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An Efficient Synthesis of Hybrid Chalcone and Acetyl Pyrazoline Derivatives as Potent Antimycobacterial and Antimicrobial Agents

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Abstract – Due to increasing microbial drug resistance, it is to more necessary to develop new antimycobacterial and antimicrobial agents. For this purpose, some new indole base chalcone (Va-e) analogs synthesised and converted into 1-acetyl-2-yrazoline (VIa-e) derivatives.Target compounds were evaluated for their antimycobacterial efficacy against Mycobacterium tuberculosis H_{37}Rv and antimicrobial activity against four common pathogenic bacterial and three common fungal strains. Structures of entire newly synthesized compounds were assigned on the basis of FTIR, ^{1}H NMR, ^{13}C NMR, LCMS as well as elemental analysis. Five derivatives (Va, Vd, Ve, VIb and VIc) displayed significant antitubercular activity. In terms of antimicrobial activity, most compounds exhibited moderate to potent activity against the bacteria and the fungal.

Keywords: 5-Methoxy-1H-indole-3-carbaldehyde, Claisen-Schmidt Condensation, 1-Acetyl pyrazoline, Antimycobacterial activity, Antimicrobial activity.

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INTRODUCTION

Physiological activities of chalcone derivatives were synthesized as series such as anti-inflammatory [1], antiviral [2], antitumor [3] , anticancer [4] and etc. The chalcone derivatives have varieties of structure and its different types of activity. In recent year bacterial infection increases and serious disease like tumor dangers for human health. In last five years, a numbers of studies shown that chalcone derivative could inhibit the increase of tumor [5-7].

Chalcones and their different types of derivative more focus due to their anti-viral,anti-microbial, anti-inflammatory activities [8, 9] and have invention of anti-cancer agents [10-12].

Pyrazole characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions. Fused heterocyclic pyrazole and its derivatives constitute an interesting class of heterocycles due to their synthetic variety and their biological activities [13].

Fungal infection and bacterial infection an important damage and a major cause of deaths. [14,15]. Recently, further investigation is modifying novel antimicrobial agents with potential efficacy [16,17]. Different antimicrobial agents have been developed by researchers in this type of disease. Yu et al. synthesized pyrazole-fused tricyclic deterrence derivatives and determined their antibacterial activity.

Knorr has been described Pyrazole moiety which comes from azoles family in 1833 [18]. Substituted Pyrazole are used as chelating reagents for many metal ions [19]. Moreover, pyrazoline containing hybrid chalcone derivatives are used as starting material for the build of condensed heterocyclic systems and express an important template for combined chemistry [20]. Therefore, the synthesis of hybrid chalcone with pyrazole and its derivatives has received an increasing attention to synthetic organic chemists and biologists. Some reviews on involvement of pyrazole nucleus as different biological agents are available in the literature [21,22]

The ratio of α,β-unsaturated ketone with hydrazine hydrate in presence of acetic acid was most useful method of pyrazoline preparation. Acetyl pyrazoline with hybrid club chalcone as one of the most scaffolds. Hence, these type of important and its biological activities shown by the pyrazoline with hybrid chalcone compound. Here with we

have carried out the synthesis and biological evaluation of pyrazoline derivatives as antimicrobial agent.

MATERIAL AND METHODS

The reagents and solvents used for reaction were of analytical reagent (AR) grade. Melting points were determined in MP80 latest model of mettle toledo. IR spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets. ^{1}H NMR and 13C NMR spectra were recorded on a Bruker Advance 400 F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using $CDCl_3$ as a solvent and TMS as an internal standard at 400 MHz frequency respectively. Chemical shifts are reported in parts per million (ppm) and coupling constant (J) are reported in Hertz. Elemental analysis was carried out by Perkin-Elmer 2400 series-II elemental analyser (Perkin-Elmer, USA). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan). TLC was run on E-Merck pre-coated 60 F254 plates and the spots were rendered visible by exposing to UV light or iodine chamber. Reference drugs antimicrobial and antitubercular activity are Ampicillin, Chloramphenicol, Ciprofloxacin, Griseofulvin, Nystatin, Rifampicin and Isoniazid used of commercial grade.

