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# Synthesis and characterization of some novel *N*-substituted derivatives of 3-(benzo[b]thiophen-2yl)-5-(4-(substituted) phenyl)-4,5-dihydro-1*H*-pyrazole and its pharmacological studies

Jeyanthi S & Muniyappan N*

Saraswathi Narayanan College of Arts and Science, Madurai Kamaraj University, Madurai 625 022, Tamil Nadu, India

E-mail: sakthisai588@gmail.com, nmuniyappan@gmail.com

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A new series of N-substituted pyrazoline derivatives bearing 2-chloro acetyl **3a-g** and benz imidazole thioether moiety **4a-g** have been synthesized from substituted benzaldehyde and 2-acetyl benzo thiophene through chalcones, followed by the cyclisation with hydrazine hydrate, acylation using chloroacetyl chloride and finally substituted with 1*H*-benzo[d]imidazole thiol. All synthesized compounds **1-4a-g** have been characterized by $^{1}$H and $^{13}$C NMR and mass spectroscopy. The present work provides major scope for the synthesis of a new series of N-substituted pyrazoline derivatives bearing 2-mercaptobenzimidazole heterocycles having various pharmacological activities associated with it like anti-oxidant, anti-inflammatory, cytotoxic properties and antimicrobial activity. Most of the compounds (**3e**, **4a**, **4b**, **4c**, **4e**, **4f** and **4g**) display a fair degree of potent, anti-oxidant, anti-inflammatory, cytotoxic and antimicrobial properties.

**Keywords**: 2-Mercaptobenzimidazole, N-Substituted pyrazolines, Anti-inflammatory, Antioxidant, Cytotoxic studies

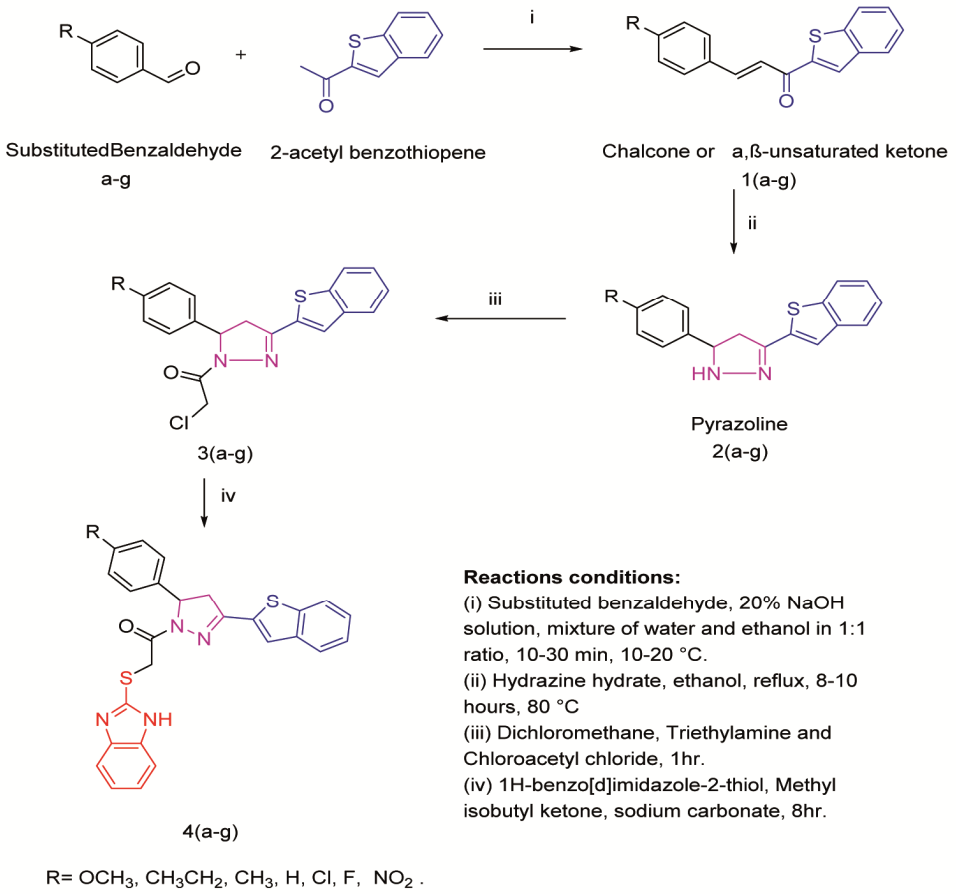
Pyrazoline, one of the heterocyclic compounds have attracted many medicinal chemists for their immense pharmacological activities associated with them[1] like anti-inflammatory[2], anti-bacterial[3], anti-fungal[4], antioxidant[4,5], anti-cancer[6], anti-convulsant[7], anti-depressant[7], anti-tuberculosis[8], and anti-amoebic[9] activities. Some of these compounds have also cox-2 inhibitor[10], recognition of transition metal ions[11] electrophotography and electro-luminescence[12]. Further pharmacological effects include cannabinoid CB1 receptor antagonists[13], anti-nociceptive[14], antidiabetic[15], anti-hyperglycaemic agents and aldose reductase inhibition[16]. They also effect ACAT inhibition[17], urotensin and somatostatin-5 receptors[17], TGF-β signal transduction inhibition[17] and neurocytotoxicity inhibitors activation[17], anti-epileptic[17], anti-trypanosomal[17], MAO-inhibitory[17]. The usefulness of pyrazoline structure provides a unique spatial configuration which allows various substitution pattern. Keeping that in mind, in our present study we intended to synthesize some novel N-substituted pyrazoline derivatives **3a-g** and with their thio ethers **4a-g**.

The synthesis of new pyrazoline derivatives **3a-g** and **4a-g** by using known methods stated below and screening of antimicrobial, anti-oxidant, anti-inflammatory and cytotoxic effects for the synthesized compounds. The reaction of α,β-unsaturated aldehydes and ketones with hydrazine became one of the most popular methods for the preparation of 2-pyrazolines[18,19]. In this method, hydrazones are formed as intermediates, which can be subsequently cyclized to 2-pyrazolines in the presence of a cyclising agent like acetic acid.

## Chemistry

The synthesis of new pyrazoline derivatives **4a-g** was carried out according to the steps shown in Scheme 1. In the initial step substituted benzaldehyde reacted with 2-acetyl benzo thiophene in presence of ethanol and water in 1:1 ratio to yield chalcone **1a-g,** which undergo cyclisation with hydrazine hydrate in ethanol at 80°C to give pyrazolines **2a-g,** the structure and yield of the compounds mentioned in Fig. 1, which on acylation with chloro-acetyl chloride in presence of triethylamine yielded compounds **3a-g,**

**List of abbreviations**: HRBC: Human red blood cell; DPPH: 2,2-diphenyl-1-picrylhydrazyl; CUPRAC: Cupric Reducing Antioxidant Capacity; MTT: (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide); MCF-7(Michigan Cancer Foundation-7): human adenocarcinoma cells; TMS: Trimethylsilane; TLC: Thin layer chromatography.




