Greener method for synthesis of indeno-N-carbothionamide pyrazole derivatives and their 
In-vitro Anti-tubercular activity.

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Abstract: In the present study a series of novel indeno-N-carbothionamide pyrazole derivatives were synthesized by the reaction of α, β-unsaturated ketones with thiosemicarbazide in polyethylene glycol-400 (PEG-400) and few drop of acetic acid. The newly synthesized compounds were characterized and confirmed by IR, ¹H-NMR, ¹³C-NMR and mass spectral data. The results obtained indicate the significant yield of indeno-N-carbothionamide pyrazole derivatives and their potent pharmacological scaffold for designing novel and broad spectrum antimycobacterials.

Keywords: indeno-N-carbothionamide pyrazole derivatives, bleaching earth clay (pH12.5), Polyethylene glycol-400(PEG-400), Antitubercular activity.

Introduction: Tuberculosis is one of the oldest diseases known to affect humans which are caused by bacteria belonging to the Mycobacterium tuberculosis species. It is observed that today one-third to one-half of the world population is infected with Mycobacterium tuberculosis leading to approximately 6% of all death globally. Tuberculosis is the leading worldwide cause of mortality resulting from an infectious bacterial agent. Mycobacterium tuberculosis is transmitted primarily through the respiratory system. Tuberculosis is a disease, which mainly affects the lungs (75-80% of the case); although in up to one-third of cases other organs are involved. It is the most prominent cause of death worldwide due to single infectious agent, and in 1993 WHO declared Tuberculosis as a global public health issue till awareness required today¹⁴.

Many heterocyclic compounds containing pyrazole-carbothionamide moiety are pharmacologically significant and possessing a huge range of target-oriented bioactivities⁵,⁶. The pyrazole derivatives make up the main skeleton of various biologically active compounds. Molecules of many modern drugs, such as anti-tubercular⁷, anticancer⁸, antiphlogistic⁹, antidiabetic¹⁰, analgesic¹¹, anti-inflammatory¹² etc., contain the pyrazole ring as structural moiety (figure 1).¹³-¹⁴ Recently carbothionamide pyrazoles and its derivatives have been proven to be an extremely useful intermediate for the synthesis of new biologically active compounds.¹⁵-¹⁶ Carbothionamide pyrazole derivatives have attracted great attention due to widespread applications in pharmaceutical¹⁷ and agrochemical industries.¹⁸-¹⁹
Polyethylene glycol (PEG-400) prompted synthesis have attracted the attention of organic chemists\textsuperscript{12-14} due to their solvating ability and aptitude to act as a phase transfer catalyst, simple work-up, eco-friendly nature and their affordable cost. PEG is non-toxic, inexpensive potentially recyclable and water soluble which facilitate it’s easy to remove from reaction product\textsuperscript{20-22}

This research work has been focused to prepare such new technique for the synthesis of indeno-N-carbothionamide pyrazole derivatives via the synthesis of α, β-unsaturated ketones by using polyethylene glycol (PEG-400) as a reaction solvent and bleaching earth clay(pH-12.5) as catalyst.

**Materials and Methods:**

**Experimental:**

All the Melting points of the synthesized compounds were recorded in open capillary tube and are uncorrected. IR spectra were recorded in KBr pallets on FTIR schimadzu spectrophotometer and 1H NMR spectra in DMSO solvent were scanned on AVANCE 300 MHz spectrometer using TMS as an internal standard. The MS were recorded on EI-Shimadzu-GC-MS spectrometer. Elemental analysis was carried out on a Carlo Ebra 106 Perkin-Elimer model 240 analyzer.