Preparation of N'-((5-acetylthiophen-2-yl) methylene)-2-chloronicotinohydrazide (III)

0.01 mol of 2-chloroisonicotinohydrazide and 0.01 mol of 5-acetylthiophene-2-carbaldehyde in 100 ml round bottom flask attached with reflux condenser. The reaction mixture heated in ethanol at reflux temperature for 5-6 hours. Reaction monitoring on TLC mobile phase ration of toluene and methanol in the ratio of (7 : 3)ml. After completion of reaction mixture poured onto water. The fallout precipitated filtered and wash with water. After dried and recrystallized from ethanol gives N'-((5-acetylthiophen-2-yl) methylene)-2-chloronicotinohydrazide (III). FTIR (KBr, vmax, cm^{-1}): 1518 (C=N streching, pyridine ring moiety), 1660 (C=O streching, amide ketone), 3369 (N-H assymetric streching), 1565 (aromatic C=C streching), 1718 (asymmetric C-O-C streching of ether linkage); 1H NMR (400 MHz, CDCl3, δ ppm): 2.5 (s, 3H, -CH3), 7.0 (s, 1H, -NH), 8.7 (s, 1H, =CH), 7.2 to 8.7 (m, 5H, 3Ar-H and 2-CH of Thiophene moiety); 13C NMR (400 MHz, CDCl3, δ ppm) : 26.4 (CH3), 148.3 (CH), 124.2 (CH), 137.9 (CH), 135.1 (C), 146.2 (C), 163.3 (CO), 125.1 (CH), 153.5 (C), 131.3 (CH), 133.4 (CH), 141.6 (C), 190.7 (CO); LCMS (m/z): 308.4 (M+1).

General method for the preparation of 2-chloro-N'-((5-(3-(substituted phenyl) acryloyl) thiophen-2-yl) methylene) nicotinohydrazide (Va-e)

Substituted aromatic aldehyde**(IVa-e)** (0.01 mol) and N'-((5-acetylthiophen-2-yl) methylene)-2-chloronicotinohydrazide(0.01 mol) **(III)** dissolved in isopropyl alcohol was taken in a 100 ml conical flask. The classical Claisen-Schmidt condensation reaction can take place.i.e. To make it alkaline, solution of 40% KOH (5ml) was added in it. Then the reaction mixture was stirred at room temperature for 24 hours on a magnetic stirrer. 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 2-chloro-N'-((5-(3-(substituted phenyl) acryloyl) thiophen-2-yl) methylene) nicotinohydrazide**(Va-e)**.

***2-chloro-N'-((5-(3-(2-fluorophenyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide* (Va)**

FTIR (KBr, $\nu_{max,}$ cm^{-1}): 1510 (C=N streching, pyridine ring moiety), 1661 (C=O streching, amide ketone), 3364 (N-H assymetric streching), 1593 (aromatic C=C streching), 1709 (asymmetric C-O-C streching of ether linkage), 1505 (C=C streching, Chalcone), 3007 (C-H Aromatic ring streching), 637 (C-F streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.8 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety), 7.9 (d, 1H, AR-CH=), 6.6 (d, 1H, -CO-CH=);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) :148.2 (CH), 124.3(CH), 138.0 (CH), 135.1 (C), 146.4 (C), 163.3 (CO), 125.2 (CH),154.7 (C), 131.6 (CH), 137.2 (CH), 145.3 (C), 180.5 (CO), 121.6 (CH), 145.1 (CH), 123.0 (C),128.3 (CH),124.2 (CH), 129.5 (CH), 115.6 (CH), 161.0 (C);LCMS (m/z): 414.4 (M+1).

***2-chloro-N'-((5-(3-(4-methoxyphenyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide* (Vb)**

FTIR (KBr, $\nu_{max,}$ cm^{-1}): 1511 (C=N streching, pyridine ring moiety),1657 (C=O streching, amide ketone), 3334 (N-H assymetric streching), 1596 (aromatic C=C streching), 1705 (assymetric C-O-C streching of ether linkage), 1510 (C=C streching, Chalcone), 3011 (C-H Aromatic ring streching), 1230 (assymetric Ar-O-C streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.8 (s, 3H, -OCH_3), 7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.8 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety), 7.9 (d, 1H, AR-CH=), 6.6 (d, 1H, -CO-CH=);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.3 (CH), 138.0 (CH), 135.1 (C), 146.4 (C), 163.3 (CO), 125.2 (CH), 154.7 (C), 131.6 (CH),

137.2 (CH), 145.3 (C), 180.5 (CO), 121.6 (CH), 145.1 (CH), 127.5 (C), 130.3 (CH),114.2 (CH), 159.6 (C), 114.3 (CH), 130.3 (CH);LCMS (m/z): 426.4 (M+1).

