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Synthesis, Characterization and Pharmacological Evaluation of Some Novel 4,5-Dihydro Pyrazole Derivatives Bearing Thiazole and Furan as Potent Antimicrobial and Anticancer Agents.

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ABSTRACT

A novel series of 4,5-dihydropyrazole derivatives (**2a-d, 3, 4a-d** and **5a-h**) were synthesized by the reacting acetophenones and 5-(2,3-dichlorophenyl)furan-2-carbaldehyde. All the newly synthesized compounds were characterized using elemental analysis and spectral data (IR, ^{1}H NMR, ^{13}C NMR and LC-MS) analysis. All the synthesized derivatives were screened for antimicrobial potential against selected gram-positive, gram-negative bacteria, yeasts, moulds and in vitro cytotoxic activity against tumor cell lines by using Mosmann's method. Doxorubicin and cisplatin was utilized as positive control to validate biological evaluation. Antimicrobial study revealed fluorinated compounds **5a, 5b, 5d** and **5f** exhibited enhanced inhibition compared to other designed derivatives. Among the fluorinated derivatives, Compound **5b** demonstrated significant activity against tested gram-positive and gram-negative bacteria and fungal species. The invitro anticancer screening of synthesized series illustrate that all the designed compounds were active, in particular tri flouro substituted Schiff base **5b** exhibited excellent anticancer activity IC50 0.32 to 0.95 µm against tested cell lines in comparison to standard drugs Cisplatin and Doxorubicin.

Keywords: 4,5-dihydropyrazole, triazole, antimicrobial, anticancer, MTT assay,

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INTRODUCTION

The prevalence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. Prudent use of antibiotics and development of novel antimicrobial agents seem to be the common strategies and action plans taken to combat this challenge. Consequently, the search for new antimicrobial agents will always remain an important task for medicinal chemists [1].

Cancer is the worldwide health problem and the most frightening disease of human [2]. Chemotherapy, either alone or as an adjunct to radiotherapy or surgery remains the treatment of choice in most of the cancers [3,4].

The current anticancer agents are mostly broad acting cytotoxic drugs. They impact structure and function of the rapidly proliferating cancer cells and arrest the cell cycle at a specific phase depending on the mechanism of action of the agents [5,6]. Due to their lack in specificity and adverse effects related to impact on rapidly dividing non-cancerous cells, there is an urgent need for identification of novel, potent, selective, and less toxic agents, which can overcome cancer resistance to drug treatment that has made many of the currently available chemotherapeutic agents ineffective [7].

The pyrazole ring emerged as a powerful scaffold used extensively in the design of compounds targeted to block the cell cycle progression in cancer cells [8]. Pharmacologic inhibitors of cyclin-dependent kinases (CDKs) have been shown to block cell cycle progression in a large variety of cell types. The use of the pyrazole ring in the design of CDKs inhibitors is demonstrated by the development of AT7519, a 4-[(2,6-dichlorobenzoyl)amino-1H-pyrazole-3-carbox amide derivative, with anti-proliferative effects in leukemia, colon and breast cancer [9]. A series of 4- arylazo-3,5-diamino-1H-pyrazole derivatives demonstrated anti-CDK kinase activities and anti-proliferative properties [10].

This renewed interest in this class of compounds and in continuation of our research [11] to furnish biologically active compounds, it was contemplated to synthesize pyrazolines clubbed with thiazole and hydrazide derivatives and studies their cytotoxicity, antimicrobial (antibacterial and antifungal) activity.

MATERIAL AND METHODS

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR 157, ^{1}H NMR and ^{13}C NMR spectra were recorded (in $CDCl_3$/DMSO-d_6) on a Bruker spectrometer at 300/400 MHz using TMS as an internal standard. Agilent LC-MS spectrometer. Elemental analysis was performed on Thermo Finnigan Flash (EA 1112 CHNS Analyzer). Dry solvents and reagents were purchased from commercial vendors. Thin layer chromatography (TLC) was performed throughout the reaction to optimize the reaction for purity and completion of reaction on Merck silica gel GF254 aluminium sheets using mixture of different polar and nonpolar solvents in varying proportions and spots were observed using iodine as visualizing agent.

EXPERIMENTAL

Chemistry

General procedure for the synthesis of 5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide (1)

Compound **1** was prepared according to the literature's [11,12]. Recrystallized from ethanol, yellowish solid (yield 69%); mp 183-185 °C; IR (KBr) ν_{max}/cm^{-1} 3436 (N-H), 1568 (C=N), 1308 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.50-3.56 (dd, 1H, *J* 17.92, 3.34, pyrazole-CH_2), 3.80-3.88 (dd, 1H, *J* 17.76, 11.40, pyrazole-CH_2), 6.07-6.11 (dd, 1H, *J* 11.36, 3.22, pyrazole-CH), 6.49-6.50 (d, 1H, *J* 3.32 ,-CH- furan), 7.11-7.12 (d, 1H, *J* 3.36, -CH-furan), 7.34-7.93 (8H, dichlorobenzene, benzylidenimin), 8.18 (s, 2H, -NH_2); LC-MS (*m/z*, %): 416 (M+1, 98.3).

***General procedure for the synthesis of 1-(4-(4-subsituted phenyl) thiazol-2-yl)-5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole derivatives* (2a-d)**

Compound **1** (0.01mol) and ethanol (20mL) mixture was stirred at 25-30°C. Commercially available phenacyl bromides (0.01mol) was added to the above mass at 25-30°C and mass was heated to reflux and stirred for 1h. Completion of reaction was confirmed by TLC, the reaction mass was cooled to 25-30°C. The product obtained was filtered and re-crystallized from suitable solvent to obtain target compounds.

Synthesis of 5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-1-(4-phenylthiazol-2-yl)-4,5-dihydro-1H-pyrazole (2a)

Recrystallized from ethanol, yellowish solid (yield 79%); mp 165-167 °C; IR (KBr) ν_{max}/cm^{-1} 1581 (C=N), 1532 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.74-3.81 (dd, 1H, *J* 17.90, 7.24, pyrazole-CH_2), 3.98-4.05 (dd, 1H, *J* 17.49, 11.85, pyrazole –CH_2), 5.86-5.90 (dd, 1H, *J* 12.24, 7.40, pyrazole-CH), 6.82-6.83 (d, 1H, *J* 3.4, -CH-furan), 7.18-7.19 (d, 1H, *J* 3.36, -CH-furan), 7.36-7.40 (s, 1H, -CH-thiazole), 7.48-7.84 (13H, dichlorobenzene, benzylidenimin, Phenyl); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.2, 60.1 (pyrazole-CH_2, CH), 101.0,105.2 (furan-C_2, C_3), 105.0 (thiazole-C_3), 127.0, 127.6 (dichlorobenzene-C_6, C_5), 127.5 (phenyl thiazole-$C_{2,6}$), 128.3,128.9, (phenyl-$C_{2,6}$, $C_{3,5}$), 128.7,129.2 (phenyl thiazole-C_4, $C_{3,5}$), 130.2 (dichlorobenzene- C_3), 131.0 (phenyl-C_4), 131.1, 133.8 (dichlorobenzene- C_2, C_4), 132.0,132.1 (bromobenzene-C_1, $C_{3,4}$), 133.0 (phenyl thiazole-C_1), 136.5 (phenyl-C_1), 138.3 (dichlorobenzene- C_1), 150.0,151.6 (furan-C_1, C_4), 150.2 (thiazole-C_4), 151.7 (pyrazole-C), 168,9(C=N); LC-MS (*m/z*, %): 517 (M+1, 99.0).

