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# Synthesis, characterisation and biological screening of *s* - triazine based chalcones and its derivatization into phenyl pyrazolines, isoxazoles

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**ABSTRACT**

Heterocyclic derivatives such as phenyl pyrazolines and isoxaxoles were prepared from *s* - triazine based chalcones. Chalcones ($A_1$ - $A_5$) are synthesised by the reaction of compound (V) with various aromatic aldehydes. Moreover, further reaction of chalcones with phenyl hydrazine hydrochloride and hydroxylamine hydrochloride in the presence of alkali gives phenyl pyrazolines ($A_6$ - $A_{10}$) and isoxazoles ($A_{11}$ - $A_{15}$) derivatives respectively. The structures of the newly synthesised compounds were confiremed by spectroscopic (IR, $^1$H NMR, $^{13}$C NMR) and elemental analysis. All the newly synthesised compounds have been screened for their antimicrobial activity against selected Gram - positive (S. aureus and S. pyogenus), Gram - negative (E. coli and P. aeruginosa) bacterial and fungal strains (C. albicans, A. niger and A. clavatus).

***Keywords:*** Chalcones; phenyl pyarazolines; isoxazoles; spectral data; elemental analysis; antimicrobial activity

## 1. INTRODUCTION

The main fears for human beings are a variety of diseases. Scientists and doctors are still struggling to find solutions with various forms of medications. Today's new medicines are results of inexorable effort made by human civilization time to time. The most common compounds of chalconoid group are the chalcones, which provide new class of medicines due to the pharmacologically active moiety and various biological activities. The chalcones are 1, 3 - diarylprop - 2 - en - 1 - one, form a broad class of compounds containing two aromatic rings bound with vinyl ketone fragment. Chalcones are useful intermediates for obtaining the variety of heterocycles [1-5]. Various chalcone derivatives are remarkable materials for their second harmonic generation [6]. They are naturally occurring plant metabolites possess a broad spectrum of biological activities such as cancer cell lines [7-8], antimitotic [9], anti-inflammatory [10], hepatoprotective [11], molluscicidal properties [12], heme oxygenase-1 [13], antimicrobial [14] etc... . So, this broad spectrum of applications encouraged us to search for another addition to the existed molecule.

Pyrazoline derivatives with a phenyl group at 5 - position show good film- forming properties, excellent features of blue photoluminescence and electroluminescence [15]. Pyrazoline derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals. Now days, a major portion of research in heterocyclic chemistry has been devoted to 2- pyrazolines containing diverse aryl groups as substituents. Pyrazoline derivatives are well known for their different biological activities such as antifeedant [16], anti- inflammatory [17], antiviral [18], antidepressant [19], antibacterial [20], antifungal [21] etc... . Many class of chemotherapeutic agents containing pyrazoline nucleus are in clinical use such as orisul (antibacterial), antipyrine (antipyretic), butazolidine (anti-inflammatory). So based on the above biological activities exhibited by the pyrazoline compounds, we reported here, the synthesis and biological screening of some novel phenyl pyrazoline derivatives.

Among heterocycles, the isoxazole unit constitutes an easily accessible nucleus that is present in a number of natural and pharmacological compounds [22], display a wide range of organic reactivities and used as an effective means of preparing new molecular scaffolds [23]. Isoxazoles have been repeatedly shown as useful synthons in organic synthesis [24]. Isoxazoles shows a broad spectrum of biological properties like fungicidal [25], antimicrobial [26], antitubercular [27], antiviral [28] etc... . So in this regard, we have synthesised some novel isoxazole derivatives and screened this compounds to antimicrobial activitiy.

## 2. EXPERIMENTAL

### 2.1. Material

All the chemicals and solvents which used for reaction were purified after getting from commercial suppliers. Melting points were taken in open capillaries using paraffin bath and were uncorrected. IR spectra were recorded on Shimadzu IR Affinity - 1 FTIR spectrometer ($V_{max}$ -1), $^{1}H$ NMR were recorded on Bruker Avance - DPX 400 MHz NMR spectrometer using $CDCl_3$ as a solvent and TMS as internal reference and $^{13}C$ NMR spectra were recorded on the same instrument at 100 MHz operating frequency using DMSO as a solvent and TMS as internal reference. The chemical shifts are expressed in parts per million (ppm) downfield from the internal standard and signals are quoted as s (singlet), d (doublet) and m (multiplate). The coupling constants (*J*) are given in Hertz (Hz). All the compounds were analyzed for carbon, hydrogen and nitrogen by the Perkin-Elmer 240 C H N elemental analyzer and the results were within ±0.4% of theoretical values. The purity of synthesised compounds were checked by thin layer chromatography conducted on Silica Gel 60 F-254 (Merck) plates of 0.25 mm thickness and the spots were located using toluene : methanol (12 : 6 v/v) eluents and visualized with UV (254 nm) light or keeping the plates in iodine chamber.

### 2.2. Method

#### 2.2.A. General procedure for the compounds (III), (IV) and (V)

Compounds (III), (IV) and (V) were prepared by the reported method [29].

