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# Synthesis, Characterisation and Pharmacological Studies of Some New Heterocyclic Hybrid Chalcones and Its Derivative - Isoxazole as Anti-Infective Agents

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***Abstract – The increase in bacterial resistance and bacterial pathogens are major health problem for the Human around the world. So, to control multidrug resistant bacteria, a hybrid novel heterocyclic molecules 2-chloro-N'-((5-(3-(substituted phenyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide (5a-h) and 2-chloro-N'-((5-(5–substitutedphenyl)isoxazol-3-yl)thiophen-2-yl)methylene)nicotinohydrazide (6a-h) are designed and synthesised. Structures of all the prepared compounds are confirmed on the basis of FTIR, $^{1}$H NMR, $^{13}$C NMR, Mass spectral data as well as elemental analysis. Proficiency of the title compounds as antimicrobial as well as antitubercular are screened against selected pathogens. Some of the newer hybrid molecules showed excellent activity and as a excellent future scope in pharmacological research.***

***Keywords:, 2-Chloroisonicotinohydrazide, Claisen-Schmidt Condensation Reaction, Chalcones, Isoxazoles, Antimicrobial Activity, Antitubercular Activity.***

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## INTRODUCTION

Various biological activity of heterocyclic compound are extensively distributed in human health. Nitrogen containing heterocyclic compound are important part in both medicinal and organic chemistry [1]. Large number of biological active compounds having common part of chalcones which containe α,β-unsaturated ketone moiety. Therefore, chalcone derivative from synthetic or nature origin exerted biological activity such as antimicrobial [2], antitumor [3], anticancer [4,5], radical scavenger [6], and inhibitor of topoisomerasel [7]. Chalcone derivative attracts many researchers to development of an efficient synthetic protocol and pharmacological activity. Further chalcone are key intermediate in the synthesis of heterocyclic compound. In this paper, we have synthesised hybrid-chalcone and isoxazole and screen for biological proficiency.

Five member heterocyclic compound isoxazole is containing nitrogen atom next to oxygen in ring system. Isoxazole forms several biological active agents and contains broad spectrum of biological activities [8].The substituted isoxazole derivatives possesses displayed antiviral[9], anticancer activities [10], anti-inflammatory, antibacterial [11], hypoglycaemic [12] and antifungal [13]. So studies of novel hybrid molecular targets for new antibacterial drugs are major field of research. Isoxazole gives various types of reaction like complexation, oxidation, reduction, carbanionic condensations, thermolysis, quaternization, protonation, photolysis, thermolysis, transformation of other heterocyclic ring system and reaction with electrophiles, grignard reagents and nuclephiles [14]. Several novel (substituted phenyl) Isoxazole derivatives are synthesized and exists herbicidal activities towards various weeds like Echinochloa, Crusgalli, SetariaViridis, Abutilon theoprastil [15-18]. Isoxazole derivative having property of inhibiting the porphyrinogen oxidase. The product have isoxazole rings have property to cure malaria, inflammatory and microbial activity [19, 20], some isoxazole compounds treat diseases like allergies, cholesterol level and viral [21-23]. Recently, the effort have been made with continuation of previous work to synthesis the biologically significant isoxazole derivatives, we demonstrate a

new strategy to employing isoxazole rings to the novel precursors as anti-infective agents.

## MATERIAL AND METHODS

Reagent used for reaction are of analytical reagent grade. Melting points are determined by open capillary method and are uncorrected. The progress of the reaction was 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 visualized using toluene : methanol eluents. 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. 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).

### Synthetic method for the preparation of N'-((5-acetylthiophen-2-yl)methylene)-2-chloronicotinohydrazide (3)

A 100 ml round bottomed flask, fitted with a reflux condenser was charged with a mixture of 2-chloroisonicotinohydrazide **(1)** (0.01 mol) and 5-acetylthiophene-2-carbaldehyde **(2)** (0.01 mol) in presence of acid catalyst in ethanol. 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 N'-((5-acetylthiophen-2-yl) methylene)-2-chloronicotinohydrazide **(3)**.

