, 6c, 6d, 7a, 7b and 7c (MIC = 250 µg/mL) showed the same potency compared to Ampicillin (MIC = 250 µg/mL) against Staphylococcus aureus. Against Streptococcus pyogenes, compounds 5b, 5e and 6d (MIC = 62.5 µg/mL) exerted the highest activity compared to Ampicillin (MIC = 100 µg/mL) and lowest to Chloramphenicol and Ciprofloxacin (MIC = 50 µg/mL) while compounds 5a, 6c, 7b and 7d (MIC = 100 µg/mL) showed comparable inhibitory effect compared to Ampicillin (MIC = 100 µg/mL) against the same organism Streptococcus pyogenes. In the case of inhibiting Gram negative bacteria, compound 7a (MIC = 50 µg/mL) and 7b (MIC = 100 µg/mL) exhibited an outstanding inhibitory effect whereas compounds 6b and 7e (MIC = 100 µg/mL) possesses the same potency compared to Ampicillin (MIC = 100 µg/mL) against Escherichia coli. Against Pseudomonas aeruginosa, compounds 5a, and 6e (MIC = 50 µg/mL), 5d and 7c (MIC = 62.5 µg/mL) exhibited excellent activity as compared to Ampicillin (MIC = 100 µg/mL). The remaining compounds showed moderate to good activity that inhibit the growth of bacterial pathogens and were found less effective than the employed standard drugs. The antibacterial activity result revealed that most of the prepared compounds showed improved activity against the Gram-positive bacteria rather than Gram-negative bacteria.

The antifungal screening data (Table I) revealed that compounds 6e (MIC = 100 µg/mL) and 5b (MIC = 200 µg/mL) displayed an excellent inhibitory effect compared to Greseofulvin (MIC = 500 µg/mL) against Candida albicans. Majority of compounds 5a, 5d, 5e, 6a, 6b, 6c, 7a, 7b, 7c and 7e (MIC = 500 µg/mL) exerted equipotent to Greseofulvin (MIC = 500 µg/mL) against Candida albicans. Against Aspergillus niger, compounds 5d, 6b, 6c and 7e (MIC = 100 µg/mL) were found to possess similar activity to Greseofulvin and Nystatin (MIC = 100 µg/mL). Compounds 5b, 5c, 6d and 7d (MIC = 100 µg/mL) showed equipotent activity compared to Greseofulvin and Nystatin (MIC = 100 µg/mL) against Aspergillus clavatus.

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A: Ampicillin, B: Chloramphenicol, C: Ciprofloxacin, D: Greseofulvin, E: Nystatin.
Antimycobacterial activity

The encouraging results of the antimicrobial screening prompted us to screen the title compounds for their in vitro antimycobacterial activity. The in vitro antimycobacterial activity of all the newly synthesised compounds was determined by using Lowenstein-Jensen medium (conventional method) against Mycobacterium tuberculosis H37Rv strain as described by Rattan. The observed results are presented in Table II 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. The results are summarised in Table III. Among the compounds screened for antimycobacterial activity, compounds 5a (MIC = 50 µg/mL), 6d (MIC = 62.5 µg/mL) and 7b (MIC = 62.5 µg/mL) were found to possess the greatest potency against Mycobacterium tuberculosis with 91, 91 and 91% inhibition respectively (Table III). Other derivatives showed moderate to poor antimycobacterial activity. Compounds 5a, 6d and 7b showed comparable activity to the standard drug. Moreover, other derivatives showed moderate to poor antimycobacterial activity.

Experimental Section

All the chemicals and solvents used for the synthesis work acquired from commercial sources were of analytical reagent (AR) grade. Melting points were determined by using open capillary tubes and are uncorrected. TLC was checked on E-Merck pre-coated 60 F254 plates and the spots were rendered visible by exposing to UV light or keeping the plates in iodine chamber. IR spectra were recorded on a Shimadzu FTIR 8401 spectrophotometer using KBr pellets. 1H and 13C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using CDCl3 as a solvent and TMS as an internal standard at 400 and 100 MHz respectively. Chemical shifts are reported in parts per million (ppm) and coupling constant (J) are reported in Hertz. The following abbreviations have been used to explain the observed multiplicities: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan). Elemental analyses were carried out by Perkin-Elmer 2400 series-II elemental analyser (Perkin-Elmer, USA). Reference drugs for antimycobacterial activity were Isoniazid and Rifampicin and for antimicrobial activity Ampicillin, Chloramphenicol, Ciprofloxacin, Griseofulvin and Nystatin of commercial grade were used.