**2.2.B. Preparation of 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {3'' - (4'''- methoxyphenyl) - 2'' - propenon - 1'' - yl} phenylamino] - s - triazine ($A_1$)**

In a round- bottomed flask, substituted acetophenone **(V)** (0.01 mol, 4.5g in 20 ml DMF) was dissolved in a dimethyl formamide and 4 - methoxybenzaldehyde (0.01 mol, 1.36g in 10 ml DMF) was added in it. To make this mixture alkaline 40% KOH(5 ml) was added as catalyst, then the reaction mixture was stirred for 24 hours at room temprature. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into crushed ice and neutralise with HCl. Finally, the product was filtered, dried and purified by recrystallization from ethanol. In the same way, the remaining compounds **($A_2$ - $A_5$)** were prepared by this method. All the synthesised compounds **($A_1$ - $A_5$)** were characterised by IR, $^1$H NMR, $^{13}$C NMR spectroscopy, the characteristic data of the entire synthesised compounds are given in spectral analysis data **(3)** and physical data are given in **Table - 1.**

**2.2.C. 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1''- phenyl 5''- (4''' - methoxyphenyl) 2'' - pyrazolin 3''- yl} phenylamino] - s - triazine ($A_6$)**

chalcone ($A_1$) (0.01 mol, 5.76g in 30 ml alcohol) and phenyl hydrazine hydrochloride (0.01mol, 1.44 g in 10 ml alcohol) was dissolved in alcohol. To make this mixture alkaline 40% KOH (5 ml) was added to the reaction mixture and refluxed for 8 hours. The progress of the reaction was monitored by using TLC. After completion of the reaction, the reaction mixture was poured into crushed and neutralise with dilute HCl, Finally, the product was filtered, washed with water, dried and recrystallization from ethanol to get product **($A_6$)** in good yield with high purity. In the same way other remaining compounds **($A_7$ - $A_{10}$)** were prepared by this method. All the synthesised compounds **($A_6$ - $A_{10}$)** were characterised by IR, $^1$H NMR, $^{13}$C NMR spectroscopy, the characteristic data of the entire synthesised compounds are given in spectral analysis data **(3)** and physical data are given in **Table - 1.**

**2.2.D. Preparation of 2 - (3' - trifluromethyphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5''- (4''' - methoxyphenyl) 2'' - isoxazol - 3''- yl} phenylamino] - s - triazine ($A_{11}$)**

Compound **($A_1$) (**0.01 mol, 5.76g in 30 ml alcohol) and hydroxylamine hydrochloride (0.01mol, 0.695g in 10 ml alcohol) was dissolved in methanol. To make this mixture alkaline 40% KOH (5 ml) was added to the reaction mixture and refluxed for 8 hours. 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. Finally, the product was filtered, washed with water, dried and recrystallization from ethanol to get product **($A_{11}$)** in good yield with high purity. In the same way other remaining compounds **($A_{12}$ - $A_{15}$)** were prepared by this method. All the synthesised compounds **($A_{12}$ - $A_{15}$)** were characterised by IR, $^1$H NMR, $^{13}$C NMR spectroscopy, the characteristic data of the entire synthesised compounds are given in spectral analysis data **(3)** and physical data are given in **Table - 1.**