Scheme 1 — Synthesis of N-substituted pyrazoline derivatives **3a-g** and **4a-g**

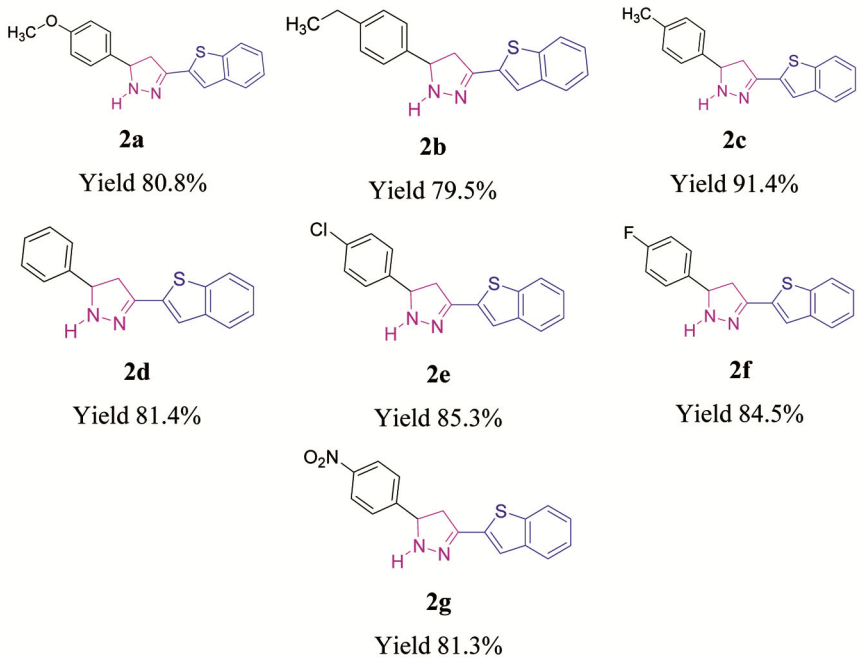


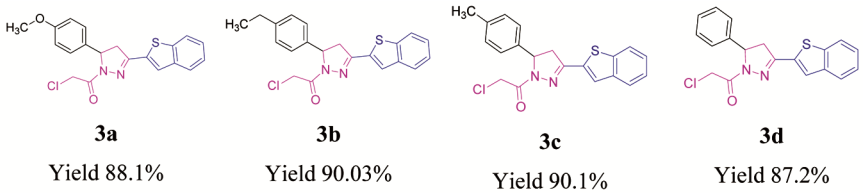

Fig. 1 — Structure of compounds **2a-g** and their yields

the structure and yield of the compounds mentioned in Fig. 2, which finally undergoes substitution with 1H-benzo[d]imidazole-2-thiol to give the new pyrazoline derivative **4a-g** the structure and yield of the compounds mentioned in Fig. 3.

All synthesized compounds were characterized by $^{1}H$ and $^{13}C$ NMR and mass spectra. In the $^{1}H$ NMR of compound **2a-g**, –NH proton appears around at δ 6.0 as broad singlet, the $CH_2$ protons of pyrazoline ring appears as a pair of doublets around at δ 3.69-3.46 and 3.16-3.01. The CH ($H_X$) proton appeared as a doublet of doublets around at δ 5.15– 4.91 ($H_X$) due to the vicinal coupling with two magnetically non-equivalent protons of the methylene group at C4 of the pyrazoline ring ($H_A$ and $H_B$). In the $^{13}C$ NMR of compound **2a-g**, The structure of the compounds **2a-g** confirmed by the disappearance of signal around at δ 183.29-178.72 assigned for C=O group and the appearance of signal around at δ 64.6-64.1 and 42.1-41.6 corresponding to the pyrazoline ring of CH and $CH_2$ respectively. Aromatic and other carbon atoms were observed at expected chemical shifts range.

The structure of the compounds **3a-g** was confirmed from its spectral data. The $CH_2$ proton of the acetyl group at position 1 of the pyrazoline ring was observed at δ 4.64-4.51 range. All the other aromatic and aliphatic protons occurred at the expected regions.

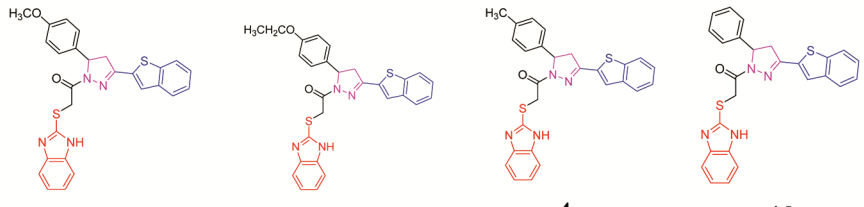
In the $^{1}H$ NMR spectra of the compounds **4a-g**, the $CH_2$ protons of the pyrazoline ring resonated as a pair of doublets between δ 4.06-3.84 and 3.36-3.27 range. The CH proton appeared as a doublet of doublet between δ 5.79-5.64 range. This is due to the vicinal coupling with two magnetic non- equivalent protons



**3a** Yield 88.1% | **3b** Yield 90.03% | **3c** Yield 90.1% | **3d** Yield 87.2%

**3e** Yield 91.3% | **3f** Yield 86.2% | **3g** Yield 91.7%

Fig. 2 — Structure of compounds **3a-g** and their yields



**4a** Yield 75.6% | **4b** Yield 81.7% | **4c** Yield 80.3% | **4d** Yield 71.8%

**4e** Yield 77.4% | **4f** Yield 75.0% | **4g** Yield 77.1%

Fig. 3 — Structure of compounds **4a-g** and their yields

of methylene group at position 4 of pyrazoline ring. The $CH_2$ proton of the acetyl group at position 1 of the pyrazoline ring were observed at δ 4.403-4.206 range as a doublet due to the geminal coupling resulted from steric structure of the compound. These geminal protons were observed as doublet due to two different possible conformations. All the other aromatic and aliphatic protons occurred at the expected regions. The ABX system of pyrazoline ring mentioned in Fig. 4 (Ref. 20).

In the $^{13}C$ spectra, the signal due to carbonyl carbon occurred between δ 168.361-168.183 range. The S-$CH_2$ carbon signal occurred around at δ 33.46-33.32 and the other aromatic and aliphatic carbons were observed at expected regions. The mass spectral data of the synthesised compound were found in full agreement with the proposed structure. All compounds gave satisfactory analytical results.

## Experimental Section

### Material and methods

The reagents were purchased from Aldrich and used without further purification and solvents were purchased from commercial suppliers and used without further purification. $^1H$ NMR and $^{13}C$ NMR spectra were obtained on a Bruker Avance instrument in $CDCl_3$ using TMS as an internal standard, operating at 300 MHz and 75 MHz respectively. Chemical shifts (δ) are expressed in ppm and coupling constants *J* are given in Hz. For $CDCl_3$ solutions the chemical shifts are reported as parts per million (δ, ppm) to residual protium or carbon of the solvents; $CDCl_3$ $\delta_H$ (δ 7.26) and $CDCl_3$ $\delta_C$ (δ 77.03). Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q =quartet, dd = doublet of doublets, m = multiplet. Melting points were obtained on a Melting point instrument. LC-MS spectra were obtained on Thermoscientific LTQ Xcallibur 2.2 LCMS spectrometer equipped with an ESI/APCI source.

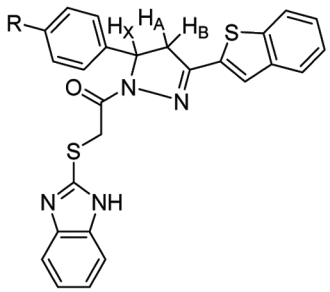



Fig. 4 — ABX system of pyrazoline ring

## General procedure for the synthesis of compounds

### 1-(Benzo[b]thiophen-2yl)-3-(4-(substituted)phenyl) prop 2-en 1-one, 1a-g

A mixture of benzaldehyde (10.0 g, 0.094 mol), 2-acetyl benzo-thiophene (14.9 g, 0.085 mol) in absolute alcohol (100.0 mL) and demineralized water (150.0 mL) was stirred under dropwise addition of 20% NaOH (19.0 mL, 0.094 mol) at 10-20°C for 30 min. The reaction mixture was stirred for 60 min at 25-30°C and the progress of the reaction was monitored by TLC. Upon reaction completion the precipitated solid was filtered, washed with 1:1 mixture of ethanol and water (50.0 mL). The crude product was precipitated using ethanol (30.0 mL) to obtain **1a-g**.