***Synthesis of 1-(4-(4-bromophenyl)thiazol-2-yl)-5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole* (2b)**

Recrystallized from ethanol, yellowish solid (yield 80%); mp 179-181 °C; IR (KBr) ν_{max}/cm^{-1} 1581 (C=N), 1532 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.73-3.80 (dd, 1H, *J* 17.92, 7.24, pyrazole-CH_2), 3.97-4.04 (dd, 1H, *J* 17.48, 11.84, pyrazole-CH_2), 5.85-5.89 (dd, 1H, *J* 12.24, 7.40, pyrazole-CH), 6.82-6.83 (d, 1H, *J* 3.4, -CH- furan), 7.18-7.19 (d, 1H, *J* 3.36Hz,-CH-furan), 7.36-7.40 (s, 1H, -CH-thiazole), 7.48-7.84 (12H, dichlorobenzene, benzylidenimin, Phenyl); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.2, 60.1 (pyrazole-CH_2, CH), 101.0,105.2 (furan-C_2, C_3), 105.0 (thiazole-C_3), 123.1 (bromo benzene-C_4), 127.0, 127.6 (dichlorobenzene-C_6, C_5), 128.2,128.8 (phenyl-$C_{1,6}$, $C_{2,4}$), 128.3 (bromo benzene-$C_{2,6}$), 130.2 (dichlorobenzene- C_3), 131.0 (phenyl-C_3), 131.1, 133.8 (dichlorobenzene-C_2, C_4), 132.0,132.1 (bromobenzene-C_1, $C_{3,5}$), 136.4 (phenyl-C_1), 138.3 (dichlorobenzene-C_1), 150.0, 151.6 (furan-C_1, C_4), 150.3 (thiazole-C_4), 151.7 (pyrazole-C), 168.9(C=N); LC-MS (*m/z*, %): 596 (M+1, 99.6).

***Synthesis of 1-(4-(4-chlorophenyl)thiazol-2-yl)-5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole* (2c)**

Recrystallized from ethanol, yellowish solid (yield 83%); mp 181-183 °C; IR (KBr) ν_{max}/cm^{-1} 1580 (C=N), 1530 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.73-3.80 (dd, 1H, *J* 17.92, 7.24, pyrazole-CH_2), 3.97-4.04 (dd, 1H, *J* 17.48, 11.84, pyrazole-CH_2), 5.85-5.89 (dd, 1H, *J* 12.24, 7.40, pyrazole-CH), 6.82-6.83 (d, 1H, *J* 3.4, -CH- furan), 7.18-7.19 (d, 1H, *J* 3.36, -CH- furan), 7.37-7.41 (s, 1H, -CH- thiazole), 7.49-7.86 (12H, dichlorobenzene, benzylidenimin, Phenyl); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.4, 60.9 (pyrazole-CH_2, CH), 101.2,105.7 (furan-C_2, C_3), 105.4 (thiazole-C_3), 123.5 (chloro benzene-C_4), 127.4, 127.8 (dichlorobenzene-C_6, C_5), 128.4,128.9 (phenyl-$C_{1,6}$, $C_{2,4}$), 128.6 (Chloro benzene-$C_{2,6}$), 130.5 (dichlorobenzene- C_3), 131.2 (phenyl-C_3), 131.4, 133.9 (dichlorobenzene- C_2, C_4), 132.2,132.4 (chloro benzene-C_1, $C_{3,5}$), 136.5 (phenyl-C_1), 138.4 (dichlorobenzene-C_1), 150.1,151.7 (furan-C_1, C_4), 150.2 (thiazole-C_4), 151.9 (pyrazole–C), 168.8(C=N); LC-MS (*m/z*, %): 551 (M+1, 99.2).

***Synthesis of 3-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)pyridine* (2d)**

Recrystallized from ethanol, yellowish solid (yield 80%); mp 182-184 °C; IR (KBr) ν_{max}/cm^{-1} 1582 (C=N), 1529 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.74-3.81 (dd, 1H, *J* 17.89, 7.20, pyrazole-CH_2), 3.98-4.06 (dd, 1H, *J* 17.43, 11.86, pyrazol-CH_2), 5.86-5.90 (dd, 1H, *J* 12.30, 7.30, pyrazole-CH), 6.83-6.84 (d, 1H, *J* 3.4Hz, -CH-furan), 7.18-7.19 (d, 1H, *J* 3.36, -CH- furan), 7.37-7.41 (s, 1H, -CH- thiazole), 7.49-7.86 (12H, dichlorobenzene,

benzylidenimin, pyridine); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.2, 60.1 (pyrazole-CH_2, CH), 101.5,105.9 (furan-C_2, C_3), 105.1 (thiazole-C_3), 124.1 (pyridine-C_4), 127.6, 127.9 (dichlorobenzene-C_6, C_5), 128.5,128.8, (phenyl-$C_{1,6}$, $C_{2,4}$), 130.6 (dichlorobenzene-C_3), 131.3 (phenyl-C_3), 131.4, 133.8 (dichlorobenzene-C_2, C_4), 133.1,134.3 (pyridine-C_2, C_3), 136.5 (phenyl-C_1), 138.4 (dichlorobenzene- C_1), 148.0, 149.1 (pyridine-C_5, C_1) 150.1,151.4 (furan-C_1, C_4), 150.4 (thiazole-C_4), 151.8 (pyrazole-C), 168.6(C=N); LC-MS (*m/z*, %): 518 (M+1, 98.9).

***General procedure for the synthesis of 2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide* (3)**

The mixture of Compound **1** (0.01mol) and ethanol (20mL) was stirred at 25-30°C, further ethyl 4-bromoacetoacetate (0.01mol) was added to the above mass, and refluxed for 1h. Completion of reaction was confirmed by TLC, reaction mass was cooled to 25-30°C and product was isolated as a residue.

The above residue (0.015mol), hydrazine hydrate (1.6mL) and absolute ethanol (20mL) mixture was stirred at 25-30°C. The mass was refluxed for 5h. Completion of reaction was confirmed by TLC and reaction mass was cooled to 25-30°C. The solids formed were filtered and recrystallized using ethanol, yellowish solid (yield 75%); mp 132-134 °C; IR (KBr) ν_{max}/cm^{-1} 1667 (C=O), 1568 (C=N), 1524 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.33 (s, 2H, thiazole-CH_2-triazole), 3.69-3.75 (dd, 1H, *J* 17.68, 6.28, pyrazole –CH_2), 3.90-3.98 (dd, 1H, *J* 17.72, 11.92, pyrazole-CH_2), 4.22 (s, 2H, -NH_2-, D_2O exchangeable), 5.74-5.78 (dd, 1H, *J* 11.84, 6.36, pyrazole-CH), 6.65 (s, 1H, -CH- thiazole), 6.66-6.67 (d, 1H, *J* 3.44, -CH- furan), 7.15 (d, 1H, *J* 3.40, -CH- furan), 7.39 (t, 1H, *J* 7.96, -CH, dichlorobenzene), 7.50-7.82 (7H, dichlorobenzene, benzylidenimin), 9.04 (s, 1H, -NH-, D_2O exchangeable); LC-MS (*m/z*, %): 513 (M+1, 98.0).

***General procedure for the synthesis of (E)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-N'-subsituted acetohydrazide derivatives* (4a-d)**

The mixture of compound **3** (0.01mol), ethanol (5V) and commercially available substituted acetophenones (0.01mol) was stirred at 25-30°C. The mass refluxed for 3h in the presence of few drops of glacial acetic acid. Completion of reaction was confirmed by TLC, the solvent was evaporated and the product was quenched into cold water, stirred for 30min and filtered. The crude solid was recrystallized in appropriate solvent systems to obtain target products.

***Synthesis of (E)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-N'-(1-p-tolylethylidene)acetohydrazide* (4a)**

Recrystallized from ethanol, off white solid (yield 84%); mp 158-160 °C; IR (KBr) ν_{max}/cm^{-1} 3320(NH), 1674 (amide C=O), 1619 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (s,3H, CH_3, methyl), 2.29 (s, 3H, CH_3, methyl benzene), 3.64 (s, 1H, pyrazole-CH_2), 3.69-3.75 (dd, 1H, *J* 17.80, 6.36, pyrazole-CH_2), 3.95 (s, 2H, thiazole-CH_2-CO-), 5.74 (m, 1H, pyrazole, -CH), 6.59 (d, 1H, *J* 2.96, -CH- furan), 6.74 (s, 1H, -CH, thiazole), 7.01 (d, 1H, *J* 2.96, -CH- furan), 7.15 (d, 2H, *J* 7.72, -2CH, methylbenzene), 7.33 (q, 1H, *J* 12.2, -CH, dichlorobenzene), 7.48-7.9 (9H, dichlorobenzene, benzylidenimin, methylbenzene), 10.51 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.7 (methyl), 21.3 (methylbenzene-methyl), 38.2, 60.1 (pyrazole-CH_2, CH), 38.6 (CH_2, aliphatic), 101.0, 105.2 (furan-C_2, C_3), 104.3 (thiazole-C_3), 127.0, 127.6 (dichlorobenzene-C_6, C_5), 128.2,128.8 (phenyl-$C_{2,6}$, $C_{3,5}$), 127.0, 129.1 (methylbenzene-$C_{2,6}$, $C_{3,5}$), 130.7 (dichlorobenzene-C_3), 131.0 (phenyl-C_4), 131.5, 133.9 (dichlorobenzene-C_2, C_4), 134.5 (methylbenzene-C_1), 136.4 (phenyl-C_1), 138.3 (dichlorobenzene-C_1), 140.7 (methylbenzene-C_4), 147.7 (C=N-NH), 150.0,151.5 (furan-C_1, C_4), 150.6 (thiazole-C_4), 151.7 (pyrazole–C), 167.5 (C=N), 171.2 (C=O); LC-MS (*m/z*, %): 628 (M+1, 98).