### 2.3. Reaction Scheme

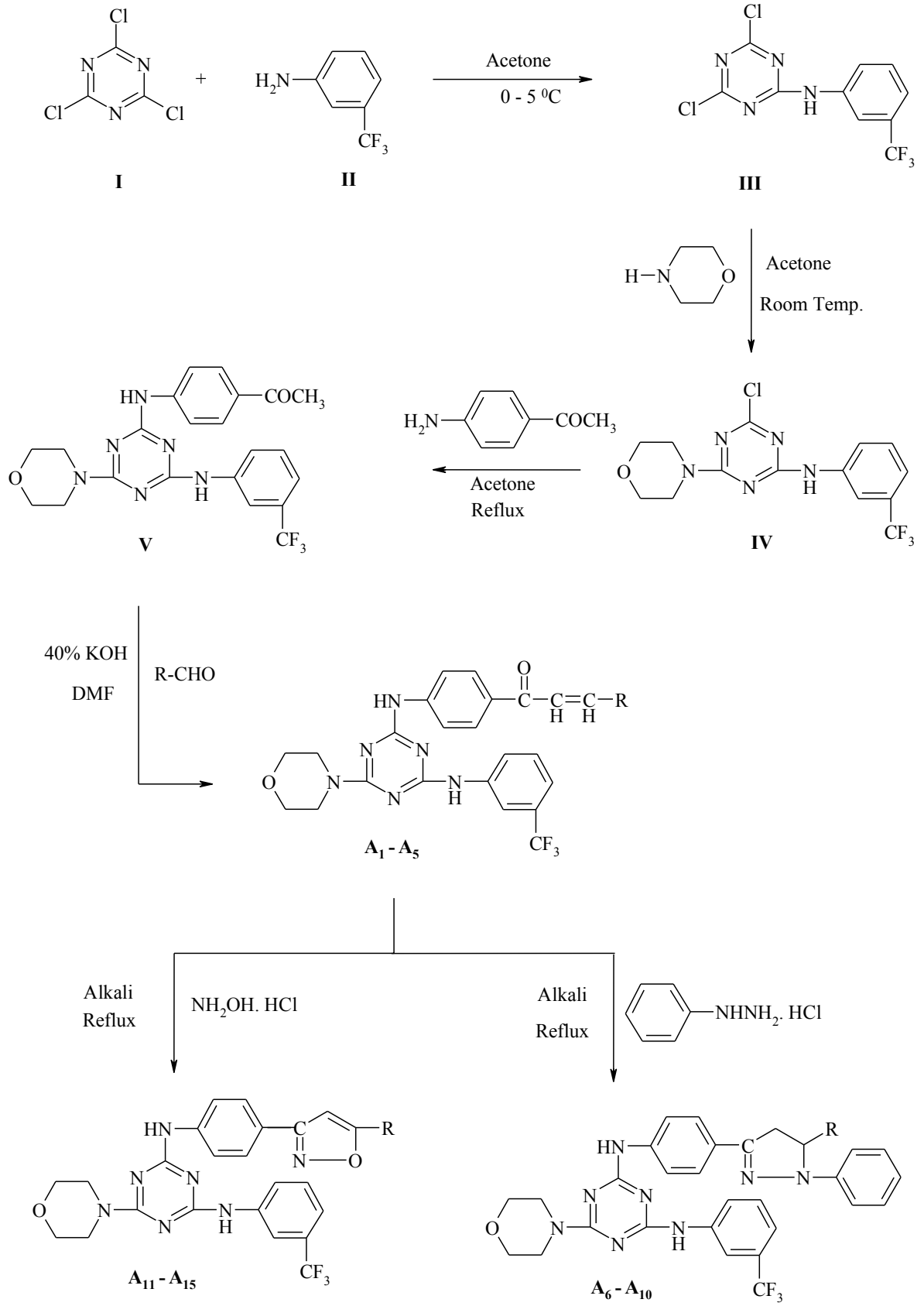

**Table 1.** The physical data of synthesised compounds $A_1$- $A_{15}$.

| Compd | R | Molecular Formula | Yield (%) | M. P $^0$C | Elemental analysis Calculated (Found) % | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| $A_1$ | 4 - Methoxy phenyl | $C_{30}H_{27}N_6F_3O_3$ | 74 | 180 | 62.50 (62.55) | 4.72 (4.73) | 14.58 (14.61) |
| $A_2$ | 4 - Chloro phenyl | $C_{29}H_{24}N_6F_3O_2Cl$ | 69 | 125 | 59.95 (59.96) | 4.16 (4.20) | 14.47 (14.45) |
| $A_3$ | 3 - Phenoxyphenyl | $C_{35}H_{29}N_6F_3O_3$ | 71 | 148 | 65.83 (65.89) | 4.57 (4.60) | 13.16 (13.12) |
| $A_4$ | 2 - Nitrophenyl | $C_{29}H_{24}N_7F_4O_3$ | 70 | 105 | 58.88 (58.84) | 4.09 (4.05) | 16.58 (16.55) |
| $A_5$ | Phenyl | $C_{29}H_{25}N_6F_3O_2$ | 65 | 108 | 63.73 (63.70) | 4.61 (4.65) | 15.38 (15.43) |
| $A_6$ | 4 - Methoxy phenyl | $C_{36}H_{33}N_8F_3O_2$ | 72 | 165 | 64.86 (64.83) | 4.98 (4.95) | 16.81 (16.79) |
| $A_7$ | 4 - Chloro phenyl | $C_{35}H_{30}N_8F_3OCl$ | 69 | 98 | 62.64 (62.83) | 4.50 (4.53) | 16.70 (16.72) |
| $A_8$ | 3 - Phenoxyphenyl | $C_{41}H_{35}N_8F_3O_2$ | 68 | 141 | 67.57 (67.55) | 4.84 (4.87) | 15.38 (15.43) |
| $A_9$ | 2 - Nitrophenyl | $C_{35}H_{30}N_9F_3O_3$ | 67 | 130 | 61.67 (61.70) | 4.43 (4.46) | 18.49 (14.47) |
| $A_{10}$ | Phenyl | $C_{35}H_{31}N_8F_3O$ | 70 | 94 | 66.03 (66.01) | 4.90 (4.87) | 17.60 (16.58) |
| $A_{11}$ | 4 - Methoxy phenyl | $C_{30}H_{26}N_7F_3O_3$ | 74 | 109 | 61.12 (61.1) | 4.44 (4.47) | 16.63 (14.62) |
| $A_{12}$ | 4 - Chloro phenyl | $C_{29}H_{23}N_7F_3O_2Cl$ | 65 | 155 | 58.64 (58.66) | 3.90 (3.87) | 16.51 (16.53) |
| $A_{13}$ | 3 - Phenoxyphenyl | $C_{35}H_{28}N_7F_3O_3$ | 71 | 123 | 64.51 (64.47) | 4.33 (4.31) | 15.05 (15.04) |
| $A_{14}$ | 2 - Nitrophenyl | $C_{29}H_{23}N_8F_3O_4$ | 73 | 117 | 57.62 (57.59) | 3.83 (3.86) | 18.54 (18.51) |
| $A_{15}$ | Phenyl | $C_{29}H_{24}N_7F_3O_2$ | 66 | 110 | 62.25 (62.21) | 4.32 (4.35) | 17.52 (17.55) |