**1-(Benzo [b ]thiophen-2yl)- 3,(4-methoxy phenyl) prop 2-en 1-one, 1a**: Yield 89.8%. Pale yellow solid. $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.90-7.85 (m, 3H), 7.67-7.62 (m, 2H), 7.50-7.39(m, 3H), 6.98-6.94 (m, 2H), 3.87 (s, 3H); $^{13}C$ NMR: δ 183.41, 161.90, 145.49, 144.25, 142.60, 139.35, 130.43, 128.46, 127.41, 127.27, 125.90, 124.98, 122.99, 118.80, 114.50 and 55.45; LC-MS: *m/z* Calcd for $C_{18}H_{14}O_2S$ [M+H]+: 295.37. Found: [M+H]+: 295.13.

**1-(Benzo [b] thiophen-2yl)-3-(4-ethyl phenyl) prop 2-en 1-one, 1b**: Yield 84.3%. Pale brown solid. $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.11 (s,1H), 7.94-7.87 (m, 3H), 7.63-7.60 (d, 2H, *J* =*8.1* Hz), 7.54-7.40 (m, 3H), 7.29-7.26 (m, 2H) 2.75-2.67 (q, 2H), 1.30-1.25 (t, 3H); $^{13}C$ NMR: δ 183.50, 147.67, 145.37, 144.51, 142.66, 139.33, 132.19, 128.76, 128.73, 128.59, 127.36, 125.97, 125.02, 122.99, 120.77, 28.92, 15.33;); LC-MS: *m/z* Calcd for $C_{19}H_{16}OS$ [M+H]+: 292.40. Found: [M+H]+: 293.12.

**1-(Benzo [b]thiophen-2yl)-3-(4-methyl phenyl) prop 2-en 1-one, 1c**: Yield 95.9%. Pale yellow solid. $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.94-7.86 (m, 3H), 7.60-7.40 (m, 5H), 7.26-7.24 (d, 2H), 2.41 (s, 3H); $^{13}C$ NMR: δ 183.49, 145.36, 144.48, 142.66, 141.38, 139.33, 131.94, 129.78, 128.71, 128.65, 127.36, 125.96, 125.018, 123.00, 120.11 and 21.59; LC-MS: *m/z* Calcd for $C_{18}H_{14}OS$ [M+H]+: 278.37. Found: [M+H]+: 278.37.

**1-(Benzo [b]thiophen-2yl)- 3 phenyl prop 2-en 1-one, 1d**: Yield 86.6%. Yellow solid. $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.95-7.88 (m, 3H), 7.70-7.67 (m, 2H), 7.58 (s, 1H), 7.53-7.40 (m, 5H). $^{13}C$ NMR: δ 183.43, 145.21, 144.38, 142.71, 139.30, 134.67, 130.79, 129.03, 128.93, 128.62, 127.45,

126.02, 125.67, 123.02 and 121.12; LC-MS: *m/z* Calcd for $C_{17}H_{12}OS$ [M+H]+: 264.34. Found: [M+H]+: 265.16.

**1-(Benzo [b ]thiophen-2yl)-3-(4-chloro phenyl) prop 2-en-1-one, 1e**: Yield 85.19%. Off-white solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.94-7.82 (m, 3H), 7.63-7.40 (m, 7H); $^{13}$C NMR: δ 183.15, 145.02, 142.87, 142.76, 139.24, 136.69, 133.56, 129.72, 129.31, 129.01, 128.77, 127.55, 126.00, 125.11, 123.03 and 121.54; LC-MS: *m/z* Calcd for $C_{17}H_{11}OClS$ [M+H]+: 298.78. Found: [M+H]+: 299.13.

**1-(Benzo [b]thiophen-2yl)-3-(4-fluoro phenyl) prop 2-en-1-one, 1f**: Yield 85.8%. Off-white solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.95-7.84 (m, 3H), 7.71-7.66 (m, 2H), 7.52-7.40 (m, 3H),7.17-7.11 (m, 2H); $^{13}$C NMR: δ 183.22, 145.09, 143.06, 142.71, 139.26, 130.45, 130.41, 130.47, 130.10, 128.91, 127.49, 126.00, 125.09, 123.02, 120.85, 120.82, 116.35, 116.06; LC-MS: *m/z* Calcd for $C_{17}H_{11}OSF$ [M+H]+: 282.33. Found: [M+H]+: 283.13.

**1-(Benzo [b]thiophen-2yl)-3-(4-nitro phenyl) prop 2-en 1-one, 1g**: Yield 85.45%. Yellow solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.94-7.85 (m, 3H), 7.70-7.65 (m, 2H), 7.51-7.40 (m, 3H), 7.16-7.12 (m, 2H); $^{13}$C NMR: δ 183.12, 145.057, 142.67, 142.46, 139.12, 136.87, 133.46, 129.62, 129.21, 129.01, 128.66, 127.45, 126.01, 125.11, 123.02 and 121.45; LC-MS: *m/z* Calcd for $C_{17}H_{11}O_3SN$ [M+H]+: 309.34. Found: [M+H]+: 310.32.

**General procedure for the preparation of compounds 2a-g**

A mixture of compound **1a** (13.0 g, 0.044 mol) in absolute alcohol (130.0 mL) and hydrazine monohydrate (4.42 g, 0.088 mol) were heated to 80°C and maintained for 8-10 hrs. The progress of the reaction was monitored by TLC. Upon reaction completion the reaction mixture was cooled to 0-5° C. The precipitated solid was filtered and washed with ethanol (20.0 mL). The crude product was purified using methanol (50.0 mL) to obtain **2a**.

**3-(Benzo[b]thiophen-2yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole, 2a**: Yield 80.76%. Pale yellow solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.86-7.78 (m, 1H), 7.75-7.69 (d, 1H), 7.41-7.24 (m, 5H), 6.94-6.86 (m, 2H), 6.01 (bs, 1H), 4.98-4.92 (dd, 1H, $J_1$=10.8 Hz, $J_2$=9.0 Hz), 3.80 (s, 3H), 3.56-3.47 (dd, 1H, $J_1$=10.8 Hz, $J_2$=6.2 Hz), 3.14-3.06 (dd, 1H, $J_1$=16.2 Hz, $J_2$=10.8 Hz); $^{13}$C NMR: δ 159.37, 147.25, 139.99, 139.72, 127.03, 134.29, 127.57, 125.19, 124.49, 123.71, 122.89, 122.38, 114.27, 64.44, 55.34 and 41.70; LC-MS: *m/z* Calcd for $C_{18}H_{16}N_2SO$ [M+H]+: 308.40. Found: [M+H]+: 309.31.

**3-(Benzo[b]thiophen-2yl)-5-(4-ethyl phenyl)-4,5-dihydro-1*H*-pyrazole, 2b**: Yield79.51%. Dark brown colour. $^1$H NMR (300MHz, $CDCl_3$): δ 7.83-7.69 (m, 2H), 7.36-7.31 (m, 4H), 7.30-7.18 (m, 3H), 6.02 (bs, 1H), 5.00-4.93 (dd, 1H, $J_1$=10.5, $J_2$= 9.0 Hz), 3.57-3.48 (dd, 1H, $J_1$=15.9 Hz, $J_2$=10.5 Hz), 3.17-3.09 (dd, 1H, $J_1$=16.2 Hz, $J_2$=8.7 Hz), 2.68-2.61 (q, 2H), 1.28-1.21 (t, 3H); $^{13}$C NMR: δ 147.21, 144.14, 140.01, 139.73, 139.49, 137.02, 128.41, 126.38, 125.19, 124.50, 123.72, 122.912, 122.39, 64.69, 41.69, 28.55 and 15.60; LC-MS: *m/z* Calcd for $C_{19}H_{18}N_2S$ [M+H]+: 306.43. Found: [M+H]+: 307.12.

