

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 (A₁ - A₅) 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 (A₆ - A₁₀) and isoxazoles (A₁₁ - A₁₅) derivatives respectively. The structures of the newly synthesised compounds were confirmed by spectroscopic (IR, ¹H NMR, ¹³C 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 pyrazolines; 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 activity.

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), ^1H 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 (multiplet). 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 $\pm 0.4\%$ 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' - trifluoromethylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {3'' - (4'''- methoxyphenyl) - 2'' - propenon - 1'' - yl} phenylamino] - s - triazine (A₁)

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 temperature. 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 (A₂ - A₅) were prepared by this method. All the synthesised compounds (A₁ - A₅) were characterised by IR, ¹H NMR, ¹³C 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' - trifluoromethylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {1''- phenyl 5''- (4''' - methoxyphenyl) 2'' - pyrazolin 3''- yl} phenylamino] - s - triazine (A₆)

chalcone (A₁) (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 (A₆) in good yield with high purity. In the same way other remaining compounds (A₇ - A₁₀) were prepared by this method. All the synthesised compounds (A₆ - A₁₀) were characterised by IR, ¹H NMR, ¹³C 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' - trifluoromethylphenylamino) - 4 - (tetrahydro - 1' , 4' - oxazine) - 6 - [4' - {5''- (4''' - methoxyphenyl) 2'' - isoxazol - 3''- yl} phenylamino] - s - triazine (A₁₁)

Compound (A₁) (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 (A₁₁) in good yield with high purity. In the same way other remaining compounds (A₁₂ - A₁₅) were prepared by this method. All the synthesised compounds (A₁₂ - A₁₅) were characterised by IR, ¹H NMR, ¹³C 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

