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Design, structural characterization, biological evaluation and molecular docking studies of methylindole bearing thiocarbamoylpyrazole moieties

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Numerous 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole derivatives have been synthesized and their structures were confirmed by elemental analysis, ^{1}HNMR, ^{13}CNMR, IR and mass spectra. The synthesized indoylpyrazole compounds were evaluated for their biological activity. The obtained results revealed clearly that compounds IVb and g displayed the highest antioxidant activity and compounds g and i which exhibited good anti-tubercular activity; whereas, the same compound IVb exhibited excellent activity against HeLa (human cervical carcinoma) cancer cell lines. Theoretical calculation of the title compounds were carried out using density functional theory method. The geometrical optimization of the prepared target compounds was theoretically analyzed. Based on the geometries the HOMO and LUMO, mulliken population analysis and reactivity indices were calculated.

Keywords: Butylated hydroxyanisole (BHA), DFT, Heterometrus laoticus cytochrome, Indole, Pyrazole

The modern lifestyle related changes of stress, wrong dietary practices smoking/alcohol consumption/drug abuse may cause overage of reactive oxygen species and free radicals formation in our body. Prevention of disease is more desirable than its successful treatment. A number of natural antioxidants reduce oxidative stress but also provide considerable protection against several cancer[1], heart diseases, and degenerative nerve diseases. The antioxidants play an important role in medical attendance. The implementation and development of synthetic or natural antioxidants are of great significance in medicinal field[2,3]. The natural antioxidants such as carotenoids, polyphenols, lipoic and ascorbic acid are derived from dietary sources and the synthetic antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) are important inhibitors of carcinogenesis, probably by way of their antioxidant function. These antioxidants indirectly bind with redox metals to prevent free radical generation[4,5]. Molecular docking is a powerful approach in molecular structure based computer assisted drug design[6]. The docking aims to achieve the possible interactions between protein and ligand molecules[7]. We get a ligand-protein complex with optimized conformation, possessing stable binding energy[8,9]. The predicted binding energy is showed in terms of hydrogen bonding and hydrophobic interaction between pyrazoles and DNA. Cancer is among the most important life-threatening diseases in the worldwide, where Tuberculosis (TB), is an opportunistic infection caused by mycobacterium species. Lethal combination of these two diseases has led to intense pressure on current chemotherapy regimes. The observed energetically favourable docked images of the3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindoleIV (a-i) with peptidyl–tRNA-hydrolase of Mycobacterium tuberculosis (PDB ID:2JRC) and cancer protein of Heterometrus laoticus cytochrome (PDBID: 2NDD)[10]. Quantum chemical computational have been proven to be an essential tool to solve interesting chemical problems such as optimize geometry, dipole moment, charge distribution and localization of the electrophilic and nucleophilic reactivity[11,12]. These computations were made by combining semi-empirical quantum mechanical method; ab initio quantum mechanical method[13] and density functional theory (DFT)[14]. DFT is a quantum mechanically computational modeling method[15] which explains properties of the molecule based on determination of the electron density of the molecule[16]. Nitrogen containing heterocyclic compounds was condensed to pyrazole ring[17] as an essential source of biologically active molecules[18].

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The indole ring and their derivatives are important applications in pharmaceuticals[19,20], fragrances, agrochemicals, pigments, material science, organic electronics and natural products. The substituent's for the second position chosen were pyrazole ring which were the privileged structures in modern medicinal chemistry[21]. Pyrazole derivatives are useful in pharmaceuticals, since many antioxidant[22] and anticancer drugs are indole pyrazole derivatives[23,24]. Due to the immense biological activities of indole associated with pyrazole compounds, we have planned to synthesize some heterocyclic compounds of indole combine with pyrazole moieties in a single molecular framework[25] to obtain a new class of highly potent bioactive compounds[26].

Materials and Methods

Materials

All the solvents and reagents used were of AR grade which were purchased from Sigma – Aldrich and Merck Specialties Pvt. Ltd. NMR spectra was recorded on Bruker Avance III, 400MHz NMR spectrometer and mass spectra on Waters UPLC - TQD mass spectrometer (ESI – MS). Nicolet 400D FTIR spectrometer was used for FTIR spectra. Melting points were determined using Digital Program Rate melting point apparatus and were uncorrected. Elemental analysis was carried out at Central Drug Research Institute, Lucknow, India.

General procedure for the synthesis of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole (IVa–i)

3-acetylindole (1.6 g, 0.01 mol) and dimethyl sulphate (1.7 g, 0.013 mol) along with base (15 mL, 2N) afforded 3-acetyl-1-methyl indole I. The reaction of 3-acetyl-1-methyl indole with an aromatic aldehyde in the presence of NaOH afforded 3-aryl-1- (1-methylindole-3-yl)-2-propen-1-one III. To a mixture of 3- (5-aryl-1-methylindole-3-yl)-2-propen-1-ones III (0.01 mol) in ethanol (30 mL) was refluxed with the appropriate quantity of thiosemicarbazide (12 mmol) in glacial acetic acid (2 mL) for 6 h, then the reaction mixture was poured into crushed ice and was kept overnight at room temperature. The precipitated product was filtered and recrystallized from ethanol.

Evaluation of antioxidant activity

For the evaluation of antioxidant capacity of the 3-(5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole compounds was measured using 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) assay. The samples were made up with methanol to different concentrations (50, 100, 250, 500 and 750 μM). 2 mL of each sample was allowed to react with 2 mL of (DPPH) stable free radical, for 30 min in dark at room temperature. The change of colour of the DPPH solution from purple to yellow is due to the presence of antioxidants. Absorbance at 517 nm was determined at various time intervals of 30 min using UV spectroscopy.

The concentration of the DPPH in the reacting solution was determined by the calibration curve plotted with absorbance of DPPH at 517 nm at various concentrations and inhibition percentage.

From the absorbance values the percentage inhibition was calculated. Butylated hydroxyanisole (BHA) was used as a standard.

$$\%\,\text{inhibition} = \frac{\text{Control absorbance - sample absorbance}}{\text{Control absorbance}} \times 100$$

Evaluation of anticancer activity

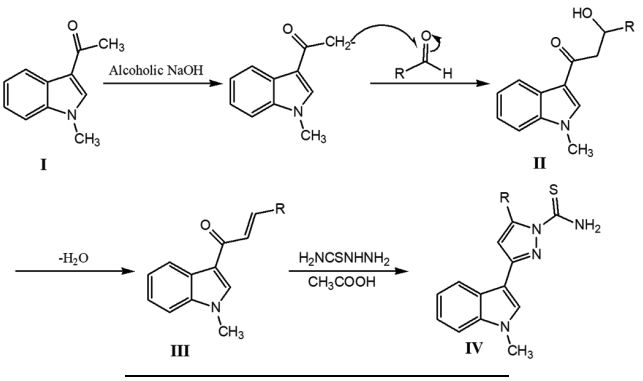
The newly synthesized compounds were also tested for the *in vitro* anticancer activity via the 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method against human cervical cancer cell lines (HeLa). Fifteen mg of MTT (Himedia, M-5655) was reconstituted in 3 mL PBS until completely dissolved and sterilized by filter sterilization. After 24 h of incubation period, the sample content in wells were removed and 3 0 μL of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO_2 incubator for 4 h. After the incubation period, the supernatant was removed and 100 μL of MTT solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm.

