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Synthesis, Characterisation and Pharmacological Studies of Some New Indole Clubbed Chalcones and Its Derivatives Pyrazoline and Pyrimidine as Antiinfective Agents

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Abstract – With the aim to discover new innovative antiinfective agents or to control multidrug resistant bacteria, a combinatorial library of some new heterocyclic derivatives pyrazoline (6a-e) and pyrimidine (7a-e) ring systems incorporate indole nucleous were designed and synthesised from chalcones. Chalcones (5a-e) react with hydrazine hydrate and guanidine hydrochloride gives 1-acetyl pyrazoline (6a-e) and 2-amino pyrimidine (7a-e) derivatives respectively. Confirmation of structures was assigned on the basis of FTIR, 1H NMR, 13C NMR spectral data as well as elemental analysis. In vitro antimicrobial as well as antitubercular proficiency of the title compounds were screened against selected pathogens. Compounds 5b, 5c, 5e, 6c, 7b and 7c exhibited excellent antimicrobial activity and said to be the most proficient members of the series.

Keywords: 5-Methoxy-1H-indole-3-carbaldehyde, 1-Acetyl pyrazoline, 2-Amino pyrimidine, Antimicrobial activity, Anti tubercular activity.

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INTRODUCTION

Antimicrobial infections have emerged as a growing threat to human health [1]. There are many reasons behind and main reason for this includes intrinsic or acquired antimicrobial resistance. Furthermore, many of the newly developed antimicrobial agents lead to serious side effects. Hence, there is a continuous need for new anti-infective drugs, which may selectively attack the microorganism without inhibiting any biochemical system of the host [2]. This has led to search for novel molecular targets for new antifungal drugs. 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, indole is a well-known for a long time and still continue the object of considerable interest mainly due to their applications in various fields [3]. Indole is an important heterocyclic system because it is built into proteins in the form of amino acid tryptophan, because it is the basis of drugs like indomethacin and because it provides the skeleton of indole alkaloids-biologically active compounds from plants including strychnine and LSD. Thus, we are focusing to synthesise novel antiinfective agents clubbed with indole core. Herein we report on the preparation of a series of new 1-acetyl pyrazoline and 2-amino pyrimidine from chalcone of 5-methoxy-1H-indole-3-carbaldehyde and substituted ketones.

Chalcone (1,3-diphenylprop-2-en-1-one) is a α, β-unsaturated carbonyl compound which is common simple scaffold found in many naturally occurring compounds. Chalcone based derivatives have gained focus since they possess simple structures and sundry pharmacological actions. A number of techniques and schemes have been reported for the synthesis of these compounds. Amongst all the stated methods, Aldol condensation and Claisen-Schmidt condensation still hold high position. Other renowned techniques include Suzuki reaction, Witting reaction, Friedel-Crafts acylation with cinnamoyl chloride, Photo-Fries rearrangement of phenyl cinnamates etc. The biological activity associated with them, including anti-inflammatory [4], antimitotic [5], anti-leishmanial [6], anti-tuberculosis [7], antimicrobial [8], anti-malarial [9] etc as well as their recognized synthetic utility in the preparation of pharmacologically-interesting heterocyclic systems like pyrazolines, which have also been largely studied owing to their pharmacological activities Pyrazolines are well-known important nitrogen containing five membered heterocyclic bioorganic molecules and used widely in the current decades due to their various biological and pharmacological activities like antimicrobial-antitubercular [10], antitumor [11], anti-inflammatory [12], antifungal [13], antidepressant [14] etc... . After the pioneering work of Fischer and Knoevenagel in the 19th century, the reaction of ketones and α, β-unsaturated aldehydes with hydrazine hydrate in acetic acid under reflux became one of the most popular methods for the preparation of pyrazolines. Among the existing various pyrazoline type derivatives (1-pyrazoline, 2-pyrazoline etc...), 1-acetylpyrazolines have been identified as one of the most promising scaffolds. Therefore, the above importance and biological activities shown by the pyrazoline compounds, herein we report, the synthesis and biological evaluation of pyrazoline derivatives as antiinfective agents.

Pyrimidine is a six member heterocyclic compound containing four carbon and two nitrogen atoms. In medicinal chemistry, pyrimidine derivatives have been very well known for their therapeutic applications. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential binding blocks of nucleic acids, DNA and RNA is one possible reason for their activity. Literature indicates that compounds having pyrimidine nucleus have wide range of therapeutic uses that include antibacterial [15], anticancer [16], anti-inflammatory [17], antiviral [18], antimalarial [19] 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 and antitubercular activity.

MATERIAL AND METHODS

All the chemical used for reaction were of analytical reagent grade. Melting points were resolute in open capillary method and are uncorrected. 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. FTIR 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. Mass spectra were scanned on a Shimadzu LC-MS 2010 spectrometer (Shimadzu, Tokyo, Japan).Elemental analysis was carried out by Perkin-Elmer 2400 series-II elemental analyser (Perkin-Elmer, USA). Reference drugs used for antimicrobial evaluation were Ampicillin, Chloramphenicol, Ciprofloxacin, Griseofulvin and Nystatin of commercial grade and for antitubercular Isoniazid and Rifampicin

Synthetic method for the preparation of 1-benzyl-5-methoxy-1H-indole-3-carbaldehyde (3)

A 100 ml round bottomed flask, fitted with a reflux condenser was charged with a mixture of 5-Methoxy-1H-indole-3-carbaldehyde **(1)** (0.01 mol), benzyl chloride **(2)** (0.01 mol) and anhydrous K_2CO_3 in dimethylformamide (DMF). Then the reaction mixture was heated under reflux temperature for 5-6 hours. After completion of the reaction as monitored by TLC, the reaction mixture was cooled, and poured onto water· The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol gives 1-benzyl-5-methoxy-1H-indole-3-carbaldehyde **(3)**.

