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Computational design, synthesis, structural analysis and biological evaluation some novel N-methylated indole incorporating pyrazole moieties

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A new method for *N*-methylation of indoles using methylating reagent dimethyl sulphate has been developed. Structures of the newly synthesized compounds have been established by elemental analysis and spectral data and evaluated as biological activity. The synthesized indoyl pyrazole compounds have been evaluated for their antioxidant and anticancer activities. The obtained results reveal clearly that compounds IVb and e display the highest antioxidant activity and compounds c and f exhibit better radical scavenging ability; whereas the same compound IVb exhibits excellent activity (IC_{50} 24µM) against HeLa (human cervical carcinoma) cancer cell lines. Theoretical calculation of the title compounds have been carried out using density functional theory method. The geometrical optimization of the prepared target compounds has been theoretically analyzed. Based on the geometries, the HOMO and LUMO, Mulliken population analysis and reactivity indices have been calculated.

Keywords: Acetyl indole, pyrazole, DFT, antioxidant, anticancer

In recent years, an interesting and accurate method for evaluating electronic interaction energies of the compounds using density functional theory (DFT) methods has been evolved, mainly because of its good trade-off between computational cost and accuracy for treating large chemical systems. The computational based quantum chemistry has become a powerful technique for the organic chemical community to analyze, elucidate their molecular geometry and to carry out the assignment of the vibrational spectra. As a result, the development of the current consideration point to clarify the DFT method was to provide for computing electronic spectra of large molecular structure of the compounds, charge transfer (CT) and excited-state properties of the molecules.

An antioxidant is a molecule that impedes or prevents the oxidation of other molecules. The oxidation reactions are of great importance to life. Many times these can be damaging too. During oxidative processes, the free radicals produced and released highly-reactive compounds generated in the body as by-products of normal processes or these may enter the body from the environment. Inhibition of the antioxidant enzymes or insufficient levels of antioxidants cause oxidative stress that may kill or damage the DNA in the cells[1-4]. Antioxidants can protect against the cell damage due to oxidative stress that free radicals cause. Oxidative stress has been linked to heart disease, cancer, arthritis, stroke emphysema, Parkinson's disease, respiratory diseases, immune deficiency and other inflammatory or ischemic conditions. Antioxidants are man-made or natural substances that may prevent some types of cell damage[5,6]. In the last few decades, the search for better antioxidants led to the synthesis and isolation of different organic molecules which showed more effective antioxidant activity in comparison with the standard antioxidants, such as β-carotene, vitamin C (ascorbic acid), Butylated hydroxyanisole (BHA)[7-9], lutein and vitamin A. Indole derivatives are very common motifs in natural products as well as drugs. Pyrazoles are well known and important nitrogen containing five membered heterocyclic compounds[10,11]. Pyrazoles are often found as key elements in structures of biologically active substances among both marketed pharmaceuticals and experimental medicines. These heterocyclic structures are useful synthetic building blocks in medicinal and organic chemistry. The insertion of indole with pyrazole structure produced new derivatives with an improved antioxidant capacity. Indolyl pyrazole compounds are very useful in medicine, because these compounds have well-known biological activities such as

anticoagulant, hepato-protective, ulcerogenic, anti-inflammatory, antifungal[12], antitumors[13], anti-HIV[14] and an interesting antioxidant activity. As a result, the development of hybrid molecules through the combination of indole and pyrazole moieties in a single molecular framework to obtain a new class of highly potent bioactive compounds is also reported[15-17].

In this paper, we report for the first time, reaction of acetyl indole with dimethyl sulphate, a new series of methylated indolyl compounds synthesized by Knoevenagel reaction. Hence, the present investigations aim towards the synthesis and biological screening of indolyl pyrazole derivatives. Computational methods predict relatively accurate molecular structure and molecular vibrations of indolyl pyrazole molecules applying the density functional theory (DFT) methods to derive information about electronic effects and evaluate the interaction between these molecules responsible for biological activity[18-21].

Experimental Section

Materials

All the reagents and solvents used were of AR grade which were purchased from Sigma – Aldrich and Merck Specialties Pvt. Ltd. NMR spectra was recorded on 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 are uncorrected. Elemental analysis was carried out at CSIR-Central Drug Research Institute, Lucknow, India.