***2-chloro-N'-((5-(3-(2,4-dichlorophenyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide* (Vc)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1509 (C=N streching, pyridine ring moiety), 1654 (C=O streching, amide ketone), 3324 (N-H assymetric streching), 1599 (aromatic C=C streching), 1709 (assymetric C-O-C streching of ether linkage), 1513 (C=C streching, Chalcone), 3016 (C-H Aromatic ring streching), 656 (assymetric C-Cl streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.8 (m, 8H, 6Ar-H and 2-CH of Thiophene moiety), 7.9 (d, 1H, AR-CH=), 6.6 (d, 1H, -CO-CH=);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.3 (CH), 138.0 (CH), 135.1 (C), 146.4 (C), 163.3 (CO), 125.2 (CH), 154.7 (C), 131.6 (CH), 137.2 (CH), 145.3 (C), 180.5 (CO), 121.6 (CH), 145.1 (CH), 131.1 (C), 130.5 (CH), 126.7 (CH), 125.5 (C), 128.7 (CH), 130.4 (C);LCMS (m/z): 465.1 (M+1).

***2-chloro-N'-((5-(3-(furan-2-yl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide* (Vd)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1509 (C=N streching, pyridine ring moiety), 1654 (C=O streching, amide ketone), 3324 (N-H assymetric streching), 1599 (aromatic C=C streching), 1709 (assymetric C-O-C streching of ether linkage), 1513 (C=C streching, Chalcone), 3016 (C-H Aromatic ring streching), 656 (assymetric C-Cl streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.2 (s, 1H, -NH), 8.6 (s, 1H, =CH),7.1 to 8.8 (m, 8H, 6Ar-H and 2-CH of Thiophene moiety), 7.9 (d, 1H, AR-CH=), 6.8 (d, 1H, -CO-CH=);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.1 (CH), 124.1 (CH), 138.2 (CH), 135.3 (C), 146.4 (C), 163.5 (CO), 125.4 (CH), 154.5 (C), 131.4 (CH), 137.3 (CH), 145.1 (C), 180.6 (CO), 127.3 (CH), 120.8 (CH), 151.6 (C), 143.8 (CH), 112.8 (CH), 113.8 (CH);LCMS (m/z): 384.9 (M-1).

***2-chloro-N'-((5-(3-(thiophen-2-yl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide* (Ve)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1514 (C=N streching, pyridine ring moiety), 1658 (C=O streching, amide ketone), 3327 (N-H assymetric streching), 1603 (aromatic C=C streching), 1714 (assymetric C-O-C streching of ether linkage), 1509 (C=C streching, Chalcone), 3012 (C-H Aromatic ring streching), 839 (C-S-C streching thiophen ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.3 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.9 (m, 8H, 6Ar-H and 2-CH of Thiophene moiety), 7.8 (d, 1H, AR-CH=), 6.9 (d, 1H, -CO-CH=);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.3(CH), 138.1 (CH), 135.2 (C), 146.6 (C), 163.4 (CO), 125.3 (CH), 154.6 (C), 131.5 (CH), 137.2 (CH), 145.3 (C), 180.4 (CO), 127.4 (CH), 134.3 (CH), 140.3 (C), 130.7 (CH), 128.1 (CH), 129.4 (CH);LCMS (m/z): 402.5 (M+1).

General method for the preparation of N'-((5-(1-acetyl-5-(substitutedphenyl)-4,5-dihydro-1H-pyrazol-3-yl)thiophen-2-yl)methylene)-2-chloronicotinohydrazide (VIa-e)

An appropriate charged of hydrazine hydrate (0.015 mol) and chalcone (**Va-e**) (0.01 mol) in a 100 ml round bottomed flask, fitted with a reflux condenser. 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 recrystallized from ethanol gives product (**VIa-e**) in good yield.