Synthetic method for the preparation of 3-(1-benzyl-5-methoxy-1H-indol-3-yl)-1-(substitutedphenyl)prop-2-en-1-one (5a-e)

By applying classical Claisen-Schmidt condensation reaction, substituted acetophenone **(4a-e)** (0.01 mol) and 1-benzyl-5-methoxy-1H-indole-3-carbaldehyde (0.01 mol) **(3)** dissolved in isopropyl alcohol in a 100 ml conical flask. To make it alkaline solution of 40% KOH (5ml) was added in it. Then the reaction mixture was stirred for 24 hours on a magnetic stirrer at room temperature. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into crushed ice, neutralized with dilute hydrochloric acid and the mixture was agitated for 4 hours a yellow solid was obtained. Finally, the product was isolated by filtration, crystallized from ethanol gives product

3-(1-benzyl-5-methoxy-1H-indol-3-yl)-(1-substitutedphenyl)prop-2-en-1-one **(5a-e)**.

Synthetic method for the preparation of 1-(3-(1-benzyl-5-methoxy-1H-indol-3-yl)-5-(substitutedphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6a-e)

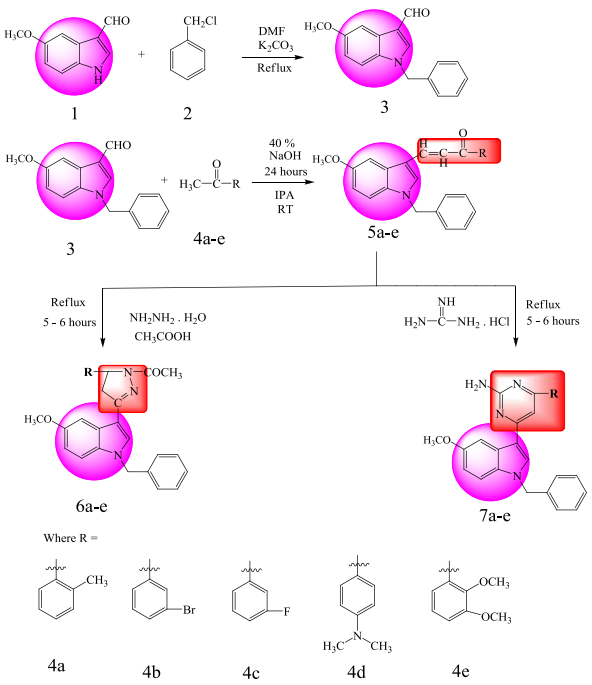
A 100 ml round bottomed flask, fitted with a reflux condenser was charged with a mixture of an appropriate chalcone (**5a-e**) (0.01 mol) and hydrazine hydrate (0.015 mol). To make the mixture acidic catalytic amount of glacial acetic acid (5 ml) was added. The reaction mixture was heated under reflux 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 ethanol gives product (**6a-e**) in good yield.

Synthetic method for the preparation of 4-(1-benzyl-5-methoxy-1H-indol-3-yl)-6-(substitutedphenyl)pyrimidin-2-amine (7a-e)

Compound (**5a-e**) (0.01 mol) condensed with guanidine 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 (10:3 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 in acetone gives product (**7a-e**) with good yield.

All the newly synthesised compounds **3**, (**5a-e**), (**6a-e**) and (**7a-e**) were characterised by IR, ^{1}H NMR, and ^{13}C NMR, LCMS 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 (**5a-e**), (**6a-e**) and (**7a-e**)

SPECTRAL ANALYSIS DATA

1-Benzyl-5-methoxy-1H-indole-3-carbaldehyde (3)

Yield 85%; m.p. 106^{0}C; Anal. Calcd. for $C_{16}H_{13}NO$: C, 81.68; H, 5.57; N, 5.95%. Found: C, 81.60; H, 5.50; N, 5.60%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3012 (aromatic =CH streching), 2925 (C-H streching of alkane), 1712 (C=O streching), 1512 (aromatic C=C streching), 1247 (C-N streching), 1220 (asymmetric C-O-C streching of ether linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.8 (s, 3H, -OC<u>H</u>$_3$), 3.9 (s, 2H, -C<u>H</u>$_2$), 10.5 (s, 1H, -C<u>H</u>O), 6.5 to 8.6 (m, 9H, 08 Ar-<u>H</u> and 1-C<u>H</u> of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 51.2 (CH_2), 54.2 (OCH_3), 103.4 (CH), 105.6 (CH), 112.4 (CH), 114.5 (CH), 118.1 (CH), 126.3 (CH),131.4 (CH), 132.5 (C), 136.0 (CH), 139.1 (CH), 133.2 (CH), 137.2 (CH), 141.8 (C), 143.2 (C), 151.2 (C), 152.4 (C-N), 175.2 (CO); LCMS (m/z): 266.1 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(2-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (5a)

Yield 82%; m.p. 136^{0}C; Anal. Calcd. for $C_{26}H_{23}NO_2$: C, 81.86; H, 6.08; N, 3.67%. Found: C, 81.80; H, 6.10; N, 3.60%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3025 (aromatic =CH streching), 2936 (C-H streching of alkane), 1654 (C=O streching), 1549 (CH=CH streching), 1510 (aromatic C=C streching), 1240 (C-N streching), 1219 (asymmetric C-O-C streching of ether linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):