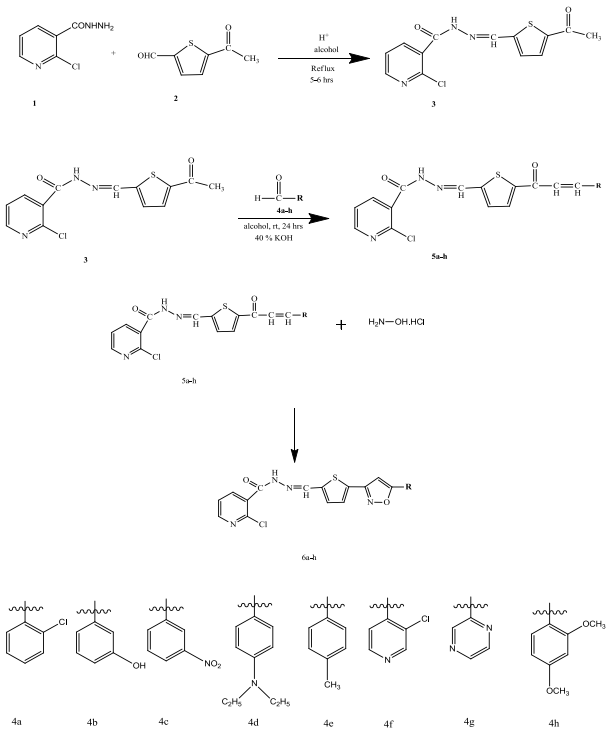
### Synthetic method for the preparation of 2-chloro-N'-((5-(3-(substituted phenyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide(5a-h)

By applying classical Claisen-Schmidt condensation reaction, substituted aldehyde **(4a-h)** (0.01 mol) and N'-((5-acetylthiophen-2-yl)methylene)-2-chloronicotinohydrazide (0.01 mol) **(3)** dissolved in isopropylalcohol 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 2-chloro-N'-((5-(3-(substituted phenyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide **(5a-h)**.

### Synthetic method for the preparation of 2-chloro-N'-((5-(5-(substitutedphenyl)isoxazol-3-yl)thiophen-2-yl)methylene)nicotinohydrazide (6a-h)

A 100 ml round bottomed flask, fitted with a reflux condenser was charged with a mixture of an appropriate chalcone (5**a-h**) (0.01 mol) and hydroxyl amine hydrochloride (0.015 mol) in 40 ml ethanol was taken in a 250 ml round bottomed flask, fitted with a reflux condenser. To make the mixture basic catalytic amount of 40 % KOH (5 ml) solution was added. Then the reaction mixture was heated under reflux for 6-7 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was then cooled and poured into crushed ice. Thus the solid mass separated out was collected by filtration, washed with water and recrystallized product **(6a-h)** from methanol.

### Reaction Scheme



**Scheme.** Methodical synthetic route for the target compounds (**5a-h**) and (**6a-h**)

## SPECTRAL ANALYSIS DATA

***N'-((5-acetylthiophen-2-yl) methylene)-2-chloronicotinohydrazide (3)***

Yield 86%; m.p. 125$^{0}$C; Anal. Calcd. for $C_{13}H_{10}ClN_3O_2S$: C, 50.73; H, 3.28; N, 13.65%.

Found: C, 50.67; H, 3.37; N, 13.71%; IR (KBr, $v_{max,}$ $cm^{-1}$): 1513 (C=N streching, pyridine ring moiety),1664 (C=O streching,amide ketone), 3364 (N-H assymetric streching), 1595 (aromatic C=C streching);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 2.5 (s, 3H, $-CH_3$), 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);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :26.4 ($CH_3$), 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).

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

Yield 82%; m.p. 156$^{0}$C; Anal. Calcd. for $C_{20}H_{13}C_{l2}N_3O_2S$: C, 55.82; H, 3.05; N, 9.77%. Found: C, 55.96; H, 3.16; N, 9.98%; IR (KBr, $v_{max,}$ $cm^{-1}$): 1510 (C=N streching, pyridine ring moiety),1661 (C=O streching,amide ketone), 3364 (N-H assymetric streching), 1595 (aromatic C=C streching), 1508 (C=C streching, Chalcone), 3012 (C-H Aromatic ring 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) :121.8 (CH), 123.9(CH), 125.2 (CH), 131.7 (CH), 127.9 (CH), 126.5 (CH), 129.2 (CH),129.8 (CH), 138.0 (CH), 145.2 (CH), 148.2 (CH), 137.6 (CH), 146.2 (C), 145.4 (C), 133.3 (C),134.3 (C),154.8 (C), 145.4 (C), 163.4 (C), 180.5 (CO);LCMS (m/z): 431.1 (M+1).

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

Yield 75%; m.p. 116$^{0}$C; Anal. Calcd. for $C_{20}H_{14}ClN_3O_3S$: C, 58.23; H, 3.43; N, 10.20%. Found: C, 58.61; H, 3.67; N, 10.43%; IR (KBr, $v_{max,}$ $cm^{-1}$): 1507 (C=N streching, pyridine ring moiety),1666 (C=O streching,amide ketone), 3361 (N-H assymetric streching), 3426 (phenolic –OH streching), 1592 (aromatic C=C streching), 1501 (C=C streching, Chalcone), 3017 (C-H Aromatic ring streching);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 5.3 (s, 1H, -OH), 7.0 (s, 1H, -NH), 8.6 (s, 1H, =CH),7.1 to 8.9 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety), 7.6 (d, 1H, AR-CH=), 6.7 (d, 1H, -CO-CH=);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) : 121.6 (CH), 123.4(CH), 125.3 (CH), 131.8 (CH), 127.5 (CH), 126.6 (CH), 129.4 (CH), 129.6 (CH), 138.2 (CH), 145.4 (CH), 148.1 (CH), 137.3 (CH), 146.4 (C), 145.6 (C), 133.1 (C), 134.7 (C), 154.9 (C), 145.2 (C), 163.1 (C), 180.3 (CO);LCMS (m/z): 412.2 (M+1).