***Synthesis of (E)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-N'-(3-methylbutan-2-ylidene)acetohydrazide* (4b)**

Recrystallized from ethanol, off white solid (yield 82%); mp 134-136 °C; IR (KBr) ν_{max}/cm^{-1} 3220 (NH), 1675 (amide C=O), 1631 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81 (m, 6H, -2CH_3, isopropyl), 1.21 (m, 1H, CH, isopropyl), 1.31 (s, 3H, -CH_3, methyl), 3.65 (s, 1H, pyrazole -CH_2), 3.69-3.75 (dd, 1H, *J* 17.80, 6.36, pyrazole-CH_2), 3.95 (s, 2H, thiazole-CH_2-CO-), 5.75 (m, 1H, pyrazole,-CH), 6.59 (d, 1H, *J* 2.96 Hz, -CH- furan), 6.74 (s, 1H, -CH, thiazole), 7.02 (d, 1H, *J* 2.96, -CH- furan), 7.33 (q, 1H, *J* 12.2, -CH, dichlorobenzene), 7.48-7.9 (7H, dichlorobenzene, benzylidenimin), 10.31 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ

19.6 (methyl), 19.8 (isopropyl), 40.3 (CH-isopropyl), 38.2, 60.1 (pyrazole-CH_2, CH), 38.6 (CH_2, aliphatic), 101.0, 105.2 (furan-C_2, C_3), 104.3 (thiazole-C_3), 127.0, 127.6 (dichlorobenzene-C_6, C_5), 128.2,128.8 (phenyl-$C_{2,6}$, $C_{3,5}$), 130.7 (dichlorobenzene-C_3), 131.0 (phenyl-C_4), 131.5, 133.9 (dichlorobenzene-C_2, C_4), 136.4 (phenyl-C_1), 138.3 (dichlorobenzene- C_1), 150.0,151.5 (furan-C_1, C_4), 150.8 (thiazole-C_4), 151.7 (pyrazole-C), 162.2(C=N-NH), 167.5 (C=N), 171.2 (C=O); LC-MS (*m/z*, %): 580 (M+1, 98.1).

***Synthesis of (E)-N'-(1-(3-chlorophenyl)ethylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide* (4c)**

Recrystallized from ethanol, off white solid (yield 86%); mp 162-164 °C; IR (KBr) ν_{max}/cm^{-1} 3310 (NH), 1674 (amide C=O), 1619 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.24 (s, 3H, -CH_3, methyl), 3.64 (s, 1H, pyrazole-CH_2), 3.69-3.75 (dd, 1H, *J* 17.78, 6.46, pyrazole-CH_2), 3.90 (s, 2H, thiazole-CH_2-CO-), 5.75 (m, 1H, pyrazole, -CH), 6.59 (d, 1H, *J* 2.98 Hz, -CH- furan), 6.75 (s, 1H, -CH, thiazole), 7.01 (d, 1H, *J* 3.0 Hz, -CH- furan), 7.15 (d, 2H, *J* 7.74 , -2CH, chlorobenzene), 7.33 (q, 1H, *J* 12.3, -CH, dichlorobenzene), 7.50-8.02 (9H, dichlorobenzene, benzylidenimin, chlorobenzene), 10.51 (s, 1H, -CO-NH-, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.5 (methyl), 38.2, 60.0 (pyrazole-CH_2, CH), 38.5 (CH_2, aliphatic), 101.0, 105.3 (furan-C_2, C_3), 104.4 (thiazole-C_3), 127.0, 127.6 (dichlorobenzene- C_6, C_5), 128.2,128.8, (phenyl- $C_{2,6}$, $C_{3,5}$), 127.3, 129.3, 130.3 (chlorobenzene-C_6, C_2, C_5), 130.7 (dichlorobenzene- C_3), 131.0 (phenyl-C_4), 131.2 (chlorobenzene-C_4), 131.5, 133.9 (dichlorobenzene-C_2, C_4), 134.4, 135.5 (chlorobenzene-C_3, C_1), 136.4 (phenyl-C_1), 138.3 (dichlorobenzene-C_1), 147.7 (C=N-NH), 150.0,151.5 (furan-C_1, C_4), 150.8 (thiazole-C_4), 151.7 (pyrazole–C), 168.7 (C=N), 171.2 (C=O); LC-MS (*m/z*, %): 648 (M+1, 98.1).

***Synthesis of (E)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-N'-(1-phenylethylidene)acetohydrazide* (4d)**

Recrystallized from ethanol, off white solid (yield 88%); mp 155-156 °C; IR (KBr) ν_{max}/cm^{-1} 3290 (NH), 1675 (amide C=O), 1620 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.23 (s, 3H, -CH_3, methyl), 3.65 (s, 1H, pyrazole –CH_2), 3.69-3.75 (dd, 1H, *J* 17.8, 6.36, pyrazole-CH_2), 3.95 (s, 2H, thiazole-CH_2-CO-), 5.75 (m, 1H, pyrazole,-CH), 6.59 (d, 1H, *J* 2.96, -CH- furan), 6.74 (s, 1H,-CH, thiazole), 7.01 (d, 1H, *J* 2.96, -CH- furan), 7.33 (q, 1H, *J* 12.2, -CH, dichlorobenzene), 7.40-7.9 (12H, dichlorobenzene, benzylidenimin, methylbenzene), 10.50 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.5 (methyl), 38.2, 60.2 (pyrazole-CH_2, CH), 38.6 (CH_2, aliphatic), 101.0, 105.3 (furan-C_2, C_3), 104.4 (thiazole-C_3), 127.0, 127.6 (dichlorobenzene-C_6, C_5), 128.2,128.8, (phenyl-$C_{2,6}$, $C_{3,5}$), 128.9, 129.2 (benzene-$C_{2,6}$, $C_{3,5}$), 130.7 (dichlorobenzene- C_3), 131.0 (phenyl-C_4), 131.3 (benzene-C_4), 131.5, 133.9 (dichlorobenzene- C_2, C_4), 134.0 (benzene-C_1), 136.5 (phenyl-C_1), 138.3 (dichlorobenzene- C_1), 147.6 (C=N-NH), 150.0,151.5 (furan-C_1, C_4), 150.8 (thiazole-C_4), 151.7 (pyrazole–C), 168.8 (C=N), 171.0 (C=O); LC-MS (*m/z*, %): 614 (M+1, 98.2).

***General procedure for the synthesis of (E)-N'-Subsituted-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide derivatives* (5a-h)**

The mixture of compound **3** (0.01mol), ethanol (5V) and commercially available substituted aldehydes (0.01mol) was stirred at 25-30°C. The mass was refluxed for 3h in the presence of few drops of glacial acetic acid. Completion of reaction was confirmed by TLC, the solvent was evaporated and the product was quenched into cold water, stirred for 30min and filtered. The crude solid was recrystallized in appropriate solvent systems to obtain target products.

***Synthesis of (E)-N'-(2,6-difluorobenzylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide* (5a)**

Recrystallized from ethanol, off white solid (yield 81%); mp 175-177 °C; IR (KBr) ν_{max}/cm^{-1} 3280 (NH), 1673 (amide C=O), 1605 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.50 (s, 1H, pyrazole-CH_2), 3.70 (dd, 1H, *J* 17.70, 6.65, pyrazole-CH_2), 3.94 (s, 2H, thiazole-CH_2-CO-), 5.70 (m, 1H, pyrazole, -CH), 6.60 (d, 1H, *J* 3.47, -CH-furan), 6.70 (s, 1H, -CH, thiazole), 6.99 (d, 1H, *J* 3.49 Hz, -CH- furan), 7.5-8.12 (11H, dichlorobenzene, benzylidenimin, diflurobenzene), 8.05 (s,1H, -CH, -N=CH-), 11.20 (s, 1H, -CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.2, 60.2 (pyrazole-CH_2, CH), 38.6(CH_2, aliphatic), 101.3, 105.4(furan-C_2, C_3), 104.5 (thiazole-C_3), 105.2, 111.3 (difluorobenzene-C_1, $C_{3,5}$), 127.0, 127.7 (dichlorobenzene-C_6, C_5), 128.2,128.8, (phenyl-$C_{2,6}$, $C_{3,5}$), 130.2 (dichlorobenzene- C_3), 131.3 (phenyl-C_4), 131.5, 133.8 (dichlorobenzene-C_2, C_4), 134.3

(difluorobenzene-C_4), 136.5 (phenyl-C_1), 138.4 (dichlorobenzene-C_1), 143 (HC=C), 150.1,151.6 (furan-C_1, C_4), 150.2 (thiazole-C_4), 151.8 (pyrazole-C), 161.3 (difluorobenzene-$C_{2,6}$), 167.6 (C=N), 171.0 (C=O); LC-MS (*m/z*, %): 636 (M+1, 98.9).