General procedure for the compounds 1, 2, 3 and 4a-e

The starting precursors 1, 2 and 3 were achieved according to literature procedure. The synthesis of the chalcones 4a-e were accomplished according to the Claisen-Schmidt condensation of substituted ketone 3 with various aromatic/ heterocyclic aldehydes under conventional protocol by the reported method affording 74-84% yield.

Preparation of 2-(3’-trifluoromethylphenylamino) -4-(tetrahydro- 1’,4’-oxazine)-6-[4’-(1’’-phenyl-5’’- (3’’-methoxyphenyl)-2’’-pyrazolin-3’’-yl]phenylamino] -1,3,5- triazine, 5a

A 100 mL round bottomed flask, fitted with a reflux condenser was charged with a mixture of an
appropriate chalcone 4a (0.01 mol, 6.0 g in 30 mL ethanol) and phenylhydrazine hydrochloride (0.01 mol, 1.4 g in 10 mL ethanol) in ethanol. The reaction proceeded with the addition of 5 mL KOH (40%) as basic solvent medium. The reaction mixture was then refluxed for 6-8 h. The progress of the reaction was monitored by using TLC. After completion of the reaction, the reaction mixture was poured into crushed ice and neutralised with dilute HCl and allowed to settle. The solid that separated out was collected by filtration, washed with hot water and purified by recrystallization from methanol to get product 5a in good yield with high purity. Similarly, the remaining compounds 6b-e were synthesised by this method.

Preparation of 2-(3'-trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-(3''-methoxyphenyl)-2''-isoxazol-3''-yl]phenylnamino]-1,3,5-triazine, 6a

Compound 4a (0.01 mol, 6.0 g in 30 mL ethanol) was condensed with hydroxylamine hydrochloride (0.01 mol, 0.6 g in 5 mL ethanol) in the presence of alkaline medium (5 mL 40% KOH) in ethanol at reflux temperature for 5-6 h in 100 mL round bottomed flask. The progress of the reaction was monitored by TLC using toluene:methanol (12:8 v/v) eluent as mobile phase. After completion of the reaction, the reaction mixture was poured into crushed ice and neutralised with dilute HCl. Finally, the product was filtered, washed with water, dried and recrystallised from methanol to get 6a in good yield with high purity. In the same way, the remaining compounds 6b-e were synthesised by the same method.

Preparation of 2-(3'-trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-(3''-methoxyphenyl)-3''-H-benzo-1'',5''-diazepin-2''-yl]phenylnamino]-1,3,5-triazine, 7a

Compound 4a (0.01 mol, 5.7 g in 30 mL ethanol) was condensed with o-phenylenediamine (0.01 mol, 1.0 g in 5 mL ethanol) in 100 mL round bottomed flask. To make the mixture acidic, 5 mL glacial acetic acid was added and the mixture refluxed for 5-6 h. The progress of the reaction was monitored by TLC using toluene:methanol (12:9 v/v) eluent as mobile phase. After completion of the reaction, the reaction mixture was poured into crushed ice and neutralised with Na$_2$CO$_3$. Finally, the product was filtered, washed with water, dried and recrystallised from methanol to get product 7a in good yield with high purity. Likewise, the remaining compounds 7b-e were synthesised by the same method.

All the synthesised compounds 5a-e, 6a-e and 7a-e were characterised by IR, $^1$H and $^{13}$C NMR as well as elemental analysis. The characterization data of all the synthesised compounds are given in the spectral analysis data.