**3-(Benzo[b]thiophen-2yl)-5-(4-methyl phenyl)-4,5-dihydro-1*H*-pyrazole, 2c**: Yield 91.39%. Pale yellow solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.83-7.78 (m, 1H), 7.74-7.68 (m, 1H), 7.356-7.24 (m, 5H), 7.18-7.15 (d, 2H, *J*=7.8 Hz), 6.02 (bs, 1H), 4.99-4.93(dd, 1H, $J_1$=10.5 Hz, $J_2$=8.7 Hz), 3.57-3.48 (dd, 1H, $J_1$=15.9 Hz, $J_2$ =10.5 Hz), 3.16-3.07 (dd, 1H, $J_1$=16.2 Hz, $J_2$=8.7 Hz), 2.44-2.34 (s, 3H); $^{13}$C NMR: δ 147.20, 140.02, 139.75, 139.29, 137.72, 137.04, 129.80, 129.59, 129.32, 128.68, 127.74, 126.32, 125.76, 125.20, 124.51, 123.74, 122.91, 122.39, 64.69, 41.73 and 21.14; LC-MS: *m/z* Calcd for $C_{18}H_{16}N_2S$ [M+H]+: 292.40. Found: [M+H]+: 293.31.

**3-(Benzo[b]thiophen-2yl)-5-phenyl)-4,5-dihydro-1*H*-pyrazole, 2d**: Yield 81.39%. Off-white solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.79-7.68 (m, 1H), 7.74-7.68 (m, 1H), 7.40-7.245 (m, 7H), 6.05 (bs, 1H), 5.01-4.95 (dd, 1H, $J_1$=10.8 Hz, $J_2$=9.0 Hz), 3.58-3.49(dd, 1H, $J_1$=16.2 Hz, $J_2$=10.8 Hz), 3.16-3.08 (dd, 1H, $J_1$=16.2 Hz, $J_2$=9.0 Hz); $^{13}$C NMR: δ 147.16, 142.27, 139.75, 136.97, 128.95, 128.68, 128.20, 127.97, 126.73, 126.42, 125.24, 124.54, 124.288, 123.77, 123,55, 122.97, 122.40, 64.91 and 41.78; LC-MS: *m/z* Calcd for $C_{17}H_{14}N_2S$ [M+H]+: 278.37. Found: [M+H]+: 279.34.

**3-(Benzo[b]thiophen-2yl)-5-(4-chloro phenyl)-4,5-dihydro-1*H*-pyrazole, 2e**: Yield 85.28%. Off-white solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.82-7.78(m, 2H), 7.36-7.30 (m, 6H), 6.05 (bs, 1H), 4.99-4.93 (dd, 1H, $J_1$=10.5 Hz, $J_2$=9.3 Hz), 3.58-3.49 (dd, 1H, $J_1$=16.2 Hz, $J_2$=10.8), 3.10-3.02 (dd, 1H, $J_1$=16.2 Hz, $J_2$=9.0 Hz,); $^{13}$C NMR: δ 147.16, 142.27, 139.75,

136.97, 128.95, 128.68, 128.20, 127.97, 126.73, 126.42, 125.24, 124.54, 124.29, 123.77, 123.55, 122.97, 122.40, 64.27 and 41.87; LC-MS: *m/z* Calcd for $C_{17}H_{13}N_2SCl$ [M+H]+: 312.82. Found: [M+H]+: 313.22.

**3-(Benzo[b]thiophen-2yl)-5-(4-fluro phenyl)-4,5-dihydro-1*H*-pyrazole, 2f**: Yield 84.53%. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.80-7.77 (m, 1H), 7.74-7.68 (m, 1H), 7.40-7.30 (m, 4H), 7.25-7.24 (d, 1H, *J*=5.4 Hz), 7.08-7.00 (m, 2H), 6.04 (bs, 1H), 5.01-4.94 (t, 1H), 3.58-3.49 (dd, 1H, $J_1$=15.9 Hz, $J_2$=10.5 Hz), 3.11-3.03 (dd, 1H, $J_1$=15.9 Hz, $J_2$=9.0 Hz,); $^{13}$C NMR: δ 164.04, 160.78, 147.19, 140.00, 139.69, 137.90, 136.79, 128.17, 128.07, 127.56, 125.30, 124.56, 123.78, 123.05, 123.39, 115.88, 115.59, 64.27 and 41.891; LC-MS: *m/z* Calcd for $C_{17}H_{13}N_2SF$ [M+H]+: 296.36. Found: [M+H]+: 297.14.

**3-(Benzo[b]thiophen-2yl)-5-(4-nitro phenyl)-4,5-dihydro-1*H*-pyrazole, 2g**: Yield 81.31%. Yellow solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.26-8.21 (m, 2H), 7.83-7.79 (m, 1H), 7.75-7.70 (m, 1H), 7.63-7.59 (m, 2H), 7.38-7.31 (m, 2H), 7.26- 7.26 (d, 1H), 6.14 (bs, 1H), 5.15-5.08 (t, 1H), 3.69-3.60(dd, 1H, $J_1$=16.2 Hz, $J_2$=10.8 Hz), 3.13- 3.04(dd, 1H, $J_1$=16.2 Hz, $J_2$ =9.9 Hz); $^{13}$C NMR: δ 149.34, 147.15, 139.69, 137.55, 136.19, 127.47, 125.50, 124.64, 124.19, 123.85, 123.41, 122.42, 64.18 and 42.13; LC-MS: *m/z* Calcd for $C_{17}H_{13}O_2SN_3$ [M+H]+: 323.37. Found: [M+H]+: 324.17.

**General procedure for the preparation compounds 3a-g**

A mixture of compound **2a** (10.0 g, 0.032 mol) in dichloromethane (150.0 mL) with triethylamine (5.9 g, 0.058 mol) was cooled to 0-5°C, chloroacetyl chloride (5.49 g, 0.048 mol) was slowly added to the reaction mixture for 10 min. starting material disappearance was checked in TLC. Upon reaction completion reaction mixture was quenched using chilled water (100.0 mL) and layer was separated organic layer was washed with 5% sodium bicarbonate solution(150.0 mL) followed by water (150.0 mL) and organic layer was dried over sodium sulphate and evaporated to dryness. The product was precipitated from the residue using methanol (100.0 mL). The precipitated product was filtered and dried to obtain compounds **3a**.

**1-(3-(Benzo[b]thiophen-2yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1yl)-2-chloroethan-1-one, 3a**: Yield 88.14%. Pale yellow solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.84-7.76 (m, 2H), 7.46-7.35 (m, 3H), 7.26-7.18 (m, 2H), 6.89-6.84 (m, 2H), 5.62-5.56 (dd, 1H, $J_1$=4.8 Hz, $J_2$=11.7 Hz), 4.56 (s, 2H), 3.89-3.79 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.78 (s, 3H), 3.33-3.26 (dd, 1H, $J_1$=17.4 Hz, $J_2$=4.5 Hz); $^{13}$C NMR: δ 149.34, 147.15, 139.69, 137.55, 136.19, 127.47, 125.50, 124.64, 124.19, 123.85, 123.41, 122.42, 64.18 and 42.13; LC-MS: *m/z* Calcd for $C_{20}H_{17}N_2SO_2Cl$ [M+H]+: 384.88. Found: [M+H]+: 385.16.

