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Synthesis, Characterisation and Antimicrobial activities of some newer heterocyclic derivatives: Acetyl Pyrazoline, Isoxazole and Amino pyrimidine bearing 1,3,5-Triazine core

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ABSTRACT

In an attempt to control multidrug resistant bacteria, a library of some new heterocyclic derivatives containing pyrazoline, isoxazole and pyrimidine ring systems bearing 1,3,5-triazine core were designed and synthesised from chalcones. Chalcones (A_I-A_V) react with hydrazine hydrate / glacial aceticacid, hydroxylamine hydrochloride / alkali and guanidine hydrochloride / alkali gives 1-acetyl pyrazoline (B_I-B_V), isoxazole (C_I-C_V) and 2-amino pyrimidine (D_I-D_V) derivatives respectively. The structures of all the newly synthesised compounds were assigned on the basis of FTIR, ^{1}H NMR, ^{13}C NMR spectral data as well as elemental analysis. *In vitro* antimicrobial proficiency of the title compounds were assessed against selected pathogens. The minimum inhibitory concentration (MIC) was determined by broth dilution method and recorded at the lowest concentration inhibiting growth of the organism. Compounds B_I, B_{II}, C_{II}, C_{III} and D_V exhibited excellent antimicrobial activity and said to be the most proficient members of the series.

Introduction

The increasing prevalence of resistant and multi-resistant bacterial strains has become a serious health problem all over the world and continues to challenge both medical and pharmaceutical communities. Thus, research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials or how to treat infections with alternative means. And therefore, it is an ongoing effort to synthesise new antimicrobial agents. Nitrogen fused heterocycles are one of the important classes of molecules that are found in a variety of natural products and biologically active compounds. Among a diverse array of nitrogen fused heterocycles, 1,3,5-triazines (or *s*- triazines) are a well known for a long time and still continue the object of considerable interest mainly due to their applications in various fields. 1,3,5-Triazine core possessing three-fold symmetry, allows for versatile modifications, uncomplicated by region chemical concerns and has proven a useful biological target [1]. 1,3,5-Triazine compounds have been studied extensively and are the subject of many reviews [2]. The triazine scaffold provides the basis for the design of molecules and structural modification of various heterocycles at the 2-, 4- and 6- positions has led to the development of several derivatives with a wide range of therapeutic activities such as trypanosomatid parasite [3], antimalarial [4], anti-HIV [5], antimicrobial [6], antileishmanial [7], autoimmune disease [8] etc… . Many chemists have synthesised effective antimicrobial agents containing 1,3,5-triazine as a core moiety [9,10]. Thus, we are focusing to synthesise novel antimicrobial agents bearing 1,3,5- triazine core. Herein we report on the preparation of a series of new 1-acetyl pyrazoline, isoxazole and 2-amino pyrimidine bearing 1,3,5- triazine core.

Pyrazolines are well-known important nitrogen containing five membered heterocyclic bioorganic molecules. These pyrazolines are used widely in the current decades due to their various biological and pharmacological activities like antitumor [11], antimicrobial [12], anti-inflammatory [13], antidepressant [14], anticancer [15] etc... . After the pioneering work of Fischer and Knoevenagel in the 19th century, the reaction of α, β-unsaturated aldehydes and ketones with hydrazine hydrate in acetic acid under reflux became one of the most popular methods for the preparation of pyrazolines [16-18]. Among the existing various pyrazoline type derivatives, 1-acetylpyrazolines have been identified as one of the most promising scaffolds. Based on the above importance and biological activities exhibited by the pyrazoline compounds, herein we report, the synthesis and biological evaluation of pyrazoline derivatives.

Isoxazole constitute a unique class of nitrogen and oxygen containing five membered heterocycles. Isoxazole forms the basis for several drugs such as Leflunomide (a disease-modifying antirheumatic drug, DMARD), Valdecoxib (a COX-2 inhibitor), and Zonisamide (an anticonvulsant). During the past years considerable evidence has accumulated to demonstrate the importance of isoxazole derivatives. They are associated with wide spectrum of biological activities such as antimicrobial [19], analgesic [20], antitubercular [21], anticancer [22] etc... . Owing to the mentioned biological activities and importance of isoxazole in medicinal prompted us to synthesise various substituted isoxazole derivatives.

Pyrimidine is a six member heterocyclic compound containing four carbon and two nitrogen atoms and is pharmacologically inactive but its synthetic derivatives possess an important role in modern medicine. Pyrimidine is the parent heterocycle of a very important group of compounds that have been extensively studied due to their importance in medicinal chemistry [23]. In medicinal chemistry, pyrimidine derivatives have been very well known for their therapeutic applications. The presence of a pyrimidine base in thymine, uracil and cytosine, which are essential building blocks of nucleic acids,

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DNA and RNA, is one possible reason for their activity. They possess broad range of pharmacological activities such as antifolate [24], anti AIDS agents [25], anti-infective agents [26], anticancer [27], antibacterial [28] etc... . In view of these observations and in continuation of our research, we report herein the synthesis of 2-amino pyrimidine derivatives from substituted chalcones, which have been found to possess an interesting profile of antimicrobial activity.

Material and Methods

The reagents and solvents used for reaction were of analytical reagent (AR) grade. Melting points were resolute in open capillary method and are uncorrected. IR spectra were recorded on a Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using $CDCl_3$ as a solvent and TMS as an internal standard at 400 MHz. 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. Elemental analysis was carried out by Perkin-Elmer 2400 series-II elemental analyser (Perkin-Elmer, USA). 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 located using toluene : methanol eluents and detection of the components were made by exposure to UV light or keeping the plates in iodine chamber. Reference drugs used for antimicrobial evaluation were Ampicillin, Chloramphenicol, Ciprofloxacin, Griseofulvin and Nystatin of commercial grade.

General procedure for the compounds (I), (II), (III) and (A_I - A_V)

Compounds (**I**), (**II**) and (**III**) were prepared by the reported method [29]. The chalcones (A_I-A_V) were prepared by the reported method [30] in good yield by a base catalysed Claisen-Schmidt condensation of appropriately substituted benzaldehydes and substituted ketone (**III**).