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

Yield 75%; m.p. 185$^{0}$C; Anal. Calcd. for $C_{20}H_{13}ClN_4O_4S$: C, 54.49; H, 2.97; N, 12.71%. Found: C, 54.36; H, 3.15; N, 12.93%; IR (KBr, $v_{max,}$ $cm^{-1}$): 1508 (C=N streching, pyridine ring moiety),1666 (C=O streching,amide ketone), 3360 (N-H assymetric streching), 1595 (aromatic C=C streching), 1511 (C=C streching, Chalcone), 3015 (C-H Aromatic ring streching), 1492 (asymetric streching nitro group);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm):7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.9 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety), 7.6 (d, 1H, AR-CH=), 6.7 (d, 1H, -CO-CH=);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) : 121.5 (CH), 123.2(CH), 124.2 (CH), 125.2 (CH), 127.5 (CH), 129.8 (CH), 131.5 (CH),134.8 (CH), 137.4(CH), 138.1 (CH), 145.3 (CH), 148.3 (CH), 137.9 (C), 134.8 (C), 145.4 (C),146.2 (C),147.6 (C), 154.8 (C), 163.3 (CO), 180.5 (CO);LCMS (m/z): 441.5 (M+1).

***2-Chloro-N'-((5-(3-(4- (diethylamino) phenyl) acryloyl) thiophen - 2 - yl) methylene) nicotinohydrazide* (5d)**

Yield 78%; m.p. 126 $^{0}$C; Anal. Calcd. for $C_{24}H_{23}ClN_4O_2S$: C, 61.73; H, 4.96; N, 12.00%. Found: C, 61.77; H, 5.13; N, 12.36%; IR (KBr, $v_{max,}$ $cm^{-1}$): 1501 (C=N streching, pyridine ring moiety),1658 (C=O streching,amide ketone), 3376 (N-H assymetric streching), 1591 (aromatic C=C streching), 1512 (C=C streching, Chalcone), 3007 (C-H Aromatic ring streching), 3050 (C-H streching aliphatic);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm):1.2 (tt, 6H, $-CH_3$), 3.4 (qq, 4H, $-CH_2$), 7.2 (s, 1H, -NH), 8.6 (s, 1H, =CH),7.1 to 8.8 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety), 7.6 (d, 1H, AR-CH=), 6.7 (d, 1H, -CO-CH=);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :13.1 ($2CH_3$), 47.2 ($2CH_2$), 121.1 (CH), 123.3(CH), 124.1 (CH), 125.3 (CH), 129.7 (CH), 111.8 (CH), 131.5 (CH),129.5 (CH), 137.5(CH), 138.3 (CH), 145.2 (CH), 148.3 (CH), 124.8 (C), 134.7 (C), 145.4 (C),146.1 (C),111.8 (C), 154.8 (C), 163.3 (CO), 180.5 (CO);LCMS (m/z): 467.3 (M+1).

***2-Chloro-N'-((5-(3-(p-tolyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide* (5e)**

Yield 78%; m.p. 105$^{0}$C; Anal. Calcd. for $C_{21}H_{16}ClN_3O_2S$: C, 61.53; H, 3.93; N, 10.25%. Found: C, 61.69; H, 4.06; N, 10.50%; IR (KBr, $v_{max,}$ $cm^{-1}$): 1519 (C=N streching, pyridine ring moiety),1651 (C=O streching,amide ketone), 3369 (N-H assymetric streching), 1599(aromatic C=C streching), 1501 (C=C streching, Chalcone), 3016 (C-H Aromatic ring streching), 3072 (C-H streching aliphatic);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm):2.3 (s, 3H, $-CH_3$), 7.2 (s, 1H, -NH), 8.6 (s, 1H, =CH),7.1 to 8.8 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety), 7.6 (d, 1H, AR-CH=), 6.7 (d, 1H, -CO-CH=);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :21.3 ($CH_3$), 121.1 (CH), 123.3(CH), 124.2 (CH), 125.3 (CH), 128.4 (CH), 128.7 (CH), 131.5 (CH),128.6 (CH), 137.5 (CH), 138.3 (CH), 145.2 (CH), 148.3 (CH), 132.3 (C), 134.7 (C), 145.4 (C),146.1 (C),128.6 (C), 154.8

(C), 163.3 (CO), 180.5 (CO);LCMS (m/z): 410.2 (M+1).

