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Synthesis And Biological Evaluation Of Some New Pyrazole, Chromen Incorporated Indole Derivatives

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ABSTRACT

Some novel indole analogues containing pyrazole and chromen systems have been synthesized and their strucure are confirmed by spectral studies. These compounds were screened for their in-vitro antioxidant, antimicrobial, anti-tuberculosis and anti-cancer activities. Compound ***4e*** *exhibited promising radical scavenging activity (RSA) (84.50%) at 50 µg/mL,* ***3e*** *exhibited good reducing power ability (FRAP) at 100 µg/mL,* ***3b*** *showed good metal chelating activity (78.89%) at concentration 100 µg/mL. Compounds* ***3b*** *and* ***4b*** *exhibited excellent MIC value of antimicrobial activity. Compounds* ***4b, 4c, 4d, 4e*** *and* ***4f*** *showed promising anti-tubercular activity against M. Tuberculosis* $H_{37}Rv$*, whereas compounds* ***3d*** *and* ***3e*** *exhibited 100% cell lysis at concentration 10 µg/mL against MDA-MB-231 (Human adenocarcinoma mammary gland) cell lines.*

Keywords: Indole, pyrazole, antioxidant, antimicrobial, anti-TB and anti-cancer activity.

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INTRODUCTION

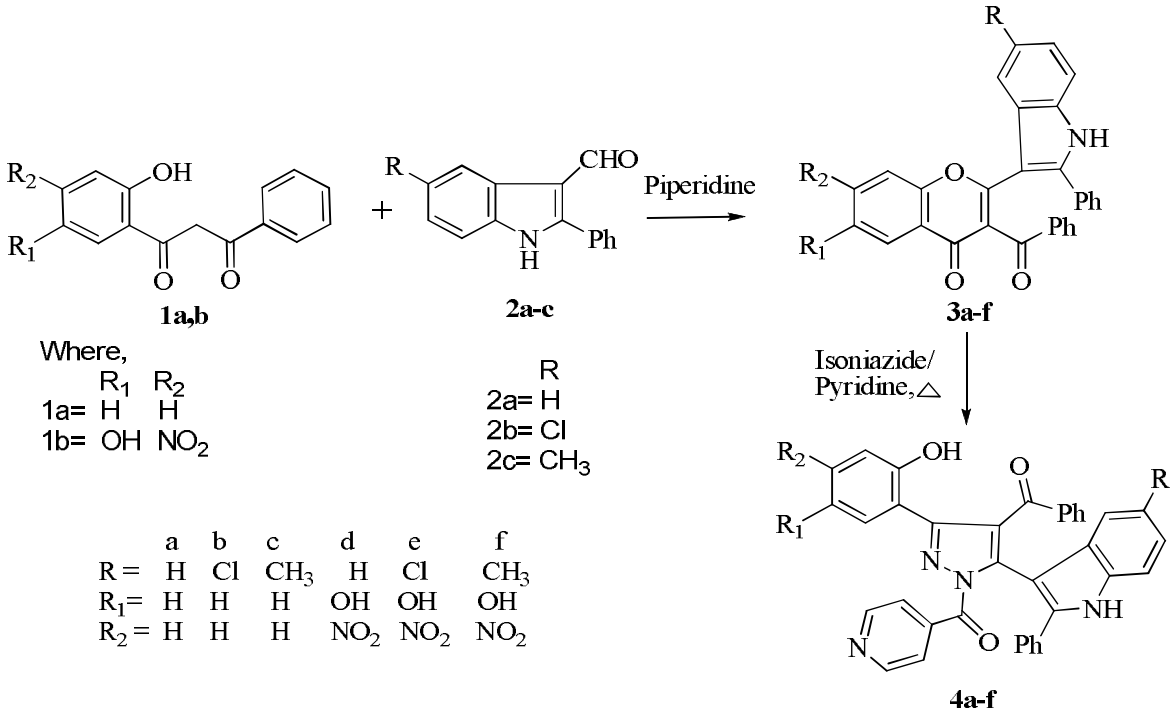
Literature survey reveales that, the biological importance of indole analogues have attractive and rewarding research targets which motivated countless researchers to study their synthesis and pharmacological properties [1-4]. Several derivatives of indole have been found to exhibit anti-inflammatory and analgesic [5], antimicrobial [6], insecticides [7], anticancer [8], HIV inhibitor [9], antioxidant [10], antituberculaosis [11], antiviral [5] and antihistaminic [12] activities.

Literature survey also revealed that pyrazole containing compounds have received considerable attention owing to their diverse chemotherapeutic potential such as, antileukemic [13,14], antitumor [15,16], anti-proliferative [17], analgesic and anti-inflammatory [18,19] activities. On the other hand chromone moiety often appears as an important structural component in both biologically active synthetic and natural compounds found to exhibit antimicrobial [20], antiviral [21], mutagenical [22], antiproliferative [23], sex pheromone [24] and antitumor [25] activities.