Spectral and analytical analysis data

Spectral and analytical data of all the synthesised compounds 5a-e, 6a-e and 7a-e are given.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-(1''-phenyl-5''-(3'''-methoxyphenyl)-2''-pyrazolin-3''-yl]phenylnamino]-1,3,5-triazine, 5a:

Yield 75%. m.p.119°C. IR (KBr): 3298 (-NH str.), 2965 (C-H str., pyrazoline), 1610 (C=N str., aromatic), 1247 (asymmetric C-O-C str., ether linkage), 1135 (OCH$_3$ str.), 1072 (C-F str.), 802 (C-N str., 1,3,5-triazine), 686 cm$^{-1}$ (C-H bend., 1,3 disubstituted benzene ring); $^1$H NMR (400 MHz, CDCl$_3$): δ 2.0 (dd, J = 11.5 and 13.0 Hz, 1H, -CH-F-CH), 3.1 (dd, J = 11.5 and 13.2 Hz, 1H, -CH-F-CH), 3.8 (s, 3H, 3-OCH$_3$), 3.6 (concealed t, 4H, -CH$_2$, oxazine ring), 3.7 (concealed t, 4H, -CH$_2$, oxazine ring), 5.2 (dd, J = 4.6 and 11.8 Hz, 1H, -CH-CH$_2$-Ar), 6.9-8.2 (m, 18H, 17 Ar-CH$_2$-Ar and 1-CH$_2$-Ar); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 37.5 (CH$_2$ methylene, pyrazoline), 45.4 (CH$_2$ oxazine), 54.1 (3-OCH$_3$), 62.6 (CH-Ar), 65.2 (CH$_2$ oxazine), 111.3 (CH), 113.4 (CH), 115.2 (CH), 116.3 (CH), 117.5 (CH), 121.1 (CH), 122.0 (CH), 124.6 (CF$_3$), 126.4 (CH), 128.7 (CH), 132.9 (C), 134.5 (CH), 135.1 (CH), 136.7 (CH), 140.2 (C), 141.6 (C), 144.3 (C), 145.5 (C), 147.9 (C), 197.7 (C=N, pyrazoline), 161.2, 163.4 and 165.8 (C=N, 1,3,5-triazine); LC-MS: m/z 666.1 (M$^+$). Anal. Calcd for C$_{36}$H$_{33}$N$_8$F$_3$O$_2$: C, 64.86; H, 4.98; N, 16.81. Found: C, 64.85; H, 4.95; N, 16.77%.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-(1''-phenyl-5''-(3'''-bromophenyl)-2''-pyrazolin-3''-yl]phenylnamino]-1,3,5-triazine, 5b:

Yield 79%. m.p.131°C. IR (KBr): 3290 (-NH str.), 2973 (C-H str., pyrazoline), 1618 (C=N str., aromatic), 1231 (asymmetric C-O-C str., ether linkage), 1083 (C-F str.), 800 (C-N str., 1,3,5-triazine), 640 cm$^{-1}$ (C-Br str.); $^1$H NMR (400 MHz, CDCl$_3$): δ 2.5 (dd, J = 10.8 and 13.6 Hz, 1H, -CH-F-CH), 3.2 (dd, J = 11.1 and 13.6 Hz, 1H, -CH-F-CH), 3.4 (concealed t, 4H, -CH$_2$, oxazine ring), 3.6 (concealed t, 4H, -CH$_2$, oxazine ring), 4.8 (dd, J = 5.3 and 12.4 Hz, 1H, -CH-CH$_2$-Ar), 7.0-8.1...
(m, 18H, 17 Ar- H and 1-NH); 13C NMR (100 MHz, CDCl3): δ 36.4 (CH2 methylene, pyrazoline), 42.1 (CH2 oxazine), 63.8 (CH-Ar), 68.3 (CH2 oxazine), 112.4 (CH), 113.5 (CH), 115.9 (CH), 116.2 (CH), 118.4 (CH), 120.1 (CH), 122.6 (CH), 123.4 (C-Br), 124.9 (CF3), 126.3 (CH), 127.1 (CH), 129.4 (C), 131.5 (CH), 132.7 (CH), 135.0 (CH), 141.3 (C), 142.8 (C), 144.3 (C), 146.7 (C), 151.2 (C=N, pyrazoline), 163.8, 166.2 and 168.3 (C=N, 1,3,5-triazine); LC-MS: m/z 714.3 (M+). Anal. Caled for C35H30N4F3OBr: C, 58.75; H, 4.22; N, 15.66. Found: C, 58.74; H, 4.25; N, 15.70%.