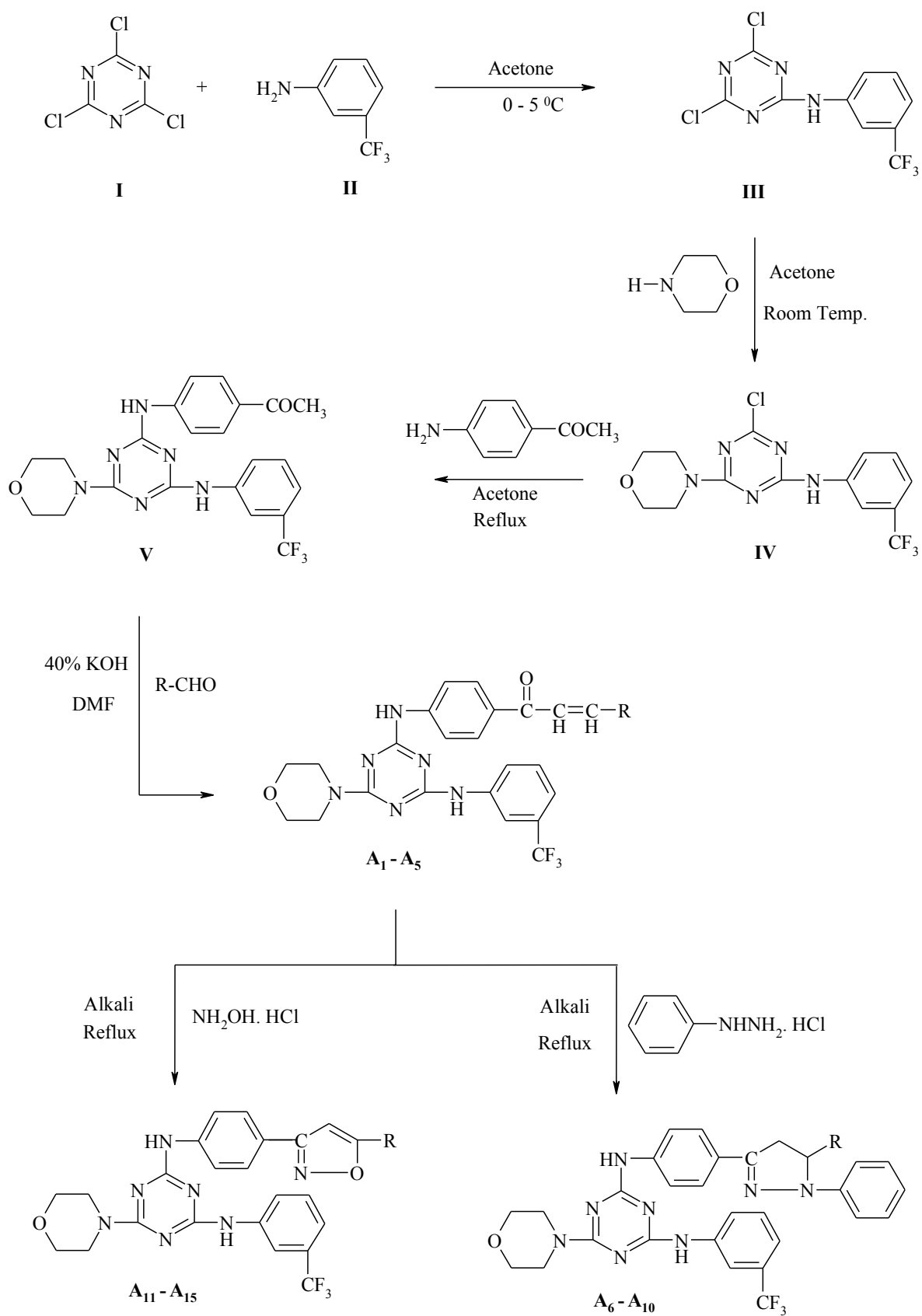


Table 1. The physical data of synthesised compounds A₁- A₁₅.

Compd	R	Molecular Formula	Yield (%)	M. P °C	Elemental analysis Calculated (Found) %		
					C	H	N
A ₁	4 - Methoxy phenyl	C ₃₀ H ₂₇ N ₆ F ₃ O 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 ₆ F ₃ O ₂ Cl	69	125	59.95 (59.96)	4.16 (4.20)	14.47 (14.45)
A ₃	3 - Phenoxyphenyl	C ₃₅ H ₂₉ N ₆ F ₃ O ₃	71	148	65.83 (65.89)	4.57 (4.60)	13.16 (13.12)
A ₄	2 - Nitrophenyl	C ₂₉ H ₂₄ N ₇ F ₄ O ₃	70	105	58.88 (58.84)	4.09 (4.05)	16.58 (16.55)
A ₅	Phenyl	C ₂₉ H ₂₅ N ₆ F ₃ O 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 ₈ F ₃ O 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 ₈ F ₃ OCl	69	98	62.64 (62.83)	4.50 (4.53)	16.70 (16.72)
A ₈	3 - Phenoxyphenyl	C ₄₁ H ₃₅ N ₈ F ₃ O ₂	68	141	67.57 (67.55)	4.84 (4.87)	15.38 (15.43)
A ₉	2 - Nitrophenyl	C ₃₅ H ₃₀ N ₉ F ₃ O ₃	67	130	61.67 (61.70)	4.43 (4.46)	18.49 (14.47)
A ₁₀	Phenyl	C ₃₅ H ₃₁ N ₈ F ₃ O	70	94	66.03 (66.01)	4.90 (4.87)	17.60 (16.58)
A ₁₁	4 - Methoxy phenyl	C ₃₀ H ₂₆ N ₇ F ₃ O 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 ₇ F ₃ O ₂ Cl	65	155	58.64 (58.66)	3.90 (3.87)	16.51 (16.53)
A ₁₃	3 - Phenoxyphenyl	C ₃₅ H ₂₈ N ₇ F ₃ O ₃	71	123	64.51 (64.47)	4.33 (4.31)	15.05 (15.04)
A ₁₄	2 - Nitrophenyl	C ₂₉ H ₂₃ N ₈ F ₃ O ₄	73	117	57.62 (57.59)	3.83 (3.86)	18.54 (18.51)
A ₁₅	Phenyl	C ₂₉ H ₂₄ N ₇ F ₃ O ₂	66	110	62.25 (62.21)	4.32 (4.35)	17.52 (17.55)

