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SYNTHESIS, CHARACTERIZATION AND SAR OF SOME CHALCONES, PHENYL PYRAZOLINES AND ISOXAZOLES CONTAINING 1,3,5-TRIAZINE SCAFFOLD AS A NEW CLASS OF ANTIMICROBIAL AND ANTITUBERCULAR AGENTS

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Abstract: A series of 1,3,5-triazine containing chalcones **(5a-e)**, phenyl pyrazolines **(6a-e)** and isoxazoles **(7a-e)** have been synthesized in order to searching of more potent antimicrobial and antitubercular agents. The structures of all the newly synthesised compounds were characterized by modern sophisticated techniques such as FTIR, ^{1}H NMR, ^{13}C NMR and LC-MS. The newly synthesized derivatives were screened for their *in vitro* antimicrobial activities against three bacterial and three fungal strains as well as *in vitro* antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain. Among the synthesized compounds, compounds **5a**, **5c**, **6b**, **6d**, **7a**, **7c** and **7d** exhibited excellent antimicrobial and antitubercular activity.

Keywords: 1,3,5-Triazine, chalcone, pyrazoline, isoxazole, antimicrobial activity, antitubercular activity.

Introduction

During the past few years, the rising population is affected with major increase in the frequency of severe infectious diseases due to the increasing number of multidrug resistant (MDR) microbial pathogens. Speedy multiplication of drug resistant strains causes a severe threat in recent years[1]. A potential approach to defeat the resistance is to design novel agents with a different mode of action so that no cross-resistance with the present therapeuticals can occur[2, 3]. So, there is a clear need for new antimicrobial and antitubercular agents with new mechanisms of action or broad-spectrum activity, to face the issues of drug-resistant microorganisms.

1,3,5-Triazine based compounds have been studied for decades due to high electron density of the triazine nitrogen atoms, allows for versatile modifications, three-fold symmetry and a wide range of biological applications. Consequences of such potential effects of triazine and an imperative need in search of new chemical entities lead us to synthesize some biologically efficient molecules.

Chalcones (1,3-diarylprop-2-en-1-ones) are important precursors of many biologically active compounds such as pyrazoline[4], isoxazole[5], pyrimidine[6], indazole[7], benzodiazepine[8] etc. Introduction of various substituents into the two aryl rings is also a subject of interest because it leads to useful structure activity relationship (SAR). A number of methods for the synthesis of chalcones are reported in the literature. The main method for the synthesis of chalcone is the classical Claisen-Schmidt condensation reaction in the presence of base. The common α, β-unsaturated carbonyl system of chalcone moiety is believed to be responsible for the broad spectrum of therapeutic activities like antimicrobial[9], antitubercular[10], antioxidant[11], anticancer[12], antimalarial[13] etc.

Pyrazolines having two adjacent nitrogen atoms in a 1,2-diazole ring are an important five membered heterocyclic bioorganic dihydropyrazole molecules. Pyrazoline and its derivatives are electron rich nitrogen heterocycles which play an important role in the diverse biological activities. Depending on the position of the double bond, three forms of pyrazoline are possible i.e. 1-pyrazoline, 2-pyrazoline and 1,3-pyrazoline. Among all the pyrazolines, 2-pyrazoline exerts the monoimino character, more stable and frequently studied one than the 1-pyrazoline and 1,3-pyrazoline[14]. The various methods for the synthesis of pyrazolines are reported in the literature. Pyrazoline ring is present in a number of pharmacologically active molecules such as Phenazone, Amidopyrine, Methampyrone (analgesic and antipyretic), Phenylbutazone, Oxyphenbutazone (anti-inflammatory), Sulfinpyrazone (uricosuric)[15] etc. Moreover, they show various various pharmacological activities such as antidepressant[16], anti-inflammatory[17], antimicrobial[18], cardiovascular[19], antimycobacterial[20] etc.

Isoxazole is a unsaturated aromatic five membered ring heterocycle containing a three carbon atoms, one oxygen atom and one nitrogen atom. Isoxazoles are important synthons for useful synthetic intermediates in organic synthesis[21]. Different methods for the synthesis of isoxazole are reported in the literature[22]. Isoxazoles and their derivatives are largely used in the area of pharmaceuticals due to wide range of biological activities such as antagonist[23], anthelmintic[24], antituberculer[25], antihypertensive[26], anticonvulsant[27], antimicrobial[28] etc. Therefore synthesis and biological activity of isoxazole containing moiety have a gain importance for the medicinal field. In view of the above mentioned knowledge of different pharmacophores and in continuation of our research[29, 30], we have designed and synthesized some new chalcones and converted into its analogues phenyl pyrazolines and isoxazoles having 1,3,5-triazine scaffold. Compounds were subjected to evaluation of their antimicrobial and antitubercular potency against various strains.

Experimental

The reagents and solvents used for reaction were of analytical reagent (AR) grade. Melting points were determined in open capillary method and are uncorrected. TLC was run on E-Merck pre-coated 60 F254 plates and the spots were rendered visible by exposing to UV light or iodine chamber. 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 400 F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using $CDCl_3$, DMSO as a solvent and TMS as an internal standard at 400 and 100 MHz frequency respectively. 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 LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan). Reference drugs for antimicrobial and antitubercular activity are Ampicillin, Chloramphenicol, Ciprofloxacin, Griseofulvin, Nystatin, Rifampicin and Isoniazid used of commercial grade.

General procedure for the compounds 1, 2, 3
The starting compounds **1**, **2** and **3** were prepared according to reported procedure [31].