General procedure for the synthesis of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole (IVa–h)

3-Acetyl indole (1.6g, 0.01mol) was reacted with dimethyl sulphate (1.7g, 0.013mol) in the presence of base (15mL, 2N) to form 3-acetyl-1-methyl indole. 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. The 3-aryl-1-(1-methylindole-3-yl)-2-propen-1-one (0.01 mol) was dissolved in glacial acetic acid (10 ml). Hydrazine hydrate (0.32 g, 0.01 mol) was added and the reaction mixture was refluxed for 8 h. The temperature was maintained at 80°C. After cooling, 50 ml water was added and the resulting precipitate was filtered. The orange yellow solid was filtered, dried and recrystallized from rectified spirit.

Evaluation of antioxidant activity

The evaluation of antioxidant activity of the samples was measured using 1,1-diphenyl-2-picryl hydrazyl radical (DPPH). 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 deep purple colour of the DPPH solution turns yellow in the presence of antioxidants. The disappearance of this radical is measured at 517 nm in a methanolic solution. Butylated hydroxyanisole (BHA) was used as a reference compound. The percentage inhibition could be calculated from the UV-Vis absorbance values.

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

Evaluation of anticancer activity

The anticancer activity of all the indolyl pyrazole derivatives was evaluated against HeLa cell lines using MTT assay. 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 30 µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken, then incubated at 37ºC 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 (Laura B. Talarico et al, 2004).

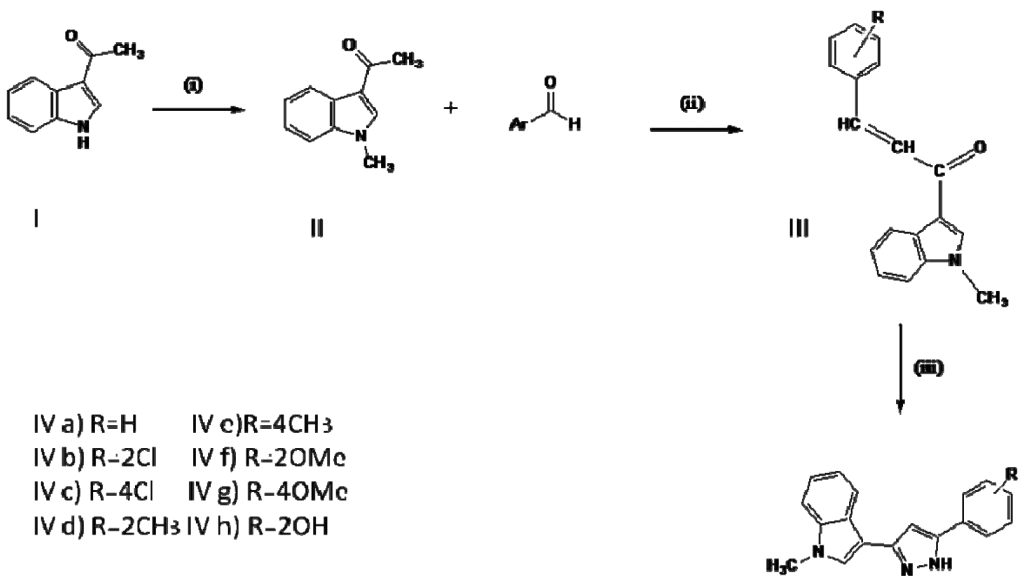
The percentage of growth inhibition was calculated using the formula:

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

Results and Discussion

Chemistry

The synthetic routes of the indolyl pyrazole prepared by the following reactions conditions and modern catalytic method pathways are cited in Scheme I. As



Reagents and Conditions: (i) $(CH_3)_2SO_4$ and NaOH, (ii) EtOH, 10% NaOH and (iii) $NH_2NH_2.H_2O$ and CH_3COOH

Scheme I

seen in Scheme I, the reaction of starting material, 3-acetyl indole(I) with dimethyl sulphate in the presence of various base is a classical method to form N-methylated indole derivatives. Dimethyl sulphate is versatile reagent and has been widely used as a methylating agent in organic synthesis. Methylation with Dimethyl sulphate has been found to be a practical method to develop N-methylated indole analogues in good yields and purity. Therefore, the development of efficient synthetic methods for the synthesis of target compounds using 3-acetyl-1-methyl indole. Next, 3 acetyl-1-methyl(II) indole treating with commercially available aromatic aldehydes, afforded 3-aryl-1-(1-methylindole-3-yl)-2-propen-1-one (III). Reaction of 3-aryl-1-(1-methylindole-3-yl)-2-propen-1-one with hydrazine hydrate along with glacial acetic acid to afford the target compound(IV).Hydrazine is a highly reactive base and reducing agent, and is widely used in organic synthesis. This reaction pathway shows that the nucleophilc attack of hydrazine hydrate at the β-carbon of the α,β-unsaturated carbonyl system leads ultimately to the pyrazole. The homogeneity of the compounds was monitored by purified by column chromatography (silica gel 60 – 150 mesh) using chloroform as the eluent to obtain pure colourless crystals of compounds.