## 3. SPECTRAL ANALYSIS DATA

**Compound $A_1$ :** 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {3" - (4"'- methoxyphenyl) - 2" - propenon - 1" - yl} phenylamino] - s - triazine. : IR (KBr) $cm^{-1}$ : 3307 (N-H str.), 3018 ( =CH ), 1624 ( -C=O ), 1578 (C=C str.), 1248 ( C -O- C str. ), 1025 ( C-F ), 807 ( C-N str. s - triazine ). $^1$H NMR ( δ ppm, $CDCl_3$ ) : 8.25 ( s, 1H, -NH ), 3.71 ( t, 8H, $-CH_2$, oxazine ring ), 3.89 ( s, 3H, p-$OCH_3$ ), 6.89 ( d, J = 9.36 Hz, lH,-CO-CH= ), 8.21 ( d, J = 8.6 Hz, 1H, Ar-CH= ), 6.98 - 8.1 ( m, 12H, Ar-H ). $^{13}$C NMR ( δ ppm, DMSO ) : 48.7, 55.5, 66.3, 111.8, 115.1, 116.1, 121.1, 121.3, 124.1, 127.9, 128.7, 129.0, 129.8, 131.8, 132.0, 133.5, 133.7, 142.7, 144.7, 145.9, 159.8, 165.7, 168.9, 176.0, 189.7.

**Compound $A_2$ :** 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {3" - (4"'- chlorophenyl) - 2" - propenon - 1" - yl} phenylamino] - s - triazine. : IR (KBr) $cm^{-1}$ : 3323 (N-H str.), 3001 ( =CH ), 1652 ( -C=O ), 1569 (C=C str.), 1232 ( C -O- C str. ), 1019 ( C- F ), 798 ( C-N str. s - triazine ), 639 ( C- Cl ). $^1$H NMR ( δ ppm, $CDCl_3$ ) : 8.35 ( s, 1H, -NH ), 3.77 ( t, 8H, $-CH_2$, oxazine ring ), 6.13 (d, J = 9.8 Hz, lH, -CO-CH= ),

7.91 ( d, J = 9.4 Hz, 1H, Ar CH= ), 7.0 - 8.2 ( m, 12H, Ar-H ). $^{13}$C NMR ( δ ppm, DMSO ) : 48.6, 63.1, 109.3, 110.9, 112.8, 113.1, 120.8, 121.6, 125.2, 125.9, 127.1, 129.1, 130.5, 131.8, 132.7, 135.0, 138.2, 141.8, 145.7, 166.0, 172.4, 179.3, 186.3.

**Compound $A_3$ :** 2 - (3' - triflurometh ylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {3" - (3"'- phenoxyphenyl) - 2" - propenon - 1" - yl} phenylamino] - s - triazine. : IR (KBr) cm$^{-1}$ : 3338 (N-H str.), 3023 ( =CH ), 1710 ( -C=O ), 1530 (C=C str.), 1225 ( C-O-C str. ), 1098 ( C-F ), 789 ( C-N str. s - triazine ). $^1$H NMR ( δ ppm, $CDCl_3$ ) : 8.1 ( s, 1H, -NH ), 3.19 ( t, 8H, -$CH_2$, oxazine ring ), 6.3 ( d, J = 8.9 Hz, lH, -CO-CH= ), 8.3 ( d, J = 9.3 Hz, 1H, Ar-CH= ), 6.9 - 7.8 ( m, 17H, Ar-H ), $^{13}$CNMR ( δ ppm, DMSO ) : 47.1, 60.4, 110.6, 111.8, 113.0, 115.9, 118.3, 120.1, 121.4, 123.6, 125.3, 129.0, 130.7, 132.1, 133.6, 134.2, 135.7, 138.3, 143.2, 145.6, 155.3, 157.1, 166.1, 169.7, 179.3, 184.7.

**Compound $A_4$ :** 2 - (3' - triflurometh ylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {3" - (2"'- nitrophenyl) - 2" - propenon - 1" - yl} phenylamino] - s - triazine. : IR (KBr) cm$^{-1}$ : 3310 (N-H str.), 3035 ( =CH ), 1706 ( -C=O ), 1540( C-$NO_2$ ), 1505 (C=C str.), 1240 ( C-O-C str. ), 1016 ( C-F ), 800 ( C-N str. s - triazine ), $^1$H NMR ( δ ppm, $CDCl_3$ ) : 8.2 ( s, 1H, -NH ), 3.83 ( t, 8H, -$CH_2$, oxazine ring ), 6.25 ( d, J = 9.2 Hz, lH, -CO-CH= ), 7.12 ( d, J = 8.8 Hz, 1H, Ar-CH= ), 6.9 - 7.9 ( m, 12H, Ar-H ). $^{13}$C NMR ( δ ppm, DMSO ) : 43.2, 67.6, 109.5, 111.2, 118.0, 121.2, 124.8, 126.0, 127.5, 128.0, 130.2, 131.5, 133.1, 133.2, 134.5, 141.8, 143.8, 147.7, 166.4, 168.1, 175.1, 188.2.