***2-Chloro-N'-((5-(3-(3-chloropyridin-4-yl) acryloyl) thiophen-2-yl) methylene) nicotinohydrazide* (5f)**

Yield 72%; m.p. 149$^0$C; Anal. Calcd. for $C_{19}H_{12}Cl_2N_4O_2S$: C, 52.91; H, 2.80; N, 12.99%. Found: C, 53.09; H, 3.02; N, 13.23%; IR (KBr, $\nu_{max,}$ cm$^{-1}$): 1502 (C=N streching, pyridine ring moiety),1680 (C=O streching,amide ketone), 3372 (N-H assymetric streching), 1601 (aromatic C=C streching), 1500 (C=C streching, Chalcone), 3019 (C-H Aromatic ring streching), 653 (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), 7.6 (d, 1H, AR-CH=), 6.7 (d, 1H, -CO-CH=);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :121.1 (CH), 124.2 (CH), 125.3 (CH), 128.4 (CH), 117.8 (CH), 148.1 (CH),128.6 (CH), 128.7 (CH), 138.3 (CH), 145.2 (CH), 148.3 (CH), 132.3 (C), 144.3 (C), 145.4 (C),146.1 (C),128.6 (C), 148.4 (C), 163.3 (CO), 180.5 (CO);LCMS (m/z): 432.4 (M+1).

***2-Chloro-N'-((5-(3-(pyrazin-2-yl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide* (5g)**

Yield 69%; m.p. 119$^0$C; Anal. Calcd. for $C_{18}H_{12}ClN_5O_2S$: C, 54.34; H,3.04; N, 17.60%. Found: C, 54.55; H, 3.33; N, 17.88%; IR (KBr, $\nu_{max,}$ cm$^{-1}$): 1503 (C=N streching, pyridine ring moiety),1669 (C=O streching,amide ketone), 3348 (N-H assymetric streching), 1595 (aromatic C=C streching), 1521 (C=C streching, Chalcone), 3028 (C-H Aromatic ring streching);$^1$H NMR (400 MHz, $CDCl_3$, δ ppm):7.2 (s, 1H, -NH), 8.6 (s, 1H, =CH),7.1 to 9.4 (m, 8H, 6Ar-H and 2-CH of Thiophene moiety), 7.6 (d, 1H, AR-CH=), 6.7 (d, 1H, -CO-CH=);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :121.1 (CH), 140.5 (CH), 125.3 (CH), 128.4 (CH), 142.6 (CH), 147.3 (CH),128.6 (CH), 128.7 (CH), 145.2 (CH), 148.3 (CH), 132.3 (C), 153.1 (C), 145.4 (C),146.1 (C),128.6 (C), 148.4 (C), 163.3 (CO), 180.5 (CO);LCMS (m/z): 397.9 (M+1).

***2-Chloro-N'-((5-(3-(2,4-dimethoxyphenyl)acryloyl)thiophen-2-yl)methylene)nicotinohydrazide*(5h)**

Yield 83%; m.p. 137$^0$C; Anal. Calcd. for $C_{22}H_{18}ClN_3O_4S$: C, 57.96; H, 3.98; N, 9.22%. Found: C, 57.79; H, 4.21; N, 9.49%; IR (KBr, $\nu_{max,}$ cm$^{-1}$): 1515 (C=N streching, pyridine ring moiety),1658 (C=O streching,amide ketone), 3361 (N-H assymetric streching), 1595 (aromatic C=C streching),1716 (asymmetric C-O-C streching of ether linkage),1522 (C=C streching, Chalcone), 3001 (C-H Aromatic ring streching);$^1$H NMR (400 MHz, $CDCl_3$, δ ppm):3.7 (ss, 6H, -OC$H_3$), 7.2 (s, 1H, -NH), 8.6 (s, 1H, =CH),7.1 to 8.8 (m, 9H, 7Ar-H and 2-CH of Thiophene moiety), 7.6 (d, 1H, AR-CH=), 6.7 (d, 1H, -CO-CH=);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :55.4 (O$CH_3$), 56.7 (O$CH_3$) 150.7 (CH), 123.3(CH), 124.2 (CH), 130.5(CH), 98.5 (CH), 128.7 (CH), 143.4 (CH),128.6 (CH), 137.5 (CH), 138.3 (CH), 145.2 (CH), 118.2 (CH), 132.3 (C), 134.7 (C), 145.4 (C),106.5 (C),128.6 (C), 154.8 (C), 163.3 (CO), 180.5 (CO);LCMS (m/z): 456.2 (M+1).