The structure of the reaction products of new derivatives (IV), were assigned by analytical and spectroscopic data. For example, The IR spectrum of compound IVa exhibited the appearance of absorption band at1331cm-1, which is ascribed to C-N vibration. The C-H band of aromatic ring appears at 3062cm-1. The C=N stretching band is observed at 1696 cm-1 and the appearance of absorption band at 1520 cm1corresponding to C═C group. The 1H NMR spectrum of compound IVa is characterized by the presence of a singlet signals at δ 3.75ppm assignable to the methyl protons of the N-CH3 group, while a multiplet at δ 6.87-7.19ppm is due to the five aryl hydrogens. The amino hydrogen of pyrazole ring appears as a well separated broad singlet at δ 12. The four hydrogen multiplet at δ 7.22-7.78 has been attributed to aryl hydrogens of the indole ring and the pyrazole hydrogen forms a one hydrogen singlet at δ6.58. The mass spectrum of the compound IVa revealed a molecular ion peak at m/z 273.13 (M+, 100.00) that are compatible with the proposed structures.

Computational Details

The density functional theory calculations of the indoyl pyrazole compounds was carried out at the B3LYP/6-311++ G(d, p) level of DFT. The optimized structure of compound (IVa) with numbering of the atoms is depicted in Figure 1 and the optimized geometry parameters such as bond lengths, bond angles and dihedral angles are listed in Table I. From the calculated values, experimental geometric

parameters agree well with almost all values. The most important Frontier Molecular Orbital (FMOs) is the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO). Frontier molecular orbitals (HOMO and LUMO) are the main orbitals which take part in chemical stability, electronic transitions, and their energy gap depicts the reactivity of the molecule.

Recently, the energy gap between HOMO and LUMO has been used to prove the bioactivity according to the structure activity relationship. The atomic orbital components of the HOMO and LUMO distribution pattern of indolyl pyrazole derivatives IV(a-e) are shown in Figure 2. The FMOs Frontier molecular orbitals (HOMO and LUMO) of compound IVa) shows that the electrons are transferring from the indole moiety towards pyrazole moiety due to HOMO-LUMO excitation. The comparison between FMOs of indolyl derivatives at the ground state HOMO is delocalized over the indole ring whereas the LUMO is placed over the pyrazole and the phenyl ring. From Table II, it is seen that compound IVb shows the maximum HOMO LUMO energy gap (0.2265eV) whereas compound IVg exhibits the minimum (−0.2340eV).

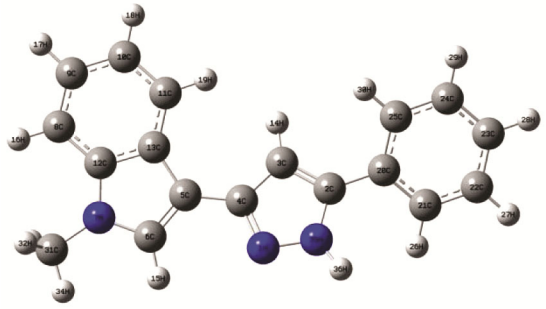


Figure 1 — Optimized structure of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole IV(a)

The proton transfer of the indolyl pyrazole compounds has been investigated here using reactivity indices. Reactivity indices such as I-Ionisation potential; A-Electron affinity; χ-Electronegativity; η-Hardness; S-Softness are complementary tools to describe the hardness, reactivity and stability of compounds and these reactivity indices are used as quantum chemical reactivity descriptor to study the path of electron transfer reaction in all the synthesized compound. Detailed HOMO and LUMO energies of compounds and the reactivity indices of compounds (IVa-h) were calculated and are listed in Table II.

Mulliken populations (R.S. Mulliken, 1955) can be used to characterize the electronic charge distribution in a molecule and the bonding, antibonding, or nonbonding nature of the molecular orbitals for particular pairs of atoms. To develop the idea of these populations, consider a real, normalized molecular orbital composed from two normalized atomic orbitals[22].