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

A 100 ml round bottomed flask, fitted with a reflux condenser was charged with a mixture of an appropriate chalcone (A_I) (0.01 mol, 6.0 gm in 30 ml ethanol) and hydrazine hydrate (0.015 mol, 0.75 gm in 5 ml ethanol). To make the mixture acidic catalytic amount of glacial acetic acid (5 ml) was added. The reaction mixture was heated under reflux temperature for 5-6 hours. The progress of the reaction was investigated by TLC using toluene: methanol (12:6 v/v) eluent as mobile phase. After completion of the reaction, the mixture was cooled to room temperature then poured into crushed ice and neutralised with Na_2CO_3. The solid mass separated was collected by filtration, washed well with hot water and recrystallised from methanol to get product (B_I) in good yield with high purity. Similarly, the remaining compounds (B_{II}-B_V) were synthesised by this given method.

Preparation of 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5''- (3, 4'''- dimethoxyphenyl) 2'' - isoxazol - 3''- yl} phenylamino] - 1,3,5- triazine (C_I)

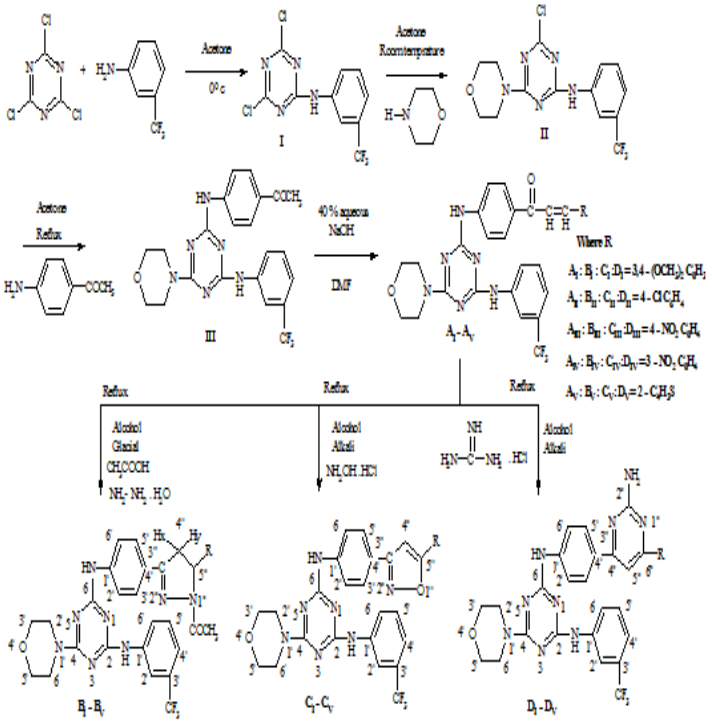
Compound (A_1) (0.01 mol, 6.0 gm in 30 ml ethanol) condensed with hydroxylamine hydrochloride (0.01mol, 0.69 gm in 5 ml ethanol) in the presence of alkaline medium (5 ml 40% KOH) in ethanol at refluxed temperature for 5-6 hours in 100 ml round bottomed flask. The progress of the reaction was monitored 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 to get product (C_I) in good yield with high purity. In the same way, the remaining compounds (C_{II}-C_V) were prepared by this given method.

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

Compound **(A_1)** (0.01 mol, 6.0 gm in 30 ml ethanol) and guanidine hydrochloride (0.015 mol, 1.43 gm in 5 ml ethanol) dissolved in ethanol was mixed in 100 ml round bottomed flask. To make this mixture alkaline 40% KOH (3 ml) was added to the reaction mixture and refluxed for 4-5 hours. The progress of the reaction was investigated 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 neutralised with dilute HCl. Finally, the product was filtered, washed with water, dried and recrystallised from methanol to get product (D_I) in good yield with high purity. Similarly, the remaining compounds (D_{II}-D_V) were prepared by this method.

All the newly synthesised compounds (A_I-A_V), (B_I-B_V) and (D_I-D_V) were characterised by IR, ^{1}H NMR, and ^{13}C NMR as well as elemental analysis. The characteristic data of the entire synthesised compounds are given in spectral analysis data.

Reaction Scheme



Methodical synthetic route for the target compounds (B_I - B_V), (C_I - C_V) and (D_I -D_V)

Spectral analysis data

Compound B_I

2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1''- acetyl 5''- (3, 4'''- dimethoxyphenyl) 2'' - pyrazolin 3''- yl} phenylamino] -1,3,5- triazine Yield 77%; m.p. 239^{0}C; Anal. Calcd. for $C_{33}H_{33}N_8F_3O_4$: C, 59.82; H, 5.02; N, 16.91%. Found: C, 59.79; H, 5.00; N, 16.87%; IR (KBr, ν_{max}, cm^{-1}): 3360 (-NH streching), 3009 (aromatic =CH streching),

2903 (C-H streching of pyrazoline moiety), 1650 & 1581 (C=O and C=N streching of pyrazoline moiety), 1547 (-NH bending), 1509 (aromatic C=C streching), 1336 (CH_3 streching of pyrazoline moiety), 1221 (asymmetric C-O-C streching of ether linkage), 1023 (C-F streching), 802 (C=N streching of 1,3,5-triazine), 689 & 838 (C-H bending of 1,3 and 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.3 (s, 3H, -COC$\underline{H}_3$), 3.2 (dd, *J* = 11.2 & 13.3 Hz, 1H, -C$\underline{H}_x$-CH), 3.6 (dd, *J* = 11.2 & 13.6 Hz, 1H, -C$\underline{H}_y$-CH), 5.6 (dd, *J* = 5.8 & 12.9 Hz, 1H, -C$\underline{H}$-CH_2-Ar), 3.7 (s, 3H, 3-OC$\underline{H}_3$), 3.9 (s, 3H, 4-OC$\underline{H}_3$), 3.73 (concealed t, 4H, -CH_2, oxazine ring), 3.84 (concealed t, 4H, -CH_2, oxazine ring), 7.0 to 8.4 (m, 12H, 11 Ar-$\underline{H}$ and 1-N$\underline{H}$); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 23.2 (CH_3, pyrazoline moiety), 38.2 (CH_2, methylene, pyrazoline moiety), 43.2 (CH_2, oxazine), 54.2 (3-OCH_3), 56.7 (4-OCH_3), 63.0 (CH-Ar), 67.1 (CH_2, oxazine), 113.3 (CH), 113.8 (CH), 114.3 (CH), 116.8 (CH), 119.2 (CH), 121.0 (CH), 124.2 (CF_3), 128.3 (CH), 130.2 (CH), 132.5 (C), 133.2 (CH), 137.2 (CH), 141.8 (C), 143.2 (C), 145.3 (C), 148.8 (C), 151.2 (C), 152.4 (C=N), 163.0, 165.8 & 169.4 (C=N, 1,3,5-triazine), 173.4 (CO pyrazoline moiety).