**Compound $A_5$ :** 2 - (3' - triflurometh ylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {3" - (phenyl) - 2" - propenon - 1" - yl} phenylamino] - s - triazine. : IR (KBr) cm$^{-1}$ : 3333 (N-H str.), 3019 ( =CH ), 1652 ( -C=O ), 1516 (C=C str.), 1249 ( C-O-C str. ), 1065 ( C-F ), 806 ( C-N str. s - triazine ). $^1$H NMR ( δ ppm, $CDCl_3$ ) : 7.9 ( s, 1H, -NH ), 3.76 ( t, 8H, -$CH_2$, oxazine ring ), 6.6 ( d, J = 9.6 Hz, lH, -CO-CH= ), 7.3 ( d, J = 9.9 Hz, 1H, Ar-CH= ), 7.1 - 8.0 ( m, 13H, Ar-H ). $^{13}$CNMR ( δ ppm, DMSO ) : 46.4, 66.2, 110.2, 112.1, 115.2, 116.6, 122.1, 124.2, 126.1, 127.8, 130.0, 131.2, 132.9, 133.5, 135.9, , 142.7, 144.3, 163.0, 167.6, 175.1, 188.0.

**Compound $A_6$ :** 2 - (3' - triflurometh ylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1"- phenyl 5"- (4"' - methoxyphenyl) 2" - pyrazolin 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3265 (-NH), 3031 (=CH str.), 2912 (C-H str., pyrazoline moiety), 1645 (C=N, pyrazoline moiety), 1256 (C-O- C), 1100 (C- F). $^1$H NMR (δ ppm, $CDCl_3$) : 2.1 (dd, 1H, -C$\underline{H}^a$-CH-), 3.2 (dd, 1H, -C$\underline{H}^b$-CH-), 5.2 (dd , 1H, -C$\underline{H}$-$CH_2$), 3.89 (s, 3H, p-OC$\underline{H}_3$), 3.69 (t, 8H, -$CH_2$, oxazine ring), 6.9 to 8.2 (m, 18H, 17 Ar-$\underline{H}$and1-N$\underline{H}$). $^{13}$C NMR (δ ppm, DMSO) : 40.6, 46.9, 57.1, 64.3, 66.3, 110.8, 112.1, 114.0, 115.6, 120.7, 123.9, 127.1, 130.0, 131.5, 133.6, 134.7, 140.4, 142.6, 144.8, 150.7, 157.9, 162.4, 164.1, 174.1.

**Compound $A_7$ :** 2 - (3' - triflurometh ylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1"- phenyl 5"- (4"' - chlorophenyl) 2" - pyrazolin 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3146 (-NH), 3054 (=CH str.), 2936 (C-H str., pyrazoline moiety), 1640 (C=N, pyrazoline moiety), 1235 (C-O- C), 1067 (C- F), 663 (C- Cl). $^1$H NMR (δ ppm, $CDCl_3$) : 2.6 (dd, 1H, -C$\underline{H}^a$-CH-), 2.9 (dd, 1H, -C$\underline{H}^b$-CH-), 5.1 (dd , 1H, -C$\underline{H}$-$CH_2$), 3.10 (t, 8H, -$CH_2$, oxazine ring), 7.0 to 8.2 (m, 18H, 17 Ar-$\underline{H}$and1-N$\underline{H}$). $^{13}$C NMR (δ ppm, DMSO) : 39.2, 46.0, 62.9, 66.7, 109.2, 111.4, 112.5, 114.6, 116.3, 120.8, 121.9, 124.0, 126.3, 129.7, 132.2, 134.4, 136.9, 142.0, 144.3, 152.5, 164.6, 166.8, 173.5.

**Compound $A_8$ :** 2 - (3' - triflurometh ylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1"- phenyl 5"- (3"' - phenoxyphenyl) 2" - pyrazolin 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3376 (-NH), 2991 (=CH str.), 2949 (C-H str., pyrazoline moiety), 1542 (C=N, pyrazoline moiety), 1246 (C-O- C), 1049 (C- F). $^1$H NMR (δ ppm, $CDCl_3$) : 2.9 (dd, 1H, -C$\underline{H}^a$-CH-), 3.2 (dd, 1H, -C$\underline{H}^b$-CH-), 4.8 (dd , 1H, -C$\underline{H}$-$CH_2$), 3.16 (t, 8H, -$CH_2$,

oxazine ring), 6.9 to 8.6 (m, 23H, 22 Ar-Hand1-NH). $^{13}$C NMR (δ ppm, DMSO): 40.0, 48.6, 61.0, 64.9, 110.5, 112.4, 114.3, 117.0, 119.6, 121.8, 123.1, 125.7, 127.0, 128.3, 130.0, 133.5, 141.4, 143.8, 149.7, 156.2, 157.1, 163.4, 165.0, 174.9.