The amount of growth inhibition was measured using the formula:

$$\%\,\text{of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

Results and Discussion

The synthetic way of the thiocarbamoyl pyrazol methyl indole synthesized by the following reactions



IV	R
A	Phenyl
B	2-chlorophenyl
C	2-hydroxyphenyl
D	2-methoxyphenyl
E	2-methylphenyl
F	2-phenylethenyl
G	4- chlorophenyl
H	4-methoxyphenyl
I	4-methylphenyl

Scheme 1

conditions and modern catalytic method pathways proposed in (Scheme 1). As seen in Scheme 1, the reaction of starting material, 3-acetyl indole with dimethyl sulphate in the presence of various bases is a classical method to form 3-acetyl-1-methyl indole[27]I. Next, 3 acetyl-1-methylindole reacting with commercially available aromatic aldehydes in the presence of sodium hydroxide which was carried out in absolute ethanol at 70°C afforded 3-aryl-1- (1-methylindole-3-yl)-2-propen-1-one (III) have been reported[28]. In this research, the 3-(5-aryl-1-methylindole-3-yl)-2-propen-1-ones (III) was treated with the appropriate quantity of thiosemicarbazide in the presence of glacial acetic acid under reflux condition for 5 h. Yielded a pale yellow precipitate which on recrystallization yield a pale yellow crystalline solid (IV), the yield of pyrazole was 50% - 71%. This reaction pathway show that proton shift from acetyl group to carbonyl carbon of the aldehyde followed by the loss of a water molecule, to give α, β unsaturated carbonyl compound. The nucleophilic attack of semithiocarbazide on α, β-unsaturated carbonyl compound at the β carbon leads to the pyrazole ring. The synthesized compounds were purified by column chromatography (silica gel 60 – 150 mesh) using chloroform as the solvent to get pure colourless crystals of compounds.

The elemental analysis showed that the molecular composition of the pale yellow crystalline solid is $C_{19}H_{16}N_4S$. The IR (KBr) spectrum of the newly synthesized compound shows peak at 3042 cm^{-1} is attributed to aromatic C-H vibration The broad band at 1612 cm^{-1} results in C=N vibration. The peak at 1, 242 cm^{-1} is attributed to thiocarbonyl band whereas NH_2 band appeared at 3, 401 cm^{-1}. In the ^1H NMR (400 MHz, DMSO d_6) spectrum; the three-hydrogen singlet at δ 2.928 has been attributed to the N-CH_3 of the indole ring. The two doublets appear one at δ 7.715 and another at δ 8.164 was ascribed to the H-4 and H-7 of the indole ring. The multiplet at δ 7.979-8.024 has been assigned to the H-5 and H-6 of the indole ring. The two singlets, one at δ 7.813 and another at δ 7.338 are assigned to the H-2 of the indole ring and CH of the pyrazole ring respectively. The two triplets at δ 7.385 and δ 8.854 are due to the three phenylhydrogen's. The two proton singlet appeared at δ7.033 for NH_2 of the pyrazole ring. The two hydrogen doublet arises at δ7.748 due to the presence of two phenyl hydrogen's. The ^{13}C NMR spectrum shows nineteen peaks, accounting for all the nineteen carbons.ESI mass spectrum showed MH+ peak at 333. This confirms the molecular mass of the compound to be 332 which in accordance with the elemental analysis data.

Computational studies

The optimization of molecular geometry and vibrational assignments of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV were performed by DFT method using the Gaussian 09 package at the Becke three parameter hybrid exchanges functional and the Lee Yang-Parr correlation functional using the 6-31G basis set. By minimizing the energy with respect to all geometrical parameters, the optimum geometry of the compounds was resolved. In order to correct the overrates, the calculated wave numbers were scaled using the scaling factor 0.962, neglecting electron interactions and anharmonicity characters of the vibration modes.

The optimized molecular geometry of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV and its parameters bond length, bond angle, molecular orbital and charge distribution on atoms were obtained from B3LYP/631G level of DFT calculations. The geometrical parameters of energy minimized structures with numbering of the atoms are depicted in (Fig. 1) and the optimized structural parameters of the molecule are listed in (Table 1).

The 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole contain indole ring, pyrazole ring and a phenyl ring. From the Table 1, the calculated optimized parameter values of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV are in close agreement with the experimental values. In all the compounds the C–S bond length is the greatest due to the larger size of sulphur atom. The bond angles of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole ranges from 105.7 to 134.8 and the planarity of the molecule is confirmed from the dihedral angles.

In 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV compounds, except 3-[5- (2-hydoxyphenyl)-1-thiocarbamoylpyrazol-3-yl]-1-methylindole IVc, the HOMO orbitals are located mainly on thiocarbamoyl group, while the LUMO orbitals are placed on pyrazole and phenyl ring. The energies of HOMO and LUMO are directly related to electro negativity, ionization potential hardness and softness and electron affinity respectively. The difference of energy between HOMO and LUMO is called energy gap and is an important stability for structure and is related with electron transfer properties. HOMO-LUMO gap is related with the bioactivity of the molecule as it explains its intramolecular charge transfer. Diagrammatic representations of HOMO-LUMO of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole are given in (Fig. 2) and the electronic parameters calculated from the energies of molecular orbitals are listed in (Table 2).

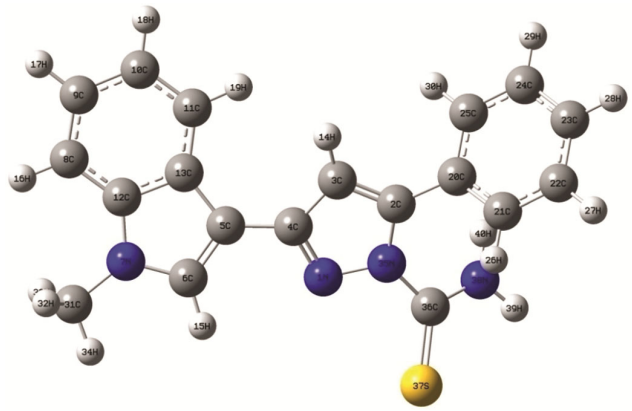


Fig. 1 — Optimized structure of 1-methyl-3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)indoleIV (a)

The Mulliken atomic charge calculation is an important aspect of a molecular system since atomic charges are related to molecular polarizability, dipole moment, molecular geometry and more a lot of properties of molecular systems. The Mulliken atomic charge 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole is shown in (Fig. 3). The electronic charge on the chelating atoms determines the bonding capability of a molecule. The atomic charges for optimized geometry 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole are calculated using B3LYP method with 6-31G set and are presented in (Table 3).