3.9 (s, 3H, -OC<u>H</u>$_3$), 4.2 (s, 2H, -C<u>H</u>$_2$), 6.5 (1H, d, *J* = 9.2 CO-C<u>H</u>=), 6.5 to 8.6 (m, 12H, 11 Ar-<u>H</u> and 1-C<u>H</u> of indole moiety), 8.2 (1H, d, *J* = 9.0 Ar-C<u>H</u>=); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 53.5 (CH_2), 56.3 (OCH_3), 105.2 (CH), 108.1 (CH), 113.5 (CH), 116.2 (CH), 119.8 (CH), 123.0 (=CH), 125.4 (CH), 132.2 (CH), 135.9 (C), 137.5 (CH), 138.2 (CH), 142.3 (CH), 143.5 (CH), 145.2 (=CH), 148.1 (C), 152.3 (C), 153.4 (C), 158.2 (C-N), 170.2 (CO); LCMS (m/z): 382.5 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(3-bomophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (5b)

Yield 75%; m.p. 145^{0}C; Anal. Calcd. for $C_{24}H_{18}BrNO$: C, 69.24; H, 4.36; N, 3.36%. Found: C, 69.20; H, 4.40; N, 3.30%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3020 (aromatic =CH streching), 2930 (C-H streching of alkane), 1660 (C=O streching), 1550 (CH=CH streching), 1513 (aromatic C=C streching), 1249 (C-N streching), 1239 (asymmetric C-O-C streching of ether linkage), 556 (C-Br streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.7 (s, 3H, -OC<u>H</u>$_3$), 3.9 (s, 2H, -C<u>H</u>$_2$), 6.3 (1H, d, *J* = 8.0 CO-C<u>H</u>=), 6.9 to 8.2 (m, 12H, 11 Ar-<u>H</u> and 1-C<u>H</u> of indole moiety), 8.5 (1H, d, *J* = 8.3 Ar-C<u>H</u>=); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 51.6 (CH_2), 59.2 (OCH_3), 108.1 (CH), 110.3 (CH), 114.6 (CH), 117.2 (CH), 120.5 (CH), 125.6 (=CH), 128.2 (CH), 131.6 (C), 134.3 (C), 135.2 (CH), 137.8 (CH), 140.1 (CH), 142.0 (CH), 143.9 (=CH), 145.2 (C), 153.4 (C), 158.2 (C), 159.3 (C-N), 168.2 (CO); LCMS (m/z): 417.3 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (5c)

Yield 75%; m.p. 145^{0}C; Anal. Calcd. for $C_{24}H_{18}FNO$: C, 81.11; H, 5.35; N, 3.94%. Found: C, 81.20; H, 5.30; N, 3.90%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3036 (aromatic =CH streching), 2935 (C-H streching of alkane), 1662 (C=O streching), 1555 (CH=CH streching), 1512 (aromatic C=C streching), 1250 (C-N streching), 1234 (asymmetric C-O-C streching of ether linkage), 1123 (C-F streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.8 (s, 3H, -OC<u>H</u>$_3$), 3.9 (s, 2H, -C<u>H</u>$_2$), 6.4 (1H, d, *J* = 7.6 CO-C<u>H</u>=), 6.7 to 8.3 (m, 12H, 11 Ar-<u>H</u> and 1-C<u>H</u> of indole moiety), 8.4 (1H, d, *J* = 7.4 Ar-C<u>H</u>=); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 52.4 (CH_2), 58.3 (OCH_3), 110.4 (CH), 112.2 (CH), 113.5 (CH), 115.6 (CH), 122.0 (CH), 123.3 (=CH), 126.1 (CH), 130.4 (C), 135.2 (C), 137.8 (CH), 139.2 (CH), 141.3 (CH), 143.2 (CH), 145.3 (=CH), 147.8 (C), 151.2 (C), 155.2 (C), 160.5 (C-N), 169.3 (CO); LCMS (m/z): 356.8 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(4-N,N-dimethylaminophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (5d)

Yield 78%; m.p. 163^{0}C; Anal. Calcd. for $C_{26}H_{24}N_2O$: C, 82.07; H, 6.36; N, 7.36%. Found: C, 82.06; H, 6.30; N, 7.30%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3030 (aromatic =CH streching), 2921 (C-H streching of alkane), 1640 (C=O streching), 1550 (CH=CH streching), 1516 (aromatic C=C streching), 1312 (-CH_3 streching), 1256 (C-N streching), 1230 (asymmetric C-O-C streching of ether linkage),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.9 (s, 3H, -C<u>H</u>$_3$), 2.0 (s, 3H, -C<u>H</u>$_3$), 3.8 (s, 3H, -OC<u>H</u>$_3$), 4.3 (s, 2H, -C<u>H</u>$_2$), 6.2 (1H, d, *J* = 7.9 CO-C<u>H</u>=), 6.9 to 8.0 (m, 12H, 11 Ar-<u>H</u> and 1-C<u>H</u> of indole moiety), 8.2 (1H, d, *J* = 7.4 Ar-C<u>H</u>=); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 36.2 (CH_3), 51.3 (CH_2), 55.2 (OCH_3), 112.5 (CH), 113.0 (CH), 115.0 (CH), 117.8 (CH), 121.5 (CH), 124.2 (=CH), 127.6 (CH), 129.3 (C), 130.2 (C), 136.0 (CH), 138.9 (CH), 139.2 (CH), 142.4 (CH), 143.2 (=CH), 146.5 (C), 150.3 (C), 153.5 (C), 161.0 (C-N), 173.5 (CO); LCMS (m/z): 381.2 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(2,3-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (5e)