***N'-((5-(1-acetyl-5-(2-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)thiophen-2-yl)methylene)-2-chloronicotinohydrazide* (VIa)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1515 (C=N streching, pyridine ring moiety), 1663 (C=O streching, amide ketone), 3358 (N-H assymetric streching), 1596 (aromatic C=C streching),1512 (C=C streching, Chalcone), 3013(C-H Aromatic ring streching), 788 ($COCH_3$streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.1 (s, 3H, -$COCH_3$), 3.7 (d, 2H, -CH_2), 4.9 (t, 1H, -CH), 7.0 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.0 to 8.6 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.2(CH), 138.1 (CH), 135.4 (C), 146.7 (C), 163.1 (CO), 125.4 (CH), 144.6 (C), 129.9 (CH), 127.5 (CH), 124.6 (C), 155.7 (C), 40.3 (CH_2), 60.9 (CH), 168.6 (CO),23.5 (CH_3),138.5 (C), 128.4 (CH), 126.7 (CH), 128.3 (CH), 128.6 (CH), 132.3 (C);LCMS (m/z): 487.3 (M+1).

***N'-((5-(1-acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)thiophen-2-yl)methylene)-2-chloronicotinohydrazide* (VIb)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1519 (C=N streching, pyridine ring moiety), 1667 (C=O streching, amide ketone), 3366 (N-H assymetric streching), 1604 (aromatic C=C streching), 1718 (asymmetric C-O-C streching of ether linkage), 1517 (C=C streching, Chalcone), 3019 (C-H Aromatic ring streching), 1128 (Streching aromatic $COCH_3$), 788 (OCH_3 streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.1 (s, 3H, -$COCH_3$), 3.7 (d, 2H, -CH_2), 3.9 (s, 3H, -$COCH_3$), 4.9 (t, 1H, -CH), 7.0 (s, 1H, -NH), 8.7 (s, 1H, =CH),6.9 to 8.6 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.4 (CH), 124.4 (CH), 138.3 (CH), 135.7 (C), 146.5 (C), 163.2 (CO), 125.1 (CH), 144.9 (C), 129.7 (CH), 127.8 (CH), 124.7

(C), 155.4 (C), 40.7 (CH_2), 65.8 (CH), 168.7 (CO), 23.6 (CH_3),134.2 (C), 126.8 (CH), 114.3 (CH), 158.2 (C), 114.2 (CH), 126.9 (CH), 55.3 (CH_3) ;LCMS (m/z): 482.6 (M+1).

***N'-((5-(1-acetyl-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)thiophen-2-yl) methylene)-2-chloronicotinohydrazide* (VIc)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1518 (C=N streching, pyridine ring moiety), 1669 (C=O streching, amide ketone), 3354 (N-H assymetric streching), 1602 (aromatic C=C streching),1516 (C=C streching, Chalcone), 3008 (C-H Aromatic ring streching), 776 ($COCH_3$ streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.0 (s, 3H, -$COCH_3$), 3.7 (d, 2H, -CH_2), 4.8 (t, 1H, -CH), 6.9 (s, 1H, -NH), 8.8 (s, 1H, =CH),7.0 to 8.7 (m, 8H, 6Ar-H and 2-CH of Thiophene moiety);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) :148.4 (CH), 124.6(CH), 138.3 (CH), 135.4 (C), 146.7 (C), 163.4 (CO), 125.3 (CH), 144.2 (C), 129.9 (CH), 127.5 (CH), 124.6 (C), 155.7 (C), 40.1(CH_2), 60.7 (CH), 168.8 (CO), 23.3 (CH_3),136.4 (C), 119.6 (CH), 126.8 (CH), 133.8 (C), 151.3 (CH), 131.2 (C);LCMS (m/z): 521.4 (M+1).