Compound B_{II}

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

Yield 63%; m.p. 153^{0}C; Anal. Calcd. for $C_{31}H_{28}N_8F_3O_2Cl$: C, 58.45; H, 4.43; N, 17.59%. Found: C,58.41; H, 4.46; N, 17.62%; IR (KBr, ν_{max}, cm^{-1}): 3378 (-NH streching), 3015 (aromatic =CH streching), 2905 (C-H streching of pyrazoline moiety), 1661 & 1579 (C=O and C=N streching of pyrazoline moiety), 1536 (-NH bending), 1520 (aromatic C=C streching), 1342 (CH_3 streching of pyrazoline moiety), 1210 (asymmetric C-O-C streching of ether linkage), 1041 (C-F streching), 849 (1,4 disubstituted benzene ring), 798 (C=N streching of 1,3,5-triazine), 682 (C-Cl streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.2 (s, 3H, -COC$\underline{H}_3$), 2.9 (dd, *J* = 10.9 & 12.7 Hz, 1H, -C$\underline{H}_x$-CH), 3.3 (dd, *J* = 10.9 & 12.5 Hz, 1H, -C$\underline{H}_y$-CH), 5.3 (dd, *J* = 5.7 & 12.5 Hz, 1H, -C$\underline{H}$-CH_2-Ar), 3.58 (concealed t, 4H, -CH_2, oxazine ring), 3.79 (concealed t, 4H, -CH_2, oxazine ring), 6.7 to 8.2 (m, 13H, 12 Ar-$\underline{H}$ and 1-N$\underline{H}$); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 22.8 (CH_3, pyrazoline moiety), 38.8 (CH_2, methylene, pyrazoline moiety), 44.0 (CH_2, oxazine), 62.3 (CH-Ar), 65.2 (CH_2, oxazine), 112.2 (CH), 113.6 (CH), 115.2 (CH), 117.1 (CH), 118.3 (CH), 120.3 (CH), 121.8 (CH), 123.3 (CF_3), 127.1 (CH), 129.6 (CH), 131.0 (C), 132.4(C), 133.4 (CH), 142.0 (C), 144.3 (C), 146.0 (C), 151.8 (C=N), 164.4, 166.2 & 168.9 (C=N, 1,3,5-triazine), 172.2 (CO pyrazoline moiety).

Compound B_{III}

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

Yield 73%; m.p. 255^{0}C; Anal. Calcd. for $C_{31}H_{28}N_9F_3O_4$: C, 57.50; H, 4.35; N, 19.47%. Found: C, 57.47; H, 4.36; N, 19.51%; IR (KBr, ν_{max}, cm^{-1}): 3246 (-NH streching), 3087 (aromatic =CH streching), 2989 (C-H streching of pyrazoline moiety), 1665 & 1571 (C=O and C=N streching of pyrazoline moiety), 1565 (-NH bending), 1505 (aromatic C=C streching), 1458 (C-NO_2 streching), 1356 (CH_3 streching of pyrazoline moiety), 1248 (asymmetric C-O-C streching of ether linkage), 1067 (C-F streching), 830 (1,4 disubstituted benzene ring) 812 (C=N streching of 1,3,5-triazine); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.6 (s, 3H, -COC$\underline{H}_3$), 3.2 (dd, *J* = 11.9 & 12.5 Hz, 1H, -C$\underline{H}_x$-CH), 3.8 (dd, *J* = 11.2 & 12.7 Hz, 1H, -C$\underline{H}_y$-CH), 5.0 (dd, *J* = 4.9 & 12.6 Hz, 1H, -C$\underline{H}$-CH_2-Ar), 3.64 (concealed t, 4H, -CH_2, oxazine ring), 3.81 (concealed t, 4H, -CH_2, oxazine ring), 7.2 to 8.5 (m, 13H, 12 Ar-$\underline{H}$ and 1-N$\underline{H}$); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 22.2 (CH_3, pyrazoline moiety), 39.3 (CH_2, methylene, pyrazoline moiety), 44.3 (CH_2, oxazine), 63.3 (CH-Ar), 66.4 (CH_2, oxazine), 110.2 (CH), 112.4 (CH), 113.3 (CH), 115.4 (CH), 117.2 (CH), 119.4 (CH), 122.0 (CH), 124.9 (CF_3), 126.5 (CH), 128.2 (CH), 132.3 (C), 133.3 (CH), 141.4 (C), 143.2 (C), 145.3 (C), 148.4 (C), 153.4 (C=N), 163.0, 165.3 & 167.2 (C=N, 1,3,5-triazine), 171.3 (CO pyrazoline moiety).

Compound B_{IV}

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

Yield 67%; m.p. 220^{0}C; Anal. Calcd. for $C_{31}H_{28}N_9F_3O_4$: C, 57.50; H, 4.35; N, 19.47%. Found: C, 57.49; H, 4.32; N, 19.46%; IR (KBr, ν_{max}, cm^{-1}): 3241 (-NH streching), 3098 (aromatic =CH streching), 2962 (C-H streching of pyrazoline moiety), 1657 & 1586 (C=O and C=N streching of pyrazoline moiety), 1551 (-NH bending), 1497 (aromatic C=C streching), 1455 (C-NO_2 streching), 1348 (CH_3 streching of pyrazoline moiety), 1236 (asymmetric C-O-C streching of ether linkage), 1045 (C-F streching), 843 (1,4 disubstituted benzene ring), 808 (C=N streching of 1,3,5-triazine); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.8 (s, 3H, -COC$\underline{H}_3$), 2.9 (dd, *J* = 12.7 & 13.4 Hz, 1H, -C$\underline{H}_x$-CH), 3.4 (dd, *J* = 12.7 & 13.4 Hz, 1H, -C$\underline{H}_y$-CH), 5.9 (dd, *J* = 4.7 & 12.8 Hz, 1H, -C$\underline{H}$-CH_2-Ar), 3.69 (concealed t, 4H, -CH_2, oxazine ring), 3.93 (concealed t, 4H, -CH_2, oxazine ring), 7.0 to 8.3 (m, 13H, 12 Ar-$\underline{H}$ and 1-N$\underline{H}$); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 22.1 (CH_3, pyrazoline moiety), 38.9 (CH_2, methylene, pyrazoline moiety), 43.8 (CH_2, oxazine), 64.5 (CH-Ar), 67.2 (CH_2, oxazine), 111.4 (CH), 113.1 (CH), 115.4 (CH), 116.0 (CH), 118.3 (CH), 120.2 (CH), 121.8 (CH), 123.3 (CF_3), 127.0 (CH), 128.8 (CH), 133.2 (C), 134.4 (CH), 143.4 (C), 144.3 (C), 145.0 (C), 149.5 (C), 150.2 (C=N), 165.3, 167.4 & 168.2 (C=N, 1,3,5-triazine), 169.2 (CO pyrazoline moiety).