Yield 78%; m.p. 163^{0}C; Anal. Calcd. for $C_{26}H_{23}O_3N$: C, 78.57; H, 5.82; N, 3.52%. Found: C, 78.80; H, 5.80; N, 3.50%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3012 (aromatic =CH streching), 2978 (C-H streching of alkane), 1646 (C=O streching), 1553 (CH=CH streching), 1510 (aromatic C=C streching), 1410 (-OCH_3 streching), 1259 (C-N streching), 1223 (asymmetric C-O-C streching of ether linkage),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.7-3.9 (m, 9H, -OC<u>H</u>$_3$), 4.1 (s, 2H, -C<u>H</u>$_2$), 5.9 (1H, d, *J* = 6.2 CO-C<u>H</u>=), 6.5 to 8.2 (m, 11H, 10 Ar-<u>H</u> and 1-C<u>H</u> of indole moiety), 8.1 (1H, d, *J* = 6.1 Ar-C<u>H</u>=); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 50.2 (CH_2), 55.4, (OCH_3), 110.5 (CH), 114.2 (CH), 116.3 (CH), 118.2 (CH), 122.1 (CH), 123.0 (=CH), 126.7 (CH), 128.0 (C), 131.4 (C), 134.2 (CH), 136.2 (CH), 138.3 (CH), 140.5 (CH), 143.1 (=CH), 149.2 (C), 152.4 (C), 155.6 (C), 162.6 (C-N), 171.6 (CO); LCMS (m/z): 398.5 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(2-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6a)

Yield 72%; m.p. 158^{0}C; Anal. Calcd. for $C_{27}H_{25}N_3O$: C, 79.58; H, 6.18; N, 10.31%. Found: C, 79.60; H, 6.20; N, 10.15%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3082 (aromatic =CH streching), 2990 (C-H streching of pyrazoline moiety), 1660 & 1575 (C=O and C=N streching of pyrazoline moiety), 1510 (aromatic C=C streching), 1355 (CH_3 streching of pyrazoline moiety), 1247 (asymmetric C-O-C streching of ether linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.4 (s, 3H, -COC<u>H</u>$_3$), 3.1 (dd, 1H, -C<u>H</u>$_x$-CH, *J* = 12.1 & 13.6 Hz), 3.4 (dd, 1H, -C<u>H</u>$_y$-CH, *J* = 12.1 & 13.6 Hz), 4.8 (dd, 1H, -C<u>H</u>-CH_2-Ar, *J* = 4.7 & 12.6 Hz), 3.9 (s, 1H, OC<u>H</u>$_3$), 7.0 to 8.0 (m, 14H, 13 Ar-<u>H</u> and 1-C<u>H</u> of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 21.2 (CH_3, pyrazoline moiety), 38.0 (CH_2, methylene, pyrazoline moiety), 40.2 (CH_3), 56.2 (OCH_3), 62.1 (CH-Ar), 112.1 (CH), 113.5 (CH), 114.0 (CH), 117.8 (CH), 119.5 (CH), 120.5 (CH), 123.4 (CH), 129.4 (CH), 131.0 (C), 136.3 (CH), 143.0 (C), 149.2 (C), 151.3 (C), 155.4 (C-OCH_3),

162.3 (C=N), 170.2 (CO pyrazoline moiety); LCMS (m/z): 310.2 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(3-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6b)

Yield 69%; m.p. 126^{0}C; Anal. Calcd. for $C_{26}H_{22}N_3BrO$: C, 66.11; H, 4.69; N, 8.90%. Found: C, 66.12; H, 4.21; N, 8.96%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3080 (aromatic =CH streching), 2995 (C-H streching of pyrazoline moiety), 1669 & 1580 (C=O and C=N streching of pyrazoline moiety), 1512 (aromatic C=C streching), 1350 (CH_3 streching of pyrazoline moiety), 1240 (asymmetric C-O-C streching of ether linkage), 559 (C-Br streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.6 (s, 3H, -COC$\underline{H}_3$), 2.9 (dd, 1H, -C$\underline{H}_x$-CH, *J* = 10.5 & 12.3 Hz), 3.2 (dd, 1H, -C$\underline{H}_y$-CH, *J* = 10.5 & 12.3 Hz), 3.8 (s, 1H, OC$\underline{H}_3$), 4.9 (dd, 1H, -C$\underline{H}$-CH$_2$-Ar, *J* = 4.1 & 12.9 Hz), 6.9 to 8.1 (m, 14H, 13 Ar-$\underline{H}$ and 1-C$\underline{H}$ of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 24.5 (CH_3, pyrazoline moiety), 34.1 (CH_2), 36.1 (CH_2, methylene, pyrazoline moiety), 53.4 (OCH_3), 60.2 (CH-Ar), 110.3 (CH), 114.2 (CH), 115.3 (CH), 116.7 (CH), 118.0 (CH), 122.4 (CH), 126.3 (CH), 130.4 (CH), 132.4 (C), 135.0 (CH), 142.7 (C), 150.6 (C), 152.1 (C), 156.9 (C-OCH_3), 161.2 (C=N), 174.3 (CO pyrazoline moiety); LCMS (m/z): 473.5 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6c)