Synthesis of (E)-N'-(2,3,4-trifluorobenzylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide **(5b)**

Recrystallized from ethanol, off white solid (yield 80%); mp 192-194 °C; IR (KBr) ν_{max}/cm^{-1} 3300 (NH), 1630 (amide C=O), 1606 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.51 (s, 1H, pyrazole-CH_2), 3.72 (dd, 1H, *J* 17.70, 6.64, pyrazole-CH_2), 3.95 (s, 2H, thiazole-CH_2-CO-), 5.70 (m, 1H, pyrazole,-CH), 6.60 (d, 1H, *J* 3.47,-CH- furan), 6.70 (s, 1H,-CH, thiazole), 6.99 (d, 1H, *J* 3.49 Hz,-CH-furan), 7.5-8.1 (10H, dichlorobenzene, benzylidenimin, triflurobenzene), 8.05 (s, 1H, -CH, -N=CH-), 11.20 (s, 1H, -CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.1, 59.9 (pyrazole-CH_2, CH), 38.5(CH_2, aliphatic), 101.3, 105.3(furan-C_2, C_3), 104.7 (thiazole-C_3), 112.9, 115.3 (trifluorobenzene-C_5, C_1), 127.0, 127.7 (dichlorobenzene-C_6, C_5), 128.0 (trifulrobenzene-C_6), 128.2,128.8 (phenyl- $C_{2,6}$, $C_{3,5}$), 130.2 (dichlorobenzene-C_3), 131.3 (phenyl-C_4), 131.5, 133.8 (dichlorobenzene-C_2, C_4), 136.5 (phenyl-C_1), 138.4 (dichlorobenzene- C_1), 140.2 (trifluorbenzene-C_3), 143 (HC=C), 148.7 (trifulorobenzene-C_2), 150.1,151.6 (furan-C_1, C_4), 150.2 (thiazole-C_4), 151.8 (pyrazole-C), 153.4 (trifulorobenzene-C_4), 167.8 (C=N), 171.6 (C=O); LC-MS (*m/z*, %): 654 (M+1, 98.0).

Synthesis of (E)-N'-(3-methoxybenzylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide **(5c)**

Re-crystallized from ethanol, off white solid (yield 85%); mp 183-185 °C; IR (KBr) ν_{max}/cm^{-1} 3270(NH), 1672 (amide C=O), 1606 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.52 (s, 1H, pyrazole-CH_2), 3.71-3.78 (dd, 1H, *J* 17.75, 6.69, pyrazole-CH_2), 3.82 (s, 3H, benzene-O-CH_3), 3.96 (s, 2H, thiazole-CH_2-CO-), 5.76 (m, 1H, pyrazole,-CH), 6.60 (d, 1H, *J* 3.48,-CH- furan), 6.71 (s, 1H,-CH, thiazole), 6.98 (d, 1H, *J* 3.49 Hz,-CH- furan), 7.4-8.23 (12H, dichlorobenzene, benzylidenimin, methoxybenzene), 8.31 (s,1H, -CH, -N=CH-), 11.68 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.3, 60.2 (pyrazole-CH_2, CH), 38.5 (CH_2, aliphatic), 55.9 (CH_3, aliphatic methyl), 101.3, 105.3 (furan-C_2, C_3), 104.7 (thiazole-C_3), 111.2, 116.6, 121.5 (methoxybenzene-C_2, C_4, C_6), 127.0, 127.8 (dichlorobenzene-C_6, C_5), 128.2,128.8 (phenyl-$C_{2,6}$, $C_{3,5}$), 129.8 (methoxybenzen-C_5), 130.2 (dichlorobenzene-C_3), 131.3 (phenyl-C_4), 131.5, 133.8 (dichlorobenzene-C_2, C_4), 136.5 (phenyl-C_1), 138.2 (methoxybenzene-C_1), 138.7 (dichlorobenzene-C_1), 143 (HC=C), 150.1,151.6 (furan-C_1, C_4), 150.3 (thiazole-C_4), 151.7 (pyrazole–C), 160.7 (methoxybenzene-C_3), 167.5 (C=N), 171.0 (C=O); LC-MS (*m/z*, %): 630 (M+1, 98.2).

Synthesis of (E)-N'-(2-(trifluoromethoxy)benzylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide **(5d)**

Recrystallized from ethanol, off white solid (yield 78%); mp 180-184 °C; IR (KBr) ν_{max}/cm^{-1} 3360 (NH), 1673 (amide C=O), 1605 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.51 (s, 1H, pyrazole-CH_2), 3.69-3.75 (dd, 1H, *J* 17.76, 6.68, pyrazole-CH_2), 3.96 (s, 2H, thiazole-CH_2-CO-), 5.75 (m, 1H, pyrazole-CH), 6.59 (d, 1H, *J* 3.48, -CH-furan), 6.71 (s, 1H, -CH, thiazole), 6.98 (d, 1H, *J* 3.49, -CH- furan), 7.3-8.01 (12H, dichlorobenzene, benzylidenimin, trifluoromethoxybenzene), 8.26 (s, 1H, -CH, -N=CH-), 11.58 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.2, 60.1 (pyrazole-CH_2, CH), 38.4 (CH_2, aliphatic), 101.3, 105.2 (furan-C_2, C_3), 104.7 (thiazole-C_3), 114.4, 116.9, 121.2 (trifluoromethoxybenzene-C_1, C_3, C_5), 121.8 (O-CF_3, aliphatic), 127.2, 127.9 (dichlorobenzene- C_6, C_5), 128.3, 128.8 (phenyl-$C_{2,6}$, $C_{3,5}$), 130.1 (trifluoromethoxybenzen-C_6), 130.6 (dichlorobenzene- C_3), 131.3 (phenyl-C_4), 131.5, 133.7 (dichlorobenzene-C_2, C_4), 132.1(trifluoromethoxybenzene-C_4), 136.6 (phenyl-C_1), 138.8 (dichlorobenzene-C_1), 143 (HC=C), 150.0, 151.5 (furan-C_1, C_4), 150.2 (thiazole-C_4), 151.7 (pyrazole-C), 160.5 (triluoromethoxybenzene-C_2), 167.3 (C=N), 171.2 (C=O); LC-MS (*m/z*, %): 684 (M+1, 98.2).

Synthesis of (E)-N'-(4-hydroxybenzylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide **(5e)**

Recrystallized from ethanol, off white solid (yield 78%); mp 183-185 °C; IR (KBr) ν_{max}/cm^{-1} 3310 (NH), 1670 (amide C=O), 1610 (amide C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.51 (s, 1H, pyrazole-CH_2), 3.69-3.75 (dd, 1H, *J* 17.76, 6.68, pyrazole –CH_2), 3.96 (s, 2H, thiazole-CH_2-CO-), 5.75 (m, 1H, pyrazole-CH), 6.59 (d, 1H, *J* 3.48, -CH- furan), 6.71 (s, 1H, -CH, thiazole), 6.98 (d, 1H, *J* 3.49, -CH-furan), 7.3-8.01 (12H, dichlorobenzene,

benzylidenimin, 4-hydroxybenzen), 8.12 (s,1H, -CH, -N=CH-), 9.10 (s, 1H, benzene-OH), 11.58 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.3, 60.1 (pyrazole-CH_2, CH), 38.2 (CH_2, aliphatic), 101.3, 105.2 (furan-C_2, C_3), 104.3 (thiazole-C_3), 116.0, 126.4 (4-hydroxybenzene-$C_{3,5}$, C_1), 127.2, 127.9 (dichlorobenzene-C_6, C_5), 128.3,128.8, (phenyl-$C_{2,6}$, $C_{3,5}$), 130.6 (4-hydroxybenzen-$C_{2,6}$), 130.6 (dichlorobenzene-C_3), 131.3 (phenyl-C_4), 131.5, 133.7 (dichlorobenzene-C_2, C_4), 136.6 (phenyl-C_1), 138.8 (dichlorobenzene-C_1), 143 (HC=C), 150.0,151.5 (furan-C_1, C_4), 150.4 (thiazole-C_4), 151.7 (pyrazole-C), 160.8 (4-hydroxybenzene-C_2), 167.1 (C=N), 171.6 (C=O); LC-MS (*m/z*, %): 616 (M+1, 98.21).