General procedure for the preparation of 2 - (3' - trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {3'' - (substitutedphenyl) - 2'' - propenon - 1'' - yl} phenylamino] - 1, 3, 5 - triazine (5a-e)
By applying classical Claisen-Schmidt condensation reaction, substituted acetophenone **(3)** (0.01 mol) and an appropriate aldehyde (0.01 mol) **(4a-e)** dissolved in DMF in a 100 ml conical flask. To make it alkaline solution of 40% KOH (5ml) was added in it. 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 a yellow solid was obtained. Finally, the product was isolated by filtration, crystallized from ethanol to get product **(5a-e)**.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {3'' - (2'''-methoxyphenyl) - 2'' - propenon - 1'' - yl} phenylamino] - 1, 3, 5 - triazine (5a):
Yellow solid, yield 80 %, mp 109-111 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3334 (NH), 3025 (=CH), 1654 (C=O), 1542 (C=C), 1433 (CH=CH), 1222 (C-O-C), 1046 (C-F), 804 (C=N), 716 (C-H, 1,2-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.5 (4H, concealed t, CH_2), 3.4 (4H, concealed t, CH_2), 3.9 (3H, s, OCH_3), 6.6 (1H, d, *J* = 9.8, CO-CH=), 6.9 - 7.8 (12H, m, Ar-H), 8.2 (1H, d, *J* = 9.8, Ar-CH=), 8.3 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 46.5 (CH_2), 52.4 (OCH_3), 66.1 (CH_2), 111.2 (CH), 113.4 (CH), 114.5 (CH), 116.2 (CH), 118.0 (CH), 121.5 (=CH), 123.1 (CH), 125.2 (CF_3), 126.1 (CH), 127.0 (C), 128.5 (CH), 130.0 (CH), 133.7 (CH), 135.3 (C), 140.6 (C), 143.5 (C), 144.2 (=CH), 147.2 (C), 155.2 (C), 163.6, 165.4 & 166.2 (C=N, 1,3,5-triazine), 172.4 (CO); LCMS (m/z): 576.3 (M^+). Anal. calcd. for $C_{30}H_{27}N_6F_3O_3$: C 62.50; H 4.72; N 14.58 %. Found, %: C 62.47; H 4.69; N 14.63 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {3'' - (4'''-methylphenyl) - 2'' - propenon - 1'' - yl} phenylamino] - 1, 3, 5 - triazine (5b):
Yellow solid, yield 76 %, mp 132-134 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3336 (NH), 3029 (=CH), 1669 (C=O), 1538 (C=C), 1463 (CH=CH), 1356 (CH_3), 1218 (C-O-C), 1040 (C-F), 800 (C=N), 812 (C-H, 1,4-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.9 (3H, s, CH_3), 3.2 (4H, concealed t, CH_2), 3.6 (4H, concealed t, CH_2), 6.7 (1H, d, *J* = 9.4, CO-CH=), 6.8 - 8.0 (12H, m, Ar-H), 8.3 (1H, d, *J* = 9.3, Ar-CH=), 8.5 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 32.6 (CH_3), 45.4 (CH_2), 65.2 (CH_2), 110.3 (CH), 112.4 (CH), 115.3 (CH), 117.8 (CH), 119.5 (CH), 122.4 (=CH), 124.3 (CH), 126.0 (CF_3), 127.2 (CH), 129.2 (C), 130.7 (CH), 132.4 (CH), 132.8 (CH), 134.3 (C), 136.7 (C), 138.2 (C), 142.8 (C), 144.8 (=CH), 146.3 (C), 166.4, 167.2 & 169.3 (C=N, 1,3,5-triazine), 169.2 (CO); LCMS (m/z): 561.3 (M^+). Anal. calcd. for $C_{30}H_{27}N_6F_3O_2$: C 64.28; H 4.85; N 14.99 %. Found, %: C 64.25; H 4.81; N 14.96 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {3'' - (4'''-fluorophenyl) - 2'' - propenon - 1'' - yl} phenylamino] - 1, 3, 5 - triazine (5c):
Yellow solid, yield 78 %, mp 100-102 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3286 (NH), 3126 (=CH), 1690 (C=O), 1585 (C=C), 1490 (CH=CH), 1256 (C-O-C), 1038 (C-F), 810 (C=N), 839 (C-H, 1,4-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4 (4H, concealed t, CH_2), 3.9 (4H, concealed t, CH_2), 6.8 (1H, d, *J* = 7.5, CO-CH=), 7.0 - 8.2 (12H, m, Ar-H),

8.4 (1H, d, *J* = 7.5, Ar-CH=), 8.5 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.5 (CH_2), 63.1 (CH_2), 108.2 (CH), 110.1 (CH), 113.4 (CH), 115.0 (CH), 117.3 (CH), 119.7 (=CH), 121.5 (CH), 124.6 (CF_3), 126.3 (CH), 128.0 (C), 131.3 (CH), 133.2 (CH), 134.5 (CH), 136.1 (C), 138.0 (C), 139.5 (C), 143.4 (C), 145.3 (=CH), 148.0 (C), 165.7, 168.1 & 170.0 (C=N, 1,3,5-triazine), 171.3 (CO); LCMS (m/z): 563.9 (M^+). Anal. calcd. for $C_{29}H_{24}N_6F_4O_2$: C 61.70; H 4.28; N 14.89 %. Found, %: C 61.65; H 4.25; N 14.93 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {3'' - (3''', 4'''-dimethylphenyl) - 2'' - propenon - 1'' - yl} phenylamino] - 1, 3, 5 - triazine (5d):
Yellow solid, yield 72 %, mp 135-137 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3340 (NH), 3032 (=CH), 1690 (C=O), 1540 (C=C), 1456 (CH=CH), 1360 (CH_3), 1223 (C-O-C), 1038 (C-F), 802 (C=N), 693 & 836 (C-H, 1,3 & 1,4-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.6 (3H, s, CH_3), 1.9 (3H, s, CH_3), 3.3 (4H, concealed t, CH_2), 3.9 (4H, concealed t, CH_2), 6.5 (1H, d, *J* = 10.5, CO-CH=), 7.2 - 8.3 (11H, m, Ar-H), 8.4 (1H, d, *J* = 10.5, Ar-CH=), 8.6 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 31.4 (CH_3), 43.2 (CH_2), 62.5 (CH_2), 111.2 (CH), 113.6 (CH), 115.2 (CH), 118.4 (CH), 120.7 (CH), 121.2 (=CH), 123.0 (CH), 125.5 (CF_3), 128.3 (CH), 130.4 (C), 131.8 (CH), 133.3 (CH), 134.0 (C), 135.2 (C), 137.8 (C), 139.1 (C), 143.2 (C), 145.5 (=CH), 147.8 (C), 165.2, 168.3 & 170.4 (C=N, 1,3,5-triazine), 172.6 (CO); LCMS (m/z): 575.6 (M^+). Anal. calcd. for $C_{31}H_{29}N_6F_3O_2$: C 64.80; H 5.08; N 14.63 %. Found, %: C 64.82; H 5.12; N 14.60 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {3'' - (3''', 4''', 5''' - trimethoxyphenyl) - 2'' - propenon - 1'' - yl} phenylamino] - 1, 3, 5 - triazine (5e):
Yellow solid, yield 81 %, mp 115-117 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3340 (NH), 3030 (=CH), 1650 (C=O), 1540 (C=C), 1430 (CH=CH), 1221 (C-O-C), 1042 (C-F), 805 (C=N), 653 & 840 (C-H, 1,3 & 1,4-disubstituted benzene ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.2 (4H, concealed t, CH_2), 3.4 (4H, concealed t, CH_2), 3.7-3.9 (9H, m, OCH_3), 6.4 (1H, d, *J* = 8.6, CO-CH=), 7.0 - 8.3 (10H, m, Ar-H), 8.4 (1H, d, *J* = 8.9, Ar-CH=), 8.5 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.3 (CH_2), 53.6 (OCH_3), 67.3 (CH_2), 112.4 (CH), 114.3 (CH), 116.1 (CH), 118.0 (CH), 120.5 (CH), 122.3 (=CH), 124.5 (CH), 126.7 (CF_3), 127.6 (CH), 129.8 (C), 130.2 (CH), 131.2 (CH), 136.2 (C), 141.8 (C), 144.7 (C), 145.3 (=CH), 148.0 (C), 150.5 (C), 153.5 (C), 162.1, 164.7 & 165.3 (C=N, 1,3,5-triazine), 168.2 (CO); LCMS (m/z): 636.4 (M^+). Anal. calcd. for $C_{32}H_{31}N_6F_3O_5$: C 60.38; H 4.90; N 13.20 %. Found, %: C 60.35; H 4.87; N 13.23 %.