***2-Chloro-N'-((5-(5-(2-chlorophenyl)isoxazol-3-yl)thiophen-2-yl)methylene nicotine hydrazide* (6a)**

Yield 76%; m.p. 154$^0$C; Anal. Calcd. for $C_{20}H_{12}Cl_2N_4O_2S$: C, 54.19; H, 2.73; N, 12.64%. Found: C, 54.47; H, 3.01; N, 12.87%; IR (KBr, $\nu_{max,}$ cm$^{-1}$): 1514 (C=N streching, pyridine ring moiety),1675 (C=O streching,amide ketone), 3368 (N-H assymetric streching), 1599 (aromatic C=C streching),3023 (C-H Aromatic ring streching), 659 (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 8.9 (m, 10H, 7Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :148.2 (CH), 124.2(CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH),144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 136.8 (C),128.2 (CH),127.4 (CH), 130.2 (CH), 129.2 (CH), 132.3 (C);LCMS (m/z): 442.8 (M-1).

***2-Chloro-N'-((5-(5-(3-hydroxyphenyl) isoxazol-3-yl) thiophen-2-yl) methylene) nicotinohydrazide* (6b)**

Yield 80%; m.p. 169$^0$C; Anal. Calcd. for $C_{20}H_{13}ClN_4O_3S$: C, 56.54; H,3.08; N, 13.13%. Found: C, 56.74; H, 3.30; N, 13.34%; IR (KBr, $\nu_{max,}$ cm$^{-1}$): 1514 (C=N streching, pyridine ring moiety),1665 (C=O streching,amide ketone), 3372 (N-H assymetric streching), 1598(aromatic C=C streching),3020 (C-H Aromatic ring streching), 3435 (phenolic –OH streching);$^1$H NMR (400 MHz, $CDCl_3$, δ ppm):5.3 (s, 1H, -OH), 7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.9 (m, 10H, 7Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.2 (CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH), 144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 131.7 (C), 117.8 (CH), 130.7 (CH), 115.8 (CH), 157.6 (C), 111.4 (CH);LCMS (m/z): 424.4 (M+1).

***2-Chloro-N'-((5-(5-(3-nitrophenyl) isoxazol-3-yl) thiophen-2-yl ) methylene) nicotinohydrazide* (6c)**

Yield 81%; m.p.176$^0$C; Anal. Calcd. for $C_{20}H_{12}ClN_5O_4S$: C, 52.93; H, 2.66; N, 15.43%. Found: C, 53.16; H, 2.87; N, 15.61%;IR (KBr, $\nu_{max,}$ cm$^{-1}$): 1514 (C=N streching, pyridine ring moiety),1670 (C=O streching,amide ketone), 3367 (N-H assymetric streching), 1598 (aromatic C=C streching),3020 (C-H Aromatic ring streching),

1497 (asymetric streching –NO group);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm):7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.7 (m, 10H, 7Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.2 (CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH), 144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 131.3 (C),131.5 (CH), 130.0 (CH), 123.8 (CH), 148.5 (C), 122.6 (CH);LCMS (m/z): 454.7 (M+1).

***2-Chloro-N'-((5-(5-(4-(diethylamino)phenyl)isoxazol-3-yl)thiophen-2-yl)methylene) nicotinohydrazide* (6d)**

Yield 79%; m.p. 132$^{0}$C; Anal. Calcd. for $C_{24}H_{22}ClN_5O_2S$: C, 60.06; H,4.62; N, 14.59%. Found: C, 60.23; H, 4.83; N, 14.72%;IR (KBr, $\nu_{max,}$ $cm^{-1}$): 1506 (C=N streching, pyridine ring moiety),1662 (C=O streching,amide ketone), 3352 (N-H assymetric streching), 1591 (aromatic C=C streching),3010 (C-H Aromatic ring streching), 3064 (C-H streching aliphatic);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 1.2 (t, 6H, $-CH_3$), 3.4 (q, 4H, $-CH_2$),7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.7 (m, 10H, 7Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :12.8 ($2CH_3$), 47.3 ($2CH_2$), 148.2 (CH), 124.2 (CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH), 144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 136.0 (C),125.7 (CH), 112.8 (CH), 149.6 (C), 112.8 (CH), 125.6 (CH);LCMS (m/z): 479.1 (M-1).

***2-Chloro-N'-((5-(5-(p-tolyl)isoxazol-3-yl)thiophen-2-yl)methylene)nicotinohydrazide* (6e)**

Yield 74%; m.p. 107$^{0}$C; Anal. Calcd. for $C_{21}H_{15}ClN_4O_2S$: C, 59.64; H, 3.58; N, 13.25%. Found: C, 59.88; H, 3.79; N, 13.50%;IR (KBr, $\nu_{max,}$ $cm^{-1}$): 1503 (C=N streching, pyridine ring moiety),1676 (C=O streching,amide ketone), 3359 (N-H assymetric streching), 1597 (aromatic C=C streching),3009 (C-H Aromatic ring streching), 3071 (C-H streching aliphatic);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 2.2 (s, 3H, $-CH_3$),7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.7 (m, 10H, 7Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :21.4 ($CH_3$), 148.2 (CH), 124.2 (CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH), 144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 123.6 (C),124.8 (CH), 129.3 (CH), 131.7 (C), 129.6 (CH), 124.7 (CH);LCMS (m/z): 423.2 (M+1).