Synthesis of (E)-N'-(2-(trifluoromethyl)benzylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide **(5f)**

Recrystallized from ethanol, off white solid (yield 83%); mp 168-170 °C; IR (KBr) ν_{max}/cm^{-1} 3250 (NH), 1673 (amide C=O), 1608 (C=N); ^1H NMR (400 MHz, DMSO-d_6) δ 3.51 (s, 1H, pyrazole-CH_2), 3.69-3.75 (dd, 1H, *J* 17.76, 6.68, pyrazole-CH_2), 3.96 (s, 2H, thiazole-CH_2-CO-), 5.75 (m, 1H, pyrazole-CH), 6.58 (d, 1H, *J* 3.48, -CH-furan), 6.70 (s, 1H, -CH, thiazole), 6.98 (d, 1H, *J* 3.49 Hz, -CH- furan), 7.3-8.0 (12H, dichlorobenzene, benzylidenimin, trifluro methylbenzene), 8.25 (s, 1H, -CH, -N=CH-), 11.48 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.2, 60.1 (pyrazole-CH_2, CH), 38.4 (CH_2, aliphatic), 101.3, 105.2 (furan-C_2, C_3), 104.7 (thiazole-C_3), 117.8 (trifluoromethylbenzene-CF_3), 122.7, 127.0 (trifluoromethylbenzene-C_1, C_3), 127.2, 127.9 (dichlorobenzene- C_6, C_5), 128.1 (trifluoromethylbenzene-C_2), 128.4, 128.7 (phenyl- $C_{2,6}$, $C_{3,5}$), 129.5 (trifluoromethylbenzen-C_6), 130.6 (dichlorobenzene- C_3), 131.3 (phenyl-C_4), 131.4, 132.1 (trifluoromethylbenzene-C_4, C_5), 131.5, 133.8 (dichlorobenzene-C_2, C_4), 136.6 (phenyl-C_1), 138.8 (dichlorobenzene-C_1), 143 (HC=C), 150.0,151.6 (furan-C_1, C_4), 150.3 (thiazole-C_4), 151.7 (pyrazole-C), 167.5 (C=N), 171.0 (C=O); LC-MS (*m/z*, %): 668 (M+1, 98.7).

Synthesis of (E)-N'-(2,4-dimethoxybenzylidene)-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide **(5g)**

Recrystallized from ethanol, off white solid (yield 82%); mp 198-200 °C; IR (KBr) ν_{max}/cm^{-1} 3300 (NH), 1674 (amide C=O), 1611 (C=N); ^1H NMR (400 MHz, DMSO-d_6) δ 3.51 (s, 1H, pyrazole-CH_2), 3.61 (s, 3H, -O-CH_3,dimethoxy benzene), 3.70-3.77 (dd, 1H, *J* 17.75, 6.69, pyrazole-CH_2), 3,84 (s, 3H, -O-CH_3, dimethoxy benzene), 3.95 (s, 2H, thiazole-CH_2-CO-), 5.75 (m, 1H, pyrazole,-CH), 6.59 (d, 1H, *J* 3.48, -CH- furan), 6.71 (s, 1H,-CH, thiazole), 6.98 (d, 1H, *J* 3.49, -CH- furan), 7.4-8.13 (12H, dichlorobenzene, benzylidenimin, dimethoxy benzene), 8.10 (s,1H, -CH, -N=CH-), 11.58 (s, 1H, - CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.2, 60.1 (pyrazole –CH_2, CH), 38.6 (CH_2, aliphatic), 55.8 (dimethoxy benzene-methyl), 101.0, 105.2 (furan-C_2, C_3), 104.7 (thiazole-C_3), 101.5, 106.7, 109.2 (dimethoxybenzene-C_3, C_5, C_1), 127.2, 127.9 (dichlorobenzene-C_6, C_5), 128.4, 128.7, (phenyl- $C_{2,6}$, $C_{3,5}$), 130.6 (dichlorobenzene-C_3), 131.3 (phenyl-C_4), 131.5, 133.8 (dichlorobenzene-C_2, C_4), 136.6 (phenyl-C_1), 133.0 (dimethoxybenzene-C_6), 138.8 (dichlorobenzene- C_1), 143 (HC=C), 150.0,151.6 (furan-C_1, C_4), 150.7 (thiazole-C_4), 151.7 (pyrazole-C), 159.5, 163.9 (dimethoxybenzene-C_3, C_4), 167.6 (C=N), 171.2 (C=O); LC-MS (*m/z*, %): 660 (M+1, 98.4).

Synthesis of (E)-N'-benzylidene-2-(2-(5-(5-(2,3-dichlorophenyl)furan-2-yl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide **(5h)**

Recrystallized from ethanol, off white solid (yield 77%); mp 169-172 °C; IR (KBr) ν_{max}/cm^{-1} 3310 (NH), 1675 (amide C=O), 1607(amide C=N); ^1H NMR (400 MHz, DMSO-d_6) δ 3.50 (s, 1H, pyrazole-CH_2), 3.72 (dd, 1H, *J* 17.70, 6.65, pyrazole-CH2), 3.96 (s, 2H, thiazole-CH_2-CO-), 5.70 (m, 1H, pyrazole-CH), 6.60 (d, 1H, *J* 3.47, -CH-furan), 6.70 (s, 1H, -CH, thiazole), 6.99 (d, 1H, *J* 3.49, -CH-furan), 7.48-8.20 (13H, dichlorobenzene, benzylidenimin, phenyl), 8.06 (s, 1H, -CH, -N=CH-), 11.22 (s, 1H, -CO-NH-, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.1, 60.0 (pyrazole-CH_2, CH), 38.3 (CH_2, aliphatic), 101.3, 105.2 (furan-C_2, C_3), 104.3 (thiazole-C_3), 127.2, 127.9 (dichlorobenzene- C_6, C_5), 128.3,128.8 (phenyl- $C_{2,6}$, $C_{3,5}$), 128.8, 129.4 (benzene-$C_{2,6}$, $C_{3,5}$), 130.7 (dichlorobenzene- C_3), 131.1 (bezene-C_4), 131.3 (phenyl-C_4), 131.5, 133.7 (dichlorobenzene-C_2, C_4), 133.8 (benzene-C_1), 136.6 (phenyl-C_1), 138.8 (dichlorobenzene-C_1), 143 (HC=C), 150.0, 151.5 (furan-C_1, C_4), 150.2 (thiazole-C_4), 151.7 (pyrazole-C), 167.4 (C=N), 171.0 (C=O); LC-MS (*m/z*, %): 600 (M+1, 98.7).

Biological protocols

Antimicrobial activity

The standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The antibacterial activity of the synthesized 4,5-dihydropyrazole derivatives **(2a-d, 3, 4a-d** and **5a-h)** were performed by broth dilution method against the following standard bacterial strains *Staphylococcus aureus* (ATCC11632), *Streptococcus faecalis* (ATCC 14506), *Bacillus subtilis* (ATCC 60511), *Klebsiella pneumoniae* (ATCC10031), *Escherichia* and *Pseudomonas aeruginosa* (ATCC10145) and antifungal activity against yeasts: *Saccharo-myces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, CT), mould: *Aspergillus niger* (ATCC 6275).

The minimal inhibitory concentrations (MIC, lg/mL) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. Test compounds (10 mg) were dissolved in dimethyl sulfoxide (DMSO, 1 mL) then diluted in culture medium (Mueller-Hintonbroth for bacteria and Sabouraud liquid medium for fungi), further progressive dilutions to obtain final concentrations of 1, 2, 4, 8, 16, 31.25, 62.5, 125, 250 and 500 lg/mL. DMSO never exceeded 1 % v/v. The tubes were inoculated with 105 cfu/mL (colony forming unit/mL) and incubated at 37°C for 24 h. The growth control consisting of media (positive control) and media with DMSO (negative control) at the same dilutions as used in the experiments were employed.