3. SPECTRAL ANALYSIS DATA

Compound A₁ : 2 - (3' - trifluoromethylphenylamino) - 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). ¹H NMR (δ ppm, CDCl₃) : 8.25 (s, 1H, -NH), 3.71 (t, 8H, -CH₂, oxazine ring), 3.89 (s, 3H, p-OCH₃), 6.89 (d, J = 9.36 Hz, 1H, -CO-CH=), 8.21 (d, J = 8.6 Hz, 1H, Ar-CH=), 6.98 - 8.1 (m, 12H, Ar-H). ¹³C 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 A₂ : 2 - (3' - trifluoromethylphenylamino) - 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). ¹H NMR (δ ppm, CDCl₃) : 8.35 (s, 1H, -NH), 3.77 (t, 8H, -CH₂, oxazine ring), 6.13 (d, J = 9.8 Hz, 1H, -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 A₃ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl_3) : 8.1 (s, 1H, -NH), 3.19 (t, 8H, -CH₂, oxazine ring), 6.3 (d, $J = 8.9$ Hz, 1H, -CO-CH=), 8.3 (d, $J = 9.3$ Hz, 1H, Ar-CH=), 6.9 - 7.8 (m, 17H, Ar-H). ^{13}C NMR (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 A₄ : 2 - (3' - trifluoromethylphenylamino) - 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-NO₂), 1505 (C=C str.), 1240 (C-O-C str.), 1016 (C-F), 800 (C-N str. s - triazine). ^1H NMR (δ ppm, CDCl_3) : 8.2 (s, 1H, -NH), 3.83 (t, 8H, -CH₂, oxazine ring), 6.25 (d, $J = 9.2$ Hz, 1H, -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 A₅ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl_3) : 7.9 (s, 1H, -NH), 3.76 (t, 8H, -CH₂, oxazine ring), 6.6 (d, $J = 9.6$ Hz, 1H, -CO-CH=), 7.3 (d, $J = 9.9$ Hz, 1H, Ar-CH=), 7.1 - 8.0 (m, 13H, Ar-H). ^{13}C NMR (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 A₆ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl_3) : 2.1 (dd, 1H, -CH^a-CH-), 3.2 (dd, 1H, -CH^b-CH-), 5.2 (dd, 1H, -CH-CH₂), 3.89 (s, 3H, p-OCH₃), 3.69 (t, 8H, -CH₂, oxazine ring), 6.9 to 8.2 (m, 18H, 17 Ar-Hand1-NH). ^{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 A₇ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl_3) : 2.6 (dd, 1H, -CH^a-CH-), 2.9 (dd, 1H, -CH^b-CH-), 5.1 (dd, 1H, -CH-CH₂), 3.10 (t, 8H, -CH₂, oxazine ring), 7.0 to 8.2 (m, 18H, 17 Ar-Hand1-NH). ^{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 A₈ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl_3) : 2.9 (dd, 1H, -CH^a-CH-), 3.2 (dd, 1H, -CH^b-CH-), 4.8 (dd, 1H, -CH-CH₂), 3.16 (t, 8H, -CH₂,