Yield 83%; m.p. 113^{0}C; Anal. Calcd. for $C_{26}H_{22}N_3FO$: C, 75.89; H, 5.39; N, 10.21%. Found: C, 75.85; H, 5.18; N, 10.18%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3078 (aromatic =CH streching), 2990 (C-H streching of pyrazoline moiety), 1645 & 1568 (C=O and C=N streching of pyrazoline moiety), 1510 (aromatic C=C streching), 1353 (CH_3 streching of pyrazoline moiety), 1222 (asymmetric C-O-C streching of ether linkage), 1106 (C-F streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.2 (s, 3H, -COC$\underline{H}_3$), 2.6 (dd, 1H, -C$\underline{H}_x$-CH, *J* = 9.5 & 10.1 Hz), 3.8 (dd, 1H, -C$\underline{H}_y$-CH, *J* = 9.6 & 10.4 Hz), 3.9 (s, 1H, OC$\underline{H}_3$), 5.2 (dd, 1H, -C$\underline{H}$-CH$_2$-Ar, *J* = 4.9 & 10.5 Hz), 6.6 to 8.2 (m, 14H, 13 Ar-$\underline{H}$ and 1-C$\underline{H}$ of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 26.2 (CH_3, pyrazoline moiety), 37.0 (CH_2), 39.0 (CH_2, methylene, pyrazoline moiety), 56.2 (OCH_3), 66.3 (CH-Ar), 111.4 (CH), 115.3 (CH), 116.5 (CH), 118.5 (CH), 119.3 (CH), 120.5 (CH), 124.2 (CH), 126.7 (CH), 129.1 (C), 131.5 (CH), 136.8 (C), 148.2 (C), 153.4 (C), 159.2 (C-OCH_3), 160.3 (C=N), 170.2 (CO pyrazoline moiety); LCMS (m/z): 412.2 (M+1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(4-N,N-dimethylaminophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6d)

Yield 76%; m.p. 136^{0}C; Anal. Calcd. for $C_{28}H_{28}N_4O$: C, 77.04; H, 6.46; N, 12.83%. Found: C, 77.10; H, 6.40; N, 12.80%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3072 (aromatic =CH streching), 2993 (C-H streching of pyrazoline moiety), 1642 & 1560 (C=O and C=N streching of pyrazoline moiety), 1512 (aromatic C=C streching), 1356 (CH_3 streching of pyrazoline moiety), 1220 (asymmetric C-O-C streching of ether linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.8 (m, 3H, CH_3), 1.9 (m, 3H, CH_3), 2.5 (s, 3H, -COC$\underline{H}_3$), 2.9 (dd, 1H, -C$\underline{H}_x$-CH, *J* = 9.9 & 13.4 Hz), 3.6 (dd, 1H, -C$\underline{H}_y$-CH, *J* = 9.0 & 13.2 Hz), 3.9 (s, 1H, OC$\underline{H}_3$), 5.6 (dd, 1H, -C$\underline{H}$-CH$_2$-Ar, *J* = 5.3 & 10.1 Hz), 6.9 to 8.1 (m, 14H, 13 Ar-$\underline{H}$ and 1-C$\underline{H}$ of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 25.3 (CH_3, pyrazoline moiety), 28.4 (CH_3), 35.4 (CH_2), 38.2 (CH_2, methylene, pyrazoline moiety), 55.1 (OCH_3), 68.4 (CH-Ar), 110.2 (CH), 113.2 (CH), 118.2 (CH), 119.0 (CH), 121.4 (CH), 122.3 (CH), 125.0 (CH), 126.8 (CH), 131.5 (C), 133.2 (CH), 139.2 (C), 144.2 (C), 152.4 (C), 160.0 (C-OCH_3), 161.1 (C=N), 169.1 (CO pyrazoline moiety); LCMS (m/z): 435.5 (M-1).

1-(3-(1-Benzyl-5-methoxy-1H-indol-3-yl)-5-(2,3-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6e)

Yield 80%; m.p. 156^{0}C; Anal. Calcd. for $C_{28}H_{27}N_3O_3$: C, 74.15; H, 6.00; N, 9.27%. Found: C, 74.20; H, 6.15; N, 9.30%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3068 (aromatic =CH streching), 2969 (C-H streching of pyrazoline moiety), 1646 & 1565 (C=O and C=N streching of pyrazoline moiety), 1519 (aromatic C=C streching), 1359 (CH_3 streching of pyrazoline moiety), 1225 (asymmetric C-O-C streching of ether linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.9 (s, 3H, -COC$\underline{H}_3$), 3.1 (dd, 1H, -C$\underline{H}_x$-CH, *J* = 8.2 & 11.5 Hz), 3.8 (dd, 1H, -C$\underline{H}_y$-CH, *J* = 8.2 & 11.8 Hz), 3.6-3.9 (m, 9H, OC$\underline{H}_3$), 5.3 (dd, 1H, -C$\underline{H}$-CH$_2$-Ar, *J* = 5.6 & 11.1 Hz), 6.6 to 8.3 (m, 13H, 12 Ar-$\underline{H}$ and 1-C$\underline{H}$ of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 24.1 (CH_3, pyrazoline moiety), 36.5 (CH_2), 39.1 (CH_2, methylene, pyrazoline moiety), 54.3 (OCH_3), 69.1 (CH-Ar), 108.2 (CH), 110.3 (CH), 116.4 (CH), 118.4 (CH), 120.5 (CH), 123.1 (CH), 126.2 (CH), 128.1 (CH), 130.2 (C), 132.4 (CH), 133.2 (C), 140.7 (C), 150.2 (C), 158.3 (C-OCH_3), 159.4 (C=N), 171.0 (CO pyrazoline moiety); LCMS (m/z): 454.3 (M+1).