Compound B_V

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

Yield 62%; m.p. 114^{0}C; Anal. Calcd. for $C_{29}H_{27}N_8F_3O_2S$: C, 57.23; H, 4.47; N, 18.41%. Found: C, 57.20; H, 4.51; N, 18.39%; IR (KBr, ν_{max}, cm^{-1}): 3289 (-NH streching), 3053 (aromatic =CH streching), 2924 (C-H streching of pyrazoline moiety), 1623 & 1573 (C=O and C=N streching of pyrazoline moiety), 1565 (-NH bending), 1486 (aromatic C=C streching), 1387 (CH_3 streching of pyrazoline moiety), 1227 (asymmetric C-O-C streching of ether linkage), 1099 (C-F streching), 802 (C=N streching of 1,3,5-triazine), 643 (C-S-C streching of sulphur linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.5 (s, 3H, -COC$\underline{H}_3$), 3.6 (dd, *J* = 11.7 & 13.8 Hz, 1H, -C$\underline{H}_x$-CH), 4.1 (dd, *J* = 12.0 & 13.8 Hz, 1H, -C$\underline{H}_y$-CH), 4.9 (dd, *J* = 5.2 & 13.6 Hz, 1H, -C$\underline{H}$-CH_2-Ar), 3.39 (concealed t, 4H, -CH_2, oxazine ring), 3.72 (concealed t, 4H, -CH_2, oxazine ring), 7.3 to 8.1 (m, 12H, 11 Ar-$\underline{H}$ and 1-N$\underline{H}$); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 24.2 (CH_3, pyrazoline moiety), 40.1 (CH_2, methylene, pyrazoline moiety), 43.1 (CH_2, oxazine), 61.2 (CH-Ar), 66.0 (CH_2, oxazine), 112.2 (CH), 114.1 (CH), 116.8 (CH), 117.4 (CH), 118.2 (CH), 121.4 (CH), 122.2 (CH), 124.4 (CF_3), 126.5 (CH), 127.2 (CH), 129.3 (CH), 132.4 (C), 142.2 (C), 143.8 (C), 151.4 (C=N), 164.2, 166.0 & 165.4 (C=N, 1,3,5-triazine), 168.4 (CO pyrazoline moiety).

Table 1. Antimicrobial activity data of synthesised compounds (B_I-B_V), (C_I-C_V) and (D_I-D_V)

Compd	Minimal bactericidal concentration MIC - µg/ml				Minimal fungicidal concentration MIC - µg/ml		
	Gram positive		Gram negative				
	S. a	S. p	E. c	P. a	C. a	A. n	A. c
B_I	200	125	100	250	1000	100	200
B_{II}	250	100	62.5	100	500	200	500
B_{III}	500	200	100	250	1000	>1000	>1000
B_{IV}	100	250	125	250	1000	>1000	>1000
B_V	250	200	125	125	>1000	250	250
C_I	100	200	125	250	500	>1000	>1000
C_{II}	250	250	125	125	500	250	250
C_{III}	200	100	250	100	500	>1000	>1000
C_{IV}	200	250	100	250	250	1000	1000
C_V	100	125	200	200	1000	1000	1000
D_I	250	200	250	125	500	250	500
D_{II}	500	200	250	200	1000	250	250
D_{III}	200	125	125	200	1000	>1000	>1000
D_{IV}	250	250	200	250	500	500	1000
D_V	62.5	125	500	125	250	1000	1000
Ampi.	250	100	100	100	-	-	-
Chlo.	50	50	50	50	-	-	-
Cipr.	50	50	25	25	-	-	-
Gris.	-	-	-	-	500	100	100
Nyst.	-	-	-	-	100	100	100

Compound C_I

2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1',4' - oxazine) - 6 - [4' - {5''- (3, 4'''- dimethoxyphenyl) 2'' - isoxazol - 3''- yl} phenylamino] - 1,3,5- triazine

Yield 68%; m.p. 126^{0}C; Anal. Calcd. for $C_{31}H_{28}N_7F_3O_4$: C, 60.10; H, 4.55; N, 15.83%. Found: C, 60.07; H, 4.59; N, 15.81%; IR (KBr, $v_{max,}$ cm^{-1}): 3154 (-NH streching), 3089 (aromatic =CH streching), 2910 (C-H streching of isoxazole moiety), 1579 (C=N streching of isoxazole moiety), 1563 (-NH bending), 1521 (aromatic C=C streching), 1260 (asymmetric C-O-C streching of ether linkage), 1041 (C-F streching), 806 (C=N streching of 1,3,5-triazine), 678 & 847 (C-H bending of 1,3 and 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.54 (s, 3H, 3-OC<u>H</u>$_3$), 3.75 (s, 3H, 4-OC<u>H</u>$_3$), 3.61 (concealed t, 4H, -CH_2, oxazine ring), 3.89 (concealed t, 4H, -CH_2, oxazine ring), 6.7 (s, 1H, -C<u>H</u>-C), 7.2 to 8.3 (m, 12H, 11 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 43.9 ($CH_{2,}$ oxazine), 55.8 (3-OCH_3), 56.4 (4-OCH_3), 67.2 ($CH_{2,}$ oxazine), 98.8 (CH, isoxazole moiety), 115.1 (CH), 116.0 (CH), 116.8 (CH), 118.2 (C), 118.9 (C), 121.1 (CF_3), 127.3 (CH), 128.2 (CH), 129.8 (CH), 131.8 (C), 139.9 (C), 142.7 (C), 158.4 (C), 159.8 (C), 162.2 (C=N, isoxazole moiety), 169.4 (C-Ar, isoxazole moiety), 166.7, 168.3 & 169.3 (C=N, 1,3,5- triazine).