2-(3′-Trifluoromethylphenylamino)-4-(tetrahydroisoquinolin-3″'-yl)phenylamino-1,3,5-triazine, 5c: Yield 67%. m.p.100°C. IR (KBr): 3312 (–NH str.), 2951 (C-H str., pyrazoline), 1624 (C=N str., pyrazoline), 1507 (C=C str., aromatic), 1234 (asymmetric C-O-C str., ether linkage), 1061 (C-F str.), 799 (C=N str., 1,3,5-triazine), 686 and 751 (C-H bend., and 1,2 disubstituted benzene ring), 696 cm⁻¹ (C-Cl str.);

1H NMR (400 MHz, CDCl3): δ 2.8 (concealed dd, 1H, –CH2–CH), 3.7 (concealed dd, 1H, –CH–CH2), 3.4 (concealed t, 4H, –CH2 oxazine ring), 3.5 (concealed t, 4H, –CH2 oxazine ring), 5.6 (dd, J = 5.7 and 12.6 Hz, 1H, –CH–CH2–Ar), 7.0-8.1 (m, 18H, 17 Ar-H and 1-NH); 13C NMR (100 MHz, CDCl3): δ 37.1 (CH2 methylene, pyrazoline), 45.3 (CH2 oxazine), 61.4 (CH-Ar), 65.4 (CH2 oxazine), 110.2 (CH), 112.7 (CH), 114.3 (CH), 115.6 (CH), 116.0 (CH), 118.3 (CH), 121.2 (CH), 123.5 (CF3), 125.8 (CH), 128.1 (CH), 129.4 (C), 130.1 (CH), 132.9 (C-Cl), 134.6 (CH), 136.2 (CH), 139.0 (C), 141.5 (C), 143.2 (C), 145.6 (C), 150.2 (C=N, pyrazoline), 160.4, 165.2 and 166.1 (C=N, 1,3,5-triazine); LC-MS: m/z 709.9 (M+). Anal. Caled for C35H30N4F3OCl: C, 62.64; H, 4.50; N, 16.70. Found: C, 62.67; H, 4.47; N, 16.68%.

2-(3′-Trifluoromethylphenylamino)-4-(tetrahydroisoquinolin-3″'-yl)phenylamino)-1,3,5-triazine, 5d: Yield 71%. m.p.129°C. IR (KBr): 3310 (–NH str.), 2941 (C-H str., pyrazoline), 1632 (C=N str., pyrazoline), 1520 (C=C str., aromatic), 1220 (asymmetric C-O-C str., ether linkage), 1082 (C-F str.), 803 (C=N str., 1,3,5-triazine), 670 cm⁻¹ (C-H bend., 1,3 disubstituted benzene ring); 1H NMR (400 MHz, CDCl3): δ 3.4 (concealed t, 4H, –CH2 oxazine ring), 3.6 (concealed t, 4H,–CH2 oxazine ring), 3.8 (dd, J = 10.4 and 11.7 Hz, 1H, –CHF–CH), 4.1 (dd, J = 10.6 and 11.9 Hz, 1H, –CHF–CH), 5.0 (dd, J = 4.9 and 11.8 Hz, 1H, –CH–CH2–Ar), 7.0-8.0 (m, 17H, 16 Ar-H and 1-NH); 13C NMR (100 MHz, CDCl3): δ 40.2 (CH2 methylene, pyrazoline), 48.3 (CH2 oxazine), 64.5 (CH-Ar), 69.1 (CH2 oxazine), 109.4 (CH), 110.2 (CH), 111.2 (CH), 114.5 (CH), 115.0 (CH), 117.1 (CH), 118.3 (CH), 120.2 (CH), 123.5 (CF3), 126.2 (CH), 128.4 (CH), 130.9 (C), 131.8 (CH), 133.5 (CH), 140.5 (C), 143.2 (C), 145.3 (C), 146.7 (C), 149.6 (C=N, pyrazoline), 151.2 (C), 165.0, 167.3 and 168.7 (C=N, 1,3,5-triazine); LC-MS: m/z 626.8 (M+). Anal. Caled for C33H29N4F3O2: C, 63.25; H, 4.66; N, 17.88. Found: C, 63.28; H, 4.63; N, 17.89%.