4-(1-Benzyl-5-methoxy-1H-indol-3-yl)-6-(2-methylphenyl)pyrimidin-2-amine (7a)

Yield 81%; m.p. 136^{0}C; Anal. Calcd. for $C_{27}H_{24}N_4O$: C, 77.12; H, 5.75; N, 13.32%. Found: C, 77.10; H, 5.70; N, 13.40%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3312 (NH_2 str. 1^0 amine of pyrimidine moiety), 3022 (aromatic =CH streching), 2998 (C-H streching of pyrimidine moiety), 1646 (C=N streching of pyrimidine moiety), 1524 (aromatic C=C streching), 1354 (CH_3 streching), 1235 (asymmetric C-O-C streching of ether linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.6 (s, 3H, C$\underline{H}_3$), 3.8 (s, 3H, OC$\underline{H}_3$), 5.0 (s, 2H,-NH_2), 7.2 to 8.0 (m, 15H, 14 Ar-$\underline{H}$ and 1-C$\underline{H}$ of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 29.2 (CH_2), 54.1 (OCH_3), 102.3 (CH, pyrimidine moiety), 113.4 (CH), 115.2 (CH), 117.3 (CH), 120.1 (CH),

123.9 (CH), 126.5 (CH), 129.2 (CH), 130.4 (CH), 132.1 (C), 134.5 (C), 151.4 (C), 156.5 (C), 161.2, 162.7, 164.5 (C, pyrimidine moiety); LCMS (m/z): 421.4 (M+1).

4-(1-Benzyl-5-methoxy-1H-indol-3-yl)-6-(3-bromophenyl)pyrimidin-2-amine (7b)

Yield 79%; m.p. 112^{0}C; Anal. Calcd. for $C_{26}H_{21}N_4BrO$: C, 64.34; H, 4.36; N, 11.54%. Found: C, 64.40; H, 4.30; N, 11.50%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3320 (NH_2 str. 1^0 amine of pyrimidine moiety), 3020 (aromatic =CH streching), 2990 (C-H streching of pyrimidine moiety), 1649 (C=N streching of pyrimidine moiety), 1526 (aromatic C=C streching), 1230 (asymmetric C-O-C streching of ether linkage), 546 (C-Br streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.9 (s, 3H, OCH_3), 5.2 (s, 2H,-NH_2), 7.1 to 8.0 (m, 15H, 14 Ar-H and 1-CH of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 27.4 (CH_2), 55.2 (OCH_3), 100.4 (CH, pyrimidine moiety), 111.3 (CH), 113.2 (CH), 115.4 (CH), 121.3 (CH), 126.2 (CH), 129.2 (CH), 131.0 (CH), 131.5 (CH), 133.4 (C), 136.2 (C), 150.2 (C), 154.2 (C), 160.5, 161.0, 164.2 (C, pyrimidine moiety); LCMS (m/z): 484.2 (M-1).

4-(1-Benzyl-5-methoxy-1H-indol-3-yl)-6-(3-fluorophenyl)pyrimidin-2-amine (7c)

Yield 74%; m.p. 178^{0}C; Anal. Calcd. for $C_{26}H_{21}N_4FO$: C, 73.57; H, 4.99; N, 13.20%. Found: C, 73.50; H, 4.80; N, 13.21%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3322 (NH_2 str. 1^0 amine of pyrimidine moiety), 3025 (aromatic =CH streching), 2992 (C-H streching of pyrimidine moiety), 1648 (C=N streching of pyrimidine moiety), 1521 (aromatic C=C streching), 1236 (asymmetric C-O-C streching of ether linkage), 1045 (C-F streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.1 (s, 3H, OCH_3), 5.9 (s, 2H,-NH_2), 6.8 to 8.2 (m, 15H, 14 Ar-H and 1-CH of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 26.2 (CH_2), 53.1 (OCH_3), 98.1 (CH, pyrimidine moiety), 110.2 (CH), 112.1 (CH), 114.2 (CH), 120.4 (CH), 128.9 (CH), 129.0 (CH), 132.2 (CH), 133.9 (CH), 135.5 (C), 137.4 (C), 138.8 (C), 152.1 (C), 158.9, 160.1, 163.5 (C, pyrimidine moiety); LCMS (m/z): 425.6 (M+1).

4-(1-Benzyl-5-methoxy-1H-indol-3-yl)-6-(4-N,N-dimethylaminophenyl)pyrimidin-2-amine (7d)

Yield 60%; m.p. 153^{0}C; Anal. Calcd. for $C_{28}H_{27}N_5O$: C, 74.81; H, 6.05; N, 15.58%. Found: C, 74.80; H, 6.25; N, 15.60%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3326 (NH_2 str. 1^0 amine of pyrimidine moiety), 3020 (aromatic =CH streching), 2990 (C-H streching of pyrimidine moiety), 1650 (C=N streching of pyrimidine moiety), 1526 (aromatic C=C streching), 1325 (CH_3 streching), 1230 (asymmetric C-O-C streching of ether linkage); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.3 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3), 5.3 (s, 2H,-NH_2), 6.7 to 8.1 (m, 15H, 14 Ar-H and 1-CH of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 22.4 (CH_2), 39.4 (CH_3), 56.2 (OCH_3), 102.5 (CH, pyrimidine moiety), 111.3 (CH), 114.3 (CH), 118.1 (CH), 122.5 (CH), 128.2 (CH), 131.3 (CH), 134.4 (CH), 135.2 (CH), 136.3 (C), 132.9 (C), 140.5 (C), 150.2 (C), 152.4, 157.0, 160.3 (C, pyrimidine moiety); LCMS (m/z): 372.1 (M-1).