Anticancer activity (MTT assay)

The synthesized 4,5-dihydropyrazole derivatives **(2a-d, 3, 4a-d** and **5a-h)** were tested in vitro for their cytotoxic properties against tumor cell lines panel consisted of Hela Hela (human cervix carcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), A2780 (human ovarian cancer cell line), BGC-823 (human gastric cancer cell line) and L02 (human normal cell line) by Mosmann's method. The MTT assay is based on the reduction of the soluble MTT (0.5 mg/mL, 100 µL), into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells [13].

The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin at 37 °C and humidified at 5% CO_2. Briefly cells were placed on 96-well plates at 100 µL total volume with density of 1–2.5 × 10^4 cells per ML and were allowed to adhere for 24 h before treatment with tested drugs in DMSO solution (10^{-5}, 10^{-6}, 10^{-7} mol/L final concentration). Triplicate wells were treated with media and agents. Cell viability was assayed after 96 h of continuous drug exposure with a tetrazolium compound. The supernant medium was removed, and 150 µL of DMSO solution was added to each well. The plates were gently agitated using mechanical plate mixer until the color reaction was uniform and the OD570 was determined using micro plate reader. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of vehicle in the MTT assay. Assays were performed in triplicate on three independent experiments. The results had good reproducibility between replicate wells with standard errors below 10%.

RESULTS AND DISCUSSION

Chemistry

The synthetic scheme utilized for synthesis of target compounds **2a-d, 3, 4a-d** and **5a-h** are outlined in Scheme **1** [11,12,14], and their analytical and physical properties were depicted in Table **1**.

The starting compound **1** was obtained by reacting commercially available acetophenone with 5-(2,3-dichlorophenyl)furan-2-carbaldehyde by schmidt condensation mechanism, which was further condensed with thiosemicarbazide in presence of ethanol medium. A series of titled compounds (**2a-d)** were concomitantly prepared by treating compound **1** with commercially available various phenacyl bromides in presence of hot ethanolic medium. Further, compound **1** was cyclized with ethyl 4-bromo acetoacetate and hydrazine hydrate to obtain crucial scaffold **3.**

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The target Schiff bases (**4a-d)** and (**5a-h)** were synthesized by condensing scaffold **3** with commercially available various aldehydes and acetophenones in presence of glacial acetic acid and ethanol.

The compounds (**5a-h)** comprising acetohydrazide structure may exist as E/Z geometrical isomers about –C=N double bond and as cis/trans amide conformers (Scheme 3). According to the literature [15-17], the compounds containing imine bond are present in higher percentage in dimethyl-d_6 sulfoxide solution in the form of geometrical E isomer about –C=N double bond. The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In this study, the spectral data were obtained in dimethyl-d6 sulfoxide solution, and no signal belonging to Z isomer was observed. On the other hand, the cis/trans conformers of E isomer were present in the dimethyl-d6 sulfoxide solution of compounds (**5a-h)** Figure **1**.

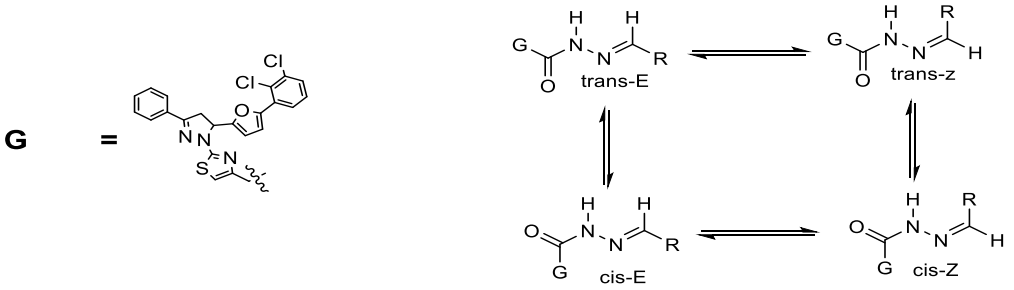
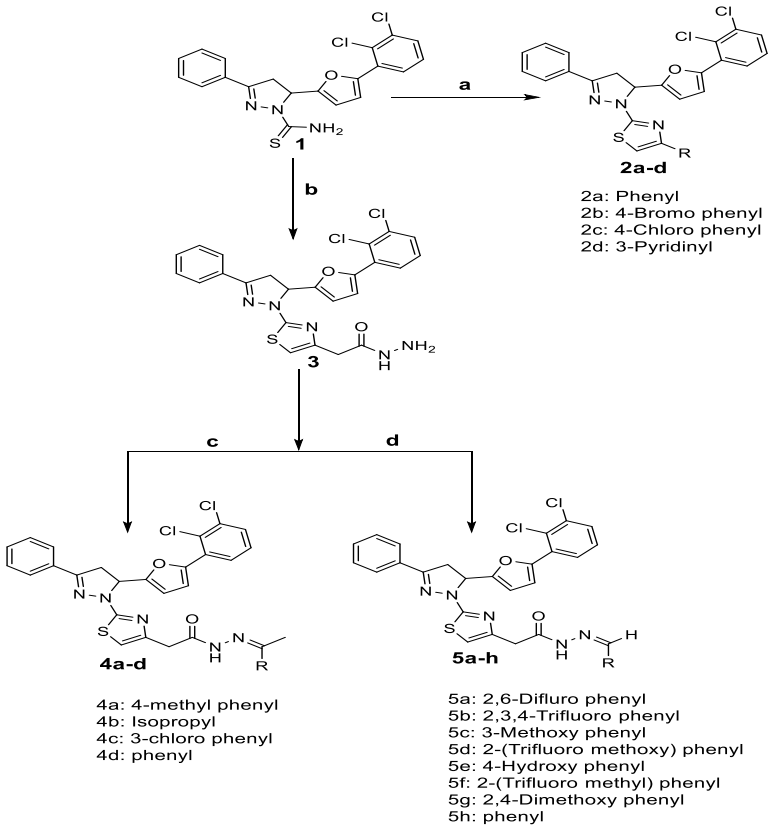


Figure 1 E/Z geometrical isomers and cis/trans confermers of 5a-h



Scheme 1. Convergent synthesis pathway of Pyroazole derivatives.
a) Subsituted phenacyl bromides, ethanol, refluxed for 1h. b) Ethyl bromo aceto acetate, ethanol, refluxed for 1h, Hydrazine hydrate, ethanol, refluxed for 5h. c) Substituted acetophenone, glacial acetic acid, ethanol, refluxed for 3h. d) Substituted aldehyde, glacial acetic acid, ethanol, refluxed for 3h.

The ^{1}H NMR spectrum of **2a-d, 3, 4a-d** and **5a-h** compounds, H_A, H_B and H_X protons of CH_2 and CH fragments of pyrazolines ring resonated as pairs were observed as doublet of doublet at δ 3.50-3.72 ppm (J_{AB} = 17.75-17.92 Hz), δ 3.80- 4.04 ppm (J_{AB} = 17.49-17.76 Hz), δ 5.65-6.07 ppm (J_{AB} = 11.36-11.74 Hz), due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position C_4 of pyrazolines ring.

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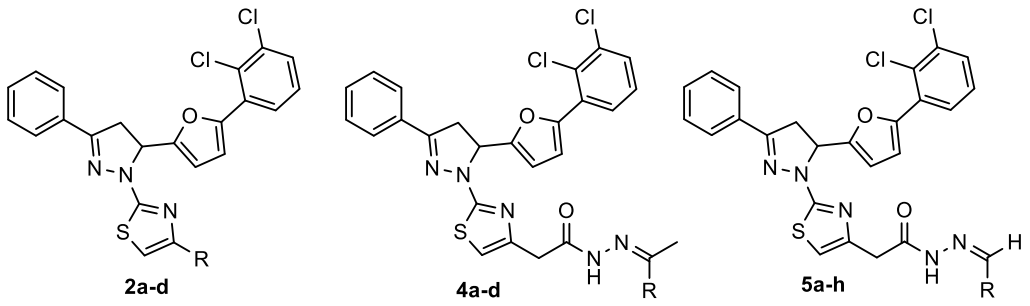
The chemical shift at δ 8.18 ppm corresponding to NH_2 protons in ^1H NMR spectrum. Additionally, C=N and C=S stretching bands at 1568 cm^{-1} and 1308 cm^{-1} respectively in IR spectrum and molecular ion peak at m/z 416 (M+1, 98.3%), which is in agreement with molecular formula $C_{20}H_{15}Cl_2N_3OS$ confirmed the formation of starting compound **1**.