2-(3′-Trifluoromethylphenylamino)-4-(tetrahydroisoquinolin-3″'-yl)phenylamino)-1,3,5-triazine, 5e: Yield 76%. m.p.165°C. IR (KBr): 3329 (–NH str.), 2982 (C-H str., pyrazoline), 1626 (C=N str., pyrazoline), 1531 (C=C str., aromatic), 1393 (CH2 str.), 1227 (asymmetric C-O-C str., ether linkage), 1091 (C-F str.), 805 (C=N str., 1,3,5-triazine), 670 and 830 cm⁻¹ (C-H bend., and 1,3 and 1,4 disubstituted benzene ring); 1H NMR (400 MHz, CDCl3): δ 2.6 (s, 3H, –CH3), 3.6 (concealed t, 4H, –CH2 oxazine ring), 3.9 (concealed t, 4H, –CH2 oxazine ring), 4.0 (concealed dd, 1H, –CHF–CH), 4.3 (concealed dd, 1H, –CHF–CH), 4.8 (concealed dd, 1H, –CH–CH2–Ar), 6.7-7.9 (m, 18H, 17 Ar-H and 1-NH); 13C NMR (100 MHz, CDCl3): δ 29.4 (CH3), 36.2 (CH2 methylene, pyrazoline), 46.4 (CH2 oxazine), 63.9 (CH-Ar), 68.0 (CH2 oxazine), 112.4 (CH), 113.2 (CH), 115.5 (CH), 116.7 (CH), 118.9 (CH), 119.0 (CH), 121.4 (CH), 124.0 (CF3), 126.3 (CH), 127.1 (CH), 128.9 (CH), 130.4 (CH), 133.2 (CH), 136.4 (CH), 138.1 (C), 140.5 (C), 142.3 (C), 144.7 (C), 148.5 (C=N, pyrazoline), 153.3 (C), 161.6, 163.7 and 165.3 (C=N, 1,3,5-triazine); LC-MS: m/z 709.9 (M+). Anal. Caled for C33H29N4F3O2: C, 65.38; H, 5.33; N, 18.55. Found: C, 65.35; H, 5.30; N, 18.51%.