General procedure for the preparation of 2 - (3' - trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1'' - phenyl - 5'' - (substitutedphenyl) - 2'' - pyrazolin - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (6a-e)
A 100 ml round bottomed flask, fitted with a reflux condenser was charged with a mixture of an appropriate chalcone **(5a-e)** (0.01 mol) and phenyl hydrazine hydrochloride (0.01 mol) in ethanol. The reaction proceeded by the addition of two drops of KOH solution to make basic medium. The reaction mixture was then refluxed for 5-6 h. The progress of the reaction was monitored by using TLC using toluene:methanol (10:4 v/v) mobile phase. After completion of the reaction, the reaction mixture was poured into crushed ice, neutralised with diluted HCl. Thus the solid mass separated was collected by filtration, washed well with hot water and recrystallized from methanol gives phenyl pyrazoline derivatives **(6a-e)**.

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

White solid, yield 78 %, mp 125-127 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3340 (NH), 3010 (=CH), 2880 (C-H), 1570 (C=N), 1512 (C=C), 1230 (C-O-C), 1146 (OCH_3), 1025 (C-F), 801 (C=N, 1,3,5-triazine), 771 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.2 (1H, dd, *J* = 11.4 & 13.5, CHx-CH), 3.4 (4H, concealed t, CH_2), 3.6 (1H, dd, *J* = 11.6 & 13.5, CHy-CH), 3.7 (4H, concealed t, CH_2), 3.9 (3H, s, OCH_3), 5.4 (1H, dd, *J* = 5.5 & 12.5, CH-CH_2-Ar), 6.9 - 8.0 (12H, m, Ar-H), 8.2 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.5 (CH_2), 42.3 (CH_2), 54.1 (OCH_3), 64.5 (CH-Ar), 66.2 (CH_2), 110.1 (CH), 112.4 (CH), 113.6 (CH), 115.1 (CH), 117.7 (CH), 119.2 (CH), 121.8 (CH), 124.2 (CF_3), 126.5 (CH), 128.7 (CH), 133.0 (C), 134.1 (CH), 138.0 (CH), 142.0 (C), 144.8 (C), 147.5 (C), 150.4 (C), 152.7 (C=N), 164.5, 165.4 & 167.1 (C=N, 1,3,5-triazine); LCMS (m/z): 666.4 (M^+). Anal. calcd. for $C_{36}H_{33}N_8F_3O_2$: C, 64.86; H, 4.98; N, 16.81 % Found: C, 64.89; H, 4.99; N, 16.83 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1'' - phenyl - 5'' - (4'''-methylphenyl) - 2'' - pyrazolin - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (6b):

White solid, yield 71 %, mp 131-133 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3346 (NH), 3035 (=CH), 2889 (C-H), 1545 (C=N), 1526 (C=C), 1360 (CH_3), 1225 (C-O-C), 1016 (C-F), 838 (C-H, 1,4 disubstituted benzene ring), 806 (C=N, 1,3,5-triazine); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.7 (3H, s, CH_3), 3.3 (1H, dd, *J* = 12.6 & 14.7, CHx-CH), 3.5 (4H, concealed t, CH_2), 3.7 (1H, dd, *J* = 12.5 & 14.6, CHy-CH), 3.8 (4H, concealed t, CH_2), 5.1 (1H, dd, *J* = 5.9 & 11.3, CH-CH_2-Ar), 7.2 - 8.1 (12H, m, Ar-H), 8.4 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 28.4 (CH_3), 38.2 (CH_2), 41.4 (CH_2), 63.1 (CH-Ar), 67.3 (CH_2), 108.3 (CH), 111.7 (CH), 113.2 (CH), 116.3 (CH), 118.0 (CH), 121.5 (CH), 123.2 (CH), 125.7 (CF_3), 126.4 (CH), 129.2 (CH), 131.3 (C), 133.3 (CH), 134.3 (C), 136.4 (CH), 140.5 (C), 143.3 (C), 146.3 (C), 150.0 (C=N), 162.2, 164.3 & 166.7 (C=N, 1,3,5-triazine); LCMS (m/z): 649.7 (M^+). Anal. calcd. for $C_{36}H_{33}N_8F_3O$: C, 66.45; H, 5.11; N, 17.22 % Found: C, 66.49; H, 5.15; N, 17.18 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1'' - phenyl - 5'' - (4'''-fluorophenyl) - 2'' - pyrazolin - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (6c):