**Procedure for the Synthesis of indeno-N-carbothionamide pyrazole derivatives (IIBa-e):**

A mixture of α, β-unsaturated ketones, (1mmol), thiosemicarbazide (1.5mmol), PEG-400 (20mL) and 4-6 drops of acetic acid was heated for 1 to 2 hours at 60-80°C temperature. After completion of reaction (monitored by TLC), the reaction mixture was cooled and poured into ice-cold water (100 mL). The obtained solid product was filtered and washed with 2x5mL water and recrystallized by ethanol to give pure product 1-(3-(4- methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-2,8-dihydrocyclopenta-[aj]indene-2carbothioamide (IIBa-e) were obtained. The PEG-400 was recovered from water by direct distillation and reused for second run by charging the same substrates. Similarly, remaining compounds of this series were also prepared by same procedure. The physical data of synthesized compounds were tabulated as in Table-1.
3-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)indenol1,2-c]pyrazole-2(4H)-carbothioamide IIBa: Pale yellow solid; mp. 160-165 oC; IR (KBr, ν, cm-1): 3442 (-NH2), 3044, 2918 (Ar-H), 1572 (C=N of pyrazole ring), 1500-1600 (Aromatic C=C), 1245 (C-N), 752 (C-Cl); 1H NMR (400 MHz, DMSO-d6) (δ, ppm): 4.2 (s, 2H, CH2), 6.8-8.8 (m, 13H, Ar-H), 8.7 (s, 1H, pyrazole), 8.8 (s, 2H, -NH2); 13C-NMR (70 MHz, DMSO-d6) (δ, ppm): 32 (-CH2), 120-140 (CH, Aromatic rings). 145-152 (C, pyrazole rings) EIMS (m/z): 467 (M+); Anal. Calcd. For C26H18ClN5S: C, 62.14; H, 3.97; Cl, 8.73; N, 17.25; S, 7.90% Found; C, 66.71; H, 3.85; Cl, 7.55; N, 14.99; S, 6.83%

3-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)indenol1,2-c]pyrazole-2(4H)-carbothioamide IIBb:
Pale yellow solid; mp. 155-158 oC; IR (KBr, ν, cm-1): 3440 (-NH2), 3040 (Ar-H), 2918 (C-H aliphatic), 1577 (C=N of pyrazole ring), 1480-1600 (Aromatic C=C), 1242 (C-N); 1H NMR (400 MHz, DMSO-d6) (δ, ppm): 2.3 (s, 3H, CH3), 4.0 (s, 2H, CH2), 6.7-8.8 (m, 13H, Ar-H), 8.6 (s, 1H, pyrazole), 8.9 (s, 2H, -NH2); 13C-NMR (70 MHz, DMSO-d6) (δ, ppm): 20.6 (-CH3), 38 (-CH2), 115-140 (CH, Aromatic rings), 145-150 (C, pyrazole rings) EIMS (m/z): 465 (M+); Anal. Calcd. For C27H21N5O2S: C, 72.46; H, 4.73; N, 15.65; S, 7.16% Found; C, 72.48; H, 4.70; N, 15.67; S, 7.13%

3-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)indenol1,2-c]pyrazole-2(4H)-carbothioamide IIBC:
Yellow solid; mp. 141-144 oC; IR (KBr, ν, cm-1): 3446 (-NH2), 2994 (Ar-H), 2933 (C-H aliphatic) 1603 (C=N of pyrazole ring), 1570-1602 (Aromatic C=C), 1260 (C-N), 1210 (C-O); 1H NMR (400 MHz, DMSO-d6) (δ, ppm): 3.8 (s, 3H, OCH3), 4.1 (s, 2H, CH2), 6.6-8.8 (m, 13H, Ar-H), 9.5 (s, 1H, pyrazole), 8.9 (s, 2H, -NH2); 13C-NMR (70 MHz, DMSO-d6) (δ, ppm): 53.7 (-OCH3), 30 (-CH2), 116-145 (CH, Aromatic rings), 135-155 (C, pyrazole rings) EIMS (m/z): 447 (M+); Anal. Calcd. For C27H21N5O2S: C, 66.73; H, 3.88; Cl, 7.58; N, 14.97; S, 6.85% Found; C, 69.44; H, 4.60; N, 15.10; O, 3.48; S, 6.90%