**1-(3-(Benzo[b]thiophen-2yl)-5-(4-ethylphenyl)-4,5-dihydro-1*H*-pyrazole-1yl)-2-chloroethan-1-one, 3b**: Yield 90.03%. brown colour solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.86-7.82(m, 1H), 7.78-7.75 (m, 1H), 7.43-7.34 (m, 3H), 7.25-7.17 (m, 4H), 5.62-5.57 (dd, 1H, $J_1$=11.7 Hz, $J_2$=4.5 Hz), 4.57 (s, 2H), 3.87-3.78(dd,1H, $J_1$=17.4 Hz, $J_2$=11.7 Hz), 3.33-3.25 (dd, 1H, $J_1$=17.7 Hz, $J_2$=4.8 Hz), 2.65-2.57 (q, 2H), 1.23-1.18 (t, 3H); $^{13}$C NMR: δ 163.87, 151.28, 144.19, 140.75, 139.23, 137.81, 134.46, 128.53, 126.62, 126.43, 125.79, 124.97, 124.41, 122.53, 60.81, 42.56, 42.24, 28.53 and 15.46; LC-MS: *m/z* Calcd for $C_{21}H_{19}N_2SOCl$ [M+H]+: 382.91. Found: [M+H]+: 383.21.

**1-(3-(Benzo[b]thiophen-2yl)-5-(4-methylphenyl)-4,5-dihydro-1*H*-pyrazole-1yl)-2-hloroethan-1-one, 3c**: Yield 90.1%. Pale brown solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.86-7.83(m, 1H), 7.78-7.75(m, 1H), 7.44-7.34 (m, 3H), 7.18-7.12 (m, 4H), 5.62-5.56 (dd, 1H, $J_1$=11.7 Hz, $J_2$=4.8 Hz), 4.56 (s, 2H), 3.88-3.79 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.32-3.25 (dd, 1H, $J_1$=17.7 Hz, $J_2$=4.8 Hz), 2.33-2.31 (s, 3H); $^{13}$C NMR: δ 163.87,151.28, 144.19, 140.75, 139.23, 137.81, 134.46, 128.53, 126.62, 126.43, 125.79, 124.97, 124.41, 122.53, 60.81, 42.56, 42.24, 28.53 and 15.46; LC-MS: *m/z* Calcd for $C_{20}H_{17}N_2SClO$ [M+H]+: 368.88. Found: [M+H]+: 369.28.

**1-(3-(Benzo[b]thiophen-2yl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-1yl)-2-chloroethan-1-one, 3d**: Yield 87.16%. Off-white solid. $^1$H NMR(300 MHz, $CDCl_3$): δ 7.84-7.79 (m, 1H), 7.79-7.76 (m, 1H), 7.46-7.36 (m, 3H), 7.27-7.23 (m, 3H), 7.06-7.00 (m, 2H), 5.64-5.58(dd, 1H, $J_1$=11.7 Hz, $J_2$=4.8 Hz), 4.62-4.52 (q, 2H), 3.92-3.82 (dd, 1H, $J_1$=17.7 Hz, $J_2$=12 Hz), 3.32-3.25 (dd, 1H, $J_1$=17.4 Hz, $J_2$=4.8 Hz); $^{13}$C NMR: δ 163.89, 151.35, 140.75, 140.59, 139.24, 134.33, 129.09, 128.14, 126.82, 126.50, 125.03, 124.41, 122.54, 61.00, 53.60, 42.59 and 42.26; LC-MS: *m/z* Calcd for $C_{19}H_{15}N_2SOCl$ [M+H]+: 354.85. Found: [M+H]+: 355.45.

**1-(3-(Benzo[b]thiophen-2yl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1yl)-2-chloroethan-1-one, 3e**: Yield 91.3%. White solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.84-7.79 (m, 1H), 7.79-7.76 (m, 1H), 7.46-7.18(m, 7H), 5.62-5.56 (dd, 1H, $J_1$=11.7 Hz, $J_2$=4.8 Hz), 4.62-4.51 (m, 2H), 3.92-3.818 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.304-3.229 (dd, 1H, $J_1$ =17.4 Hz, $J_2$ =4.8 Hz); $^{13}$C NMR: δ 163.979, 151.163, 140.777, 139.171, 139.033, 134.114, 133.977, 129.255, 127.311, 126.845, 126.567, 125.048, 124.471, 122.549, 60.377, 42.450 and 42.118; LC-MS: *m/z* Calcd for $C_{19}H_{14}N_2SOCl_2$ [M+H]+: 389.29. Found: [M+H]+: 390.59.

**1-(3-(Benzo[b]thiophen-2yl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1yl)-2-chloroethan-1-one, 3f**: Yield 86.17%. White solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.825-7.780 (m, 1H), 7.777-7.751 (m, 1H), 7.439 (s,1H), 7.415-7.316 (m, 5H), 7.309-7.230 (m, 1H), 5.645-5.590 (m, 1H), 4.578 (s, 2H), 3.894-3.797 (dd, 1H, $J_1$=17.4 Hz, $J_2$=11.7 Hz), 3.328-3.254 (dd, 1H, $J_1$=17.4 Hz, $J_2$=4.8 Hz); $^{13}$C NMR: δ 164.052, 163.941, 160.781, 151.166, 140.768, 139.188, 136.423, 136.380, 134.203, 127.740, 126.794, 126.541, 125.036, 124.453, 122.546, 116.617, 115.828, 60.330, 42.516 and 42.163; LC-MS: *m/z* Calcd for $C_{19}H_{14}N_2SOFCl$ [M+H]+: 372.84. Found: [M+H]+: 373.25.

**1-(3-(Benzo[b]thiophen-2yl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1yl)-2-chloroethan-1-one, 3g**: Yield 91.65%. Yellow solid. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.23-8.18 (m, 2H), 7.87-7.74 (m, 2H), 7.47-7.26 (m, 5H), 5.72-5.66 (dd, 1H, $J_1$=12.0 Hz, $J_2$=5.4 Hz), 4.65-4.51(q, 2H), 3.99-3.89 (dd, 1H, $J_1$=17.7 Hz, $J_2$=12.0 Hz), 3.32-3.25 (dd, 1H, $J_1$=17.4 Hz, $J_2$=5.1 Hz); $^{13}$C NMR: δ 164.226, 151.062, 147.698, 147.337, 140.84, 139.09, 133.69, 127.08, 126.89, 125.12, 124.52, 124.46, 122.58, 60.32, 42.34 and 41.91; LC-MS: *m/z* Calcd for $C_{19}H_{14}N_3SClO_3$ [M+H]+: 399.95. Found: [M+H]+: 400.01.

**General procedure for the preparation of compounds 4a-g**

A mixture of compound **3a** (1.0g, 0.0026mol) and 1H-benzo[d]imidazole-2-thiol (0.410 g, 0.0027 mol) in methyl isobutyl ketone (15.0 mL) with sodium carbonate (0.33g, 0.0031mol) was heated to 75-80°C and maintained for 8hr, starting material disappearance was checked in TLC. Upon reaction completion reaction mixture was filtered and washed with water (15.0 mL) and methyl isobutyl ketone (20.0 mL). The product was recrystallized from methanol (10.0 mL). The precipitated product was filtered and dried to obtain compounds **4a**.

**2(-(1*H*-Benzo[d]imidazole-2yl)thio-1-(3-(benzo[b]thiophen-2yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-yl)-ethan-1-one, 4a**: Yield 75.64%. White solid. m.p.234-237°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 11.34 (bs, 1H), 7.87-7.84 (d, 1H, *J*=7.5 Hz), 7.79-7.76 (m, 1H), 7.65-7.65 (d, 1H, *J*=1.8 Hz), 7.63-7.14(m, 8H), 6.87-6.83 (m, 2H), 5.66-5.61 (dd, 1H, $J_1$=11.4 Hz, $J_2$=4.5 Hz), 4.37-4.22 (q, 2H), 3.94-3.84 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.73(s, 3H), 3.35-3.27 (dd, 1H, $J_1$=17.7 Hz, $J_2$=4.5 Hz); $^{13}$C NMR: δ 168.18, 159.46, 152.42, 149.51, 143.87, 140.93, 139.13, 134.90, 134.05, 132.39, 127.13, 126.82, 126.61, 125.02, 124.49, 122.59, 122.42, 122.00, 118.48, 114.56, 110.60, 60.59, 55.28, 43.09 and 33.46; LC-MS: *m/z* Calcd for $C_{27}H_{22}N_4S_2O_2$ [M+H]+: 498.62. Found: [M+H]+: 499.13.