The two protons of furan ring appeared as two doublet at δ 6.82 ppm and δ 7.18 ppm with coupling constant J = 3.4 Hz and J = 3.36 Hz respectively and disappearance of NH_2 at δ 8.18 ppm in ^1H NMR spectrum. Further, LC-MS data and strong absorption band at 1581cm^{-1} (C=N), 1532 cm^{-1} (C=C) in IR spectrum established the synthesis of titled compounds (**2a-d)**.

The ^1H NMR spectrum of compound **3**, depict NH_2, NH protons at δ 4.22 ppm and δ 9.04 ppm respectively, additionally LCMS data, elemental analysis and strong absorption band at 1667cm^{-1} corresponding to carbonyl group in IR spectrum authenticated the formation of scaffold **3**.

The absence of characteristic peaks corresponding to NH_2 of compound **3** at δ 4.22 ppm and appearance of aromatic hydrogen absorptions in range of δ 7.48- 8.20 ppm and methyl group at δ 2.22-2.24 ppm in ^1H NMR spectrum confirmed the formation of designed compounds (**4a-d)** and (**5a-h).** Further, IR, LC-MS and elemental analysis data confirmed formation of target Schiff bases.



Pharmacological activity and Structure Activity Relationship

Antimicrobial activity

The results of antimicrobial testing of designed compounds **(2a-d, 3, 4a-d** and **5a-h)** against selected gram-positive, gram-negative bacteria, yeasts, moulds are illustrated in Table **2**.

The antimicrobial activity of series (**2a-d)** displayed comparable antimicrobial activity against tested bacterial and fungal species in comparison to reference drugs**.** Among the series, compound **2c** comprising 4-Cl substitution depicted excellent antimicrobial activity against both gram-negative and gram-positive organisms than their antifungal inhibition.

The antimicrobial activity of compound **3** displayed excellent inhibition against tested bacterial and fungal species with MIC 4–16 µ/ml. Several recent experiments indicated that the incorporation of hydrophobic moieties into the framework of acetohydrazide enhance penetration of drug into tissues of mammalian host and into the waxy cell wall of bacterium. This strategy of drug design has been proposed as a vehicle for controlled study of growth cycle of pathogen as well as for augmenting fundamental drug activity.

Table 1: Analytical and Physico-chemical data of synthesized compounds

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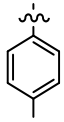
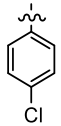
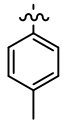
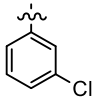
Compound	R	Molecular Formula	M.W[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	%Analysis of C, H, N found (calc.)[c]		
						C	H	N
1	NA	$C_{20}H_{15}Cl_2N_3OS$	416.32	183-185 °C/ ethanol	69	57.70(58.0)	3.63(3.70)	10.09(10.20)
2a		$C_{28}H_{19}Cl_2N_3OS$	516.44	165-167 °C/ ethanol	79	65.12(65.18)	3.71(3.19)	8.14(8.23)
2b		$C_{28}H_{18}BrCl_2N_3OS$	595.34	179-181°C/ ethanol	80	56.49(56.84)	3.05(3.12)	7.06(7.12)
2c		$C_{28}H_{18}Cl_3N_3OS$	550.89	181-183°C/ ethanol	83	61.05(61.12)	3.29(3.31)	7.63(7.67)
2d		$C_{27}H_{18}Cl_2N_4OS$	517.43	182-184 °C/ ethanol	80	62.67(62.75)	3.51(3.65)	10.83(10.86)
3	NA	$C_{24}H_{19}Cl_2N_5O_2S$	512.41	132-134°C/ ethanol	75	56.26(56.34)	3.74(3.84)	13.67(13.78)
4a		$C_{33}H_{27}Cl_2N_5O_2S$	628.57	158-160°C/ ethanol	84	63.06(63.13)	4.33(4.35)	11.14(11.21)
4b		$C_{29}H_{27}Cl_2N_5O_2S$	580.53	134-136°C/ ethanol	82	60(60.07)	4.69(4.70)	12.06(12.15)
4c		$C_{32}H_{24}Cl_3N_5O_2S$	648.99	162-164°C/ ethanol	86	59.22(60.0)	3.73(3.78)	10.79(10.81)

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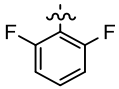
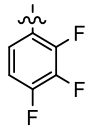
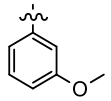
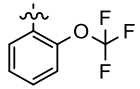
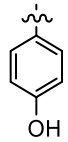
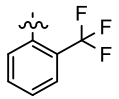
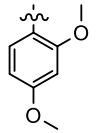
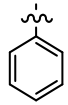
4d		$C_{32}H_{25}Cl_2N_5O_2S$	614.54	155-156°C/ ethanol	88	62.54(62.61)	4.10(4.12)	11.40(11.41)
5a		$C_{31}H_{21}Cl_2F_2N_5O_2S$	636.5	175-177°C/ ethanol	81	58.5(58.65)	3.33(3.42)	11(11.1)
5b		$C_{31}H_{20}Cl_2F_3N_5O_2S$	654.49	192-194°C/ ethanol	80	56.89(56.90)	3.08(3.13)	10.7(10.78)
5c		$C_{32}H_{25}Cl_2N_5O_3S$	630.54	183-185°C/ ethanol	85	60.95(60.98)	4.00(4.06)	11.11(11.14)
5d		$C_{32}H_{22}Cl_2F_3N_5O_3S$	684.51	180-184°C/ ethanol	78	56.15(56.24)	3.24(3.34)	10.23(10.45)
5e		$C_{31}H_{23}Cl_2N_5O_3S$	616.52	183-185°C/ ethanol	78	60.39(60.45)	3.76(3.85)	11.36(11.45)
5f		$C_{32}H_{22}Cl_2F_3N_5O_2S$	668.52	168-170°C/ ethanol	83	58.07(58.11)	3.54(3.57)	10.26(10.28)
5g		$C_{33}H_{27}Cl_2N_5O_4S$	660.57	198-200°C/ ethanol	82	60.00(60.03)	4.12(4.15)	10.6(10.65)
5h		$C_{31}H_{23}Cl_2N_5O_2S$	600.52	169-172°C/ ethanol	77	62.00(62.0)	3.86(3.96)	11.66(11.67)

[a] Molecular weight of the compound

[b] Melting point of the compound at their decomposition

[c] Elemental analysis of C, H, and N were within ±0.4% of theoretical value

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In line with the above discussion, We synthesized various Schiff bases **(4a-d & 5a-h)** possessing enhanced lipophilicity, further to study structure activity relationship of the designed derivatives, various electron donating and withdrawing groups were substituted in various position of phenyl ring. Halogens which are known for inductive, conjugative, steric and/or electronic properties were introduced at various positions which effects biological activity directly (i.e., antimicrobial).

Among the series, fluorinated compounds **5a, 5b, 5d** and **5f** exhibited enhanced inhibition compared to other derivatives among tested anti-bacterial and fungal species. In recent times, it is reported that incorporation of fluorine atom into heterocycles provides compounds with enhanced biological properties. The enhanced biological activity of fluorinated heterocycles is due to accumulation of fluorine on carbon and causing increased oxidative and thermal stability. Hence fluorinated drugs due to their inherent characteristics of being metabolically non-degradable and increased lipid solubility are utilized to enhance the rate of drug absorption and there in vivo transport.

Further, SAR results among synthesized fluorine derivatives (**5a, 5b, 5d** and **5f**) confirmed compound **5b** consisting 2,3,4 tri flouro substitution is most potent of the series with MIC ranging from 0.5 to 4 µg/ml. The pharmacological results confirm that replacement or alteration in positions and number of fluorine atoms results in forfeiture of antimicrobial activity.

Table 2: Antimicrobial activity expressed as MIC (µg/mL).