White solid, yield 70 %, mp 115-117 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3350 (NH), 3040 (=CH), 2856 (C-H), 1535 (C=N), 1521 (C=C), 1216 (C-O-C), 1010 (C-F), 830 (C-H, 1,4 disubstituted benzene ring), 804 (C=N, 1,3,5-triazine); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.2 (1H, dd, *J* = 11.2 & 13.4, C<u>H</u>x-CH), 3.4 (4H, concealed t, C<u>H</u>$_2$), 3.6 (1H, dd, *J* = 11.6 & 13.9, C<u>H</u>y-CH), 3.7 (4H, concealed t, C<u>H</u>$_2$), 5.3 (1H, dd, *J* = 6.3 & 10.7, C<u>H</u>-CH_2-Ar), 6.8 - 8.2 (12H, m, Ar-<u>H</u>), 8.3 (1H, s, N<u>H</u>); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 39.5 (CH_2), 46.3 (CH_2), 62.5 (CH-Ar), 69.1 (CH_2), 110.4 (CH), 112.6 (CH), 114.0 (CH), 116.1 (CH), 119.3 (CH), 121.2 (CH), 124.4 (CH), 126.3 (CF_3), 128.2 (CH), 130.5 (CH), 132.1 (C), 134.9 (CH), 137.8 (CH), 141.6 (C), 144.1 (C), 147.8 (C), 150.4 (C), 152.4 (C=N), 163.4, 165.2 & 167.2 (C=N, 1,3,5-triazine); LCMS (m/z): 653.2 (M^+). Anal. calcd. for $C_{35}H_{30}N_8F_4O$: C, 64.22; H, 4.61; N, 17.12 % Found: C, 64.19; H, 4.58; N, 17.14 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1'' - phenyl - 5'' - (3''', 4'''-dimethylphenyl) - 2'' - pyrazolin - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (6d):
White solid, yield 76 %, mp 121-123 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3349 (NH), 3040 (=CH), 2890 (C-H), 1550 (C=N), 1529 (C=C), 1363 (CH_3), 1226 (C-O-C), 1019 (C-F), 657 & 832 (C-H, 1,3 & 1,4 disubstituted benzene ring), 802 (C=N, 1,3,5-triazine); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.6 (3H, s, CH_3), 2.4 (3H, s, CH_3), 3.4 (1H, dd, *J* = 11.9 & 15.5, CHx-CH), 3.6 (4H, concealed t, CH_2), 3.8 (1H, dd, *J* = 11.9 & 14.7, CHy-CH), 3.8 (4H, concealed t, CH_2), 5.3 (1H, dd, *J* = 7.3 & 12.6, CH-CH_2-Ar), 7.0 - 8.3 (11H, m, Ar-H), 8.5 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 26.2 (CH_3), 36.4 (CH_2), 40.3 (CH_2), 62.2 (CH-Ar), 65.1 (CH_2), 111.2 (CH), 113.4 (CH), 115.8 (CH), 118.2 (CH), 120.4 (CH), 122.0 (CH), 124.4 (CH), 126.6 (CF_3), 128.3 (CH), 130.0 (CH), 132.1 (C), 134.5 (CH), 135.7 (C), 136.2 (CH), 138.2 (C), 140.1 (C), 142.2 (C), 147.2 (C=N), 161.2, 163.4 & 165.6 (C=N, 1,3,5-triazine); LCMS (m/z): 663.5 (M^+). Anal. calcd. for $C_{37}H_{35}N_8F_3O$: C, 66.86; H, 5.30; N, 16.86 % Found: C, 66.84; H, 5.33; N, 16.90 %.

2 - (3' - Trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1'' - phenyl - 5'' - (3''', 4''', 5'''-trimethoxyphenyl) - 2'' - pyrazolin - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (6e):
White solid, yield 65 %, mp 124-126 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3345 (NH), 3013 (=CH), 2875 (C-H), 1565 (C=N), 1505 (C=C), 1226 (C-O-C), 1142 (OCH_3), 1021 (C-F), 800 (C=N, 1,3,5-triazine), 770 & 836 (C-H, 1,3 & 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.9 (1H, dd, *J* = 10.2 & 12.1, CHx-CH), 3.2 (4H, concealed t, CH_2), 3.4 (1H, dd, *J* = 10.2 & 11.2, CHy-CH), 3.5 (4H, concealed t, CH_2), 3.7 - 3.9 (9H, m, OCH_3), 5.2 (1H, dd, *J* = 5.0 & 11.2, CH-CH_2-Ar), 6.8 - 8.2 (10H, m, Ar-H), 8.3 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 39.4 (CH_2), 43.2 (CH_2), 55.2 (OCH_3), 62.3 (CH-Ar), 64.6 (CH_2), 111.2 (CH), 113.9 (CH), 114.5 (CH), 116.8 (CH), 118.9 (CH), 120.0 (CH), 122.4 (CH), 124.4 (CF_3), 127.2 (CH), 129.2 (CH), 131.3 (C), 133.4 (CH), 135.2 (CH), 139.5 (C), 143.2 (C), 145.2 (C), 148.9 (C), 151.6 (C=N), 161.4, 163.7 & 166.3 (C=N, 1,3,5-triazine); LCMS (m/z): 727.3 (M^+). Anal. calcd. for $C_{38}H_{37}N_8F_3O_4$: C, 62.80; H, 5.13; N, 15.42 % Found: C, 62.83; H, 5.09; N, 15.45 %.

General procedure for the preparation of 2 - (3' - trifluoromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5'' - (substitutedphenyl) - 2'' - isoxazol - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (7a-e)
Compound **(5a-e)** (0.01 mol) condensed with hydroxylamine hydrochloride (0.01mol) in the presence of alkaline medium (5 ml 40% KOH) in ethanol at refluxed temperature for 5-6 hours 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 afforded isoxazole **(7a-e)**.

2 - (3' - Trifluoromethylphenylamino) - 4 -(tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5'' - (2''' -methoxyphenyl) - 2'' - isoxazol - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (7a):
White solid, yield 82 %, mp 118-120 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3240 (NH), 3026 (=CH), 2825 (C-H), 1531 (C=N), 1526 (C=C), 1240 (C-O-C), 1142 (OCH_3), 1022 (C-F), 804 (C=N, 1,3,5-triazine), 783 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.6 (4H, concealed t, CH_2), 3.8 (4H, concealed t, CH_2), 3.9 (3H, s, OCH_3), 6.4 (1H, s, C<u>H</u>-C), 6.8

- 7.9 (12H, m, Ar-H), 8.3 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.4 (CH_2, oxazine), 56.4 (OCH_3), 68.2 (CH_2), 98.3 (CH), 113.3 (CH), 115.4 (CH), 117.8 (CH), 119.4 (C), 120.3 (C), 123.3 (CF_3), 126.3 (CH), 128.4 (CH), 130.3 (CH), 133.4 (C), 135.2 (C), 140.4 (C), 154.2 (C), 158.1 (C=N), 160.0 (C-Ar), 160.2, 163.7 & 165.6 (C=N, 1,3,5-triazine); LCMS (m/z): 588.3 (M^+). Anal. calcd. for $C_{30}H_{26}N_7F_3O_3$: C, 61.12; H, 4.44; N, 16.63 % Found: C, 61.14; H, 4.46; N, 16.60 %.

2 - (3' - Trifluoromethylphenylamino) - 4 -(tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5'' - (4''' -methylphenyl) - 2'' - isoxazol - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (7b):
White solid, yield 82 %, mp 118-120 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3356 (NH), 3012 (=CH), 2820 (C-H), 1546 (C=N), 1516 (C=C), 1356 (CH_3), 1219 (C-O-C), 1036 (C-F), 802 (C=N, 1,3,5-triazine), 780 (C-H, 1,2 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.9 (3H, s, CH_3), 3.3 (4H, concealed t, CH_2), 3.5 (4H, concealed t, CH_2), 6.5 (1H, s, C<u>H</u>-C), 6.9 – 8.0 (12H, m, Ar-H), 8.2 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 29.4 (CH_3), 40.4 (CH_2, oxazine), 66.3 (CH_2), 101.4 (CH), 107.2 (CH), 112.6 (CH), 114.3 (CH), 116.8 (C), 122.6 (C), 124.0 (CF_3), 126.1 (CH), 129.3 (CH), 131.2 (CH), 134.6 (C), 135.3 (C), 136.3 (C), 138.3 (C), 154.2 (C=N), 159.3 (C-Ar), 161.6, 164.5 & 166.5 (C=N, 1,3,5-triazine); LCMS (m/z): 574.6 (M^+). Anal. calcd. for $C_{30}H_{26}N_7F_3O_2$: C, 62.82; H, 4.56; N, 17.09 % Found: C, 62.80; H, 4.51; N, 17.11 %.