2-(3′-Trifluoromethylphenylamino)-4-(tetrahydroisoquinolin-3″'-yl)phenylamino)-1,3,5-triazine, 5f: Yield 79%. m.p.140°C. IR (KBr): 3304 (–NH str.), 2914 (C-H str., isoxazole), 1598 (C=N str., isoxazole), 1504 (C=C str., aromatic), 1254 (asymmetric C-O-C str., ether linkage), 1161 (OCH3 str.), 1069 (C-F str.), 800 (C=N str., 1,3,5-triazine), 679 cm⁻¹ (C-H bend., 1,3 disubstituted benzene ring); 1H NMR (400 MHz, CDCl3): δ 3.7 (concealed t, 4H,–CH2 oxazine ring),
2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-{5''-(2''-isoxazol-3''-yl)phenylamino]-1,3,5-triazine, 6b: Yield 73%. m.p.90°C. IR (KBr): 3328 (-NH str.), 2932 (C-H str., aromatic), 1226 (asymmetric C-O-C str., ether linkage), 1035 (C-F str.), 801 (C=N str., 1,3,5-triazine), 682 (C-H bend., 1,3 disubstituted benzene ring); 1H NMR (400 MHz, CDCl3): δ 3.9 (concealed t, 4H, -CH2, oxazine ring), 3.4 (concealed t, 4H, -CH2, oxazine ring), 6.3 (1H, s, -CH=), 6.9- 8.1 (m, 13H, 12 Ar-H and 1-NH); 13C NMR (100 MHz, CDCl3): δ 46.8 (CH2 oxazine), 65.2 (CH2 oxazine), 101.2 (CH, isoxazole), 112.8 (CH), 114.7 (CH), 116.5 (CH), 117.6 (C), 118.0 (C), 122.3 (C-Br), 111.0 (CH), 114.7 (CH), 116.5 (CH), 117.6 (C), 118.0 (C), 122.3 (C-Br), 123.8 (CF3), 125.4 (CH), 127.0 (CH), 129.6 (CH), 131.4 (C), 134.1 (C), 140.3 (C), 161.6 (C=N, isoxazole), 162.4 (C-Br), 164.6, 166.2 and 167.0 (C=N, 1,3,5-triazine); LC-MS: m/z 599.3 (M⁺). Anal. Calcd for C31H27N3F3O3: C, 61.02; H, 4.41; N, 16.66%.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-{5''-(2''-furanyl)-2''-isoxazol-3''-yl)phenylamino]-1,3,5-triazine, 6d: Yield 68%. m.p.113°C. IR (KBr): 3319 (-NH str.), 2940 (C-H str., isoxazole), 1633 (C=N str., isoxazole), 1514 (C=C str., aromatic), 1238 (asymmetric C-O-C str., ether linkage), 1098 (C-F str.), 799 (C=N str., 1,3,5-triazine), 691 cm⁻¹ (C-H bend., 1,3 disubstituted benzene ring); 1H NMR (400 MHz, CDCl3): δ 3.9 (concealed t, 4H, -CH2, oxazine ring), 3.4 (concealed t, 4H, -CH2, oxazine ring), 6.6 (1H, s, -CH=), 6.9-7.9 (m, 12H, 11 Ar-H and 1-NH); 13C NMR (100 MHz, CDCl3): δ 40.2 (CH2 oxazine), 62.5 (CH2 oxazine), 102.1 (CH, isoxazole), 108.3 (CH), 112.0 (CH), 113.7 (CH), 115.6 (CH), 118.0 (C), 119.8 (C), 125.4 (CF3), 127.6 (CH), 130.2 (CH), 131.4 (C), 133.9 (C), 142.0 (C), 154.3 (C), 162.3 (C=N, isoxazole), 163.9 (C-Ar), 164.1, 165.8 and 167.3 (C=N, 1,3,5-triazine); LC-MS: m/z 549.3 (M⁺). Anal. Calcd for C29H20N3F3O3: C, 59.02; H, 4.03; N, 17.84. Found: C, 59.01; H, 4.02; N, 17.83%.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-{5''-(4''-N,N-dimethylphenyl)-2''-isoxazol-3''-yl)phenylamino]-1,3,5-triazine, 6e: Yield 72%. m.p.107°C. IR (KBr): 3382 (-NH str.), 2927 (C-H str., isoxazole), 1637 (C-N str., aromatic), 1364 (C-C str., aromatic), 1075 (C-F str.), 802 (C=N str., 1,3,5-triazine), 689 and 825 cm⁻¹ (C-H bend., 1,3 and 1,4 disubstituted benzene ring); 1H NMR (400 MHz, CDCl3): δ 2.6 (s, 3H, -CH3), 3.1 (concealed t, 4H, -CH2, oxazine ring), 3.3 (concealed t, 4H, -CH2, oxazine ring), 6.0 (1H, s, -CH=), 6.9-8.4 (m, 13H, 12 Ar-H and 1-NH); 13C NMR (100 MHz, CDCl3): δ 39.5 (CH3), 44.2 (CH2 oxazine), 66.4 (CH2 oxazine), 100.4 (CH, isoxazole), 111.0 (CH), 113.2 (CH), 115.4 (CH), 116.8 (C), 119.5 (C), 124.6 (CF3), 127.8 (CH), 129.0 (CH), 130.2 (CH), 131.3 (C), 134.7 (C), 140.2 (C), 151.9 (C), 161.8 (C=N, isoxazole), 162.4 (C-Ar), 163.0, 165.7 and 167.5 (C=N, 1,3,5-triazine); LC-MS: m/z 601.6 (M⁺). Anal. Calcd for C31H26N3F3O3: C, 61.79; H, 4.85; N, 18.60. Found: C, 61.78; H, 4.81; N, 18.57%.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-{5''-(3''-methoxyphenyl)-2''-isoxazol-3''-yl)phenylamino]-1,3,5-triazine, 7a: Yield 75%. m.p.115°C. IR (KBr): 3382