***N'-((5-(1-acetyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)thiophen-2-yl)methylene)-2-chloronicotinohydrazide*(VId)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1511 (C=N streching, pyridine ring moiety), 1663 (C=O streching, amide ketone), 3351 (N-H assymetric streching), 1612 (aromatic C=C streching),1514 (C=C streching, Chalcone), 3008 (C-H Aromatic ring streching), 779 ($COCH_3$ streching), 1129 (C-O-C streching furan ring); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.1 (s, 3H, -$COCH_3$), 3.7 (d, 2H, -CH_2), 4.8 (t, 1H, -CH), 6.9 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.0 to 8.6 (m, 8H, 6Ar-H and 2-CH of Thiophene moiety);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.6 (CH), 124.8(CH), 138.1 (CH), 135.7 (C), 146.5 (C), 163.4 (CO), 125.5 (CH),144.3 (C), 129.7 (CH), 127.6 (CH), 124.8 (C), 155.5 (C), 38.8 (CH_2), 51.3 (CH), 168.3 (CO), 22.9 (CH_3),151.0 (C), 141.5 (CH), 110.1 (CH), 109.2 (CH);LCMS (m/z): 442.3 (M+1).

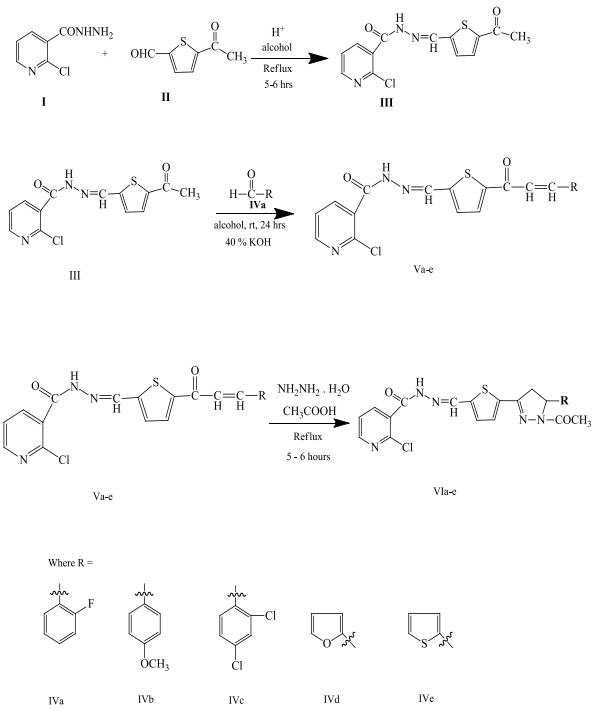
***N'-((5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)thiophen-2-yl)methylene)-2-chloronicotinohydrazide* (VIe)**

FTIR (KBr, $v_{max,}$ cm^{-1}): 1514 (C=N streching, pyridine ring moiety), 1660 (C=O streching, amide ketone), 3344 (N-H assymetric streching), 1618 (aromatic C=C streching),1504 (C=C streching, Chalcone), 3012 (C-H Aromatic ring streching), 782 ($COCH_3$ streching), 687 (C-S streching); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.0 (s, 3H, -$COCH_3$), 3.7 (d, 2H, -CH_2), 4.8 (t, 1H, -CH), 6.9 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.0 to 8.6 (m, 8H, 6Ar-H and 2-CH of Thiophene moiety);^{13}C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.7 (CH), 124.5 (CH), 138.2 (CH), 135.5 (C), 146.6 (C), 163.3 (CO), 125.2 (CH), 144.2 (C), 129.4 (CH), 127.8 (CH), 124.6 (C), 155.3 (C), 41.5 (CH_2), 50.6 (CH), 168.6 (CO), 23.5 (CH_3), 127.5 (C), 128.1 (CH), 127.3 (CH), 129.3 (CH);LCMS (m/z): 457.1 (M-1).

RESULT AND DISCUSSION

Chemistry

The reaction between compound **(I)** and compound **(II)** in presence of acid catalyst found compound **(III)** react with different aldehyde **(IVa)** are represented drawing in **Scheme 1.** The key intermediate aldehyde **(Iva)** is subjected to react with compound **(III)** with 40 % KOH in presence of alcohol form compound **(Va-e) .** The formation of new hybrid chalcone derivative were total characterised by the spectroscopic techniques such as FTIR, ^{1}H NMR, ^{13}C NMR and LCMS. As an example, In the IR spectrum of compound **Va**, a strong absorption band is observed at 1520-1540 cm^{-1} and 1660-1700 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 aromatic ring containC=C functional was observed at 1565-1600 cm^{-1} respectively.