2 - (3' - Trifluoromethylphenylamino) - 4 -(tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5'' - (4''' -fluorophenyl) - 2'' - isoxazol - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (7c):
White solid, yield 68 %, mp 145-147 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3323 (NH), 3065 (=CH), 2862 (C-H), 1590 (C=N), 1554 (C=C), 1236 (C-O-C), 1025 (C-F), 809 (C=N, 1,3,5-triazine), 836 (C-H, 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4 (4H, concealed t, CH_2), 3.5 (4H, concealed t, CH_2), 6.7 (1H, s, C<u>H</u>-C), 7.0 - 8.2 (12H, m, Ar-H), 8.3 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 42.6 (CH_2, oxazine), 64.2 (CH_2), 103.5 (CH), 108.9 (CH), 111.5 (CH), 113.2 (CH), 115.0 (C), 119.2 (C), 123.8 (CF_3), 125.2 (CH), 127.5 (CH), 129.4 (CH), 133.0 (C), 136.2 (C), 137.8 (C), 139.1 (C), 151.3 (C=N), 157.2 (C-Ar), 163.4, 165.2 & 167.3 (C=N, 1,3,5-triazine); LCMS (m/z): 576.3 (M^+). Anal. calcd. for $C_{29}H_{23}N_7F_4O_2$: C, 60.31; H, 4.01; N, 16.98 % Found: C, 60.28; H, 4.05; N, 16.95 %.

2 - (3' - Trifluoromethylphenylamino) - 4 -(tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5'' - (3''' , 4''' - dimethylphenyl) - 2'' - isoxazol - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (7d):
White solid, yield 73 %, mp 119-121 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3360 (NH), 3016 (=CH), 2826 (C-H), 1629 (C=N), 1525 (C=C), 1360 (CH_3), 1236 (C-O-C), 1038 (C-F), 800 (C=N, 1,3,5-triazine), 659 & 834 (C-H, 1,3 & 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.6 (3H, s, CH_3), 1.9 (3H, s, CH_3), 3.5 (4H, concealed t, CH_2), 3.9 (4H, concealed t, CH_2), 6.8 (1H, s, C<u>H</u>-C), 7.0 - 8.0 (11H, m, Ar-H), 8.3 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 27.3 (CH_3), 41.5 (CH_2, oxazine), 64.5 (CH_2), 102.3 (CH), 109.5 (CH), 113.1 (CH), 115.4 (CH), 117.3 (C), 121.4 (C), 123.3 (CF_3), 124.2 (CH), 125.6 (CH), 130.3 (CH), 132.3 (C), 134.5 (C), 136.0 (C), 139.2 (C), 152.3 (C=N), 157.2 (C-Ar), 163.2, 165.4 & 167.3 (C=N, 1,3,5-triazine); LCMS (m/z): 587.6 (M^+). Anal. calcd. for $C_{31}H_{28}N_7F_3O_2$: C, 63.37; H, 4.80; N, 16.69 % Found: C, 63.40; H, 4.84; N, 16.63 %.

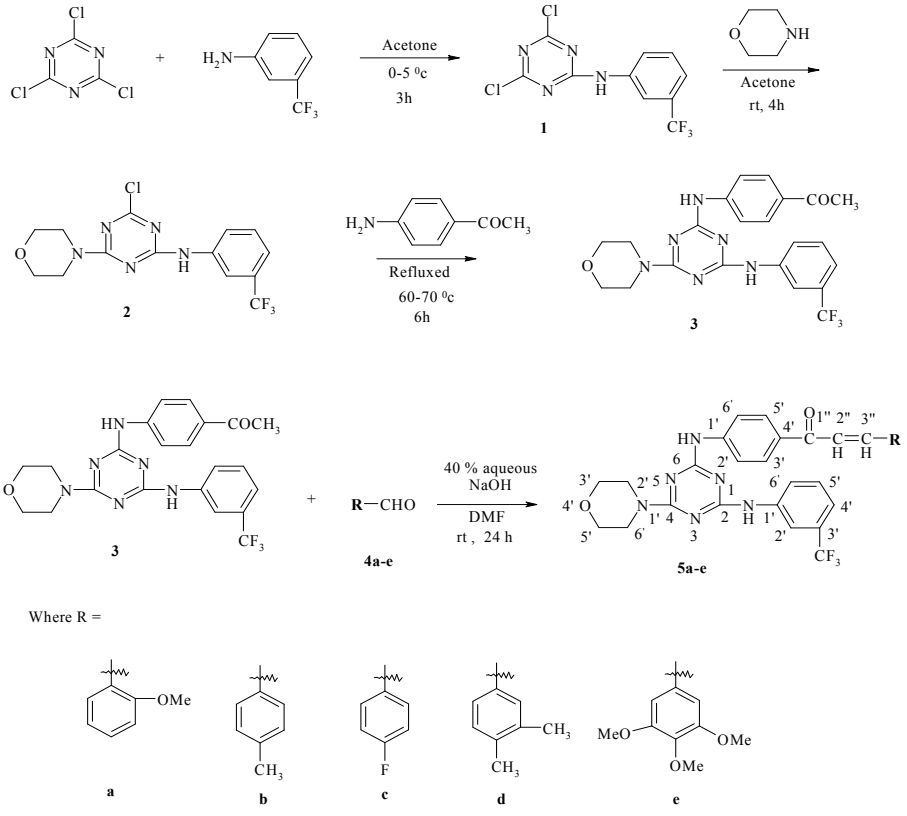
2 - (3' - Trifluoromethylphenylamino) - 4 -(tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5'' - (3''' , 4''', 5''' - trimethoxyphenyl) - 2'' - isoxazol - 3'' - yl} phenylamino] - 1, 3, 5 - triazine (7e):

White solid, yield 77 %, mp 124-126 ^{0}C. IR (KBr, ν_{max}, cm^{-1}): 3244 (NH), 3029 (=CH), 2818 (C-H), 1633 (C=N), 1525 (C=C), 1238 (C-O-C), 1140 (OCH_3), 1029 (C-F), 800 (C=N, 1,3,5-triazine), 659 & 834 (C-H, 1,3 & 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.2 (4H, concealed t, CH_2), 3.4 (4H, concealed t, CH_2), 3.6 - 3.9 (9H, m, OCH_3), 6.9 (1H, s, CH-C), 7.1 - 8.2 (12H, m, Ar-H), 8.4 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 46.3 (CH_2, oxazine), 55.5 (OCH_3), 69.4 (CH_2), 99.2 (CH), 110.2 (CH), 116.3 (CH), 119.2 (CH), 120.1 (C), 122.2 (C), 124.5 (CF_3), 129.3 (CH), 131.4 (CH), 134.5 (C), 136.3 (C), 138.2 (C), 147.4 (C), 149.3 (C), 153.2 (C), 156.3 (C=N), 162.2 (C-Ar), 164.9, 166.7 & 169.3 (C=N, 1,3,5-triazine); LCMS (m/z): 649.2 (M^+). Anal. calcd. for $C_{32}H_{30}N_7F_3O_5$: C, 59.17; H, 4.65; N, 15.09 % Found: C, 59.21; H, 4.69; N, 15.05 %.