(-NH str.), 1595 (C=N str., benzodiazepine), 1506 (C=C str., aromatic), 1246 (asymmetric C-O-C str., ether linkage), 1155 (OCH₃ str.), 1023 (C-F str.), 659 (C-H bend., 1.3 substituted benzene ring), 800 cm⁻¹ (C=N str., 1,3,5-triazine); ¹H NMR (400 MHz, CDCl₃): δ 2.3 (dd, J = 6.3 and 13.5 Hz, 1H, CH₂, diazepine), 2.6 (dd, J = 6.3 and 13.9 Hz, 1H, CH₂, diazepine), 3.4 (concealed t, 4H, -CH₂, oxazine ring), 3.6 (concealed t, 4H, -CH₂, oxazine ring), 3.8 (s, 3H, 3-OCH₃), 6.7-8.2 (m, 17H, 16 Ar-H and 1-NH); ¹³C NMR (100 MHz, CDCl₃): δ 37.4 (CH₂, methylene, diazepine), 46.8 (CH₂ oxazine), 56.8 (3-OCH₃), 66.7 (CH₂ oxazine), 110.2 (CH), 113.7 (CH), 115.1 (CH), 118.4 (CH), 119.8 (CH), 121.5 (CH), 124.0 (CF₂), 127.8 (C), 128.3 (CH), 131.1 (CH), 132.8 (C), 133.1 (CH), 135.7 (CH), 138.3 (C), 141.0 (C), 143.6 (C), 147.2 (C), 153.1 (C=N, diazepine), 158.4 (C), 166.4, 169.1 and 171.4 (C=N, 1,3,5-triazine). LC-MS: m/z 664.7 (M⁺). Anal. Calcd for C₃₅H₃₃N₇F₃O₂: C, 65.05; H, 4.70; N, 16.86. Found: C, 65.09; H, 4.75; N, 16.89%.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-(3''-bromophenyl)-3''H-benzo-1',5''-diazepin-2''-yl]phenylamino]-1,3,5-triazine, 7b: Yield 71%. m.p.98°C. IR (KBr): 3378 (-NH str.), 1645 (C≡N str., benzodiazepine), 1506 (C=C str., aromatic), 1228 (asymmetric C-O-C str., ether linkage), 1009 (C-F str.), 664 (C-H bend., 1.3 substituted benzene ring), 810 (C≡N str., 1,3,5-triazine), 588 cm⁻¹ (C-Br str.): ¹H NMR (100 MHz, CDCl₃): δ 2.4 (dd, J = 6.8 and 13.4 Hz, 1H, CH₂, diazepine), 2.1 (dd, J = 6.4 and 13.4 Hz, 1H, CH₂, diazepine), 3.5 (concealed t, 4H, -CH₂, oxazine ring), 3.6 (concealed t, 4H, -CH₂, oxazine ring), 7.0-8.1 (m, 17H, 16 Ar-H and 1-NH); ¹³C NMR (100 MHz, CDCl₃): δ 37.6 (CH₂, methylene, diazepine), 46.4 (CH₂ oxazine), 64.2 (CH₂ oxazine), 111.8 (CH), 112.5 (CH), 114.2 (CH), 116.0 (CH), 118.6 (CH), 121.6 (C-Br), 123.7 (CH), 124.5 (CF₂), 126.4 (C), 129.0 (CH), 131.8 (CH), 132.5 (C), 133.9 (CH), 135.0 (CH), 137.2 (C), 142.3 (C), 144.0 (C), 146.9 (C), 152.7 (C≡N, diazepine), 163.1, 165.7 and 169.8 (C=N, 1,3,5-triazine). LC-MS: m/z 712.3 (M⁺). Anal. Calcd for C₃₅H₃₃N₇F₃O₂Br: C, 58.92; H, 4.95; N, 15.70. Found: C, 58.88; H, 4.98; N, 15.67%.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-(3''-furanyl)-3''H-benzo-1',5''-diazepin-2''-yl]phenylamino]-1,3,5-triazine, 7d: Yield 75%. m.p.95°C. IR (KBr): 3361 (-NH str.), 1642 (C≡N str., benzodiazepine), 1513 (C=C str., aromatic), 1237 (asymmetric C-O-C str., ether linkage), 1064 (C-F str.), 683 (C-H bend., 1.3 substituted benzene ring), 807 cm⁻¹ (C≡N str., 1,3,5-triazine); ¹H NMR (400 MHz, CDCl₃): δ 2.9 (dd, J = 7.2 and 12.8 Hz, 1H, -CH₂, diazepine), 2.7 (dd, J = 7.2 and 12.6 Hz, 1H, CH₂, diazepine), 4.1 (concealed t, 4H, -CH₂, oxazine ring), 4.2 (concealed t, 4H, -CH₂, oxazine ring), 6.9-8.1 (m, 16H, 15 Ar-H and 1-NH); ¹³C NMR (100 MHz, CDCl₃): δ 38.3 (CH₂, methylene, diazepine), 48.3 (CH₂ oxazine), 65.5 (CH₂ oxazine), 109.4 (CH), 111.6 (CH), 113.0 (CH), 114.2 (CH), 116.3 (CH), 118.0 (CH), 120.3 (CH), 124.8 (CF₂), 126.9 (C), 128.1 (CH), 130.0 (CH), 131.3 (C), 133.7 (CH), 135.6 (CH), 137.0 (C), 140.1 (CH), 144.7 (C), 146.2 (C), 152.4 (C≡N, diazepine), 163.1, 165.7 and 167.3 (C=N, 1,3,5-triazine). LC-MS: m/z 625.3 (M⁺). Anal. Calcd for C₃₅H₃₃N₇F₃O₂S: C, 63.46; H, 4.35; N, 17.94. Found: C, 63.41; H, 4.31; N, 17.98%.