Scheme 1. Methodical synthetic route for the target compounds (**Va-e**) and (**VIa-e**)

The ^{1}H NMR spectrum of compound **Va** showed a doublet at δ 6.6ppm for the -CO-CH= and at δ 7.1 ppm for the one proton from amino compound and one proton at δ 8.7 ppm of =CH. The other remaining seven aromatic and two protons of Thiophene moiety appeared as a multiplet signal at δ 7.1-8.8 ppm. Finally, the ^{13}C NMR spectra was recorded in $CDCl_3$. The compound **(Va)** spectral signals were in good agreement with the proposed structure. In the ^{13}C NMR spectrum of

compound **Va**, the most deshielded signal that appeared at δ 180.5 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 145.1 ppm. The signals for aromatic carbons appeared between at δ 115.6-163.3 ppm in the ^{13}C spectrum.

In IR spectrum of compound VIa, starching vibration of the C=O functional group of acetyl group is observed at 1663 cm^{-1} with strong absorption band attached at N_1 position in pyrazoline ring. A stretching band for the C=N functionality of pyrimidine ring moiety and –N-H streching frequency observed at 1515 and 3358 cm^{-1}.

The ^{1}H NMR spectrum of compound **VIa** showed a singlet at δ 2.1 ppm for the $COCH_3$ protons. The amide group proton at δ 7.0 ppm and at δ8.7 ppm of schiff-base contain one proton of =CH. The other remaining seven aromatic and two protons of Thiophene moiety appeared as a multiplet signal at δ 7.1-8.8 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 **VIa**, the shielded signal at δ 23.5 and 40.3 ppm was assigned to the methyl and methylenecarbon of pyrazoline ring. The most deshielded signal that appeared at δ 163 ppm was assigned to the carbonyl carbon of the amide group attached with the pyrazoline unit. The signals for aromatic carbons appeared between δ 110.5-151.7 ppm in the ^{13}C spectrum.

Table 1. The physical data of synthesised compoundsIII, (Va-e) and (VIa-e)

Compd	Molecular Formula	Yield	Melting Point °C	Elemental Analysis					
				% of C		% of H		% of N	
				Calcd.	Found	Calcd.	Found	Calcd.	Found
III	$C_{13}H_{10}ClNO_2S$	78	145	50.73	50.78	3.28	3.42	13.65	13.79
Va	$C_{28}H_{21}ClFNO_2S$	81	169	58.04	58.23	3.17	3.36	10.15	10.45
Vb	$C_{21}H_{21}ClNO_2S$	76	136	59.22	59.35	3.79	3.88	9.87	10.11
Vc	$C_{29}H_{24}Cl_2N_2O_2S$	75	178	51.69	51.76	2.60	2.85	9.04	9.32
Vd	$C_{30}H_{24}ClNO_3S$	78	200	56.95	56.22	3.13	3.29	10.89	11.01
Ve	$C_{28}H_{21}ClNO_2S_2$	80	113	53.79	53.96	3.01	3.24	10.46	10.69
VIa	$C_{30}H_{24}ClFN_2O_2S$	83	131	56.23	56.46	3.65	3.88	14.90	15.17
VIb	$C_{23}H_{24}ClNO_2S$	80	126	57.32	57.49	4.18	4.38	14.53	14.68
VIc	$C_{31}H_{27}Cl_2N_3O_2S$	73	100	50.73	50.99	3.10	3.34	13.45	13.61
VId	$C_{32}H_{27}ClNO_3S$	79	159	54.36	54.58	3.65	3.76	15.85	16.06
VIe	$C_{30}H_{24}ClNO_2S_2$	75	161	52.45	52.69	3.52	3.68	15.29	15.54

In vitro antimicrobial activity

Antimicrobialactivity [23] was screened against Staphylococcus aureus(MTCC 96)Streptococcus pyogenes(MTCC 442), Escherichia coli MTCC 443,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 microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [20]. All the synthesised compounds (**Va-e**) and (**VIa-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 2**.