Results and discussion

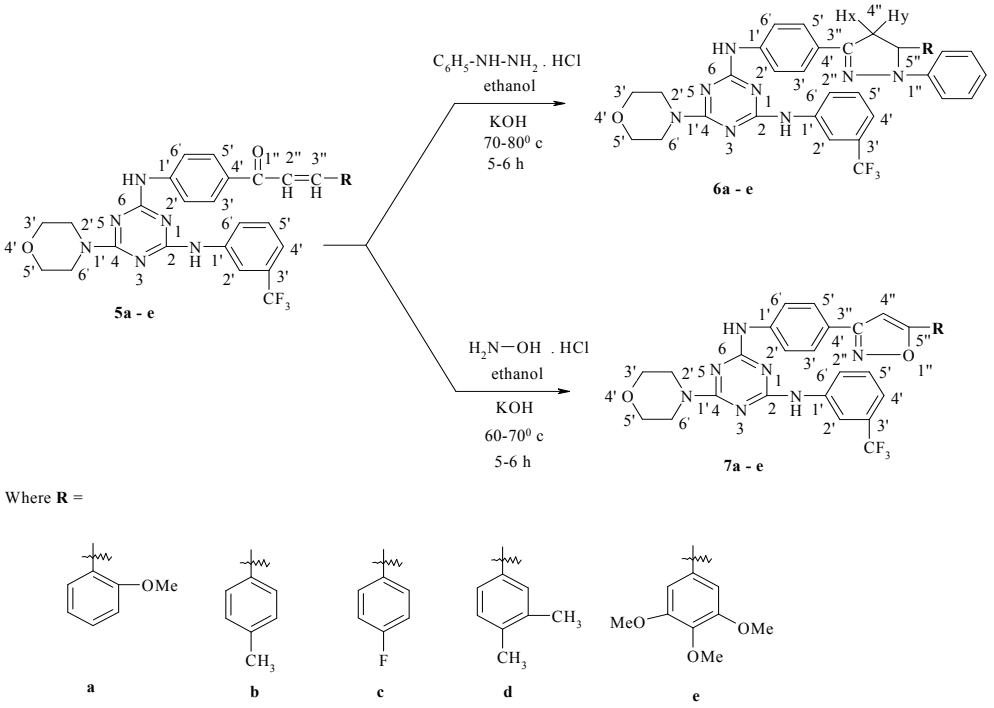
Chemistry

The synthetic strategies adopted to obtain the starting precursors **(1)**, **(2)**, **(3)** and chalcones **(5a-e)** as shown in **Schemes 1.** The key intermediate chalcones **(5a-e)** were synthesized in excellent yields by condensing various appropriate benzaldehyde **(4a-e)** with the substituted acetophenone **(3)** in the presence of potassium hydroxide under Claisen-Schmidt reaction conditions. These chalcones were subjected to cycloaddition condensation reactions using phenyl hydrazine hydrochloride and hydroxylamine hydrochloride give the corresponding 1-phenyl-2-pyrazoline **(6a-e)** and isoxazole **(7a-e)** derivatives respectively, in good yields as systematic path depicted in **Schemes 2.**



Scheme1. Synthetic route for the starting precursor **1**, **2**, **3** and **(5a-e)**

The structures of all the synthesized compounds are confirmed from their spectral and analytical data. As an example, in the IR spectrum of compound **5a**, a strong absorption band is observed at 1433 and 1654 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 1,2 disubstituted benzene ring were appeared at 716 cm^{-1}. The C=N stretching of 1,3,5-triazine nucleus and C=C functionality of aromatic ring were observed at 804 and 1542 cm^{-1} respectively. The ^{1}H NMR spectrum of compound **5a** showed a doublet at δ 6.6 (*J* = 9.8 Hz) ppm for the -CO-C<u>H</u>= proton and at δ 8.2 (*J* = 9.8 Hz) ppm for the Ar-C<u>H</u>= proton of α, β- unsaturated carbonyl group protons. The other remaining seventeen aromatic protons appeared as a multiplet signal at δ 6.9-7.8 ppm. Finally, the ^{13}C NMR spectrum of the compound **5a** was recorded in $CDCl_3$ and the spectral signals were in good agreement with the proposed structure. The most deshielded signal that appeared at δ 172.4 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 121.5 and 144.2 ppm. The signals for aromatic carbons appeared between at δ 111.2-155.2 ppm in the ^{13}C spectrum.



Scheme 2. Systematic path for the synthesis of **(6a-e)** and **(7a-e)**

IR spectrum of phenyl pyrazoline, compound **6a** exerted a strong stretching absorption band for the C=N functionality of pyrazoline unit and C=C functionality of aromatic ring were observed at 1570 and 1512 cm^{-1} respectively. The C_4"-H stretching of pyrazoline ring were appeared at 2880 cm^{-1}. The C=N stretching of 1,3,5-triazine core and aromatic C-H bending vibrations were observed at 801 and 3010 cm^{-1} respectively. The ^{1}H NMR spectrum of compound **6a** exhibited a pro-chiral methylene protons C_4"-H of pyrazoline appeared as two distinct doublet of a doublet at δ 3.2 ppm (*J* = 11.4 and 13.5 Hz) and at δ 3.6 ppm (*J* = 11.6 and 13.5 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 doublet of a doublet at δ 5.4 ppm (*J* = 5.5 and 12.5 Hz) due to C<u>H</u>-CH_2-Ar proton. The other remaining seventeen aromatic protons appeared as a multiplet signal at δ 6.9-8.0 ppm. Finally, the ^{13}C NMR spectrum of the cyclized product **6a** was recorded in $CDCl_3$ and the spectral signals were in good agreement with the proposed structures. The shielded signal at δ 40.5 ppm was assigned to the methylene carbon of pyrazoline ring. The signals for aromatic carbons exerted between at δ 110.1-150.4 ppm in the ^{13}C spectrum. The IR spectrum of compound **7a** exhibited the disappearance of absorption at 1654 cm^{-1} corresponding to >C=O group of chalcone and exhibited a strong absorption band at 1531 cm^{-1} due to the C=N functionality of isoxazole unit. The C-H functionality of isoxazole unit was observed at 2825 cm^{-1}. The aromatic C=C stretching, C-H bending vibrations for 1,2-disubstituted benzene ring appeared at 1526 and 783 cm^{-1} respectively. The C=N stretching of 1,3,5-triazine core was observed at 804 cm^{-1}. The ^{1}H NMR spectrum of compound **7a** showed chiral C_4"-H proton of isoxazole ring which appeared as singlet at δ 6.4 for C<u>H</u>-C proton. The other remaining twelve aromatic protons resonate as a multiplet signal at δ 6.8-7.9. Finally, the ^{13}C NMR spectrum of compound **7a** showed a signal at δ 98.3, 158.1 and 160.0 due to the -CH, C=N and C-Ar carbon of isoxazole moeity which was also assigned to the isoxazole unit. The signals for aromatic carbons appeared between δ 113.3-154.2 in the ^{13}C NMR spectrum. Moreover, there are no absorptions in the region of 1600-1700 cm^{-1} in IR spectra of compound **6a** and **7a** which indicating the absence of a C=O group of chalcone moiety in these structures and further confirmed the cyclization of chalcone in to phenyl pyrazoline and iosxazoles derivatives. In addition, distinctive singlet was observed around at δ 3.7-4.0 for methoxy group of aryl ring attached to chalcone, pyrazoline and isoxazole unit and singlet around δ 8.2-8.5 stands for secondary amine attached with 1,3,5-triazine.