Compounds	Gram-positive organisms[a]			Gram-negative organisms[a]			Fungi[a]		
	Sa	Sf	Bs	Kp	Ec	Pa	Sc	Ct	An
1	8	31.25	8	4	62.5	16	62.5	31.25	125
2a	8	62.5	62.5	8	16	62.5	8	8	4
2b	8	62.5	62.5	62.5	8	62.5	16	4	4
2c	**2**	**4**	**4**	**1**	**1**	**1**	**4**	**4**	**4**
2d	16	31.25	31.25	16	16	8	31.25	16	16
3	**16**	**4**	**8**	**4**	**8**	**16**	**16**	**4**	**16**
4a	16	125	125	125	125	125	125	125	62.5
4b	**16**	**31.25**	**31.25**	**16**	**4**	**4**	**16**	**16**	**31.25**
4c	16	16	8	31.25	16	125	125	125	62.5
4d	31.25	62.5	8	62.5	31.25	62.5	62.5	125	125
5a	**16**	**8**	**16**	**16**	**8**	**8**	**4**	**8**	**16**
5b	**2**	**2**	**1**	**4**	**1**	**4**	**1**	**1**	**0.5**
5c	125	125	16	16	125	31.25	8	16	16
5d	16	16	4	16	8	16	8	31.25	8
5e	125	125	31.25	31.25	125	125	16	62.5	8
5f	16	8	31.25	16	125	8	8	31.25	8
5g	31.25	125	31.25	16	8	125	125	125	125
5h	31.25	16	62.5	31.25	31.25	8	8	125	125
Ciprofloxacin	≤5	≤5	≤1	≤1	≤1	>5	-	-	-
Norfloxacin	<5	<5	≤1	≤1	≤1	>5	-	-	-
Flucanozole	-	-	-	-	-	-	≤1	≤1	≤1

[a]The screening organisms. Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632, Sa), *Streptococus faecalis* (ATCC 14506, Sf), and *Bacillus subtilis* (ATCC 60511, Bs).
[b]The screening organisms. Gram-negative bacteria: *Klebsiella penumoniae* (ATCC 10031, Kp), *Escherichia coli* (ATCC 10536, Ec), and *Pseudomonas aeruginosa* (ATCC 10145, Pa).
[c]The screening organisms. Yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, Ct), mould: *Aspergillus niger* (ATCC 6275, An).

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Anticancer activity

The titled compounds (**2a-d, 3, 4a-d and 5a-h**) were evaluated for their in vitro cytotoxic activity against tumor cell lines panel consisting Hela (human cervix carcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), A2780 (human ovarian cancer cell line), BGC-823 (human gastric cancer cell line) and L02 (human normal cell line) by using Mosmann's method. Doxorubicin and cisplatin was utilized as positive control to validate biological evaluation and their results are illustrated in Table **3**.

The initial cytotoxicity results of designed compounds endorse compound **3** is potent with IC 50 values 1.3 to 4.2 µg/ml among tested cancerous cell and not affecting the normal cell line. Literature survey confirms, Schiff bases are considered to be among the most important group of compounds in medicinal chemistry due to their preparative accessibility, structural variety and specific cytotoxicity [18]. Fluoro compounds have also been traditionally associated with potent antitumor properties. Other than the well-established fluoro-nucleosides such as 5-fluoro uracil, the fluorine containing anticancer molecules include flutamide, an anti-androgen which was launched in 1983 for the treatment of prostate cancer and fluorinated anthracycline antibiotics, steroids, Vitamin D_3 analogs and fluorine containing taxoids have been shown to be much more effective than their parent analogs [19].

Table 3: Cytotoxicity of synthesized compounds against human tumor cells (IC50 ±SD, µM).

Compounds	Human tumor cells					Human normal cells
	Hela	A549	MCF-7	A2780	BGC-823	L02
1	**4.02±0.43**	**0.65±0.32**	**0.61±0.86**	**0.94±0.09**	**0.87±0.76**	>40
2a	5.92±0.87	2.11±0.62	2.10±0.64	3.82±0.54	2.34±0.62	>40
2b	4.68±0.43	3.87±0.37	2.60±0.48	5.32±1.25	4.51±2.32	>40
2c	6.24±0.87	3.24±0.24	**1.02±0.54**	2.98±1.87	2.93±0.47	>40
2d	6.21±0.34	2.87±0.52	**1.11±0.34**	3.44±0.70	1.76±0.71	>40
3	**4.23±0.23**	2.18±0.45	**1.65±0.44**	**2.12±0.32**	1.34±0.11	>40
4a	6.23±0.41	3.44±0.23	4.32±0.11	3.14±0.23	**1.04±0.34**	>40
4b	**6.82±0.34**	4.06±0.14	2.98±0.34	**2.17±0.33**	**1.02±0.43**	>40
4c	**3.94±0.47**	3.01±0.50	2.23±0.50	3.09±1.34	2.01±0.04	>40
4d	**2.89±0.24**	**1.43±0.35**	2.45±0.52	3.18±1.52	1.11±1.11	>40
5a	**1.23±0.21**	1.82±0.24	1.60±0.54	1.93±0.20	0.78±0.54	>40
5b	**0.95±0.19**	**0.35±0.56**	**0.32±0.43**	**0.95±0.87**	**0.65±0.32**	>40
5c	**4.87±1.22**	2.67±0.43	**1.65±0.54**	3.21±1.87	1.98±0.22	>40
5d	**1.02±0.35**	1.02±0.87	1.87±0.35	2.28±0.34	0.34±0.23	>40
5e	**5.54±0.32**	2.87±0.54	2.21±1.65	3.89±1.27	2.01±0.63	>40
5f	**1.23±0.54**	1.98±0.33	1.22±1.14	1.12±0.45	0.41±0.29	>40
5g	6.62±0.56	3.26±0.95	3.22±2.05	4.35±0.36	6.11±0.73	>40
5h	**4.45±0.34**	3.34±0.90	3.67±0.12	4.12±0.34	5.43±0.73	>40
Doxorubicin (control)	1.03±0.22	0.67±0.13	0.73±0.25	0.95±0.31	1.08±0.15	>40
Cisplatin (Control)	5.65±0.21	1.83±0.62	1.85±0.46	2.39±0.47	0.98±0.25	>40

[a] Mean value ±SD (standard deviation from three experiments).
[b] Boldface: IC50 ≤ the control, (IC_{50} , µg mL^{-1})

The structure–activity relationship studies have shown that Schiff bases containing substituted phenyl group exhibited interesting anticancer properties. It has been observed that various substitutions (including chloro, hydroxyl, bromo, methoxy, phenolic etc.,) on various position of phenyl ring, have significant effect on

cytotoxicity of these compounds. Among the Schiff bases (**4a-d** & **5a-h**) fluorinated compound **5a** (2,6-F) exhibited excellent inhibition then other fluorinated derivatives 5b (2,3,4-F), 5d (2-OCF3) and 5f (2-CF3).

Edwards et al [20]. Confirmed that, more the methoxy groups in a compound, is beneficial for antimitotic activity against Hela cells. This found contrast in case of compound **5g** (1,4-OCH3), with (IC50 6.62µm) which might be due to the more bulky nature of the compound. With increasing the size of halogen from fluorine to chlorine **4c** (2-Cl) caused loss of cytotoxic activity.

Among the designed series compound **5b** exhibited excellent cytotoxic activity with IC50 0.32 to 0.95 µm against tested cell lines in comparison to standard drugs Cisplatin and Doxorubicin.

CONCLUSION

In the present study four series of novel pyrazole derivatives (**2a-d, 3, 4a-d** and **5a-h**) were designed and synthesized. The entire target compounds were investigated for their antimicrobial potential against selected gram-positive, gram-negative bacteria, yeasts, moulds. Further, titled compounds were evaluated for their in vitro cytotoxic activity against tumor cell lines panel consisting Hela (human cervix carcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), A2780 (human ovarian cancer cell line), BGC-823 (human gastric cancer cell line) and L02 (human normal cell line) by using Mosmann's method. Doxorubicin and cisplatin was utilized as positive control to validate biological evaluation.

Antimicrobial study revealed fluorinated compounds **5a, 5b, 5d** and **5f** exhibited enhanced inhibition compared to other designed derivatives. Among the fluorinated derivatives, Compound **5b** demonstrated significant activity against tested gram-positive and gram-negative bacteria and fungal species. The invitro anticancer screening of synthesized series illustrate that all the designed compounds were active, in particular tri flouro substituted Schiff base **5b** exhibited excellent anticancer activity IC50 0.32 to 0.95 µm against tested cell lines in comparison to standard drugs Cisplatin and Doxorubicin.

The promising invitro antimicrobial and anticancer activity of fluro substituted di hydro pyrazole derivatives make them certainly promising molecules for further lead optimization in the development of novel antimicrobial and anticancer agents.

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