Vibrational studies of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole

The theoretical vibrational frequencies of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole are predicted by quantum mechanical calculations and compared with the experimental data. The experimental and calculated vibrational spectra of 1-methyl-3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)indole IV (a) are

Table 1 — Optimized geometrical parameters of 1-methyl-3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)indoleIV (a)

Atom	Bond length (Å)	Atom	Bond angle (°)	Atom	Dihedral angle (°)
N_1-C_4	1.3465	N_1-C_4-C_3	110.68	N_{35}-C_2-C_{20}-C_{25}	138.67
C_4-C_3	1.4305	C_4-C_3-H_{14}	127.39	C_{20}-C_{25}-C_{24}-H_{29}	179.96
C_3-H_{14}	1.0771	H_{14}-C_3-C_2	125.41	C_{23}-C_{22}-C_{21}-H_{26}	-178.64
C_3-C_2	1.3812	C_3-C_2-N_{35}	105.74	C_{21}-C_{22}-C_{23}-H_{28}	179.82
C_2-N_{35}	1.4045	C_2-N_{35}-N_1	111.25	S_{37}-C_{36}-N_{38}-H_{40}	175.02
C_4-C_5	1.4500	N_1-C_4-C_5	120.19	C_6-C_5-C_4-C_3	-162.83
C_5-C_6	1.3834	C_4-C_5-C_6	124.22	C_{31}-N_7-C_6-C_5	-179.49
C_6-H_{15}	1.0785	C_5-C_6-H_{15}	128.5	C_{13}-C_5-C_4-N_1	-164.17
C_6-N_7	1.3846	H_{15}-C_6-N_7	122.6	H_{17}-C_9-C_8-C_{12}	-179.97
C_7-C_{31}	1.4565	C_6-N_7-C_{31}	125.9	C_8-C_9-C_{10}-H_{18}	179.36
N_{35}-C_{36}	1.4148	C_{31}-H_{22}-H_{33}	35.5	N_7-C_{12}-C_{13}-C_{11}	178.73
C_{36}-S_{37}	1.7042	C_{31}-H_{32}-H_{34}	35.5	H_{18}-C_{10}-C_{11}-C_{13}	-179.99
C_{36}-N_{38}	1.3543	C_{31}-N_7-C_{12}	125.5	H_{29}-C_{24}-C_{23}-C_{22}	179.96
N_7-C_{12}	1.3945	N_7-C_{12}-C_8	125.4	C_{25}-C_{20}-C_{21}-H_{26}	178.58
C_{12}-C_8	1.0852	C_{12}-C_8-H_{16}	120.5	C_4-N_1-N_{35}-C_{36}	-176.30
C_8-H_{16}	1.4117	C_{12}-C_8-C_9	113.8	H_{34}-C_{31}-N_7-C_{12}	179.83

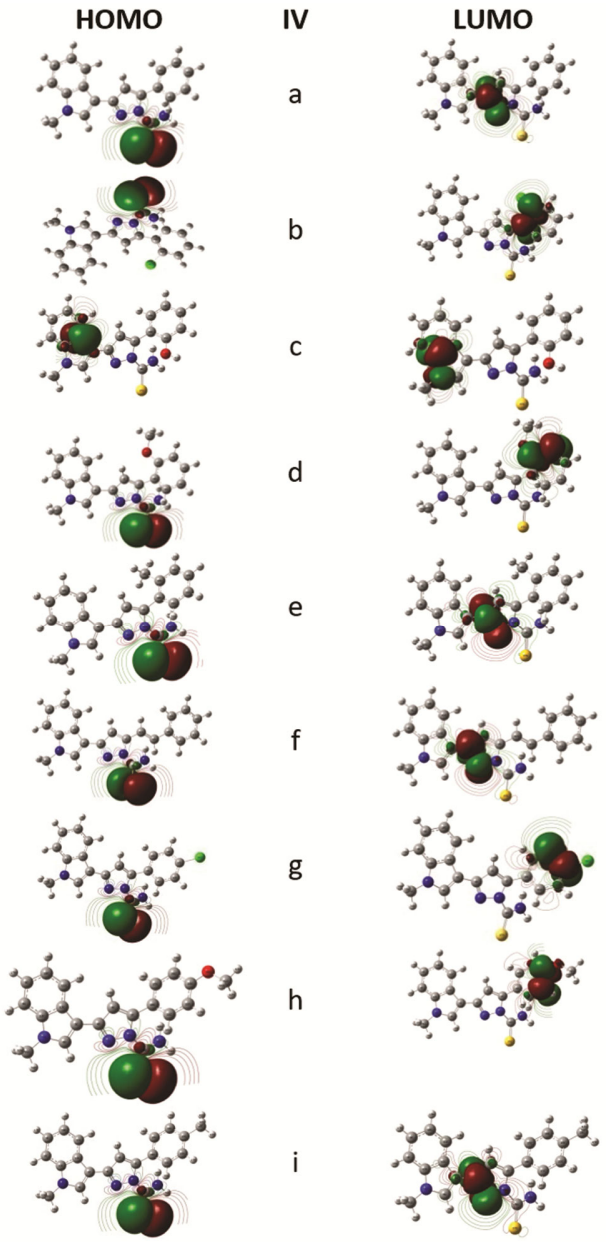


Fig. 2 — HOMO and LUMO of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV (a-i)

given in (Figs 4 & 5) and the vibrational assignments of 3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole compound were shown in (Table 4).

The thiocarbonyl group of N-H stretching vibrations are observed experimentally at 3401 cm^{-1} and theoretically observed at 3453 cm^{-1}. The bands observed between 3073-3184 cm^{-1} is assigned to aromatic C-H stretching vibrations which is experimentally observed at 3156 cm^{-1} and 3042 cm^{-1}. The vibrational analysis shows C=N stretching vibrations at 1566 cm^{-1} and these are in close agreement with the experimental values 1576 cm^{-1}.