Furthermore, the mass spectrum of compounds **5a**, **6a** and **7a** showed M^+ peak at m/z 576.3, 666.4 and 588.3 (100%) respectively along with other fragment peaks, which further supported the structure of compounds **5a**, **6a** and **7a**. The obtained elemental analysis values are also in good agreement with theoretical data.

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[32]. The results are summarised in **Table 1**.

The antibacterial screening of chalcone **(5a-e)**, 1-phenyl pyrazoline **(6a-e)** and isoxazole derivatives **(7a-e)** pointed out that in Gram-positive bacteria, compounds **5b**, **6b**, **7b** (MIC = 62.5 µg/ml) showed an outstanding inhibitory effect against *Staphylococcus aureus* as compared to Ampicillin (MIC = 250 µg/ml) and admirable to Chloramphenicol and Ciprofloxacin (MIC = 50 µg/ml). Compounds **5c**, and **7a** (MIC = 100 µg/ml), and **7c** (MIC = 125 µg/ml) and **7e** (MIC = 200 µg/ml) showed appreciable activity to Ampicillin (MIC = 250 µg/ml) while compounds **5a**, **5d**, **6c**, **6d** and **7d** found to posses comparable 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 **5c** (MIC = 50 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 **5b**, **6b**, **7a**, and **7d** (MIC = 100 µg/ml), **5e**, **6d** and **7b** (MIC = 125 µg/ml) exerted significant 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 **6c**, **7c** and **7d** (MIC = 62.5 µg/ml) demonstrated excellent activity compared to Ampicillin (MIC = 100 µg/ml) while compounds **5a**, **5b** and **7a** (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 **6d** (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 **6a**, **6c**, **7a**, **7b**, **7c** and **7d** (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*. 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 **5b**, and **7a** (MIC = 100 µg/ml) and **6a** and **7e** (MIC = 250 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 **5a**, **5d**, **5e**, **6b**, **6d**, **6e** and **7d** (MIC = 500 µg/ml) showed the same potency as Griseofulvin (MIC = 500 µg/ml) against *Candida albicans*. Compounds **5d** (MIC = 100 µg/ml) depicted equipotent to Griseofulvin (MIC = 100 µg/ml) and Nystatin (MIC = 100 µg/ml) against *Aspergillus niger*. Compounds **6d** and **7c** (MIC = 100 µg/ml) found equipotent to Griseofulvin (MIC = 100 µg/ml) and Nystatin (MIC = 100 µg/ml) against *Aspergillus clavatus*.

Table 1 *In vitro* antimicrobial and antitubercular activity of the synthesized compounds **(5a-e)**, **(6a-e)** and **(7a-e)**

Entry	Antimicrobial activity (MIC) µg/ml							Antitubercular activity
	Antibacterial activity				Antifungal activity			
	Gram Positive Bacteria		Gram Negative Bacteria		Fungus			% Inhibition at 250 µg/ml
	S. aureus	*S. pyogenes*	*E. coli*	*P. aerug.*	*C. albican*	*A. niger*	*A. clavatus*	*M. tuberculosis* $H_{37}Rv$
5a	250	200	100	200	500	1000	>1000	89
5b	62.5	100	100	200	100	>1000	>1000	56
5c	100	50	200	250	1000	>10000	>1000	90
5d	250	250	200	250	500	100	>1000	34
5e	500	125	125	125	500	250	200	70
6a	250	200	200	100	250	>1000	>1000	76
6b	62.5	100	125	200	500	>1000	>1000	86
6c	250	250	62.5	100	>1000	>1000	>1000	93
6d	250	125	250	50	500	250	100	46

6e	200	200	200	250	500	1000	500	57
7a	100	100	100	100	100	>1000	>1000	64
7b	62.5	125	250	100	1000	>1000	>1000	79
7c	125	250	62.5	100	1000	>1000	100	81
7d	250	100	62.5	100	500	>1000	>1000	96
7e	200	200	200	200	250	>1000	>1000	23
A	250	100	100	100	–	–	–	–
B	50	50	50	50	–	–	–	–
C	50	50	25	25	–	–	–	–
D	–	–	–	–	500	100	100	–
E	–	–	–	–	100	100	100	–
F	–	–	–	–	–	–	–	99
G	–	–	–	–	–	–	–	98

A: Ampicillin, B: Chloramphenicol, C: Ciprofloxacin, D: Greseofulvin, E: Nystatin, F: Isoniazid and G: Rifampicin.

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* $H_{37}Rv$ strain[33]. The observed results are presented in **Table 1** in the form of inhibition (%), relative to that of standard antitubercular drugs isoniazid and rifampicin. Compounds demonstrating more than 80% 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 **5a** (MIC = 100 µg/ml), **5c** (MIC = 50 µg/ml), **6b** (MIC = 62.5 µg/ml), **6c** (MIC = 62.5 µg/ml), **7c** (MIC = 62.5 µg/ml) and **7d** (MIC = 62.5 µg/ml) were found to possess the greatest potency against *Mycobacterium tuberculosis* with 89, 90, 86, 93, 81 and 96 % inhibition respectively **(Table 2)**. Other derivatives showed moderate to poor antitubercular activity.