Antimicrobial screening data of compounds chalcone (**Va-e**) and 1- acetyl pyrazoline (**VIa-e**) shows that compound **VIb** and **VIe** showed an outstanding inhibitory effect i.e. MIC = 50 and 62.5 µg/ml against Staphylococcus aureusas compared ampicillin (MIC = 250 µg/ml) and moderate to chloramphenicol and ciprofloxacin (MIC = 50 µg/ml) whereas compounds**Vd** and **VIc** (MIC = 100 µg/ml) showed better activity compared to ampicillin (MIC = 250 µg/ml) and poor to chloramphenicol and ciprofloxacin (MIC = 50 µg/ml) against Staphylococcus aureus.

In the case of pathogenic Streptococcus pyogenes, compound **Ve** (MIC = 62.5 µg/ml) showed an outstanding inhibitory effect whereas compound **Vb, VIa, VIa** and **VIe** (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).

Against Gram negative bacteria, compound **Ve** (MIC = 50 µg/ml) showed maximum activity against Escherichia coli as compared to ampicillin while compounds **va,Vc, VIc** and **VIe** (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 **Ve, VIb** and **VIC** (MIC = 100 µg/ml) showed excellent activity 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 **Vb** (MIC = 250 µg/ml), **Vd** (MIC = 100 µg/ml), **VIa** (MIC = 200 µg/ml), **VIb** (MIC = 100 µg/ml), **VIc** (MIC = 250 µg/ml), **VId** (MIC = 200 µg/ml) and **VIe** (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 **Va** and **Vc** (MIC = 500 µg/ml) showed the same potency as griseofulvin (MIC = 500 µg/ml) against Candida albicans. Compound **Vb, VIb** and **VIc** (MIC = 100 µg/ml) showed equipotent to griseofulvin (MIC = 100 µg/ml) and nystatin (MIC = 100 µg/ml) against Aspergillus niger. While compound **Ve** and **VIe** (MIC = 100

µg/ml) were found to be active against the fungal pathogen Aspergillus clavatus.

Table 2. Antimicrobial activity data of synthesised compounds (Va-e) and (VIa-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. e
Va	125	200	100	250	500	1000	500
Vb	250	100	250	200	250	100	500
Vc	200	125	100	250	500	>1000	250
Vd	100	200	125	200	100	250	500
Ve	125	62.5	50	100	>1000	100	100
VIa	250	100	200	200	200	500	>1000
VIb	50	200	125	100	100	100	>1000
VIc	100	125	100	100	250	100	250
VId	125	200	125	200	200	500	>1000
VIe	62.5	100	100	125	100	>1000	100
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 antimycobacterial 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 [204The observed results are presented in **Table 3** 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 **Va** (MIC = 62.5 µg/ml), **Vd** (MIC = 62.5 µg/ml), **Ve** (MIC = 50 µg/ml) **VIb** (MIC = 62.5µg/ml) and **VIc** (MIC = 62.5 µg/ml) were found to possess the greatest potency against Mycobacterium tuberculosis with **89, 82, 91, 86** and **90** % inhibition respectively (**Table 3**). Other derivatives showed moderate to poor antitubercular activity.

Table 3. In vitro antitubercular activity (% inhibition) of the synthesized compounds(Va-e) and (VIa-e) at concentration 250 µg/ml

Compd	Inhibition (%)
Va	89
Vb	76
Vc	70
Vd	82
Ve	91
VIa	76
VIb	86
VIc	90
VId	73
VIe	64
Rifampicin	98

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

Compd	Inhibition (%)	MIC (µg/ml)
Va	89	62.5
Vd	82	62.5
Ve	91	50
VIb	86	62.5
VIc	90	50
Isoniazid	99	0.20
Rifampicin	98	40

CONCLUSION

A new class of chalcone and its derivatives, as a novel class of antitubercular and antimcrobial agents was synthesized. The newly synthesized novel heterocycles showed good antitubercular and antimicrobial activities against both drug-sensitive and drug-resistant strains of Mycobacterium tuberculosis as well as antimicrobial species. These results make new indole clubbed chalcone, pyrazoline and pyrimidine derivatives interesting lead molecules for further synthetic and biological evaluation.