The total overlap population for any pair of atoms in a molecule is in general made up of positive and negative contributions. If the total overlap population between two atoms is positive, they are bonded; if negative, they are antibonded. From the Mulliken population analysis, the atomic charge values were obtained (Figure 3). The Mulliken atomic charges of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole IVa are

Table I — Optimized geometrical parameters of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole IV(a)

Atom	Bond length (Å)	Atom	Bond angle (°)	Atom	Dihedral angle (°)
N_1C_4	1.3583	$N_1C_4C_3$	110.49	$N_1C_4C_3H_{14}$	179.95
C_4C_3	1.4242	$C_4C_3H_{14}$	127.25	$C_4C_3H_{14}C_2$	179.56
C_3H_{14}	1.0764	$C_4C_3C_2$	106.43	$H_{14}C_3C_2N_{34}$	−157.56
C_3C_2	1.3927	$C_3C_2N_{35}$	105.39	$C_3C_2N_{34}H_{35}$	−177.76
C_4C_5	1.4535	$C_2N_{35}H_{36}$	127.85	$C_3C_2N_{34}N_1$	179.18
C_5C_6	1.3817	$N_1C_4C_5$	120.20	$N_1C_4C_5C_6$	−178.12
C_6H_{15}	1.0782	$C_3C_4C_6$	129.28	$C_4C_5C_6H_{15}$	−179.99
C_6N_7	1.3885	$C_4C_5C_6$	124.64	$C_5C_6H_{15}N_7$	−179.59
N_7C_{31}	1.4553	$C_5C_6H_{15}$	128.51	$N_7C_{12}C_8H_{16}$	−159.05
N_7C_{12}	1.3921	$C_5C_6N_7$	110.34	$C_{12}C_8H_{16}C_9$	179.95
$C_{12}C_{13}$	1.4288	$C_6N_7C_{31}$	125.98	$C_8H_{16}C_9H_{17}$	178.51
$C_{12}C_8$	1.4004	$N_7C_{31}H_{32}$	111.02	$C_{12}C_8C_9C_{10}$	−179.87
C_8H_{16}	1.0852	$C_{31}H_{32}H_{33}$	35.023	$H_{17}C_9C_{10}H_{18}$	−179.96
C_8C_9	1.3944	$C_{31}N_7C_{12}$	125.44	$H_{18}C_{10}C_{11}H_{19}$	−179.34
C_9H_{17}	1.0853	$N_7C_{12}C_8$	129.76	$C_{10}C_{11}H_{19}C_{13}$	−179.99
C_9C_{10}	1.4120	$C_{12}C_8H_{16}$	121.46	$C_{10}C_{11}C_{13}C_{12}$	−60.18

presented in Table III. The Mulliken atom charge is positive for all hydrogen atoms. Nitrogen atoms possess negative charge and chlorine atoms carry positive charge. For example compound IVa the Mulliken atom charges are −0.268 (N1) for nitrogen and +0.175 (H15) for hydrogen atom.

From Table II reveals that the compound IVc has the lowest value of Hardness (η), i.e, 0.1118eV, whereas