3-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)indenol1,2-c]pyrazole-2(4H)-carbothioamide IIBd:
Yellow solid; mp. 167-170 oC; IR (KBr, ν, cm-1): 3429 (-NH2), 3048 (Ar-H), 1596 (C=N of pyrazole ring), 1590-1600 (Aromatic C=C), 1530 (-N-O), 1240 (C-N); 1H NMR (400 MHz, DMSO-d6) (δ, ppm): 4.1 (s, 2H, CH2), 6.8-9.0 (m, 13H, Ar-H), 9.1 (s, 1H, pyrazole), 9.2 (s, 2H, -NH2); 13C-NMR (70 MHz, DMSO-d6) (δ, ppm): 28.4 (-CH2), 114-144 (CH, Aromatic rings), 140-154 (C, pyrazole rings) EIMS (m/z): 481 (M+); Anal. Calcd. For C26H18ClN5O2S: C, 65.26; H, 3.79; N, 17.56; O, 6.69; S, 6.70% Found; C, 65.28; H, 3.80; N, 17.54; O, 6.71; S, 6.72%

3-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)indenol1,2-c]pyrazole-2(4H)-carbothioamide IIBe:
Yellow solid; mp. 158-160 oC; IR (KBr, ν, cm-1): 3446 (-NH2), 3050 (Ar-H), 1595 (C=N of pyrazole ring), 1510-1604 (Aromatic C=C), 1235 (C-N); 1H NMR (400 MHz, DMSO-d6) (δ, ppm): 4.2 (s, 2H, CH2), 6.8-8.4 (m, 9H, Ar-H), 8.6 (s, 2H, -NH2), 3.2 (s, 3H, CH3); 13C-NMR (70 MHz, DMSO-d6) (δ, ppm): 13 (-CH3), 33 (-CH2), 115-142 (CH, Aromatic rings), 133-140 (C, pyrazole rings) EIMS (m/z): 406 (M+); Anal. Calcd. For C21H16ClN5S: C, 62.14; H, 3.97; Cl, 8.73; N, 17.25; S, 7.90% Found; C, 62.16; H, 3.96; Cl, 8.76; N, 17.24; S, 7.93%

Results and Discussion:

Chemistry

we have reported greener synthesis of some novel indeno-N-carbothioamide pyrazole derivatives. A new series of such a carbothioamide pyrazole derivatives were synthesized from cyclic α, β- unsaturated ketones. These cyclic α, β-unsaturated ketones were synthesized by Claisen-Schmidt Condensation of indan-1-one with different het/araldehydes in the presence of a catalytic amount of bleaching earth (10 mol% of pH 12.5) and PEG-400 as green reaction solvent. The reaction time, yield and melting point of α, β-Unsturated ketones (IIBa-e) are observed.
Scheme-1: Synthesis of indeno-N-carbothionamide pyrazole derivatives (IIBa-e):

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Het/Ar</th>
<th>Mol. Formula</th>
<th>Yield %</th>
<th>M. P. (°C)</th>
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<tbody>
<tr>
<td>1</td>
<td>IIBa</td>
<td>Cl/C</td>
<td>C_{26}H_{18}ClN_{3}S</td>
<td>90</td>
<td>160-165</td>
</tr>
<tr>
<td>2</td>
<td>IIBb</td>
<td>H_{3}C/N</td>
<td>C_{27}H_{21}N_{5}S</td>
<td>93</td>
<td>155-158</td>
</tr>
<tr>
<td>3</td>
<td>IIBc</td>
<td>H_{3}CO/C</td>
<td>C_{27}H_{21}N_{5}S</td>
<td>95</td>
<td>141-144</td>
</tr>
<tr>
<td>4</td>
<td>IIBd</td>
<td>O_{2}N/C</td>
<td>C_{26}H_{18}N_{6}O_{2}S</td>
<td>98</td>
<td>167-170</td>
</tr>
<tr>
<td>5</td>
<td>IIBe</td>
<td>OHC/C</td>
<td>C_{21}H_{16}ClN_{3}S</td>
<td>83</td>
<td>158-160</td>
</tr>
</tbody>
</table>