Compound C_{II}

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

Yield 65%; m.p. 165^{0}C; Anal. Calcd. for $C_{29}H_{23}N_7F_3O_2Cl$: C, 58.64; H, 3.90; N, 16.51%. Found: C, 58.66; H, 3.87; N, 16.53%; IR (KBr, $v_{max,}$ cm^{-1}): 3195 (-NH streching), 3078 (aromatic =CH streching), 2913 (C-H streching of isoxazole moiety), 1565 (C=N streching of isoxazole moiety), 1557 (-NH bending), 1526 (aromatic C=C streching), 1248 (asymmetric C-O-C streching of ether linkage), 1054 (C-F streching), 801 (C=N streching of 1,3,5- triazine), 839 (C-H bending of 1,4 disubstituted benzene ring), 689 (C-Cl streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.42 (concealed t, 4H, -CH_2, oxazine ring), 3.64 (concealed t, 4H, -CH_2, oxazine ring), 6.2 (s, 1H, -C<u>H</u>-C), 7.0 to 8.4 (m, 13H, 12 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 44.2 ($CH_{2,}$ oxazine), 66.3 ($CH_{2,}$ oxazine), 99.2 (CH, isoxazole moiety), 114.2 (CH), 116.3 (CH), 117.8 (CH), 119.1 (C), 119.8 (C), 120.2 (CH), 122.3 (CH), 123.7 (CF_3), 126.4 (CH), 128.0 (CH), 130.1 (CH), 131.3 (C), 132.2 (C), 137.6 (C), 141.8 (C), 163.4 (C=N, isoxazole moiety), 168.2, (C-Ar, isoxazole moiety), 166.2, 169.4 & 170.0 (C=N, 1,3,5-triazine).

Compound C_{III}

2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1',4' - oxazine) - 6 - [4' - {5''- (4'''- nitrophenyl) 2'' - isoxazol - 3''- yl} phenylamino] - 1,3,5- triazine

Yield 75%; m.p. 155^{0}C; Anal. Calcd. for $C_{29}H_{23}N_8F_3O_4$: C, 57.62; H, 3.83; N, 18.54%. Found: C, 57.65; H, 3.79; N, 18.49%; IR (KBr, $v_{max,}$ cm^{-1}): 3210 (-NH streching), 3021 (aromatic =CH streching), 2929 (C-H streching of isoxazole moiety), 1583 (C=N streching of isoxazole moiety), 1541 (-NH bending), 1519 (aromatic C=C streching), 1448 (C-NO_2 streching), 1231 (asymmetric C-O-C streching of ether linkage), 1065 (C-F streching), 805 (C=N streching of 1,3,5- triazine), 851 (C-H bending of 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.58 (concealed t, 4H, -CH_2, oxazine ring), 3.79 (concealed t, 4H, -CH_2, oxazine ring), 6.5 (s, 1H, -C<u>H</u>-C), 6.9 to 8.1 (m, 13H, 12 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 44.2 ($CH_{2,}$ oxazine), 66.3 ($CH_{2,}$ oxazine), 99.2 (CH, isoxazole moiety), 114.2 (CH), 116.3 (CH), 117.8 (CH), 119.1 (C), 119.8 (C), 120.2 (CH), 122.3(CH), 123.7 (CF_3), 126.4 (CH), 128.0 (CH), 130.1 (CH), 131.3 (C), 132.2 (C), 137.6 (C), 141.8 (C), 163.4 (C=N, isoxazole moiety), 168.2, (C-Ar, isoxazole moiety), 166.2, 169.4 & 170.0 (C=N, 1,3,5-triazine).

Compound C_{IV}

2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1',4' - oxazine) - 6 - [4' - {5''- (3'''- nitrophenyl) 2'' - isoxazol - 3''- yl} phenylamino] - 1,3,5- triazine

Yield 70%; m.p. 131^{0}C; Anal. Calcd. for $C_{29}H_{23}N_8F_3O_4$: C, 57.62; H, 3.83; N, 18.54%. Found: C, 57.59; H, 3.82; N, 18.57%; IR (KBr, $v_{max,}$ cm^{-1}): 3213 (-NH streching), 3026

(aromatic =CH streching), 2933 (C-H streching of isoxazole moiety), 1596 (C=N streching of isoxazole moiety), 1537 (-NH bending), 1525 (aromatic C=C streching), 1430 (C-NO_2 streching), 1236 (asymmetric C-O-C streching of ether linkage), 1058 (C-F streching), 803 (C=N streching of 1,3,5-triazine), 630 (C-H bending of 1,3 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.81 (concealed t, 4H, -CH_2, oxazine ring), 4.03 (concealed t, 4H, -CH_2, oxazine ring), 6.2 (s, 1H, -C<u>H</u>-C), 7.0 to 8.3 (m, 13H, 12 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 44.2 (CH_2, oxazine), 66.3 (CH_2, oxazine), 99.2 (CH, isoxazole moiety), 114.2 (CH), 116.3 (CH), 117.8 (CH), 119.1 (C), 119.8 (C), 120.2 (CH), 122.3(CH), 123.7 (CF_3), 126.4 (CH), 128.0 (CH), 130.1 (CH), 131.3 (C), 132.2 (C), 137.6 (C), 141.8 (C), 163.4 (C=N, isoxazole moiety), 168.2, (C-Ar, isoxazole moiety), 166.2, 169.4 & 170.0 (C=N, 1,3,5-triazine).