2-(3'-Trifluoromethylphenylamino)-4-(tetrahydro-1',4'-oxazine)-6-[4'-(3''-N,N-dimethylphenyl)-3''H-benzo-1',5''-diazepin-2''-yl]phenylamino]-1,3,5-triazine, 7e: Yield 58%. m.p.104°C. IR (KBr): 3350 (-NH str.), 1638 (C≡N str., benzodiazepine), 1529 (C=C str., aromatic), 1389 (CH₂ str.), 1232 (asymmetric
C-O-C str., ether linkage), 807 cm\(^{-1}\) (C=C str., benzene ring), 807 cm\(^{-1}\) (C=N str., 1,3,5-triazine); \(^{1}\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.9 (s, 3H, -CH\(_3\)), 2.4 (concealed dd, 1H, -CH\(_2\)-, diazepine), 2.6 (concealed dd, 1H, CH-F, oxazine ring), 3.3 (concealed t, 4H, -CH\(_2\)-, oxazine ring), 6.7-8.2 (m, 17H, 16 Ar-H and 1-NH); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 31.4 (CH\(_3\)), 38.0 (CH\(_2\)-, oxazine), 46.1 (CH\(_2\)-, oxazine), 65.4 (CH\(_2\)-, oxazine), 110.5 (CH), 112.7 (CH), 114.3 (CH), 116.9 (CH), 118.3 (CH), 121.2 (CH), 123.5 (CF\(_3\)), 125.1 (C), 127.0 (CH), 130.8 (CH), 132.4 (C), 134.8 (CH), 136.2 (CH), 138.0 (C), 140.6 (C), 142.4 (C), 144.2 (C), 150.7 (C=N, diazepine), 154.2 (C), 165.3, 167.8 and 169.0 (C=N, 1,3,5-triazine); LC-MS: m/z 677.2 (M\(^+\)). Anal. Calcd for C\(_{37}\)H\(_{30}\)N\(_5\)F\(_3\)O: C, 65.61; H, 5.09; N, 18.60. Found: C, 65.61; H, 5.05; N, 18.55%.

**Conclusion**

In conclusion, we have presented a facile and efficient route to synthesise phenyl pyrazoline, isoxazole and benzodiazepine derivatives in good yield. The method reported in this investigation is effective in giving excellent conversion to the product, less energy consuming and apparently substituent insensitive. All the synthesised compounds have been screened for their biological activity with the aim of discovering innovative structural leads serving as potent antimycobacterial and antimicrobial agents. The results indicate that all the derivatives exhibit appreciable antimicrobial activity. Among the fifteen newly synthesized compounds, analogues 5a, 5b, 5d, 6b, 6d, 6e, 7c and 7e possessing electron withdrawing atom/group such as methoxy, chloro and bromo at the meta or para position have been identified as the most potent antibacterial agents. As for antifungal activity, compounds 5b and 6e show excellent antifungal activity. Compounds 5a, 6d and 7b display excellent antimycobacterial activity. Moreover the obtained results indicate that compounds 6d and 7d bearing the furanyl ring in the moiety show the highest antimycobacterial and antimicrobial activity.

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