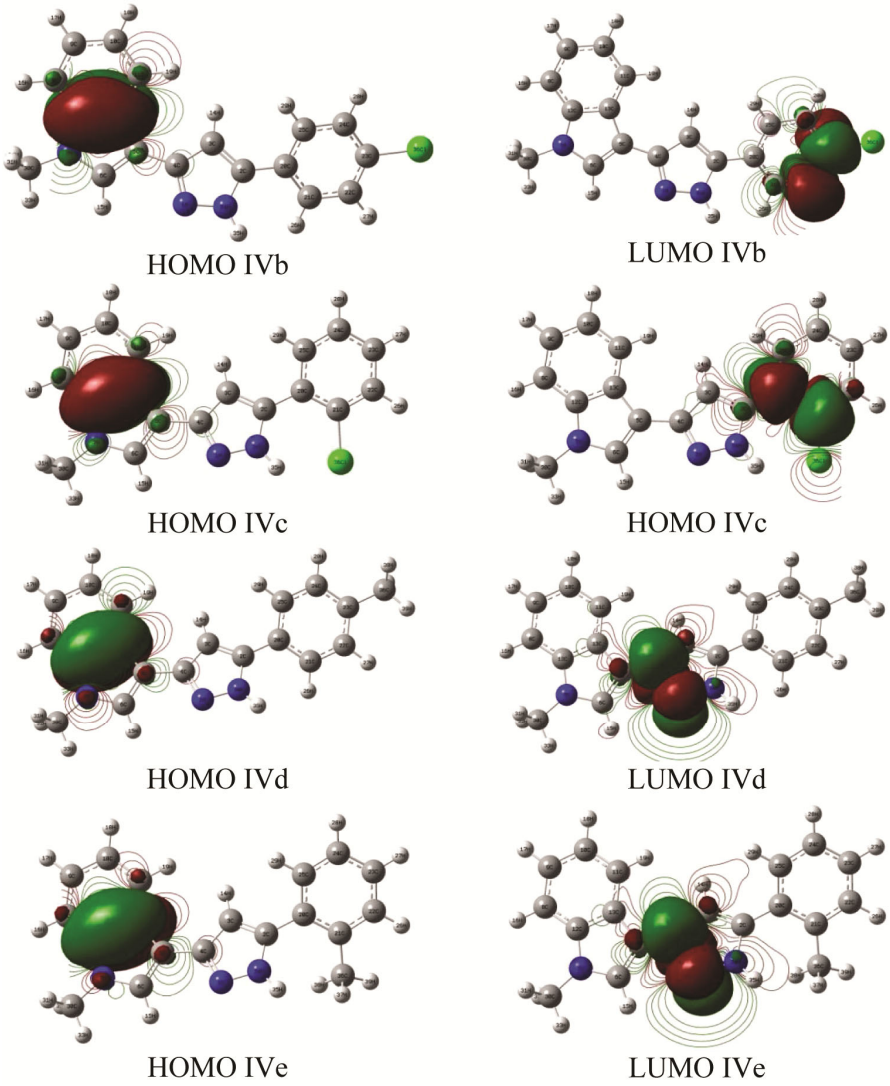


Figure 2 — HOMO and LUMO of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole IV(a)

Table II — Energies of HOMO-LUMO of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole IV(a-h)

Parametres (a.u)	B3LYP/631G							
	IVa	IVb	IVc	IVd	IVe	IVf	IVg	IVh
HOMO	−0.2256	−0.2295	−0.2273	−0.2246	−0.2351	−0.2240	−0.2209	−0.2237
LUMO	0.0126	−0.0030	−0.0037	0.0145	0.0137	0.0155	0.0130	0.0817
HOMO-LUMO	−0.2383	0.2265	0.2236	−0.2392	−0.2489	−0.2396	−0.2340	−0.3054
I	0.2256	0.2295	0.2273	0.2246	0.2351	0.2240	0.2209	0.2237
A	−0.0126	0.0030	0.0037	−0.0145	−0.0137	−0.0155	−0.0130	−0.0817
χ	0.1064	0.1163	0.1155	0.1050	0.1106	0.1042	0.1039	0.0709
η	0.1191	0.1132	0.1118	0.1196	0.1244	0.1198	0.1170	0.1527
S	4.1955	4.4146	4.4716	4.1799	4.0171	4.1722	4.2733	3.2742

I-Ionisation potential; A-Electron affinity; χ-Electronegativity; η-Hardness; S-Softness

IVh has the highest value (0.1527eV). Compound IVe has the highest Ionisation potential (I) value (0.2351eV) among compounds a-h while IVg has the lowest chemical potential value (0.2209eV). The result reveals that the compound with highest Electron affinity (A) value was IVc, whose value is 0.0037 eV whereas IVa has the lowest value -0.0126eV.In addition, among the set of compounds, IVb has the highest Electronegativity (χ) value (0.1163eV) while IVh has the lowest value (0.0709eV). IVc (4.4716) has the highest value of softness of the compound and the lowest value is IVh (3.2742).

Evaluation of Antioxidant activity

The indolyl pyrazole derivatives were tested for their free radical scavenging activities using DPPH assay. The antioxidant activity of the synthesized compounds were also determined with measuring the IC_{50} (μM) values in Table IV. The data revealed that the indolyl pyrazole moieties showed potent DPPH radical scavenging activity, comparable to that of standard Butylated hydroxyanisole (BHA). The highest fee radical scavenging activity was achieved for compound IVb and their IC_{50} value is 28μM; whereas IVe was the second of DPPH list and their IC_{50} values were found to be 39μM because compound having substitution with electron-withdrawing groups enhanced antioxidant activity against DPPH free radicals. Moreover, compounds IVf and IVc were showed moderate scavenging activity with IC_{50} values 48 and 147μM. The IC_{50} value of standard (BHA) was established 624μM.