***2-Chloro-N'-((5-(5-(3-chloropyridin-4-yl)isoxazol-3-yl)thiophen-2-yl)methylene) nicotinohydrazide* (6f)**

Yield 60%; m.p. 159$^{0}$C; Anal. Calcd. for $C_{19}H_{11}Cl_2N_5O_2S$, C, 51.36; H, 2.50; N, 15.76%. Found: C, 51.64; H, 2.73; N, 15.87%;IR (KBr, $\nu_{max,}$ $cm^{-1}$): 1519 (C=N streching, pyridine ring moiety),1659 (C=O streching,amide ketone), 3315 (N-H assymetric streching), 1595 (aromatic C=C streching),3013 (C-H Aromatic ring streching), 661 (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 8.7 (m, 9H, 6Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.2 (CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH), 144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 143.4 (C),120.4 (CH), 148.5 (CH), 148.2 (CH), 130.1 (CH);LCMS (m/z): 445.1 (M+1).

***2-Chloro-N'-((5-(5-(pyrazin-2-yl)isoxazol-3-yl)thiophen-2-yl)methylene)nicotinohydrazide* (6g)**

Yield 69%; m.p. 138$^{0}$C; Anal. Calcd. for $C_{18}H_{11}ClN_6O_2S$: C, 52.62; H,2.70; N, 20.46%. Found: C, 52.81; H, 2.91; N, 20.72%;IR (KBr, $\nu_{max,}$ $cm^{-1}$): 1505 (C=N streching, pyridine ring moiety),1666 (C=O streching,amide ketone), 3354 (N-H assymetric streching), 1599 (aromatic C=C streching),1711 (asymmetric C-O-C streching of ether linkage),1515 (C=C streching, Chalcone), 3011 (C-H Aromatic ring streching);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm):7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.7 (m, 9H, 6Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) : 148.2 (CH), 124.2 (CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH), 144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 152.4 (C),144.8 (CH), 142.3 (CH), 144.6 (CH);LCMS (m/z): 411.3 (M+1).

***2-Chloro-N'-((5-(5-(2,4-dimethoxyphenyl)isoxazol-3-yl)thiophen-2-yl)methylene)nicotinohydrazide* (6h)**

Yield 69%; m.p. 146$^{0}$C; Anal. Calcd. for $C_{22}H_{17}ClN_4O_4S$: C, 56.35; H, 3.65; N, 11.95%. Found: C, 56.58; H, 3.75; N, 12.21%;IR (KBr, $\nu_{max,}$ $cm^{-1}$): 1504 (C=N streching, pyridine ring moiety),1669 (C=O streching,amide ketone), 3368 (N-H assymetric streching), 1595 (aromatic C=C streching),1709 (asymmetric C-O-C streching of ether linkage),1513 (C=C streching, Chalcone), 3016 (C-H Aromatic ring streching);$^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 3.8 (s, 6H, $-OCH_3$),7.1 (s, 1H, -NH), 8.7 (s, 1H, =CH),7.1 to 8.7 (m, 10H, 7Ar-H and 2-CH of Thiophene moiety, 1-CH isoxazole moiety);$^{13}$C NMR (400 MHz, $CDCl_3$, δ ppm) :55.5 ($OCH_3$), 56.2 ($OCH_3$)148.2 (CH), 124.2 (CH), 138.2 (CH), 135.1 (C), 146.2 (C), 163.2 (CO), 125.3 (CH), 144.1 (C), 140.0 (C), 127.5 (CH), 128.3 (C), 150.1 (CH), 98.2 (CH), 169.2 (C), 106.9 (C),127.4 (CH), 107.3 (CH), 161.4 (C), 98.7 (CH), 158.4 (C);LCMS (m/z): 469.5 (M+1).

## RESULT AND DISCUSSION

### Chemistry

All these new heterocyclic derivatives **(5a-h)** and **(6a-h)** were characterised by means of spectroscopic techniques FT-IR, $^{1}$H NMR,$^{13}$C NMR and LCMS. FTIR spectrum of compound **5a**, C=O and CH = CH functionality of chalcone moiety were observed at 1661 and 1508 cm$^{-1}$ further the $^{1}$H NMR spectrum of same compound exerted a doublet at δ 76.6 and 7.9 ppm for the -CO-CH= and Ar-CH= of α, β proton. the, The most deshielded signal appeared in $^{13}$C NMR spectrum of compound **5a** at δ 170.2, 148.2 and 137.6 ppm was mainly due to the carbonyl carbon and CH = CH functionality of the chalcone moiety.