**Compound $A_9$ :** 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1"- phenyl 5"- (2"' - nitrophenyl) 2" - pyrazolin 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3302 (-NH), 3006 (=CH str.), 3000 (C-H str., pyrazoline moiety), 1589 (C=N, pyrazoline moiety), 1486(C-$NO_2$), 1231 (C-O- C), 1061 (C- F). $^1$H NMR (δ ppm, $CDCl_3$) : 2.2 (dd, 1H, -CH$^a$-CH-), 2.5 (dd, 1H, -CH$^b$-CH-), 5.3 (dd , 1H, -CH-$CH_2$), 3.46 (t, 8H, -$CH_2$, oxazine ring), 7.0 to 8.3 (m, 18H, 17 Ar-Hand1-NH). $^{13}$C NMR (δ ppm, DMSO) : 38.0, 48.8, 65.7, 66.2, 111.2, 112.6, 115.0, 117.6, 118.4, 120.9, 122.2, 123.8, 126.1, 129.6, 131.9, 132.5, 140.7, 142.0, 145.1, 150.9, 163.0, 166.4, 173.8.

**Compound $A_{10}$ :** 2 - (3' - trifluromethylphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {1"- phenyl 5"- (phenyl) 2" - pyrazolin 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3398 (-NH), 3102 (=CH str.), 2965 (C-H str., pyrazoline moiety), 1560 (C=N, pyrazoline moiety), 1221 (C-O- C), 1098 (C- F). $^1$H NMR (δ ppm, $CDCl_3$) : 2.4 (dd, 1H, -CH$^a$-CH-), 2.6 (dd, 1H, -CH$^b$-CH-), 5.7 (dd , 1H, -CH-$CH_2$), 3.78 (t, 8H, -$CH_2$, oxazine ring), 7.2 to 8.4 (m, 19H, 18 Ar-Hand1-NH). $^{13}$C NMR (δ ppm, DMSO): 37.2, 46.6, 63.4, 68.6, 108.5, 110.2, 112.4, 114.1, 116.6, 118.0, 120.5, 124.3, 127.0, 128.3, 130.7, 131.4, 141.2, 144.9, 149.2, 162.5, 163.1, 174.8.

**Compound $A_{11}$ :** 2 - (3' - trifluromethyphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5"- (4"' - methoxyphenyl) 2" - isoxazol - 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3304 (-NH), 3089 (=CH str.), 834 (C-H bending), 1608 (C=N str., isoxazolemoiety), 1253 (C-O- C), 1068 (C- F). $^1$H NMR (δ ppm, $CDCl_3$) : 3.79 (s, 3H, p-O$CH_3$), 3.56 (t, 8H, -$CH_2$, oxazine ring), 6.69 (1H, s, -CH=), 6.7 to 8.1 (m, 13H, 12 Ar-H and1-NH). $^{13}$C NMR (δ ppm, DMSO) : 48.1, 56.7, 66.8, 98.4, 116.0, 116.9, 118.2, 121.3, 124.4, 127.0, 128.3, 129.8, 131.8, 138.9, 142.7, 160.6, 162.2, 165.7, 168.9, 169.3, 176.0.

**Compound $A_{12}$ :** 2 - (3' - trifluromethyphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5"- (4"' - chlorophenyl) 2" - isoxazol - 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3373 (-NH), 3019 (=CH str.), 815 (C-H bending), 1645 (C=N str., isoxazolemoiety), 1249 (C-O- C), 1066 (C- F), 661 (C- Cl). $^1$H NMR (δ ppm, $CDCl_3$) : 3.47 (t, 8H, -$CH_2$, oxazine ring), 6.56 (1H, s, -CH=), 6.9 to 8.2 (m, 13H, 12 Ar-H and1-NH). $^{13}$C NMR (δ ppm, DMSO) : 48.0, 66.8, 98.6, 112.1, 114.8, 120.6, 122.5, 123.7, 125.8, 127.9, 132.2, 133.8, 138.2, 142.9, 164.1, 166.3, 169.4, 173.2, 176.0.

**Compound $A_{13}$ :** 2 - (3' - trifluromethyphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5"- (3"' - phenoxyphenyl) 2" - isoxazol - 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3390 (-NH), 3080 (=CH str.), 843 (C-H bending), 1640 (C=N str., isoxazolemoiety), 1250 (C-O- C), 1049 (C- F), $^1$H NMR (δ ppm, $CDCl_3$) : 3.72 (t, 8H, -$CH_2$, oxazine ring), 6.45 (1H, s, -CH=), 6.7 to 8.3 (m, 18H, 17 Ar-H and1-NH). $^{13}$C NMR (δ ppm, DMSO) : 47.9, 67.2, 99.4, 110.2, 112.5, 118.3, 121.4, 122.6, 124.2, 126.4, 127.0, 128.7, 132.1, 134.4, 137.2, 143.2, 156.7, 165.1, 168.8, 170.2, 171.5, 173.0.