Compound C_V

2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1',4' - oxazine) - 6 - [4' - {5''- (2'''- thienyl) 2'' - isoxazol - 3''- yl} phenylamino] - 1,3,5- triazine

Yield 64%; m.p. 117^{0}C; Anal. Calcd. for $C_{27}H_{22}N_7F_3O_2S$: C, 57.34; H, 3.92; N, 17.34%. Found: C, 57.37; H, 3.94; N, 17.37%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3312 (-NH streching), 3039 (aromatic =CH streching), 2967 (C-H streching of isoxazole moiety), 1546 (C=N streching of isoxazole moiety), 1520 (-NH bending), 1517 (aromatic C=C streching), 1240 (asymmetric C-O-C streching of ether linkage), 1091 (C-F streching), 795 (C=N streching of 1,3,5-triazine), 653 (C-S-C streching of sulphur linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.39 (concealed t, 4H, -CH_2, oxazine ring), 4.66 (concealed t, 4H, -CH_2, oxazine ring), 6.9 (s, 1H, -C<u>H</u>-C), 7.7 to 8.6 (m, 12H, 11 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 44.2 (CH_2, oxazine), 66.3 (CH_2, oxazine), 99.2 (CH, isoxazole moiety), 114.2 (CH), 116.3 (CH), 117.8 (CH), 119.1 (C), 119.8 (C), 120.2 (CH), 122.3(CH), 123.7 (CF_3), 126.4 (CH), 128.0 (CH), 130.1 (CH), 131.3 (C), 132.2 (C), 137.6 (C), 141.8 (C), 163.4 (C=N, isoxazole moiety), 168.2, (C-Ar, isoxazole moiety), 166.2, 169.4 & 170.0 (C=N, 1,3,5-triazine).

Compound D_I

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

Yield 76%; m.p. 131^{0}C; Anal. Calcd. for $C_{32}H_{30}N_9F_3O_3$: C, 59.55; H, 4.68; N, 19.53%. Found: C, 59.53; H, 4.69; N, 19.50%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3313 (NH_2 str. 1^0 amine of pyrimidine moiety), 3025 (aromatic =CH streching), 2999 (C-H streching of pyrimidine moiety), 1647 (C=N streching of pyrimidine moiety), 1571 (-NH bending), 1525 (aromatic C=C streching), 1234 (asymmetric C-O-C streching of ether linkage), 1081 (C-F streching), 812 (C=N streching of 1,3,5- triazine), 658 & 830 (C-H bending of 1,3 and 1,4 disubstituted benzene ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.72 (s, 3H, 3-OC<u>H</u>$_3$), 3.87 (s, 3H, 4-OC<u>H</u>$_3$), 3.42 (concealed t, 4H, -CH_2, oxazine ring), 3.61 (concealed t, 4H, -CH_2, oxazine ring), 5.06 (s, 2H,-NH_2), 7.2 to 8.3 (m, 13H, 12 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 47.1 (CH_2, oxazine), 54.2 (3-OCH_3), 57.4 (4-OCH_3), 65.2 (CH_2, oxazine), 101.2 (CH, pyrimidine moiety), 112.2 (CH), 116.1 (CH), 116.6 (CH), 121.2 (CH), 123.8 (CF_3), 125.2 (CH), 128.3 (CH), 128.7 (CH), 129.5 (CH), 131.3 (C), 137.4 (C), 142.2 (C), 157.3 (C), 158.0 (C), 162.4, 163.8, 165.2 (C, pyrimidine moiety), 164.3, 167.9 & 169.8 (C=N, 1,3,5-triazine).

Compound D_{II}

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

Yield 69%; m.p. 150^{0}C; Anal. Calcd. for $C_{30}H_{25}N_9F_3OCl$: C, 58.12; H, 4.06; N, 20.33%. Found: C, 58.10; H, 4.10; N, 20.34%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3426 (NH_2 str. 1^0 amine of pyrimidine moiety), 3065 (aromatic =CH streching), 3012 (C-H streching of pyrimidine moiety), 1660 (C=N streching of pyrimidine moiety), 1545 (-NH bending), 1519 (aromatic C=C streching), 1220 (asymmetric C-O-C streching of ether linkage), 1112 (C-F streching), 843 (C-H bending of 1,4 disubstituted benzene ring), 803 (C=N streching of 1,3,5-triazine), 672 (C-Cl streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.76 (concealed t, 4H, -CH_2, oxazine ring), 3.94 (concealed t, 4H, -CH_2, oxazine ring), 5.26 (s, 2H,-NH_2), 6.8 to 8.2 (m, 14H, 13 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 46.5 (CH_2, oxazine), 66.1 (CH_2, oxazine), 100.8 (CH, pyrimidine moiety), 112.9 (CH), 115.3 (CH), 117.1 (CH), 120.2 (CH), 124.1 (CF_3), 125.4 (CH), 127.6 (CH), 128.0 (CH), 130.2 (CH), 132.5 (C), 133.6 (C), 138.0 (C), 143.1 (C), 161.2, 162.4, 164.5 (C, pyrimidine moiety), 165.4, 168.3 & 170.3 (C=N, 1,3,5-triazine).

Compound D_{III}

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

Yield 75%; m.p. 149^{0}C; Anal. Calcd. for $C_{30}H_{25}N_{10}F_3O_3$: C, 57.14; H, 3.99; N, 22.21%. Found: C, 57.11; H, 3.95; N, 22.17%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3412 (NH_2 str. 1^0 amine of pyrimidine moiety), 3026 (aromatic =CH streching), 3019 (C-H streching of pyrimidine moiety), 1628 (C=N streching of pyrimidine moiety), 1589 (-NH bending), 1574 (aromatic C=C streching), 1439 (C-NO_2 streching), 1239 (asymmetric C-O-C streching of ether linkage), 1100 (C-F streching), 834 (C-H bending of 1,4 disubstituted benzene ring), 806 (C=N streching of 1,3,5-triazine); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.52 (concealed t, 4H, -CH_2, oxazine ring), 3.81 (concealed t, 4H, -CH_2, oxazine ring), 4.92 (s, 2H,-NH_2), 6.9 to 8.3 (m, 14H, 13 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 45.0 (CH_2, oxazine), 64.2 (CH_2, oxazine), 101.4 (CH, pyrimidine moiety), 111.5 (CH), 113.8 (CH), 116.2 (CH), 118.5 (CH), 123.7 (CF_3), 126.5 (CH), 127.9 (CH), 129.0 (CH), 131.5 (CH), 133.2 (C), 137.5 (C), 142.9 (C), 147.8 (C), 163.5, 164.3, 165.9 (C, pyrimidine moiety), 166.2, 168.4 & 170.5 (C=N, 1,3,5-triazine).