**2(-(1*H*-Benzo[d]imidazole-2yl)thio-1-(3-(benzo[b]thiophen-2yl)-5-(4-ethylphenyl)-4,5-dihydro-1*H*-pyrazole-1-yl)ethan-1-one, 4b**: Yield 81.72%. White solid. m.p.230-233°C. $^1$H NMR (300 MHz, $CDCl_3$): δ δ 11.37 (bs, 1H), 7.87-7.75 (m, 2H), 7.65-7.63 (d, 1H), 7.46-7.40 (m, 3H), 7.38-7.37 (d, 1H), 7.35-7.14 (m, 6H), 5.69-5.64 (dd, 1H, $J_1$=11.4 Hz, $J_2$=4.5 Hz), 4.38-4.22 (q, 2H), 3.95-3.85 (dd, 1H, $J_1$=17.4 Hz, $J_2$=11.4 Hz), 3.36-3.28 (dd, 1H, $J_1$=17.7 Hz, $J_2$ =4.5 Hz), 2.63-2.56 (q, 2H), 1.21-1.16 (t, 3H); $^{13}$C NMR: δ 168.29, 152.48, 149.52, 144.40, 143.89, 140.94, 139.12, 137.49, 134.89, 134.02, 128.72, 127.15, 126.62, 125.38, 125.38, 125.02, 124.49, 122.60, 122.43, 122.00, 118.53, 110.61, 60.83, 43.16, 33.36, 28.48 and 15.32; LC-MS: *m/z* Calcd for $C_{28}H_{24}N_4OS_2$ [M+H]+: 496.65. Found: [M+H]+: 497.15.

**2(-(1*H*-Benzo[d]imidazole-2yl)thio-1-(3-(benzo[b]thiophen-2yl)-5-(4-methylphenyl)-4,5-dihydro-1*H*-pyrazole-1-yl)ethan-1-one, 4c**: Yield 80.25%. White solid. m.p.237-240°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 11.36 (bs, 1H), 7.87-7.76 (m, 2H,), 7.65-7.63 (d, 1H), 7.47-7.35 (m, 4H), 7.30 -7.13 (m, 6H), 5.68-5.62 (dd, 1H, $J_1$=11.4 Hz, $J_2$=4.5 Hz), 4.38-4.21 (q, 2H), 3.96-3.86 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.55-3.28 (dd, 1H, $J_1$=17.7 Hz, $J_2$=4.8 Hz), 2.29 (s, 3H); $^{13}$C NMR: δ 168.31, 152.43, 149.50, 140.95, 139.13, 138.16, 137.33, 134.02, 129.90, 127.13, 126.62, 125.36, 125.02, 124.48, 122.59, 122.01, 118.54, 110.60, 60.86, 43.17, 33.33 and 21.10; LC-

MS: *m/z* Calcd for $C_{27}H_{22}N_4S_2O$ [M+H]+: 482.62. Found: [M+H]+: 483.25.

**2(-(1*H*-Benzo[d]imidazole-2yl)thio-1-(3-(benzo [b]thiophen-2yl)-5-phenyl)-4,5-dihydro-1*H*-pyrazole-1-yl)ethan-1-one, 4d**: Yield 71.84%. White solid. m.p.229-232°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 11.26 (bs, 1H), 7.87-7.78 (m, 2H,), 7.65-7.63 (d, 1H), 7.47-7.30 (m, 5H), 7.30-7.12 (m, 6H), 5.71-5.65 (dd, 1H, $J_1$=11.4 Hz, $J_2$=4.5 Hz), 4.37-4.24 (q, 2H), 3.99-3.90 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.55-3.28 (dd, 1H, $J_1$=17.4 Hz, $J_2$ =4.8 Hz); $^{13}$C NMR: δ 168.35, 152.46, 149.40, 141.04, 139.23, 138.06, 137.23, 124.50, 122.60, 122.00, 118.34, 110.60, 60.84, 43.11, 33.40; LC-MS: *m/z* Calcd for $C_{26}H_{20}N_4S_2O$ [M+H]+: 468.59. Found: [M+H]+: 469.05.

**2(-(1H-Benzo[d]imidazole-2yl)thio-1-(3-(benzo [b]thiophen-2yl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole 1-yl)ethan 1-one, 4e**: Yield 77.38%. white solid. m.p.253-255°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 11.16 (bs, 1H), 7.88-7.77 (m, 2H), 7.67-7.61 (m, 1H), 7.49 (s, 1H), 7.47-7.15 (m, 9H), 5.70-5.64(dd, 1H, $J_1$=11.7 Hz, $J_2$=4.8 Hz), 4.37-4.24(q, 2H), 4.00-3.90 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.35-3.27(dd, 1H, $J_1$=17.4 Hz, $J_2$=4.8 Hz); $^{13}$C NMR: δ 168.36, 139.07, 138.72, 134.23, 129.47, 127.31, 126.94, 126.74, 125.09, 124.53, 122.61, 122.16, 118.62, 110.54, 60.40,43.021 and 33.38; LC-MS: *m/z* Calcd for $C_{26}H_{19}N_4S_2OCl$[M+H]+: 503.04. Found: [M+H]+: 504.14.

**2(-(1*H*-Benzo[d]imidazole-2yl)thio-1-(3-(benzo [b]thiophen-2yl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl)ethan-1-one, 4f**: Yield 74.97%. White solid. m.p.233-236°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 11.20 (s, 1H), 7.88-7.85 (d, 1H, *J*=7.8 Hz), 7.80-7.79 (d, 1H, *J*=1.5 Hz), 7.78-7.77 (d, 1H, *J*=1.5), 7.66-7.63 (m, 1H), 7.50-7.25 (m, 5H), 7.21-7.16 (m, 2H), 7.07-7.00 (m, 2H), 5.71-5.65 (dd, 1H, $J_1$=11.4 Hz, $J_2$=4.5 Hz), 4.37-4.24 (q, 2H), 3.99-3.90 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.35-3.28 (dd, 1H, $J_1$=17.4 Hz, $J_2$=4.5 Hz). $^{13}$C NMR: δ 168.34, 152.32, 149.28, 143.88, 140.97, 139.08, 136.06, 134.83, 133.79, 127.38, 127.29, 127.27, 126.73, 125.08, 124.52, 122.62, 122.55, 122.10, 118.60, 116.37, 116.08, 110.54, 60.35, 43.11 and 33.38; LC-MS: *m/z* Calcd for $C_{26}H_{19}N_4S_2OF$[M+H]+: 486.58. Found: [M+H]+: 487.75.