Isoxazole was confirmed by screened the compound **6a** for FTIR, a strong absorption band was observe at 1603 cm$^{-1}$which corresponds to the stretching vibration of the C-N functionality of of isoxazoloe ring. $^{1}$H NMR of isoxazole derivative 6a to 6h showed the CH proton of isoxazole ring at between 5.5 to 6.9 ppm as well as carbon NMR also depicted the C=N functionality of isoxazole ring at 157.6 δ ppm. Additionally, mass spectrum of all the compounds showed molecular ion peak $M^{+}$ corresponding to their exact mass which is in agreement with its proposed structure. The obtained elemental analysis values are in good agreement with theoretical data.

### *In vitro* antimicrobial and antitubercular activity

All the synthesised compounds were screened for their antibacterial and antitubercular activity using standard broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [24] against Staphylococcus aureus MTCC 96 andStreptococcus pyogenes MTCC 442, Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC 441, Candida albicans MTCC 227, Aspergillusniger MTCC 282 and Aspergillusclavatus MTCC 1323. The minimal inhibitory concentration (MIC) of all the synthesised compounds are summarised in **Table 1**. The same all compounds screened for *in vitro* antitubercular activity by using Lowenstein-Jensen medium (conventional method) against *Mycobacterial tuberculosis* $H_{37}$Rv strain [25]. The compunds who has more than 80 % zone of inhibition in primary scrring are selected for MIC and data obtained are summerized in **Table 2**.

**Table - 1. Antimicrobial activity data of synthesised compounds (5a-h) and (6a-h)**

| 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 | 100 | 62.5 | 125 | 100 | 100 | 100 | 500 |
| 5b | 125 | 62.5 | 200 | 125 | 125 | 100 | 250 |
| 5c | 250 | 125 | 200 | 200 | 500 | >1000 | 500 |
| 5d | 200 | 200 | 250 | 125 | >1000 | 500 | 500 |
| 5e | 250 | 125 | 500 | 125 | >1000 | 500 | 500 |
| 5f | 62.5 | 50 | 125 | 50 | 125 | 100 | 500 |
| 5g | 50 | 62.5 | 62.5 | 100 | 100 | 100 | 250 |
| 5h | 100 | 125 | 125 | 100 | >1000 | 250 | 500 |
| 6a | 125 | 200 | 125 | 250 | 500 | 125 | 250 |
| 6b | 250 | 200 | 100 | 200 | 500 | 250 | 500 |
| 6c | 250 | 500 | 200 | 250 | 500 | 500 | >1000 |
| 6d | 250 | 500 | 250 | 200 | >1000 | 500 | >1000 |
| 6e | 250 | 125 | 125 | 125 | 500 | >1000 | >1000 |
| 6f | 125 | 200 | 100 | 100 | 100 | 125 | 125 |
| 6g | 100 | 100 | 62.5 | 50 | 125 | 100 | 100 |
| 6h | 250 | 125 | 62.5 | 62.5 | 250 | 500 | 250 |
| Ampi. | 125 | 100 | 100 | 100 | - | - | - |
| Chlo. | 50 | 50 | 50 | 50 | - | - | - |
| Cipr. | 50 | 50 | 25 | 25 | - | - | - |
| Gris. | - | - | - | - | 500 | 100 | 100 |
| 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.: Aspergillusniger, A. c.: Aspergillusclavatus.*Ampi: Ampicillin, Chlo.: Chloramphenicol, Cipr.: Ciprofloxacin, Gris.: Greseofulvin, Nyst.: Nystatin. '-': not tested.

**Table 2.** In vitro antitubercular activity (% inhibition) and MIC of the synthesized compounds **(5a-h)** and **(6a-h)**

| Compd | Inhibition (%) | MIC |
|---|---|---|
| 5a | 94 | 62.5 |
| 5b | 68 | - |
| 5c | 23 | - |
| 5d | 46 | - |
| 5e | 57 | - |
| 5f | 87 | 100 |
| 5g | 96 | 100 |
| 5h | 91 | 62.5 |
| 6a | 81 | 100 |
| 6b | 89 | 62.5 |
| 6c | 70 | - |
| 6d | 68 | - |
| 6e | 75 | - |
| 6f | 83 | 100 |
| 6g | 86 | 100 |
| 6h | 76 | - |
| Isoniazid | 99 | 40 |
| Rifampicin | 98 | 0.20 |

'-': not tested.