Molecular docking studies

Molecular docking is a powerful approach in molecular structure based computer assisted drug design. The docking aims to achieve the possible interactions between protein and ligand molecules. We get a ligand-protein complex with optimized conformation, possessing stable binding energy[29]. The predicted binding energy is showed in terms of hydrogen bonding and hydrophobic interaction between pyrazoles and DNA. The target protein in required format was taken from Protein Data Bank (PDB). The ligands were loaded in the PDB format using Open babel GUI (C) 2006. The geometry optimization of the compounds was done by Gaussian '09 software with density functional theory at the B3LYP/631G level of theory[30,31]. Proper organization of the ligand in the particular groove of the target DNA is furnished by PyRx-Python prescription 0.80[32]. Cancer is among the most important life-threatening diseases in the worldwide, where Tuberculosis (TB), is an opportunistic infection caused by mycobacterium species. Lethal combination of these two diseases has led to intense pressure on current chemotherapy regimes. The observed energetically favourable docked images of the 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV with peptidyl –tRNA hydrolase of Mycobacterium

Table 2 — Energies of HOMO-LUMO of 1-methyl-3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)indoleIV (a-i)

Paramerers (a.u)	IV a	IVb	IV c	IV d	IV e	IV f	IV g	IV h	IV i
E_{HOMO}	-0.1901	-0.1944	-0.1893	-0.1869	-0.1872	-0.1926	-0.1966	-0.1887	-0.1886
E_{LUMO}	0.0063	-0.0140	-0.0129	0.0058	0.0071	0.0053	-0.0110	0.0063	0.0080
ΔE	-0.1964	0.1804	0.1763	-0.1927	-0.1944	-0.1979	0.1856	-0.1950	-0.1966
Ionization Potential (I)	0.1901	0.1944	0.1893	0.1869	0.1872	0.1926	0.1966	0.1887	0.1886
Electron affinity (A)	-0.0063	0.0140	0.0129	-0.0058	-0.0071	-0.0053	0.0110	-0.0063	-0.0080
Electronegativity (χ)	0.0919	0.1042	0.1011	0.0905	0.0900	0.0936	0.1038	0.0912	0.0902
Hardness (η)	0.0982	0.0902	0.0881	0.0963	0.0972	0.0989	0.0928	0.0975	0.0983
Softness (S ()	5.0911	5.5410	5.6711	5.1886	5.1440	5.0510	5.3876	5.1261	5.0849

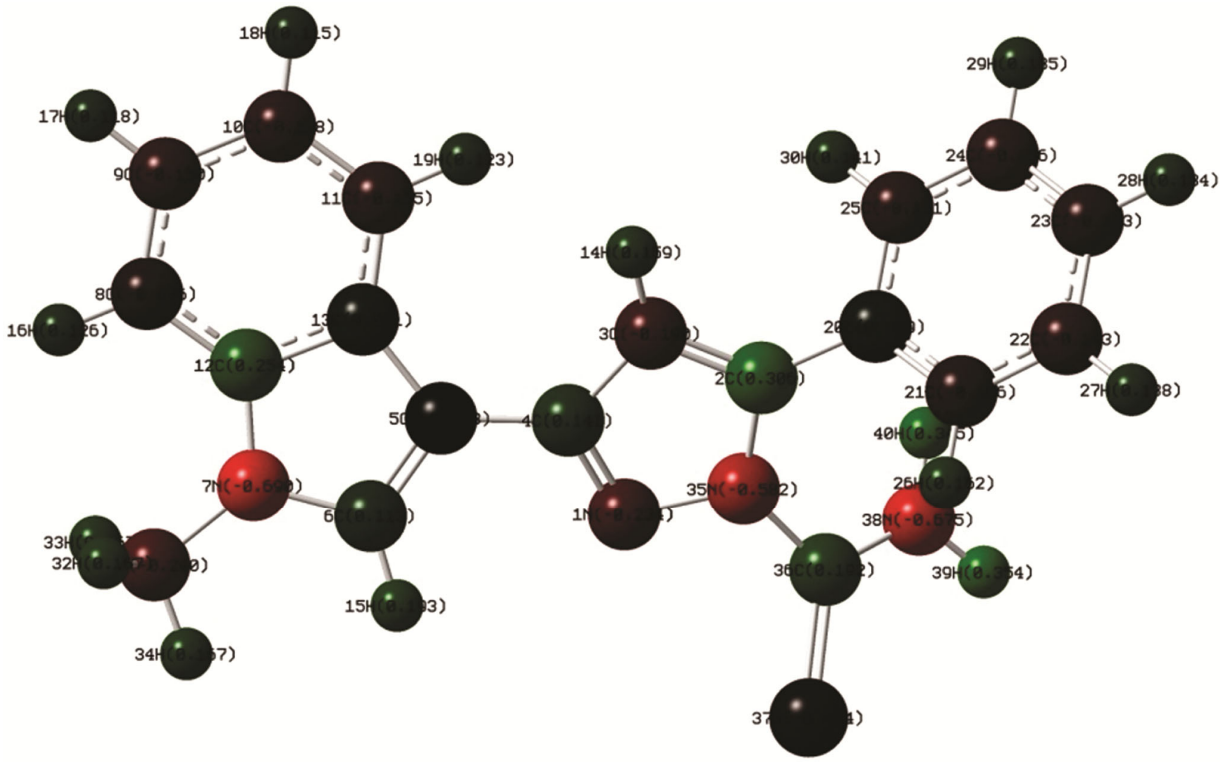


Fig. 3 — Mulliken charge distribution of 1-methyl-3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)indoleIV (a)

tuberculosis (PDB ID: 2JRC) and cancer protein of Heterometrus laoticus cytochrome (PDB ID: 2NDD) are shown in (Fig. 6). The docking scores and H-bond between the proteins of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole are given in (Table 5).

An orally administration drug should be small and moderately lipophilic. Lipinski's rule of five or thumb is a simple tool to determine whether a compound has biological or pharmacological activity that would make it an orally active drug in humans. The rule evaluated with the drug's pharmacokinetics in the human body. The data of lipinski's rule is given in (Table 6). The 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole obey the Lipinski rule of five hence, the compounds are docked into the active site of cell division protein peptidyl –tRNA hydrolase and Heterometrus laoticus cytochrome (PDB code: 2JRCand 2NDD). From the result, the 3-[5- (2-phenylethenyl)-1H-pyrazol-3-yl]-1-methylindole 14f have the highest docking score and 3-[5- (2-methoxyphenyl)-1H-pyrazol-3-yl]-1-methylindole 14d has the lowest docking score against both TB and cancer proteins.