4-(1-Benzyl-5-methoxy-1H-indol-3-yl)-6-(2,3-dimethoxyphenyl)pyrimidin-2-amine (7e)

Yield 69%; m.p. 123^{0}C; Anal. Calcd. for $C_{28}H_{26}N_4O_3$: C, 72.09; H, 5.62; N, 12.01%. Found: C, 72.15; H, 5.20; N, 12.10%; IR (KBr, $\nu_{max,}$ cm^{-1}): 3421 (NH_2 str. 1^0 amine of pyrimidine moiety), 3063 (aromatic =CH streching), 2987 (C-H streching of pyrimidine moiety), 1656 (C=N streching of pyrimidine moiety), 1520 (aromatic C=C streching), 1225 (asymmetric C-O-C streching of ether linkage), 1125 (OCH_3 streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.6-4.0 (m, 9H, OCH_3), 5.0 (s, 2H,-NH_2), 6.8 to 8.2 (m, 14H, 13 Ar-H and 1-CH of indole moiety); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 20.5 (CH_2), 59.2 (OCH_3), 104.3 (CH, pyrimidine moiety), 110.2 (CH), 113.5 (CH), 117.2 (CH), 120.4 (CH), 122.6 (CH), 130.8 (CH), 133.7 (CH), 135.6 (CH), 137.2 (C), 139.5 (C), 142.3 (C), 152.4 (C), 153.3, 156.6, 161.1 (C, pyrimidine moiety); LCMS (m/z): 467.2 (M+1).

RESULT AND DISCUSSION

Chemistry

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 synthetic route used to synthesise the unreported title compounds **(5a-e), (6a-e)** and **(7a-e)** is illustrated in reaction scheme. The formation of all these new heterocyclic derivatives were fully characterised by means of spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and LCMS. As an example, in the IR spectrum of compound **5a**, a strong absorption band is observed at 1549 and 1654 cm^{-1} which corresponds to the stretching vibration of the CH = CH and C=O functionality of α, β- unsaturated carbonyl group of chalcone moiety. The =CH and C=C functionality of aromatic ring were observed at 1510 and 3025 cm^{-1} respectively. The ^{1}H NMR spectrum of compound **5a** showed a doublet at δ 6.5 (J = 9.2 Hz) ppm for the -CO-CH= and at δ 8.2 (J = 9.0 Hz) ppm for the Ar-CH= of α, β unsaturated carbonyl group protons. The other remaining twelve aromatic and indole protons appeared as a multiplet signal at δ 6.5-8.3 ppm. Finally, the ^{13}C NMR spectra of the compound **5a** was recorded in $CDCl_3$ and the spectral signals were in good agreement with the proposed structure. In the ^{13}C NMR spectrum of compound **5a**, the most deshielded signal that appeared at δ 170.2 ppm was assigned to the carbonyl carbon of the chalcone moiety. The signal for CH = CH functionality of α, β- unsaturated carbonyl group was appeared at δ 123.0 and 145.2 ppm. The signals for aromatic carbons appeared between at δ 105.2-153.4 ppm in the ^{13}C spectrum.

In the IR spectrum of compound **6a**, a strong absorption band is observed at 1660 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 1575 and 1510 cm^{-1} respectively. The C_4''-H stretching of pyrazoline ring was observed at 2990 cm^{-1}. A strong absorption band was observed at 1355 cm^{-1} due to the presence of the CH_3 group. The ^{1}H NMR spectrum of compound **6a** showed a singlet at δ 2.4 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 3.1 ppm (*J* = 12.1 & 13.6 Hz) and at δ 3.4 ppm (*J* = 12.1 & 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 4.8 ppm (*J* = 4.7 & 12.6 Hz) due to CH-CH_2-Ar proton. The other remaining fourteen aromatic protons appeared as a multiplet signal at δ 7.0-8.0 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 **6a**, the shielded signal at δ 38.0 and 40.2 ppm was assigned to the methylene and methyl carbon of pyrazoline ring. The most deshielded signal that appeared at δ 170.2 ppm was assigned to the carbonyl carbon of the acetyl group attached with the pyrazoline unit. The signals for aromatic carbons appeared between δ 112.1-162.3 ppm in the ^{13}C spectrum.

The IR spectrum of compound **7a** showed a strong characteristic band at 1646 cm^{-1} and 3312 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 2998 cm^{-1}. The aromatic C=C stretching was observed at 1524 cm^{-1} respectively. The ^{1}H NMR spectrum of compound **7a** showed a sharp singlet at δ 5.0 due to the NH_2 protons, and it also showed a sharp singlet at δ 7.4 due to HC=C, which confirmed the cyclisation of the chalcone into a pyrimidine ring. The other remaining fifteen aromatic protons resonate as a multiplet signal at δ 7.2-8.0 ppm. ^{13}C NMR spectrum of compound **7a** showed a signal at 102.3 due to the –CH carbon of pyrimidine ring and signal at δ 161.2, 162.7 and 164.5 ppm assigned to the C=N carbon of pyrimidine ring which assigned the pyrimidine unit. The signals for aromatic carbons appeared between δ 113.4-156.5.0 ppm in the ^{13}C spectrum. The obtained elemental analysis values are in good agreement with theoretical data. Further, mass spectra of all the title compounds showed molecular ion peak M^+ corresponding to their exact mass which is in agreement with its proposed structure.