**2(-(1*H*-Benzo[d]imidazole-2yl)thio-1-(3-(benzo [b]thiophen-2yl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl)ethan-1-one, 4g**: Yield 77.08%. White solid. m.p.238-241°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 10.93 (s, 1H), 8.21-8.18 (d, 2H, *J*=8.7 Hz), 7.89-7.86(d, 2H, *J*=7.8 Hz), 7.81-7.78 (d, 1H, *J*=6.9 Hz), 7.67-7.42(m, 5H), 7.40-7.14 (m, 3H), 5.80-5.74 (dd, 1H, $J_1$=5.1 Hz, $J_2$=12.0 Hz), 4.40-4.29 (q, 2H), 4.07-3.97 (dd, 1H, $J_1$=17.7 Hz, $J_2$=11.7 Hz), 3.36-3.29 (dd, 1H, $J_1$=17.7 Hz, $J_2$=5.1 Hz); $^{13}$C NMR: δ 168.33, 147.03, 139.01, 134.75, 127.52, 126.90, 126.60, 125.17, 124.60, 122.69, 122.65, 122.24, 118.64, 110.44, 60.31, 42.80 and 33.45; LC-MS: *m/z* Calcd for $C_{26}H_{19}N_5S_2O_3$ [M+H]+: 513.59. Found: [M+H]+: 514.39.

## Results and Discussion

### Pharmacology

#### *Anti-inflammatory study*

HRBC stabilization method was used for the estimation of *in-vitro* anti-inflammatory activity[21]. Steroidal anti-inflammatory agents usually lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. The results indicated that all the compounds, at various concentrations showed significant anti-inflammatory activity and showed a moderate anti-inflammatory activity when compared with the standard and the results are given in Table 1. Compounds **4g** exhibits significant activity against the standard in all concentrations and **4a** showed higher activity than the standard with 15 µg/mL concentration (Fig. 5).

#### *Antioxidant study*

DPPH free radical scavenging method[22], reducing power assay[23], nitric oxide radical scavenging method, $H_2O_2$ scavenging method, and CUPRAC assay method[24] were used for the estimation of

Table 1 — Anti-inflammatory activity of samples with different concentration

| Test Sample | Sample at different concentrations (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 5 | 10 | 15 |
| | Inhibition (%) | | | Absorption (nm) | | |
| **4a** | 71.28 | 73.10 | 93.95 | 1.723 | 1.614 | 1.563 |
| **4b** | 70.11 | 72.91 | 74.38 | 1.793 | 1.625 | 1.537 |
| **4c** | 73.45 | 76.41 | 76.71 | 1.593 | 1.415 | 1.397 |
| **4d** | 69.61 | 71.41 | 71.88 | 1.823 | 1.715 | 1.687 |
| **4e** | 68.28 | 69.83 | 70.76 | 1.903 | 1.810 | 1.754 |
| **4f** | 66.96 | 69.43 | 70.18 | 1.982 | 1.834 | 1.789 |
| **4g** | 79.10 | 80.16 | 81.61 | 1.254 | 1.190 | 1.103 |
| Diclofenac | 84.11 | 85.61 | 87.46 | 0.952 | 0.861 | 0.750 |

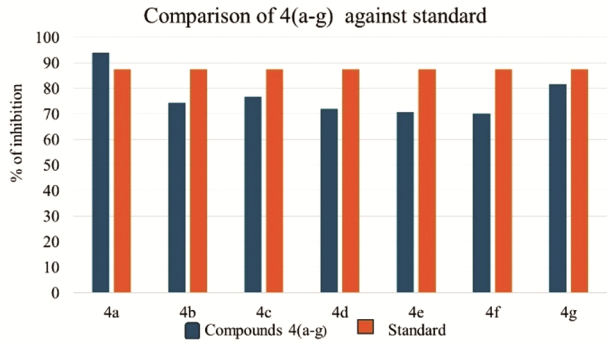



Fig. 5 — Comparison of anti-inflammatory activity of compounds **4a-g** and std with different concentrations

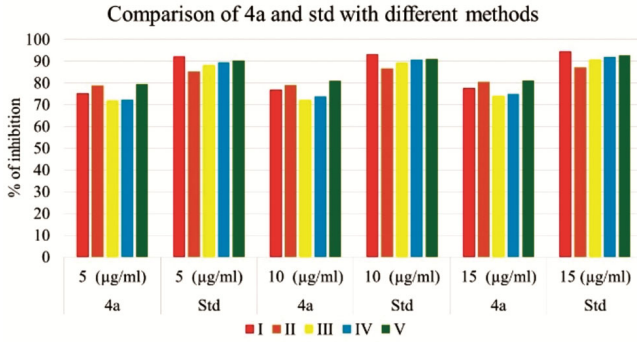



Fig. 6 — Effect of antioxidant activity of **4a** and std with various methods at different concentrations

Table 2 — Antioxidant activity of samples with different concentration

| Test drug | Concentration (µg/mL) | Inhibition (%) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| **4a** | 5 | 75.2 | 78.8 | 71.8 | 72.2 | 79.4 |
| | 10 | 76.9 | 79.0 | 72.0 | 73.7 | 80.8 |
| | 15 | 77.5 | 80.4 | 73.9 | 75.0 | 81.2 |
| **4b** | 5 | 68.6 | 65.8 | 77.8 | 62.2 | 71.4 |
| | 10 | 69.2 | 66.5 | 78.6 | 63.4 | 72.6 |
| | 15 | 70.4 | 67.6 | 79.9 | 64.7 | 74.0 |
| **4c** | 5 | 71.0 | 70.9 | 60.0 | 63.4 | 79.2 |
| | 10 | 73.2 | 72.3 | 61.5 | 64.1 | 80.0 |
| | 15 | 74.0 | 73.7 | 62.2 | 65.3 | 81.5 |
| **4d** | 5 | 65.5 | 68.5 | 72.6 | 67.2 | 69.4 |
| | 10 | 66.3 | 69.2 | 73.2 | 68.9 | 70.1 |
| | 15 | 67.6 | 70.7 | 74.0 | 69.7 | 72.8 |
| **4e** | 5 | 62.0 | 60.4 | 65.4 | 63.0 | 68.0 |
| | 10 | 63.8 | 61.5 | 66.0 | 63.9 | 69.3 |
| | 15 | 64.1 | 62.0 | 67.9 | 65.1 | 70.2 |
| **4f** | 5 | 78.1 | 72.6 | 71.8 | 73.2 | 77.4 |
| | 10 | 79.0 | 73.5 | 73.0 | 74.9 | 78.0 |
| | 15 | 79.9 | 74.0 | 74.5 | 75.4 | 79.3 |
| **4g** | 5 | 61.5 | 76.5 | 60.5 | 72.2 | 65.0 |
| | 10 | 62.0 | 77.6 | 62.0 | 73.5 | 66.3 |
| | 15 | 63.1 | 78.6 | 63.4 | 74.9 | 67.8 |
| Ascorbic acid (std) | 5 | 92.2 | 85.3 | 88.0 | 89.5 | 90.2 |
| | 10 | 93.0 | 86.7 | 89.3 | 90.6 | 91.0 |
| | 15 | 94.6 | 87.0 | 90.6 | 91.9 | 92.6 |

1=DPPH free radical scavenging method, 2=Reducing power assay, 3=Nitric oxide radical scavenging method, 4=$H_2O_2$ Scavenging method, 5=CUPRAC assay

antioxidant activity of all the compounds. All the samples showed good antioxidant activity with various methods at different concentration in each method and the results are given in Table 2. The results indicated that all the samples, at various concentrations (5, 10 and 15 µg/mL) showed significant antioxidant activity in each method. Compounds **4a, 4b, 4c, 4f and 4g** exhibits good antioxidant activity in all the methods with 15µg/mL. Compound **4a** exhibited significant actvity in all the methods when compared to the standard (Fig. 6).