Evaluation of antioxidant activity

The indolyl thiocarbamoyl pyrazole derivatives were assayed to determine their free radical scavenging activities using DPPH method. From the data revealed that the indole pyrazole moieties showed potent antioxidant ability, comparable to that of standard Butylated hydroxyanisole (BHA). The IVb and IVg were most active and their IC_{50} value is 73 µM and 68 µM; whereas IVe was the second of DPPH list because the 2nd and 4th positions of chloro or methoxy groups enhanced antioxidant activity

Table 3 — Mulliken charge distribution of 1-methyl-3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)indoleIV (a)

Atom	Mulliken Atomic Charge	Atom	Mulliken Atomic Charge
N1	-0.234	H16	0.126
C2	0.306	H17	0.118
C3	-0.190	H18	0.115
C4	0.141	H19	0.123
C5	-0.008	C20	0.019
C6	0.113	C21	-0.106
N7	-0.690	C22	-0.143
C8	-0.086	C23	-0.113
C9	-0.150	C24	-0.136
C10	-0.138	C25	-0.131
C11	-0.135	H26	0.162
C12	0.254	H27	0.138
C13	0.021	H28	0.134
H14	0.159	H29	0.135
H15	0.193	H30	0.141

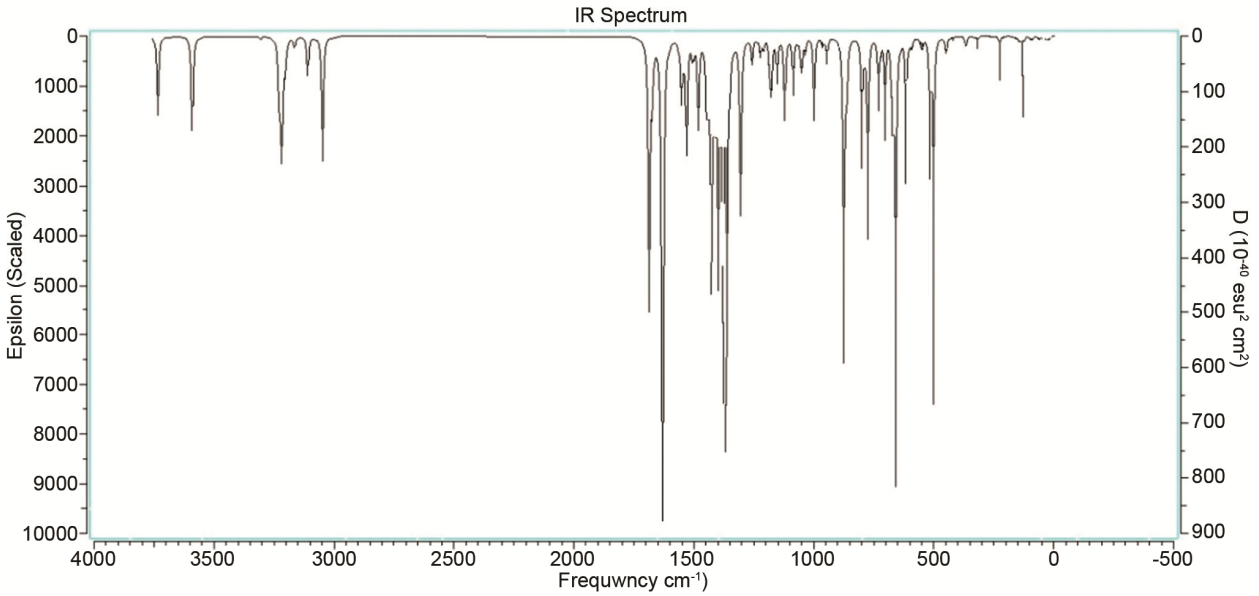


Fig. 4 — Theoretical vibrational spectrum of 3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole Iva

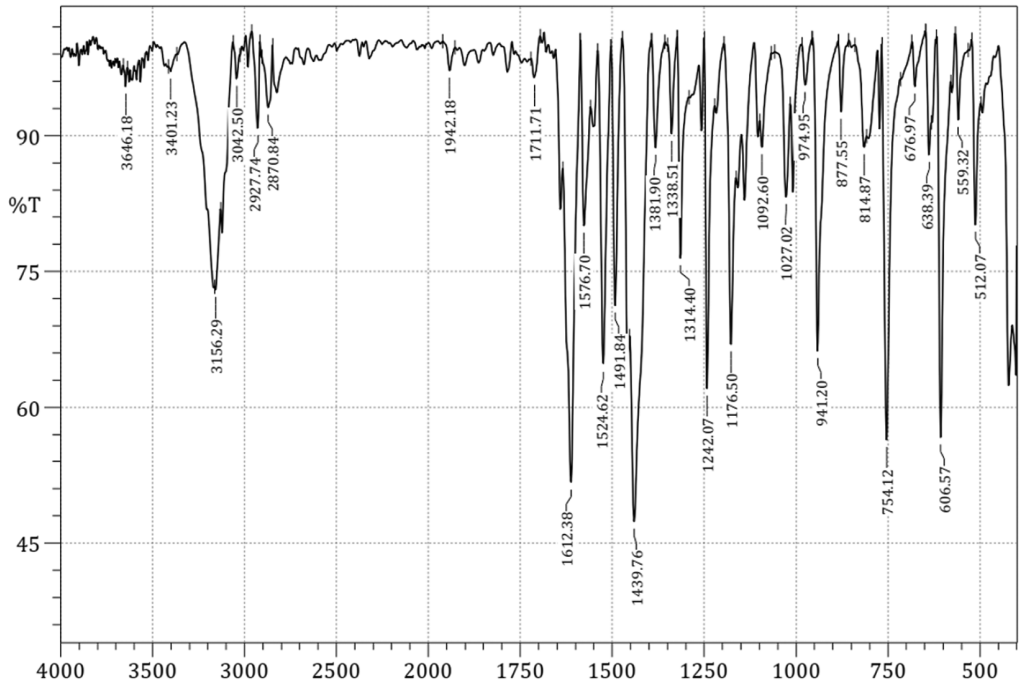


Fig. 5 — Experimental FT-IR spectrum of 3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IVa

against DPPH free radicals. The standard (BHA) IC_{50} value was established 624 μM (Table 7).

Evaluation of Anti-tubercular activities

A new indolylpyrazole bearing thiocarbamoyl compounds which showed highest docking scores were screened for their anti-tubercular activity. The result reveals that the compounds 3-[5- (4-chlorophenyl)-1-thiocarbamoylpyrazol-3-yl]-1-methylindole IVg and 3-[5- (4-methylphenyl)-1-thiocarbamoylpyrazol-3-yl]-1-methylindole IVi which exhibited good anti-tubercular activity. The pyrazole derivative with the paramethyl group was showed to be very active (Fig. 7 and Table 8).