Compound D_{IV}

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

Yield 71%; m.p. 171^{0}C; Anal. Calcd. for $C_{30}H_{25}N_{10}F_3O_3$: C, 57.14; H, 3.99; N, 22.21%. Found: C, 57.17; H, 4.02; N, 22.19%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3409 (NH_2 str. 1^0 amine of pyrimidine moiety), 3031 (aromatic =CH streching), 3015 (C-H streching of pyrimidine moiety), 1636 (C=N streching of pyrimidine moiety), 1583 (-NH bending), 1565 (aromatic C=C streching), 1446 (C-NO_2 streching), 1232 (asymmetric C-O-C streching of ether linkage), 1097 (C-F streching), 671 (C-H bending of 1,3 disubstituted benzene ring), 802 (C=N streching of 1,3,5-triazine); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.69 (concealed t, 4H, -CH_2, oxazine ring), 4.09 (concealed t, 4H, -CH_2, oxazine ring), 5.57 (s, 2H,-NH_2), 7.3 to 8.1 (m, 14H, 13 Ar-<u>H</u> and 1-N<u>H</u>); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 44.9 (CH_2, oxazine), 63.2 (CH_2, oxazine), 101.0 (CH, pyrimidine moiety), 110.8 (CH), 112.5 (CH), 115.1 (CH), 117.8 (CH),

124.2 (CF_3), 125.7 (CH), 126.6 (CH), 128.1 (CH), 130.3 (CH), 132.8 (C), 136.6 (C), 142.1 (C), 146.4 (C), 162.8, 165.6, 167.0 (C, pyrimidine moiety), 169.4, 170.2 & 172.6 (C=N, 1,3,5-triazine).

Compound D_V

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

Yield 62%; m.p. 135^{0}C; Anal. Calcd. for $C_{28}H_{24}N_9F_3OS$: C, 56.85; H, 4.09; N, 21.31%. Found: C, 56.83; H, 4.11; N, 21.34%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3379 (NH_2 str. 1^0 amine of pyrimidine moiety), 3089 (aromatic =CH streching), 3031 (C-H streching of pyrimidine moiety), 1613 (C=N streching of pyrimidine moiety), 1565 (-NH bending), 1559 (aromatic C=C streching), 1226 (asymmetric C-O-C streching of ether linkage), 1083 (C-F streching), 796 (C=N streching of 1,3,5- triazine), 641 (C-S-C streching of sulphur linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.32 (concealed t, 4H, -CH_2, oxazine ring), 3.53 (concealed t, 4H, -CH_2, oxazine ring), 5.79 (s, 2H,-NH_2), 7.0 to 8.4 (m, 13H, 12 Ar-H and 1-NH); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 46.9 (CH_2, oxazine), 66.2 (CH_2, oxazine), 99.6 (CH, pyrimidine moiety), 112.5 (CH), 113.8 (CH), 114.1 (CH), 118.6 (CH), 124.5 (CF_3), 126.8 (CH), 128.9 (CH), 131.2 (CH), 134.5 (C), 138.6 (C), 142.0 (C), 162.2, 163.0, 165.9 (C, pyrimidine moiety), 167.5, 168.9 & 171.2 (C=N, 1,3,5-triazine).

Result and discussion

Chemistry

The synthetic route used to synthesise the unreported title compounds **(B_I-B_V), (C_I-C_V)** and **(D_I-D_V)** is illustrated in reaction scheme. The aim of the present study was to develop an efficient protocol with good to excellent yields in a short span of time without formation of any side product. The formation of all these new heterocyclic derivatives were fully characterised by means of spectroscopic techniques such as FT-IR, ^{1}H NMR, and ^{13}C NMR. As an example, in the IR spectrum of compound **B_I**, a strong absorption band is observed at 1650 cm^{-1} which corresponds 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 is observed at 1581 and 1509 cm^{-1} respectively. The C_4''-H stretching of pyrazoline ring was observed at 2903 cm^{-1}. A strong absorption band was observed at 1336 cm^{-1} due to the presence of the CH_3 group. The aromatic C-H bending vibrations for 1,3 and 1,4 disubstituted benzene ring were observed at 689 and 838 cm^{-1} respectively. The C=N stretching of 1,3,5-triazine core were observed at 802 cm^{-1}. The ^{1}H NMR spectrum of compound **B_I** showed a singlet at δ 2.3 ppm for the $COCH_3$ protons. The pro-chiral methylene protons C_4''-H of pyrazoline appeared as two distinct doublets of a doublet at δ 3.2 ppm (J = 11.2 & 13.3 Hz) and at δ 3.6 ppm (J = 11.2 & 13.6 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 5.6 ppm (J = 5.8 & 12.9 Hz) due to CH-CH_2-Ar proton. The other remaining eleven aromatic protons appeared as a multiplet signal at δ 7.0-8.2 ppm. Finally, the ^{13}C NMR spectra of the cyclised product were recorded in $CDCl_3$ and the spectral signals were in good agreement with the proposed structures. In the ^{13}C NMR spectrum of compound **B_I**, the shielded signal at δ 23.2 and 38.2 ppm was assigned to the methyl and methylene carbon of pyrazoline ring. The most deshielded signal that appeared at δ 173.4 ppm was assigned to the carbonyl carbon of the acetyl group attached with the pyrazoline unit. The signals for aromatic carbons appeared between δ 113.3-151.2 ppm in the ^{13}C spectrum.

The IR spectrum of compound **C_I** exhibited the disappreance of absorption at 1660 cm^{-1} corresponding to >C=O group of chalcone and exhibited absorptions at a strong band at 1579 cm^{-1} due to the C=N of isoxazole unit. The C-H functionality of isoxazole unit was observed at 2910 cm^{-1}. The aromatic C=C stretching, C-H bending vibrations for 1,3 and 1,4 disubstituted benzene ring were appeared at 1521, 678 and 847 cm^{-1} respectively. The C=N stretching of 1,3,5-triazine core were observed at 806 cm^{-1}. The ^{1}H NMR spectrum of compound **C_I** showed chiral C_4''-H proton of isoxazole ring appeared as singlet at δ 6.7 ppm for CH-C proton. The other remaining eleven aromatic protons appeared as a multiplet signal at δ 7.2-8.1 ppm. ^{13}C NMR spectrum of compound **C_I** showed a signal at 98.8, 162.2 and 169.4 due to the –CH carbon, C=N carbon and C-Ar of isoxazole moeity which assigned the isoxazole unit. The signals for aromatic carbons appeared between δ 115.1-159.8 ppm in the ^{13}C spectrum.