**Compound $A_{14}$ :** 2 - (3' - trifluromethyphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5"- (2"' - nitrophenyl) 2" - isoxazol - 3"- yl} phenylamino] - s - triazine : IR (KBr) cm$^{-1}$ : 3311 (-NH), 3079 (=CH str.), 824 (C-H bending), 1631 (C=N str., isoxazolemoiety), 1481(C-$NO_2$), 1228 (C-O- C), 1097 (C- F). $^1$H NMR (δ ppm, $CDCl_3$) : 3.81 (t, 8H, -$CH_2$, oxazine ring), 6.51 (1H, s, -CH=), 6.8 to 8.3 (m, 13H, 12 Ar-H and1-NH). $^{13}$C NMR (δ ppm, DMSO) : 48.7, 65.2, 96.4, 111.5, 115.7, 118.2, 121.0, 124.6, 126.5, 128.2, 131.4, 134.6, 137.0, 141.1, 146.8, 165.8, 167.5, 170.6, 172.1, 174.5.

**Compound $A_{15}$ :** 2 - (3' - trifluromethyphenylamino) - 4 - (tetrahydro - 1', 4' - oxazine) - 6 - [4' - {5"- (phenyl) 2" - isoxazol - 3"- yl} phenylamino] - s - triazine : IR (KBr) $cm^{-1}$ : 3356 (-NH), 3032 (=CH str.), 867(C-H bending), 1608 (C=N str., isoxazolemoiety), 1257 (C-O- C), 1067 (C- F). $^1H$ NMR (δ ppm, $CDCl_3$) : 3.24 (t, 8H, $-CH_2$, oxazine ring), 6.41 (1H, s, -CH=), 6.8 to 8.1 (m, 14H, 13 Ar-H and1-NH). $^{13}C$ NMR (δ ppm, DMSO) : 46.1, 66.3, 98.2, 112.5, 114.1, 120.3, 123.1, 125.8, 127.2, 129.0, 131.7, 133.2, 138.4, 142.8, 164.6, 166.4, 169.0, 172.7, 174.2.

## 4. RESULT AND DISCUSSION

### 4.1. Antimicrobial evaluation

All the newly synthesised compounds were screened for antibacterial and antifungal activity by Broth dilution method [30] against a panel of selected Gram - positive (S. aureus MTCC 96 and Streptococcus pyogenes MTCC 442) and Gram - negative bacteria (E. coli MTCC 443 and P. aeruginosa MTCC 441) and selected fungal strains (C. albicans MTCC 227, A. niger MTCC 282 and A calavatus MTCC 1323). DMSO was used as a solvent. Ampicillin and Chloramphenicol was used as a standard drug for antibacterial activity while Greseofulvin and Nystatin was used as a standard drug for antifungal activity. The results are showed in Table - 2.

### 4.1.A. Antibacterial Activity

From the screening results (Table-2), it has been observed that, In Gram positive bacterial strains compounds $A_2$, $A_5$, $A_{15}$ (MIC =100 µg/ml) and $A_8$, $A_9$, $A_{10}$ (MIC =125 µg/ml) exhibited excellent activity, compounds $A_1$, $A_3$, $A_7$ and $A_{11}$ (MIC =200 µg/ml) showed significant activity while compounds $A_4$, $A_6$, $A_{12}$, $A_{13}$ and $A_{14}$ (MIC =250 µg/ml) exhibited equipotent activity against S. aureus (MTCC 96) compared to Ampicillin (MIC = 250 µg/ml). Compounds $A_7$ and $A_{10}$ (MIC =100 µg/ml) exhibited equipotent activity, whereas compounds $A_2$, $A_3$, $A_4$, $A_9$ and $A_{14}$ (MIC =200 µg/ml) were moderately active against S. pyogenes (MTCC 442) compared to Ampicillin (MIC = 100 µg/ml). In Gram negative bacterial strains compound $A_2$ (MIC = 62.5 µg/ml) exhibited excellent activity against E. coli (MTCC 443) compared to Ampicillin (MIC = 100 µg/ml) and modest to Chloramphenicol (MIC = 50 µg/ml) while compounds $A_3$, $A_7$, $A_8$ (MIC = 100 µg/ml) and $A_4$, $A_5$, $A_{12}$ and $A_{14}$ (MIC = 125 µg/ml) showed equipotent activity against E. coli (MTCC 443) compared to Ampicillin (MIC = 100 µg/ml). Compound $A_7$ (MIC = 62.5 µg/ml) exhibited excellent activity against P. aeruginosa (MTCC 441) compared to Ampicillin (MIC = 100 µg/ml) and comparable to Chloramphenicol (MIC = 50 µg/ml), whereas compounds $A_4$, $A_6$ (MIC = 100 µg/ml) and $A_2$, $A_{12}$ (MIC = 125 µg/ml) showed equipotent activity against P. Aeruginosa (MTCC 441) compared to Ampicillin (MIC = 100 µg/ml), while all other compounds were showed low to moderately active against all selected organisms.