Table 3 — Antibacterial activity of all the compounds

| Pathogen | Standard (mm)[a)] | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **4a** | **4b** | **4c** | **4d** | **4e** | **4f** | **4g** |
| 1 | 43 | – | – | – | – | – | – | – |
| 2 | 39 | – | 14 | 14 | 12 | 10 | 10 | 10 |
| 3 | 43 | 14 | 16 | 13 | 17 | 16 | 13 | 10 |
| 4 | 43 | – | 10 | 13 | 10 | – | 10 | – |

1=*Klebsiella pneumoniae, 2=Escherichia coli, 3=Staphylococcus aureus, 4=Bacillus subtilis.*
[a)] streptomycin sulphate

Table 4 — Anti-fungal activity of all the compounds

| Pathogens | Standard (mm)[b)] | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **4a** | **4b** | **4c** | **4d** | **4e** | **4f** | **4g** |
| 1 | 15 | 20 | 15 | 18 | 19 | 23 | 21 | 19 |
| 2 | 11 | – | – | – | – | – | – | – |
| 3 | 10 | –- | – | – | – | – | – | – |
| 4 | 16 | 13 | 16 | 14 | 14 | 13 | 16 | 14 |

1= *Aspergillus fumigatus, 2= Asperigillus flavus, 3= C.albicans, 4= Mucor sp.*[b)] amphotericin B

***Antimicrobial study***

The antibacterial activity for all the compounds were screened against the clinical pathogens *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*[25]. The antifungal activity for all the compounds were screened against the clinical pathogens for *Aspergillus flavus*, *Aspergillus fumigates, Candida albicans* and *Mucor species*[25]. The zone of inhibition was calculated, and the results are given in Table 3 and Table 4 respectively.

When compared to the standard (Table 3) the antibacterial activity of all the compounds were very low at 300 µL concentrations and the compounds showed no activity against *Klebsiella pneumoniae*.

When compared to the standard (Table 4) antifungal activity of all the compounds **4a**, **4b**, **4c**, **4d**, **4e**, **4f** and **4g** were fantastic against *Aspergillus fumigatus*, moderate and equal activity against *Mucor sp*, and no activity against *Aspergillus flavus, and C. albicans*.

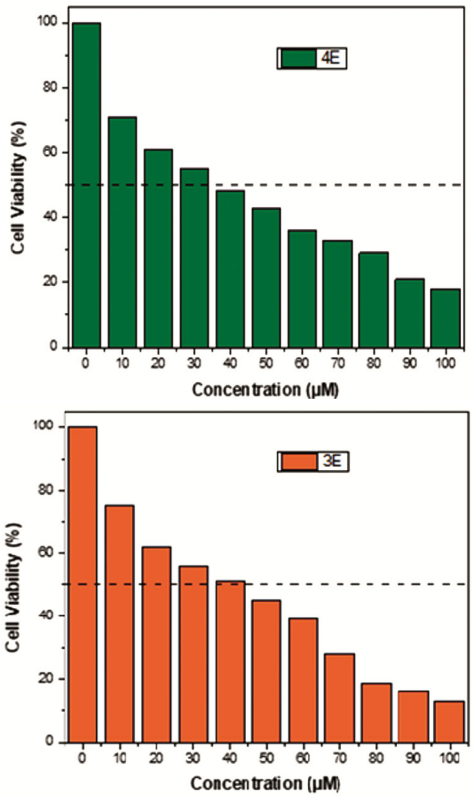



Fig. 7 — Effect of varying concentrations of **4e** and **3e** on cytotoxicity of MCF-7 (Breast cancer) cell lines as determined by MTT assay

Table 5 — Effect of varying concentrations of **3e** and **4e** on cytotoxicity of MCF-7 (Breast cancer) cell lines as determined by MTT assay

| Blank | MCF-7 cells | | | |
|---|---|---|---|---|
| | **3e** | | **4e** | |
| | Mean | Viability | Mean | Viability |
| 0 | 0 | 100 | 0 | 100 |
| 10 | 25 | 75 | 29 | 71 |
| 20 | 38 | 62 | 39 | 61 |
| 30 | 44 | 56 | 45 | 55 |
| 40 | 49 | 51 | 52 | 48 |
| 50 | 55 | 45 | 57 | 43 |
| 60 | 61 | 39 | 64 | 36 |
| 70 | 72 | 28 | 67 | 33 |
| 80 | 81 | 19 | 71 | 29 |
| 90 | 84 | 16 | 79 | 21 |
| 100 | 87 | 13 | 82 | 18 |
| IC50 | 43.25± 0.05 µM | | 38.75± 0.05 µM | |

***Cytotoxic study***

Each one sample was taken from **3a-g** and **4a-g** respectively to study cytotoxic potential of the compound as a representative study for the other compounds. The effects of varying concentrations and comparison of the compound **3e** and **4e** on cytotoxicity of MCF-7 cell lines by MTT Assay method are given in Table 5 and (Fig. 7 and Fig. 8)

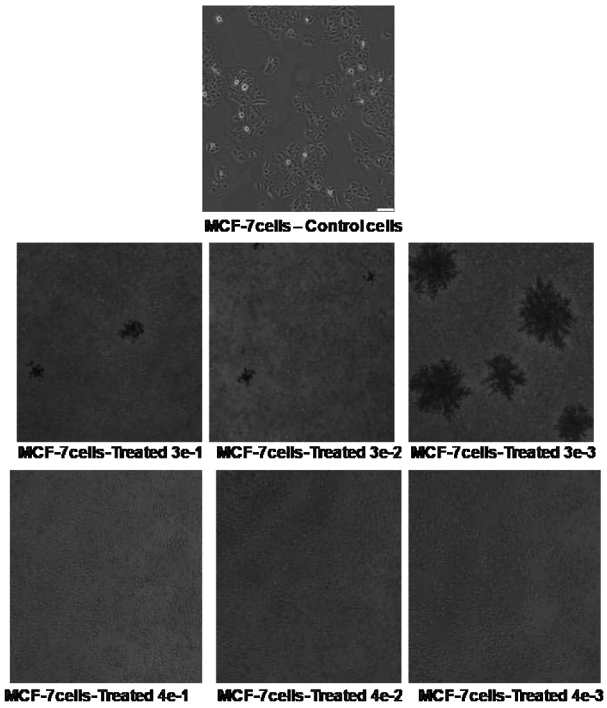



Fig. 8 — Effect of **3e** and **4e** on cytotoxicity of MCF-7 (Breast cancer) cell lines as determined by MTT assay

respectively[26,27]. It is observed from the results of cytotoxic effects that the potency of **3e** in inhibiting a biological process is greater than **4e**, which is evident from the greater IC50 value of **3e** compared to **4e**. The compound **3e** without any further substituent group is sufficient in inhibiting anti-cancer activity. The reason behind the difference in IC50 values of **3e** and **4e** requires further investigations and the derivatives **3a-g** as well.

## Conclusion

With the scope of developing potent antioxidant, anti-inflammatory, anti-microbial and cytotoxic studies, a series of N-substituted novel pyrazoline derivatives were synthesised. All the synthesised compounds were characterised by mass, $^{1}$H NMR and $^{13}$C spectra. All the derivatives 4(a-g) were screened for their anti-microbial, anti-inflammatory, antioxidant and cytotoxic studies. Compound 4a showed higher anti-inflammatory activity than the standard. Compounds 4a, 4b, 4c, 4f and 4g exhibits good antioxidant activity in all the methods and compound 4a exhibited significant actvity in all the methods when compared to the standard. In terms of antimicrobial study antifungal activity of all the compounds 4a, 4b, 4c, 4d, 4e, 4f and 4g were fantastic against *Aspergillus fumigatus*, moderate and equal against *Mucor sp*, compared to the

standard. The antibacterial activity of all the compounds was very low and the compounds showed no activity against *Klebsiella pneumoniae.*

In cytotoxic study the potency of **3e** is greater than **4e** which is evident from the greater IC50 value of **3e** compared to **4e**. The compound **3e** without any further substituent group is sufficient in inhibiting anti-cancer activity. Further studies are necessary in order to understand the relation between the derivatives and the activity in cytotoxic studies, which could lead to the design of more potent anti-cancer agents for the therapeutic use and other applications.

## Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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