Evaluation of Anticancer activity

The compounds which have highest antioxidant activities (IVb, IVe and IVf) were screened for their

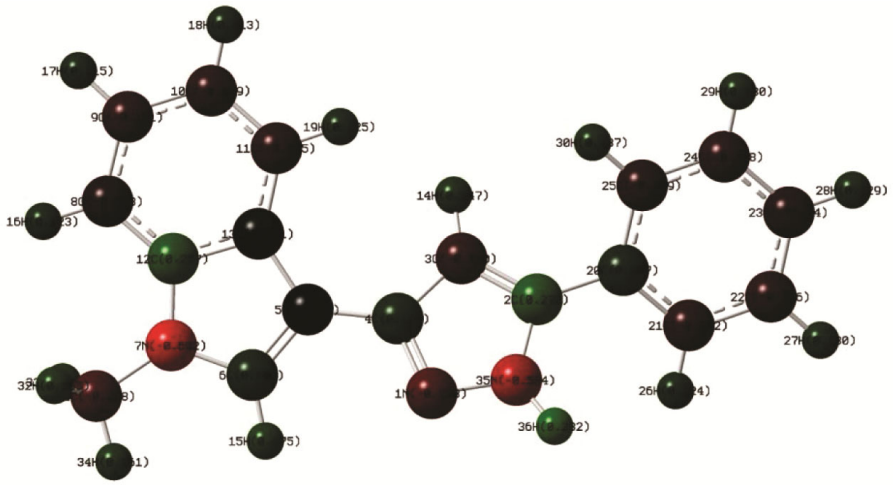


Figure 3 — Mulliken charge distribution of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole (IVa)

Table III — Mulliken charge distribution of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole IV(a)

Atom	Mulliken Atomic Charge	Atom	Mulliken Atomic Charge
N1	−0.268	H16	0.123
C2	0.272	H17	0.115
C3	−0.170	H18	0.113
C4	0.108	H19	0.125
C5	0.002	C20	0.097
C6	0.102	C21	−0.142
N7	−0.692	C22	−0.136
C8	−0.088	C23	−0.114
C9	−0.151	C24	−0.138
C10	−0.139	C25	−0.129
C11	−0.135	H26	0.124
C12	0.257	H27	0.130
C13	0.011	H28	0.129
H14	0.147	H29	0.130
H15	0.175	H30	0.137

Table IV — DPPH radical scavenging activity of 3-(1-methyl-1*H*-indol-3yl)-5 phenylpyrazole (IVa-h)

Antioxidant Activity	
Tested compounds	IC_{50}(μM)
IVa	235
IVb	28
IVc	147
IVd	246
IVe	39
IVf	48
IVg	257
IVh	340
BHA	624

BHA (Butylated hydroxyanisole

Table V — Anticancer activity of 3-(1-methyl-1*H*-indol-3yl)-5 phenylpyrazole (IVb,c and f)

Anticancer Activity	
Tested compounds	IC_{50}(μM) (HeLa)
IVb	24.149
IVe	25.509
IVf	49.197

anticancer activity. The indoyl pyrazole derivatives were evaluated for their anticancer activity via the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method against human cancer cell lines i.e. human cervical malignant cells (HeLa). The anticancer activities of the indolyl pyrazole compounds IVb,e and f along with that of the reference drug Doxorubicin IC_{50} = 1.654 ±0.25 are shown in Table III. Table V indicates that compound IVb exhibited pronounced inhibitory action against HeLa and their IC_{50} value is 24.149 μM and compounds IVe and f displayed good anticancer activity against HeLa cell lines as expressed by IC_{50} values of 25.509μM and 49.197μM

Conclusion

In conclusion, a new series of 3-(1-methyl-1*H*-indol-3yl)-5-phenylpyrazole derivatives were successfully synthesized by the reaction of 3-acetyl-1-methyl indole with different aromatic aldehydes. All synthesized compounds were screened for their antioxidant and anticancer activity: the compound with highest antioxidant activity was IVb, whose IC_{50} value is 31μM; however the same compound IVb, exhibited excellent activity with IC_{50} 24μM against HeLa (human cervical carcinoma) cancer cell lines. The optimized geometrical parameters were calculated at B3LYP/6-31G(d,p) basis set. Compounds with lower HOMO–LUMO band gap have higher biological activities. The feasibilities of hydrogen bonding were explained by Mulliken atomic charge analysis.

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