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REFERENCES

1. F. Herencia, M. Ferrándiz, and A. Ubeda (1998). Bioorganic and Medicinal Chemistry Letters, 8, pp. 1169-1174.

2. L. R. Dong, D.Y. Hu and Z. X. Wu (2017). Chinese Chemical Letters, 28, pp. 1566-1570.

3. C. Jin, Y. J. Liang and H. He (2013). Biomedicine & Pharmacotherapy, 67, pp. 215-217.

4. D. K. Mahapatra, S. K. Bharti and V. Asati (2015). European Journal of Medicinal Chemistry, 46, pp. 69-114.

5. M. F. A. Mohamed, M. S. A. Shaykoon and M. H. Abdelrahman (2017). Bioorganic Chemistry, 72, pp. 32-41.

6. H. Wang, M. Wang, et. al. (2015). European Journal of Medicinal Chemistry, 92, pp. 439-448.

7. G. C. Wang, J. Qiu., X. W. Xiao, et. al. (2018). Bioorganic Chemistry, 76, pp. 249-257.

8. C. Zhuang, W. Zhang, C. Sheng, W. Zhang, C. Xing, Z. Miao (2017). Chalcone: A privileged structure medicinal chemistry, 117, pp. 7762-7810.

9. P. Singh, A. Anand, V. Kumar (2017). Eur. J. Med. Chem., 85, pp. 758-777.

10. D.K. Mahapatra, S.K. Bharti, V. Asati (2015). Eur. J. Med. Chem., 98, pp. 69-114.

11. C. Karthikeyan, N.S. Moorthy, S. Ramasamy, U. Vanam, E. Manivannan, D. Karunagaran, P., Anticancer Drug Discov., 2015, 110, pp. 97-115.

12. H. Mirzaei, S. Emami (2016). Eur. J. Med. Chem., 121, pp. 610-639

13. El. Emary, T.I. (2006). J. Chin. Chem. Soc., 53, pp. 391-401.

14. J. Rangaswamy, H. V. Kumar, S. T. Harini, N. Naik (2012). Bioorg. Med. Chem. Lett., 22, pp. 4773-7.

15. A. Solanki, S. B. Kumar, A. A. Doshi, C. Ratna Prabha (2013). Polyhedron, 63, pp. 147-155.

16. A. P. Keche, G. D. Hatnapure, R. H. Tale, A. H. Rodge, V. M. Kamble (2012). Bioorg. Med. Chem. Lett., 22, 6611-5.

17. E. Yamuna, R. A. Kumar, M. Zeller, K. J. Rajendra Prasad (2012). Eur. J. Med. Chem., pp. 228-38.

18. N. Gokhan-Kelekci, S. Yabanoglu, E. Kupeli, U. Salgin, O. Ozgen, G. Ucar, E. Yesilada, E. Kendi, A. Yesilada, A. A. Bilgin (2007). Bioorg. Med. Chem., 15, pp. 5775–5786.

19. P. Singh, K. Paul, W. Holzer (2006). Bioorg. Med. Chem., 14, pp. 5061–5071.

20. U. Tiwari, C. Ameta, M. K. Ranwal, R. Ameta, P. B. Punjabi (2013). Ind. J. Chem. Sect. B., 52, pp. 432–439.

21. H. Singh & V. K. Kapoor (1996). Medicinal and Pharmaceutical Chemistry, pp. 278–282.

a) J. Liu, Z. Zhao, Z. Meng-Yue, Hai.-Liang. Xin (2013). Mini Rev. Med. Chem., 13, pp. 1957–1966.

b) A. Chauhan, P. K. Sharma, N. Kaushik (2013). Int. J. ChemTech Res., 3, pp. 11–17

c) H. Kumar, D. Saini, S. Jain, N. Jain (2013). Eur. J. Med. Chem., 70, pp. 248–258.

d) A. Jamwal, A. Javed, V. Bhardwaj (2013). J. Pharm. Biol. Sci., 3, pp. 114–123.

22. National Committee for Clinical Laboratory Standards. 2000. Methods for Dilution, antimicrobial susceptibility tests for bacteria that grow aerobically approved standard, (M7A5) (5th edition). National Committee for Clinical Laboratory Standards, Wayne, PA.

23. A. Rattan, Antimicrobials in laboratory medicine. B.L. Churchill, Livingstone, New Delhi, 2000, pp. 85-105.

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