The antibacterial screening of compounds hybrid chalcone (**5a-h**), compound **5f** and **5g** showed an outstanding inhibitory effect i.e. MIC = 62.5 and 50 µg/ml against Staphylococcus aureusas compared ampicillin (MIC = 250 µg/ml) and moderate to chloramphenicol and ciprofloxacin (MIC = 50 µg/ml) while in isoxazole series compounds, compounds **6a, 6f** and **6g** exhibited good activity against same Staphylococcus aureusas compared ampicillin (MIC = 250 µg/ml). In the case of inhibiting Streptococcus pyogenes, compound **5a, 5b, 5c, 5e, 5f, 5h, 6e, 6g** and **6h** 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 **5g, 6g** and **6h** (MIC = 62.5 µg/ml) showed excellent activity against Escherichia coli as compared to ampicillin while remaining compounds poor 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). Against Pseudomonas aeruginosa, Compound **5a, 5d, 5e, 5f, 5g, 5h, 6e, 6f, 6g and 6h** found to possesses better to ampicillin (MIC = 100 µg/ml) and modest to chloramphenicol (MIC = 50 µg/ml) and ciprofloxacin (MIC = 25 µg/ml).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.

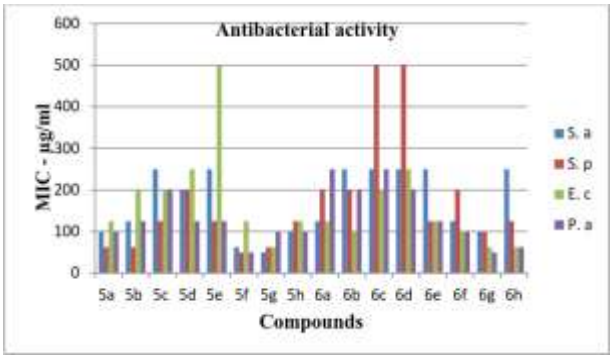



**Figure-1. Antibacterial activity**

In vitro antifungal activity data summarized in **table 1** shows that, it is found that compounds **5a, 5b,5f, 5g,6f and 6g** displayed good antifungal activity against Candida albicans as compared to griseofulvin (MIC = 500 µg/ml) and nystatin (MIC = 100 µg/ml). Compound **5a, 5b, 5f, 5g, 6a, 6f and 6g** showed better to griseofulvin (MIC = 100 µg/ml) and nystatin (MIC = 100 µg/ml) against Aspergillus niger. While compound **6g** was found to be active against the fungal pathogen Aspergillus clavatus.

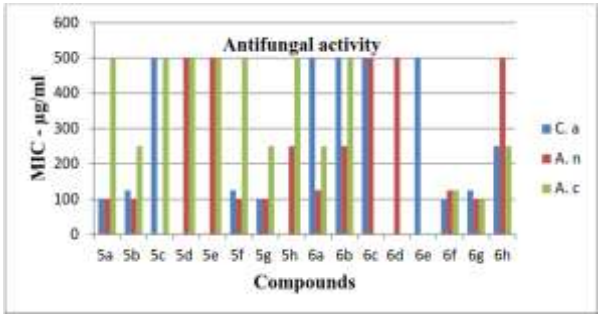



**Figure-2. Antifungal activity**

The antitubercular activities summarized in **table 2** `indicate that the compounds **5a**, **5f**, **5g**, **5h**, **6a**, **6b**, **6f** and **6h** observed more than 80% inhibition in preliminary test and that compounds are selected for screening for MIC and showed good zone of inhibition such as **62.5**, **100**, **100**, **62.5**, **100** and **100** µg/ml respectively.

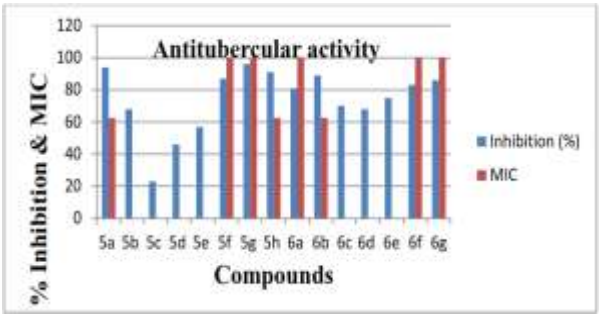



**Figure-3. Antitubercular activity**

## CONCLUSION

In this study, some new hybrid chalcone-imines clubbed compounds and their derivatives isozxazoles have been synthesized and act as a powerful template for making a potent antimicrobial and antitubercular agents as anti-infective agents. In addition to that each compound had been shown an excellent antimicrobial and antitubercular activity. Among the various analogues, compounds **5a**, **5f**, **5g**, **6a**, **6b** and **6f** had electron donating groups such as Cl,-OH present in the part of the molecular structure and offered as excellent antiinfective agents. Therefore, there is a need for further study of the above mentioned compounds for the development of the novel a typical antiinfective agents.

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