The IR spectrum of compound **D_I** showed a strong characteristic band at 1647 cm^{-1} and 3313 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 2999 cm^{-1}. The aromatic C=C stretching, C-H bending vibrations for 1,3 and 1,4 disubstituted benzene ring were appeared at 1525, 658 and 830 cm^{-1} respectively. The C=N stretching of 1,3,5-triazine core were observed at 812 cm^{-1}. The ^{1}H NMR spectrum of compound **D_I** showed a sharp singlet at δ 5.06 due to the NH_2 protons, and it also showed a sharp singlet at δ 7.52 due to HC=C, which confirmed the cyclisation of the chalcone into a pyrimidine ring. The other remaining twelve aromatic protons resonate as a multiplet signal at δ 7.2-8.0 ppm. ^{13}C NMR spectrum of compound **D_I** showed a signal at 101.2 due to the –CH carbon of pyrimidine ring and signal at δ 163.8 and 165.2 ppm assigned to the C=N carbon of pyrimidine ring which assigned the pyrimidine unit. The signals for aromatic carbons appeared between δ 112.2-158.0 ppm in the ^{13}C spectrum. There are no absorptions in the region of 1600-1700 cm^{-1} in IR spectra of compound **C_I** and **D_I** which indicating the absence of a C=O group of chalcone moiety in these structures and further confirmed the cyclisation of chalcone in to its derivatives. Moreover, distinctive singlet around δ 3.54-3.87 ppm stands for methoxy group of aryl ring attached to pyrazoline, isoxazole and pyrimidine unit, singlet around δ 8.1-8.5 ppm stands for secondary amine attached with 1,3,5-triazine which confirmed the presence of 1,3,5-triazine. The obtained elemental analysis values are in good agreement with theoretical data.

In vitro 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 were used as the standard antifungal drugs. The minimal inhibitory concentration (MIC) of all the synthesised compounds was determined by the broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS)[31]. All the synthesised compounds **(B_I-B_V),** **(C_I-C_V)** and **(D_I-D_V)** were screened for

their antibacterial and antifungal activities in three sets against bacteria and fungi used in the present protocol. The results are summarised in **Table 1**.

The antibacterial screening of compounds 1- acetyl pyrazoline (**B_I-B_V**), isoxazole (**C_I-C_V**) and 2-amino pyrimidine derivatives (**D_I-D_V**) pointed out that compound **D_V** showed an outstanding inhibitory effect i.e. MIC = 62.5 µg/ml against Staphylococcus aureus as compared ampicillin (MIC = 250 µg/ml) and equipotent to chloramphenicol and ciprofloxacin (MIC = 50 µg/ml) while compounds **B_{IV}**, **C_I** and **C_V** (MIC = 100 µg/ml) exhibited good activity compared to ampicillin (MIC = 250 µg/ml) and modest to chloramphenicol and ciprofloxacin (MIC = 50 µg/ml) against Staphylococcus aureus. In the case of inhibiting Streptococcus pyogenes, compounds **B_{II}**, **C_{III}** (MIC = 100 µg/ml), **B_I**, **D_{III}**, **C_V** and **D_V** (MIC = 125 µg/ml) were found to be comparable to ampicillin (MIC = 100 µg/ml) and moderate to chloramphenicol and ciprofloxacin (MIC = 50 µg/ml). Whereas in the case of inhibiting Gram negative bacteria, compound **B_{II}** (MIC = 62.5 µg/ml) showed maximum activity against Escherichia coli as compared to ampicillin while compounds **B_I**, **B_{III}** and **C_{IV}** (MIC = 100 µg/ml) showed similar activity against Escherichia coli upon comparison with the standard drug ampicillin and lowest to chloramphenicol (MIC = 50 µg/ml) and ciprofloxacin (MIC = 25 µg/ml). Compounds **B_{II}** and **C_{III}** (MIC = 100 µg/ml), **B_V**, **C_{II}**, **B_{IV}**, **D_I** and **D_V** (MIC = 125 µg/ml) found to possesses equivalent to ampicillin (MIC = 100 µg/ml) and modest to chloramphenicol (MIC = 50 µg/ml) and ciprofloxacin (MIC = 25 µg/ml) against Pseudomonas aeruginosa. Compounds **B_I**, **C_{III}**, **C_{IV}** and **D_{III}** (MIC = 200 µg/ml) exhibited significant activity to ampicillin (MIC = 200 µg/ml) while compounds **B_{II}**, **B_V**, **C_{II}**, **D_I** and **D_{IV}** (MIC = 250 µg/ml) found similar to ampicillin (MIC = 200 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-positive bacteria rather than Gram-negative bacteria.

From in vitro antifungal activity data, it is found that compounds **C_{IV}** and **D_V** (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). Compounds **B_{II}**, **C_I**, **C_{II}**, **C_{III}**, **D_I**, **D_V** and **B_{IV}** showed the same potency as griseofulvin (MIC = 500 µg/ml) against Candida albicans. Compound **B_I** (MIC = 100 µg/ml) showed equipotent to griseofulvin (MIC = 100 µg/ml) and nystatin (MIC = 100 µg/ml) against Aspergillus niger. While none of the compounds were found to be active against the fungal pathogen Aspergillus clavatus.

S. a.: Staphylococcus aureus, S. p.: Streptococcus pyogenes, E. c.: Escherichia coli, P. a.: Pseudomonas aeruginosa, C. a.: Candida albicans, A. n.: Aspergillus niger, A. c.: Aspergillus clavatus. Ampi: Ampicillin, Chlo.: Chloramphenicol, Cipr.: Ciprofloxacin, Gris.: Greseofulvin, Nyst.: Nystatin. '-': not tested.

Conclusion

A series of pyrazoline, isoxazole and pyrimidine derivatives carrying a 1,3,5- triazine core have been synthesised in good yield and screened for their biological activity with the aim of discovering innovative structure leads serving as potent antimicrobial agents. The results indicated that all the derivatives exhibited appreciable antibacterial activities. Among the fifteen newly synthesised compounds, analogs **B_I, B_{II}, C_{II}, C_{III}** and **D_V** 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 **B_I** was 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. A close look at the SAR (structure activity relationship) of these compounds clearly indicates the influence of substituents on pyrazoline, isoxazole and pyrimidine ring. These finding conclude that the titled compounds have the properties to kill the microbes in some extent when compared with standard drug. These results suggest that chalcones and their derivatives have an opportunity to behave as generation of new antimicrobial agents and have excellent scope for further development as commercial antimicrobial agents.

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