Table 2 *In vitro* antitubercular activity data of the synthesized compounds exhibiting greater inhibition against *M. tuberculosis* $H_{37}Rv$ (MICs, µg/ml)

Entry	% Inhibition	MIC (µg/ml)
5a	89	100
5c	90	50
6b	86	62.5
6c	93	62.5
7c	81	62.5
7d	96	62.5

A: Isoniazid and B: Rifampicin

Structure activity relationship (SAR)

The substitution pattern of the aryl ring in all derivatives are observed to affect biological activities. The electronic nature of the substituents led to significant variation in all kinds of pharmacological activities. Compounds containing electron withdrawing group (-F) increase antimicrobial and antitubercular activity in chalcone, pyrazoline and isoxazole derivatives

(compound **5c**, **6c** and **7c**) Compounds containing electron releasing group (-CH_3 and -OCH_3) affect more antimicrobial and antitubercular activity. The SAR study indicates that compounds containing electron releasing groups at the para position increases antibacterial activity while the presence of electron withdrawing group at the same position lead to enhanced antifungal activity.

Conclusion

With the aim of discover innovative structure leads serving as potent antimicrobial and antitubercular agents, a new series of chalcone, 1-phenyl pyrazoline and isoxazole derivatives bearing 1,3,5-triazine nucleus. 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 isoxazole ring, the activities changed drastically. Among the fifteen newly synthesised compounds, analogues **5b**, **6b**, **6c**, **6d**, **7a** and **7d** possessing electron withdrawing atom/group such as methoxy, fluoro and methyl at the meta or para position were identified as the most potent antibacterial agents and compound E3 and F3 were found to be the most effective antifungal agent. The results described here merit further investigations in our laboratory using a forward chemical genetic approach in finding lead molecules as antimicrobial agents. Compounds **5a**, **5c**, **6b**, **6c**, **7c** and **7d** displayed excellent antitubercular activity. Consequently, the compounds proved to be worthy for further modifications to obtain more efficacious antimicrobial and antitubercular compounds.

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References

1. Overbye K. M., Barrett J. F. *Drug Discov. Today*, **2005**, 10, 45.
2. Vicini P., Geronikaki A., Anastasia K., Incerti M., Zani F. *Bioorg. Med. Chem.*, **2006**, 14, 3859.
3. Mcdonald L. C. *Clin. Infect. Dis.*, **2006**, 42, S65.
4. Maleki B., Azarifar D., Moghaddam M. K., Hojati S. F., Gholizadeh M., Salehabadi H. *J. Serb. Chem. Soc.*, **2009**, 74, 1371.
5. Solankee A., Patel K., Patel R. *Indian J. Chem.*, **2013**, 52B, 671.
6. Sharma M., Chaturvedi V., Manju Y. K., Bhatnagar S., Srivastava K., Puri S. K., Chauhan P. M. S. *Eur. J. Med. Chem.*, **2009**, 44, 2081.
7. Sapnakumari M., Narayana B., Sarojini B. K., Madhu L. N. *Med. Chem. Res.*, **2014**, 23, 2368.
8. Solankee A., Tailor R. ILCPA, **57**, 2015, 13.
9. Solankee A., Tailor R. *Chem. Sci. Trans.*, **2015**, 4, 1057.
10. Gupta R. A., Kaskhedikar S. G. *Med. Chem. Res.*, **2013**, 22, 3863.
11. Bandgar B. P., Jalde S. S., Adsul L. K., Shringare S. N., Lonikar S. V., Gacche R. N., Dhole N. A., Nile S. H., Shirfule A. L., *Med. Chem. Res.*, **2012**, 21, 4512.
12. Kumar D., Kumar N. M., Akamatsu K., Kusaka E., Harada H., Ito T., *Bioorg. Med. Chem.*, **2010**, 20, 3916.

13. Tomar V., Bhattacharjee G., Kamaluddin, Rajakumar S., Srivastava K., Puri S. K., *Eur. J. Med. Chem.*, **2010**, 45, 745.
14. Yusuf M., Jain P. *Arabian J. Chem.*, **2014**, 7, 553.
15. Rahman Md. A., Siddiqui A. A. *Int. J. Pharm. Sci. Drug Res.*, **2010**, 2, 165.
16. Ozdemir Z., Kandilci H. B., Gumusel B., Calis U., Bilgin A. A. *Eur. J. Med. Chem.*, **2007**, 42, 373.
17. Kelekci N. G., Yabanoglu S., Kupeli E., Salgin U., Ozgen O., Ucar G., Yesilada E., Kendi E., Yesilada A., Bilgin A. A., *Bioorg. Med. Chem.*, **2007**, 15, 5775.
18. Solankee A., Kapadia K., Ciric A., Sokovic M., Doytchinova I., Geronikaki A. *Eur. J. Med. Chem.*, **2010**, 45, 510.
19. Malhotra V., Pathak S., Nath R., Mukerjee D., Shanker K. *Indian J. Chem.*, **2002**, 41B, 1310.
20. Solankee A., Tailor R. *Chemistry International*, **2016**, 2, 189.
21. Kashima C. *Heterocycles*, **1979**, 12, 1343.
22. Hu F., Szostak M. *Adv. Synth. Catal.*, **2015**, 357, 2583
23. Yamamoto T., Fujita K., Asari S., Chiba A., Kataba Y., Ohsumi K., Ohmuta N., Iida Y., Ijichi C., Iwayama S., Fukuchi N., Shoji M. *Bioorg. Med. Chem. Lett.*, **2007**, 17, 3736.
24. Banerjee A. K., Bandyopadhyay S., Gayen A. K., Sengupta T., Das A. K., Chatterjee G. K., Chaudhuri S. K. *Arzneimittelforschung*, **1994**, 44, 863.
25. Mao J., Yuan H., Wang Y., Wan B., Pieroni M., Huang Q., Breemen R. B. V., Kozikowski A. P., Franzblau S. G. *J. Med. Chem.*, **2009**, 52, 6966.
26. Rahman M. U., Rathore A., Siddiqui A. A., Parveen G., Shahar Y. M. *BioMed Res. Int.*, **2014**, 2014, 739056.
27. Soman S. S., Soni J. N., Patel T. B. *Med. Chem. Res.*, **2014**, 23, 3803.
28. Solankee A., Tailor R. *ILCPA*, **47**, 2015, 109.
29. Solankee A., Tailor R., Kapadia K. *Chemistry Journal*, **2016**, 6, 80.
30. Solankee A., Tailor R., Kapadia K. *Indian J. Chem.*, **2016**, 55B, 1277.
31. Solankee A., Patel K., Patel R. *E-J. Chem.*, **2012**, 9, 1897.
32. *National Committee for Clinical Laboratory Standards (NCCLS)*, 940, West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA. *Performance standards for antimicrobial susceptibility testing, twelfth informational supplement* [1-56238-454-6, M100-S12 (M7)], **2002**.
33. Rattan A. Antimicrobials in Laboratory Medicine, 5th edn. (Churchill Livingstone, New Delhi), **2000**, 85.

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