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) [20]. All the synthesised compounds (**5a-e**), (**6a-e**) and (**7a-e**) 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 chalcone (**5a-e**), 1- acetyl pyrazoline (**6a-e**) and 2-amino pyrimidine derivatives (**7a-e**) pointed out that compound **7c** showed an outstanding inhibitory effect i.e. MIC = 62.5 µg/ml against Staphylococcus aureus as compared ampicillin (MIC = 250 µg/ml) and moderate to chloramphenicol and ciprofloxacin (MIC = 50 µg/ml) while compounds **5c** and **6c** (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, compound **5c** showed an outstanding inhibitory effect i.e. MIC = 50 µg/ml compounds while **6b** and **6e** (MIC = 100 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 **5b, 6c** and **7c** (MIC = 50 µg/ml) showed maximum activity against Escherichia coli as compared to ampicillin while compounds **5c, 6b, 6e, 7b** and **7d** (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). Compound **6b** (MIC = 50 µg/ml) showed excellent activity while compounds **5b, 5d, 5e, 6c, 6e, 7b** and **7c** (MIC = 100 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. 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 **6b** and **7b** (MIC = 100 µg/ml) displayed highest antifungal activity against Candida albicans as compared to griseofulvin (MIC = 500 µg/ml) and equivalent to nystatin (MIC = 100 µg/ml). Compounds **5a** and **7d** showed the same potency as griseofulvin (MIC = 500 µg/ml) against Candida albicans. Compound **5c** (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.

Table - 1. Antimicrobial activity data of synthesised compounds (5a-e), (6a-e) and (7a-e)

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
5a	200	125	250	200	500	>1000	500
5b	125	200	50	100	250	1000	500
5c	100	50	100	125	200	100	200
5d	200	125	125	100	1000	500	500
5e	125	500	62.5	100	>1000	>1000	>1000
6a	250	200	125	200	250	>1000	1000
6b	200	100	100	50	100	200	500
6c	100	200	50	100	200	>1000	200
6d	500	200	125	250	>1000	>1000	1000
6e	250	100	100	100	>1000	500	>1000
7a	125	500	250	200	>1000	1000	500
7b	50	200	100	100	100	1000	>1000
7c	62.5	125	50	100	125	500	200
7d	250	200	100	250	500	500	500
7e	500	200	200	200	1000	>1000	1000
Ampi.	125	100	100	100	-	-	-
Chlo.	50	50	50	50	-	-	-
Cipr.	50	50	25	25	-	-	-
Gris.	-	-	-	-	500	500	500
Nyst.	-	-	-	-	100	100	100

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.

In Vitro Antitubercular Activity

The encouraging results of the antimicrobial screening prompted us to screen the title compounds for their *in vitro* antitubercular activity. The *in vitro* antitubercular activity of all the newly synthesized compounds were determined by using Lowenstein-Jensen medium (conventional method) against *Mycobacterial tuberculosis* H37Rv strain [21]. The observed results are presented in **Table 2** in the form of inhibition (%), relative to that of standard antitubercular drugs isoniazid and rifampicin. Compounds demonstrating more than 90% inhibition in the primary screening were retested at lower concentration (MIC) in a Lowenstein–Jensen medium and evaluated for their MIC values. Among the compounds screened for antitubercular activity, compounds **5a** (MIC = 62.5 µg/ml), **5c** (MIC = 50 µg/ml), **5e** (MIC = 100 µg/ml) **6a** (MIC = 62.5 µg/ml), **6d** (MIC = 50 µg/ml), **7a** (MIC = 62.5 µg/ml) and **7c** (MIC = 62.5 µg/ml) were found to possess the greatest potency against Mycobacterium tuberculosis with **86**, **90**, **80**, **82**, **81**, **92** and **80** % inhibition respectively (**Table 3**). Other derivatives showed moderate to poor antitubercular activity.

Table 2: In vitro antitubercular activity (% inhibition) of the synthesized compounds (5a-e), (6a-e) and (7a-e) at concentration 250 µg/ml

Compd	Inhibition (%)
5a	86
5b	78
5c	90
5d	54
5e	80
6a	82
6b	74
6c	35
6d	81
6e	77
7a	92
7b	79
7c	80
7d	54
7e	46
Isoniazid	99
Rifampicin	98

Table 3: In vitro antitubercular activity of compounds exhibiting greater inhibition

Compd	Inhibition (%)	MIC (µg/ml)
5a	86	62.5
5c	90	50
5e	80	100
6a	82	62.5
6d	81	50
7a	92	100
7c	80	62.5
Isoniazid	99	0.20
Rifampicin	98	40

CONCLUSION

Indole clubbed chalcone, pyrazoline and pyrimidine derivatives have been synthesised in good yield and screened for their biological activity with the aim of discovering innovative structure leads serving as potent antimicrobial and antituberculaar agents. The results indicated that all the derivatives exhibited appreciable antibacterial activities. Among the fifteen newly synthesised compounds, analogs

5b, **6a**, **6e** and **7b** possessing electron withdrawing atom/group such as methyl, bromo and fluoro at the ortho or meta position were identified as the most potent 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. Moreover, compounds **5a, 5c**, **5e**, **6a**, **6d**, **7a**, **7c** displayed excellent anti tubercular activity.

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