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 A₉ : 2 - (3' - trifluoromethylphenylamino) - 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-NO₂), 1231 (C-O- C), 1061 (C- F). ^1H NMR (δ ppm, CDCl₃) : 2.2 (dd, 1H, -CH^a-CH-), 2.5 (dd, 1H, -CH^b-CH-), 5.3 (dd, 1H, -CH-CH₂), 3.46 (t, 8H, -CH₂, 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 A₁₀ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl₃) : 2.4 (dd, 1H, -CH^a-CH-), 2.6 (dd, 1H, -CH^b-CH-), 5.7 (dd, 1H, -CH-CH₂), 3.78 (t, 8H, -CH₂, 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 A₁₁ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl₃) : 3.79 (s, 3H, p-OCH₃), 3.56 (t, 8H, -CH₂, oxazine ring), 6.69 (1H, s, -CH=), 6.7 to 8.1 (m, 13H, 12 Ar-Hand1-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 A₁₂ : 2 - (3' - trifluoromethylphenylamino) - 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). ^1H NMR (δ ppm, CDCl₃) : 3.47 (t, 8H, -CH₂, oxazine ring), 6.56 (1H, s, -CH=), 6.9 to 8.2 (m, 13H, 12 Ar-Hand1-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 A₁₃ : 2 - (3' - trifluoromethylphenylamino) - 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), ^1H NMR (δ ppm, CDCl₃) : 3.72 (t, 8H, -CH₂, oxazine ring), 6.45 (1H, s, -CH=), 6.7 to 8.3 (m, 18H, 17 Ar-Hand1-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 A₁₄ : 2 - (3' - trifluoromethylphenylamino) - 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-NO₂), 1228 (C-O- C), 1097 (C- F). ^1H NMR (δ ppm, CDCl₃) : 3.81 (t, 8H, -CH₂, oxazine ring), 6.51 (1H, s, -CH=), 6.8 to 8.3 (m, 13H, 12 Ar-Hand1-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 A₁₅ : 2 - (3' - trifluoromethylphenylamino) - 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., isoxazole moiety), 1257 (C-O-C), 1067 (C-F). ¹H NMR (δ ppm, CDCl₃) : 3.24 (t, 8H, -CH₂, oxazine ring), 6.41 (1H, s, -CH=), 6.8 to 8.1 (m, 14H, 13 Ar-H and 1-NH). ¹³C 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. clavatus* 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 A₂, A₅, A₁₅ (MIC =100 µg/ml) and A₈, A₉, A₁₀ (MIC =125 µg/ml) exhibited excellent activity, compounds A₁, A₃, A₇ and A₁₁ (MIC =200 µg/ml) showed significant activity while compounds A₄, A₆, A₁₂, A₁₃ and A₁₄ (MIC =250 µg/ml) exhibited equipotent activity against *S. aureus* (MTCC 96) compared to Ampicillin (MIC = 250 µg/ml). Compounds A₇ and A₁₀ (MIC =100 µg/ml) exhibited equipotent activity, whereas compounds A₂, A₃, A₄, A₉ and A₁₄ (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 A₂ (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 A₃, A₇, A₈ (MIC = 100 µg/ml) and A₄, A₅, A₁₂ and A₁₄ (MIC = 125 µg/ml) showed equipotent activity against *E. coli* (MTCC 443) compared to Ampicillin (MIC = 100 µg/ml). Compound A₇ (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 A₄, A₆ (MIC = 100 µg/ml) and A₂, A₁₂ (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 A₂ (MIC = 250 µg/ml) and A₇ (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

$\mu\text{g/ml}$), while compounds A₁, A₅, A₆, A₁₀, A₁₂, A₁₃ and A₁₅ (MIC = 500 $\mu\text{g/ml}$) showed comparable activity against *C. Albicans* (MTCC 227) compared to Greseofulvin (MIC = 500 $\mu\text{g/ml}$). Compounds A₁ (MIC = 200 $\mu\text{g/ml}$) and A₂ (MIC = 100 $\mu\text{g/ml}$) exhibited equipotent activity against *A. niger* (MTCC 282) compared to Greseofulvin (MIC = 100 $\mu\text{g/ml}$) and Nystatin (MIC = 100 $\mu\text{g/ml}$). None of the compounds showed promising antifungal activity against *A. clavatus* (MTCC 1323).

Table 2. Antibacterial and antifungal activity data of compounds A₁ - A₁₅.

Comp	Minimal bactericidal concentration $\mu\text{g/ml}$				Minimal fungicidal concentration $\mu\text{g/ml}$		
	Gram positive		Gram negative				
	<i>S. aureus</i> MTCC-96	<i>S. pyogenus</i> MTCC-442	<i>E. coli</i> MTCC-443	<i>P. aerug</i> MTCC-441	<i>C. albicans</i> MTCC-227	<i>A. niger</i> MTCC-282	<i>A. clavatus</i> 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, ¹H NMR, ¹³C 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 **A₂**, **A₇** and **A₁₂** were found more active than the remaining compounds due to the presence 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 findings concluded that the titled compounds have the properties to kill the microbes in some extent when compared with standard drug. These results suggest that chalcone and their derivatives have an opportunity to behave as broad spectrum antimicrobial agents and have excellent scope for further development as commercial antimicrobial agents.

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