Table-1: Physico-chemical data of newly synthesized N-carbothionamide pyrazole derivatives (IIBa-e):
Focusing on our aim to find the greener way to synthesis of pharmacologically active compounds as indeno-N-carbothionamide pyrazole derivatives by using few drops of acetic acid and PEG-400 as green solvent. Experimentally the condensations occur smoothly followed by the Michael addition of thiosemicarbazone to giving corresponding indeno-N-carbothionamide pyrazole derivatives (IIBa-e) as the products shown in Scheme-1. After completion of reaction, highly pure products having good yield can be obtained simply by recrystallization from aqueous acetic acid. The reaction time, yield and melting point of indeno-N-carbothionamide pyrazole derivatives (IIBa-e) are presented in Table-1.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Compounds</th>
<th>Antimycobacterial activity measured in MIC (µg/mg) value against</th>
</tr>
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<tr>
<td></td>
<td>IIBa</td>
<td>M.tuberculosis 11.5</td>
</tr>
<tr>
<td></td>
<td>IIBb</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>IIBc</td>
<td>10.5</td>
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<tr>
<td></td>
<td>IIBd</td>
<td>9</td>
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<tr>
<td></td>
<td>IIBe</td>
<td>8.5</td>
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<tr>
<td></td>
<td>Rifampicin</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Iosniazid</td>
<td>1.83</td>
</tr>
</tbody>
</table>

MIC- Minimum inhibitory concentration, Mycobacterial pathogens used as: Mycobacterium tuberculosis (MTB), Mycobacterium Phlei (Mp) and Mycobacterium Smegmatis (Ms)

Table-2: Showing in-vitro antitubercular activity of new N-carbothionamide pyrazolederivatives. (IIBa-e):

Antitubercular study:

The disc diffusion method is used for screening of in-vitro antitubercular activity of newly synthesized N-carbothionamide pyrazole derivatives and their results are shown in Table-2. The newly synthesized N-carbothionamide Pyrazole derivatives were evaluated for antimycobacterial activity against various mycobacterial strain like Mycobacterium tuberculosis (MTB), Mycobacterium Phlei (Mp) and Mycobacterium Smegmatis (Ms) by measuring their minimum inhibitory concentration (MIC). The compounds such as IIBc, IIBe, IIBf and IIBi were showed promising antitubercular activity against all mycobacteria as compared to standard drug such as Rifampicin and Isoniazid. The compound like IIBc, IIBd and IIBe were more active against Mycobacterium tuberculosis (MTB) and the compounds such as IIBa, IIBc and IIBd were strongly active against Mycobacterium Pheli (Mp) and Mycobacterium Smegmatis (Ms). The compounds like IIBa and IId were a somewhat less active than one or more
mycobacterial pathogen

*In-vitro* antitubercular activity results that The N-carbothionamide pyrazole compounds as IIBc, and IIBe were showed promising antitubercular activity against all mycobacteria as compared to standard drug such as Rifampicin and Isoniazid. It can be concluded from above explanation that the antimycobacterial activity increases due to compounds containing –NO₂ and –CSNH₂ functional groups, also by substituted pyrazole ring.

**Conclusion:**
In summary, we have developed an efficient and eco-friendly methodology for the synthesis of indeno-N-carbothionamide pyrazole derivatives using bleaching earth clay (pH 12.5) as catalyst and polyethylene glycol-400 (PEG-400) as alternative green reaction solvent. The synthesized compounds were confirmed by the spectral analysis and further evaluated for their antitubercular activity. The antitubercular activity revealed that most of the compounds showed moderate to good activity. Due to the presence of functional groups like –NO₂ and –CSNH₂, as well as pyrazole ring in the synthesized compounds posses significant antitubercular activity.

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**References:**