Evaluation of Anticancer activity

The newly synthesized compounds which have highest anti-oxidant activity were also tested for the

in vitro anticancer activity against human cervical tumor cell lines (HeLa) by 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. From the MTT test, cytotoxic effects of the compounds were examined and IC_{50} values were calculated. From the results, the compound 3-[5- (2-chlorophenyl)-1-thiocarbamoylpyrazol-3-yl]-1-methylindole IVb exhibited powerful growth inhibition activity against human cervical tumor cell lines with IC_{50} of 26.043 µg/mL (Fig. 8 and Table 9). The compound -methylindole IVh exhibited remarkable antitumor activity and their IC_{50} value is 94.064 µg/mL.

Table 4 — Vibrational assignments of 3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole

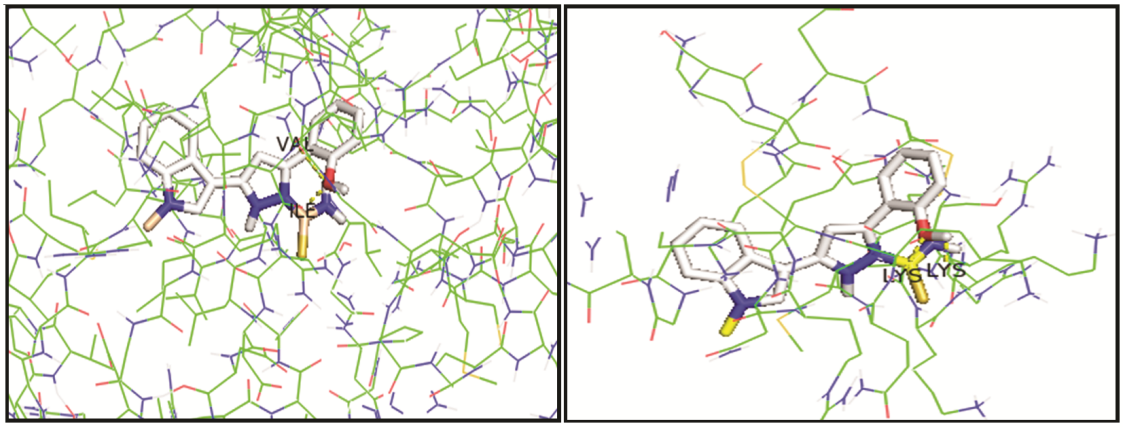
Mode	Calculated IR frequency (cm^{-1}) scaled	Intensity	Assignment	Type
101	3592	46	N_{38}-H_{39}	N-H Stretching
100	3453	66	N_{38}-H_{40}	N-H Stretching
99	3184	1	C_3-H_{14}	C-H Stretching
96	3094	46	C_8-H_{16}	C-H Stretching
94	1609	32	C_8-C_{12},	C-C Stretching
93	1566	39	C_9-C_8-C_{12}	C -C-C Bending
89	1491	39	H_{27}-C_{22}-C_{23}	H-C-C Bending
85	1390	58	N_1-C_4	N-C Stretching
82	1297	14	C_{20}-C_{25}	C-C Stretching
79	1209	15	H_{14}-C_3-C_4	H-C-C Bending
78	1177	0.004	H_{27}-C_{22}-C_{23}	H-C-C Bending
75	1027	2	C_{21}-C_{22}-C_{23}	C -C-C Bending
67	995	8	C_{23}-C_{24}-C_{25}	C -C-C Bending
32	960	38	N_1-N_{35}	N-N Stretching
49	908	11	N_{35}-C_2,	N-C Stretching
40	840	129	H_{39}-N_{38}-C_{36},	H-N-C Bending
32	763	2	C_8-C_{12}	C-C Stretching

Table 5 — Docking score of 3- (5-aryl-1-thiocarbamoy lpyrazol-3-yl)-1-methylindole IV

Compound IV	Docking score KJ mol^{-1}		H-bond	
	2JRC	2NDD	2JRC	2NDD
a	-9.4	-5.5	-	CYS-2
b	-9.0	-5.3	VAL-8	CYS-2
c	-9.3	-5.4	VAL-92	LYS-14
			ILE-93	LYS-18
d	-9.1	-5.2	-	CYS-15
				MET-16
				GLN-17
e	-9.0	-5.3	-	-
f	-9.6	-5.4	VAL-8	MET-16
			VAL-133	CYS-2
g	-9.9	-5.6	-	CYS-15
h	-9.6	-5.5	-	CYS-15
i	-10	-5.7	VAL-8	CYS-2

Table 6 — Lipinski's rule of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV

Compound IV	Mol. wt<500	HB Donar<5	HB Acceptor <10	Log P <5	Molecular Refractivity (40-130)
a	332	2	2	3.6104	101.098
b	366	2	2	3.4919	103.177
c	348	3	3	3.3160	102.763
d	362	2	3	3.6190	107.650
e	346	2	2	3.9189	105.835
f	358	2	2	4.1138	111.032
g	366	2	2	3.4919	103.177
h	362	2	3	3.6190	107.650
i	346	2	2	3.9189	105.835



IVc bound with 2JRC protein **IVc bound with 2NDD protein**

Fig. 6 — Docking image of 3-[5- (2-hydoxyphenyl)-1-thiocarbamoylpyrazol-3-yl]-1-methylindole IVc

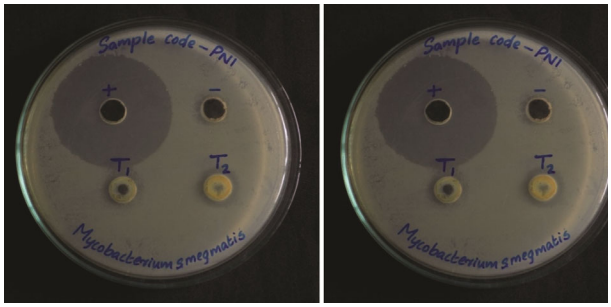


Fig. 7 — Anti-tubercular images of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV

Table 7 — Antioxidant activities of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV

Compound IV	IC_{50} value (μM)
A	392
B	73
C	174
D	299
E	192
F	256
G	68
H	91
I	367
BHA	624

Table 8 — Anti-tubercular activities of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV

Organisms		Mycobacterium smegmatis (mm)
compound	Concentration of samples	Positive control-Gentamycin 80 mcg
24 g	Gentamycin	41
	Negative control	-
	T1 (400 mcg)	17
	T2 (800 mcg)	20
24 i	Gentamycin	52
	Negative control	-
	T1 (400 mcg)	11
	T2 (800 mcg)	18

Table 9 — Anticancer activities of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole IV

Concentration (μg/ mL)	Percentage viability	
	IVb	IVh
6.25	92.34	90.42
12.5	77.57	80.85
25	51.75	73.4
50	38.25	52.12
100	16.68	29.78
IC_{50} (μg/ mL)	26.043	94.064