### 4.1.B. Antifungal Activity

From the screening results (Table-2), it has been observed that, compound $A_2$ (MIC = 250 µg/ml) and $A_7$ (MIC = 100 µg/ml) exhibited excellent activity against C. albicans (MTCC 227) compared to Greseofulvin (MIC = 500 µg/ml) and equipotent to Nystatin (MIC = 100

µg/ml), while compounds $A_1$, $A_5$, $A_6$, $A_{10}$, $A_{12}$, $A_{13}$ and $A_{15}$ (MIC = 500 µg/ml) showed comparable activity against C. Albicans (MTCC 227) compared to Greseofulvin (MIC = 500 µg/ml). Compounds $A_1$ (MIC = 200 µg/ml) and $A_2$ (MIC = 100 µg/ml) exhibited equipotent activity against A. niger (MTCC 282) compared to Greseofulvin (MIC = 100 µg/ml) and Nystatin (MIC = 100 µg/ml). None of the compounds showed promising antifungal activity against A. clavatus (MTCC 1323).

**Table 2.** Antibacterial and antifungal activity data of compounds $A_1$ - $A_{15}$.

| Comp | Minimal bactericidal concentration µg/ml | | | | Minimal fungicidal concentration µg/ml | | |
|---|---|---|---|---|---|---|---|
| | Gram positive | | Gram negative | | | | |
| | S. aureus MTCC-96 | S. pyogenus MTCC-442 | E. coli MTCC-443 | P. aerug MTCC-441 | C. albicans MTCC-227 | A. niger MTCC-282 | A. clavatus MTCC-1323 |
| $A_1$ | 200 | 250 | 200 | 250 | 500 | **200** | 500 |
| $A_2$ | **100** | 200 | **62.5** | **125** | **250** | **100** | 500 |
| $A_3$ | 200 | 200 | **100** | 200 | 1000 | 500 | 500 |
| $A_4$ | 250 | 200 | **125** | **100** | 1000 | 500 | >1000 |
| $A_5$ | **100** | 250 | **125** | 200 | 500 | 1000 | >1000 |
| $A_6$ | 250 | 250 | 200 | **100** | 500 | 1000 | 1000 |
| $A_7$ | 200 | **100** | **100** | **62.5** | **100** | 1000 | 500 |
| $A_8$ | **125** | 250 | **100** | 250 | 1000 | 1000 | 1000 |
| $A_9$ | **125** | 200 | 250 | 500 | 1000 | 1000 | 1000 |
| $A_{10}$ | **125** | **100** | 200 | 250 | 500 | >1000 | >1000 |
| $A_{11}$ | 200 | 500 | 200 | 200 | 1000 | 500 | 1000 |
| $A_{12}$ | 250 | 250 | **125** | **125** | 500 | 250 | 250 |
| $A_{13}$ | 250 | 250 | 500 | 500 | 500 | 500 | 500 |
| $A_{14}$ | 250 | 200 | **125** | 250 | 1000 | >1000 | >1000 |
| $A_{15}$ | **100** | 250 | 250 | 250 | 500 | 1000 | 1000 |
| A | 250 | 100 | 100 | 100 | - | - | - |
| B | 50 | 50 | 50 | 50 | - | - | - |
| C | - | - | - | - | 500 | 100 | 100 |
| D | - | - | - | - | 100 | 100 | 100 |

Where A= Ampicillin, B = Chloramphenicol (Standard Drugs for antibacterial activity)
C = Greseofulvin, D = Nystatin (Standard Drugs for antifungal activity)

## 5. CONCLUSION

In outline, we have synthesised some bioactive chalcones and convert them into pyrazoline and isoxazole moiety by using conventional method. The method adopted for the synthesis of pharmacologically important molecules in this investigation is simple, efficient, and inexpensive. The IR, $^{1}$H NMR, $^{13}$C NMR spectral analysis and elemental analysis of all

the newly synthesised compounds confirmed that purity of the entire synthesised compound is good.

All the synthesised compounds were screened for antimicrobial activity. Majority of the synthesised compounds were found to potentially active against both selected Gram positive, Gram negative organisms and selected fungal organisms. From the results of antibacterial and antifungal activity, it can be concluded that compounds **$A_2$**, **$A_7$** and **$A_{12}$** were found more active then the remaining compounds due to the present of chlorine atom. So overall it was revealed that the no substitution on phenyl ring showed no inhibition of the tested bacteria while the compounds that showed some inhibition was due to the presence of substitution of methoxy, chloro, phenoxy and nitro group on some position of the phenyl ring. These finding conclued that the titled compounds have the properties to kill the microbes in some extent when compared with standared drug. These result suggest that chalcone and their derivatives have an opportunity to behave as broad spectrum antimicrobial agents and have exellent scope for further development as commercial antimicrobial agents .

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