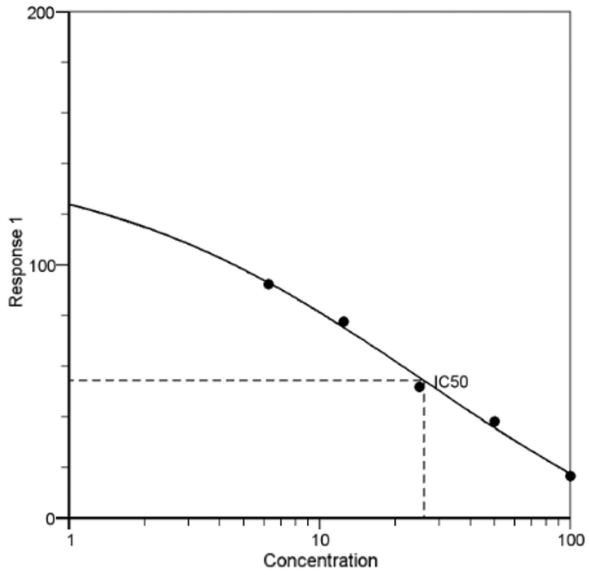


Fig. 8 — Plot of %viability *vs* concentration of 3-[5- (2-chlorophenyl)-1-thiocarbamoylpyrazol-3-yl]-1-methylindole IVb

Conclusion

In conclusion, a series of new compounds of 3- (5-aryl-1-thiocarbamoylpyrazol-3-yl)-1-methylindole have been synthesized and their structures were confirmed by elemental analysis, ^{1}HNMR, ^{13}CNMR, IR and mass spectra. The electronic structure calculations and vibrational frequencies of the energy minimized targeted molecules were calculated using Gaussian '09 software. The optimized DFT data calculated by Gaussian '09 software reveal a better agreement with the theoretical ones. The biomedical applications of the compounds such as antioxidant activity, anti TB activity, anticancer activity and molecular docking property were determined. Among the synthesized compounds, IVb (IC_{50} =73 μM), 24 g (IC_{50}=68 μM) & IVh (IC_{50}=91 μM) possess excellent antioxidant effectiveness when compared to the IC_{50} value of standard Butylated hydroxyanisole (BHA). The compounds IVg and IVi has the highest docking score with 2JRC and 2NDD and are most active against both TB and cancer protein. The compound 3-[5- (2-chlorophenyl)-1-thiocarbamoylpyrazol-3-yl]-1-methylindole IVb exhibited powerful growth inhibition activity against human cervical tumor cell lines with IC_{50} of 26.043 μg/mL.

Spectral data

1-methyl-3- (5-phenyl-1-thiocarbamoylpyrazol-3-yl)indoleIVa:

Pale yellow solid; yield 67%; mp.: 208–210°C; IR (KBr) cm-1: 3042 (C-H), 1242 (thiocarbonyl band 1612 (C=N) 3401 (NH_2); 1HNMR (DMSO-d6): δ 2.928 (s, 3H, N-CH_3), 7.813 (s, 1H, H-2 of indole), 8.785 (d,

J=1.6Hz, 1H, H-4 of indole), 7.979-8.024 (m, 2H, H-5 & H-6 of indole), 8.767 (d, J=1.6Hz, 1H, H-7 of indole), 7.338 (s, 1H, pyrazole), 7.033 (s, 2H, thiocarbamoyl), 7.748 (d, J=12.4Hz, 2H, 2ArH), 8.854 (t, J=4.4Hz, 2H, ArH), 7.385 (t, J=10Hz, 1H, ArH), *m/z*:332.11 (100.0%), 333.11 (20.5%), 334.11 (4.5%) Anal.Calcd. for $C_{19}H_{16}N_4S$ (332.4): C, 68.65; H, 4.85; N, 16.85%; Found: C, 68.63; H, 4.83; N, 16.85%.

1-methyl-3-[5- (2-chlorophenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVb:

Pale yellow solid; yield 68%; mp.: 206°C; IR (KBr) cm-1: 3154 (C-H), 1239 (thiocarbonyl band 1640 (C=N) 3434 (NH_2); 1HNMR (DMSO-d6): δ 2.732 (s, 3H, N-CH_3), 7.402 (s, 1H, H-2 of indole), 7.327 (d, J=3.6Hz, 1H, H-4 of indole), 8.555-8.623 (m, 2H, H-5 & H-6 of indole), 7.353 (d, J=2.4Hz, 1H, H-7 of indole), 7.378 (s, 1H, pyrazole), 7.295 (s, 2H, thiocarbamoyl), 7.185-7.230 (m, 4H, ArH).*m/z*:366.07 (100.0%), 368.07 (32.0%), 367.07 (20.5%), 369.07 (6.6%), 368.07 (4.5%), 368.08 (2.0%), 370.06 (1.4%); Anal. Calcd. For $C_{19}H_{15}ClN_4S$ (366.87): C, 62.20.; H, 4.12; N, 15.27%; Found: C, 62.20; H, 4.15; N, 15.28%.

1-methyl-3-[5- (2-hydoxyphenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVc:

Pale yellow solid; yield 57%; mp.: 228°C; IR (KBr) cm-1: 3159 (C-H), 1241 (thiocarbonyl band 1614 (C=N), 1514 (C=C); 1HNMR (DMSO-d6): δ 2.812 (s, 3H, N-CH_3), 7.213 (s, 1H, H-2 of indole), 7.340 (d, J=3.2Hz, 1H, H-4 of indole), 7.171-7.181 (m, 2H, H-5 & H-6 of indole), 7.416 (d, J=2.8Hz, 1H, H-7 of indole), 7.114 (s, 1H, pyrazole), 7.029 (s, 2H, thiocarbamoyl), 8.204-8.233 (m, 4H, ArH), 7.033 (s, 1H, -OH).6.84 (s, 1H, CH) *m/z*:348.10 (100.0%), 349.11 (20.5%), 350.10 (4.5%), 350.11 (2.0%), 349.10 (1.5%); Anal. Calcd.for $C_{19}H_{16}N_4OS$ (348.45): C, 65.50; H, 4.63; N, 16.08%; Found: C, 65.52; H, 4.62; N, 16.05%.

1-methyl-3-[5- (2-methoxyphenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVd:

Pale yellow solid; yield 59%; mp.: 216°C; IR (KBr) cm-1: 3159 (C-H), 1279 (thiocarbonyl band 1610 (C=N) 1520 (C=C); 1HNMR (DMSO-d6): δ 2.726 (s, 3H, N-CH_3), 7.145 (s, 1H, H-2 of indole), 7.166 (d, J=2.4Hz, 1H, H-4 of indole), 7.179-7.193 (m, 2H, H-5 & H-6 of indole), 7.227 (d, J=2.4Hz, 1H, H-7 of indole), 7.095 (s, 1H, pyrazole), 6.922 (s, 2H, thiocarbamoyl), 8.187-8.195 (m, 4H, ArH), 3.797 (s, 3H, -OCH_3).*m/z*:362.12 (100.0%), 363.12 (21.6%), 364.12 (4.5%), 363.13 (2.2%); Anal. Calcd. For $C_{20}H_{18}N_4OS$ (362.45): C, 66.28; H, 5.01; N, 15.46%; Found: C, 66.24; H, 5.03; N, 15.44%.

1-methyl-3-[5- (2-methylphenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVe:

Pale yellow solid; yield 61%; mp.: 218°C IR (KBr) cm-1: 3137 (C-H), 1244 (thiocarbonyl band 1605 (C=N) 1511 (C=C); 1HNMR (DMSO-d6): δ 2.806 (s, 3H, N-CH_3), 7.142 (s, 1H, H-2 of indole), 7.165 (d, J=2Hz, 1H, H-4 of indole), 7.202-7.218 (m, 2H, H-5 & H-6 of indole), 7.237 (d, J=4Hz, 1H, H-7 of indole), 7.138 (s, 1H, pyrazole), 7.121 (s, 2H, thiocarbamoyl), 8.161-8.172 (m, 4H, ArH), 2.359 (s, 3H, -CH_3). *m/z*:346.13 (100.0%), 347.13 (21.5%), 348.12 (4.5%), 348.13 (2.2%); Anal. Calcd. For $C_{20}H_{18}N_4S$ (346.45): C, 69.34; H, 5.24; N, 16.17%; Found: C, 69.33; H, 5.26; N, 16.17%.

1-methyl-3-[5- (2-phenylethenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVf:

Pale yellow solid; yield 60%; mp.: 208°C; IR (KBr) cm-1: 3130 (C-H), 1302 (thiocarbonyl band 1609 (C=N) 1555 (C=C); 1HNMR (DMSO-d6): δ 2.708 (s, 3H, N-CH_3), 7.225 (s, 1H, H-2 of indole), 7.237 (d, J=3.2Hz, 1H, H-4 of indole), 7.442-7.458 (m, 2H, H-5 & H-6 of indole), 7.510 (d, J=2.4Hz, 1H, H-7 of indole), 7.193 (s, 1H, pyrazole), 7.188 (s, 2H, thiocarbamoyl), 8.210-8.316 (m, 4H, ArH), 6.537 (d, J=4.8Hz 2H, -CH=).*m/z*:358.13 (100.0%), 359.12 (22.7%), 360.12 (4.5%), 360.13 (2.5%), 361.12 (1.0%)Anal. Calcd.for$C_{21}H_{18}N_4S$ (358.46): C, 70.36; H, 5.08; N, 15.63%; Found: C, 70.33; H, 5.08; N, 15.62%.

1-methyl-3-[5- (4-chlorophenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVg:

Pale yellow solid; yield 62%; mp.: 208°C; IR (KBr) cm-1: 3130 (C-H), 1302 (thiocarbonyl band 1612 (C=N) 1554 (C=C); 1HNMR (DMSO-d6): δ 2.614 (s, 3H, N-CH_3), 7.230 (s, 1H, H-2 of indole), 7.087 (d, J=1.6Hz, 1H, H-4 of indole), 8.284-8.293 (m, 2H, H-5 & H-6 of indole), 7.474 (d, J=1.2Hz, 1H, H-7 of indole), 7.209 (s, 1H, pyrazole), 7.180 (s, 2H, thiocarbamoyl), 8.154 (d, J=1.6Hz, 2H, 2ArH), 7.686 (d, J=4.8Hz, 2H, ArH). *m/z*:366.07 (100.0%), 368.07 (32.0%), 367.07 (20.5%), 369.07 (6.6%), 368.08 (2.0%), 370.06 (1.4%); Anal. Calcd.for$C_{19}H_{15}ClN_4S$ (366.87): C, 62.20; H, 4.12; N, 15.27%; Found: C, 62.19; H, 4.15; N, 15.29%.

1-methyl-3-[5- (4-methoxyphenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVh:

Pale yellow solid; yield 59%; mp.: 216–218°C; IR (KBr) cm-1: 3155 (C-H), 1276 (thiocarbonyl band 1601

(C=N) 1520 (C=C)1HNMR (DMSO-d6): δ 2.819 (s, 3H, N-CH_3), 7.123 (s, 1H, H-2 of indole), 7.138 (d, J=1.6Hz, 1H, H-4 of indole), 7.225-7.229 (m, 2H, H-5 & H-6 of indole), 7.449 (d, J=2.4Hz, 1H, H-7 of indole), 7.105 (s, 1H, pyrazole), 6.933 (s, 2H, thiocarbamoyl), 7.805 (d, J=3.2Hz, 2H, 2ArH), 8.474 (d, J=2.4Hz, 2H, ArH), 3.821 (s, 3H, -OCH_3*m/z*:362.12 (100.0%), 363.12 (21.6%), 364.12 (4.5%), 363.12 (1.5%); Anal. Calcd. For $C_{20}H_{18}N_4OS$ (362.45): C, 66.28; H, 5.01; N, 15.46%; Found: C, 66.28; H, 5.02; N, 15.46%.

1-methyl-3-[5- (4-methylphenyl)-1-thiocarbamoylpyrazol-3-yl]indoleIVi:

Pale yellow solid; yield 58%; mp.: 220°C; IR (KBr) cm-1: 3156 (C-H), 1239 (thiocarbonyl band 1640 (C=N) 3438 (NH_2)1HNMR (DMSO-d6): δ 2.854 (s, 3H, N-CH_3), 7.155 (s, 1H, H-2 of indole), 7.182 (d, J=1.6Hz, 1H, H-4 of indole), 7.212-7.227 (m, 2H, H-5 & H-6 of indole), 7.324 (d, J=2Hz, 1H, H-7 of indole), 7.136 (s, 1H, pyrazole), 7.050 (s, 2H, thiocarbamoyl), 8.183-8.195 (m, 4H, ArH), 2.443 (s, 3H, -CH_3); *m/z*: 346.13 (100.0%), 347.13 (21.6%), 348.12 (4.5%), 348.13 (2.2%), 347.12 (1.5%); Anal. Calcd. For $C_{20}H_{18}N_4S$ (346.45): C, 69.34; H, 5.24; N, 16.17%; Found: C, 69.35; H, 5.23; N, 16.19%.

Conflict of interest

All authors declare no conflict of interest.

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