Isoniazid (isonicotinylhydrazide, INH) even though synthesized in 1912 by Mayer and Mally [26] its activity against tuberculosis was first reported in 1950 and introduced as a drug for tubrculosis in 1952 [27]. Since then it has become a frontline drug for TB treatment. Later, clinically it has been showed that when TB patients are treated with INH, it react with Vit-B_6 to form hydrazone leading to Vit-B_6 deficiency. Therefore several analogues of INH have been synthesized and tested for anti-TB activity [28-30]. In view of the above findings and in continuation of our research on the synthesis of biologically active molecules [31-34], in present investigation we report the synthesis, antioxidant, antimicrobial, antitubercular and anti-cancer activities of the title compounds.

MATERIALS AND METHODS

A typical synthetic strategy employed to obtain the title compounds (**3a-f**) and (**4a-f**) is depicted in scheme 1. The key intermediates 3-benzoyl-2-(5-substituted 2-phenyl-1*H*-indol-3-yl)-4*H*-chromen-4-ones (**3a-f**) were prepared by cyclocondensation of 1-(2-hydroxyphenyl)-3-phynylpropan-1,3-dione (**1a-b**) [35] with 2,5-disubstituted indole-3-carboxaldehydes (**2a-c**) [36] using catalytic amount of piperidine in ethanol under reflux condition. Compounds (**3a-f**) on reaction with isoniazide in pyridine at reflux temperature afforded [4-benzoyl-3-(2-hydroxyphenyl)-5-(2-phenyl-1*H*-indole-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl(pyridine-4-yl]methanones (**4a-f).** The IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies are used to ascertain the structures of these previously unknown compounds.



Scheme 1 Schematic pathway for the synthesis of compounds **3** & **4**

Experimental Procedure: All the reagents are obtained commercially and used by further purification using standard procedures. Melting points are determined by an open capillary method and are uncorrected. Purity of the compounds is checked by thin layer chromatography using silica gel-G coated Al plates (Merck) and spots are visualized by exposing the dry plates in iodine vapors. The IR (KBr pellet) spectra are recorded on a Perkin-Elmer (Spectrum ONE) FT-IR Spectrometer. The ^{1}H and ^{13}C NMR (DMSO-d_6) spectra are recorded with a BRUKER NMR 500 and 125 MHz spectrometers respectively, and

the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements are carried out by Electron Impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer.

Substituted 1-(2-hydroxyphenyl)-3-phenyl-3-phenylpropan-1,3-diones (1a-c) are prepared by literature method [35]. **2,5-distubstituted indol-3-carboxaldehydes (2a-c)** are prepared by literature method [36].

General procedure for synthesis of 3-benzoyl -2-(5-substituted 2-phenyl-1H-indol-3-yl)-4H-chromen-4-ones (3a-f): A mixture of compound **1** (0.01 mol) and 2-phenyl-1*H*- indol-3-carboxaldehyde **2** (0.01 mol) containing 4-5 drops piperidine in ethanol (25 mL) was refluxed on a water bath for 6-8 h. The progress of reaction was monitored by TLC. After cooling, the reaction mixture was decomposed in ice-cold water. The product separated was filtered, washed with cold water, dried and recrystallized from ethanol-acetic acid (6:4) mixture.

3-Benzoyl-2-(2-phenyl-1H-indol-3-yl)-4H-chromen-4-one (3a): Yield: 81.6%, m.p. 163-164 °C; Rf 0.76 benzene: ethyl acetate: (3:2) mixture; FTIR (KBr) cm^{-1}: 3,306 (indole NH), 1,628 (C=O), 1,579 (C=O), 1,035 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.72 (s,1H, indole NH); 6.94-8.30 (m, 18H, Ar-H). Anal.% $C_{30}H_{19}NO_3$: C, 81.62; H, 4.34; N, 3.17. Found: C, 81.63; H, 4.30; N, 3.15.

3-Benzoyl-2-(5-chloro-2-phenyl-1H-indol-3-yl)-4H-chromen-4-one (3b): Yield: 69.70% m.p. 244-46 °C; Rf 0.72 benzene: ethyl acetate: (3:2) mixture; FTIR (KBr) cm^{-1}: 3,330 (indole-NH), 1,665 (C=O), 1,610 (C=O) 1,028 (C-O-C), 777 (C-Cl); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.74 (s, 1H, indole NH); 6.97-8.26 (m, 17H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 193.47 (C=O), 185.99 (C=O), 162.12, 150.52, 136.00, 135.95, 134.93, 131.06, 129.82, 129.51, 127.47, 127.41, 126.88, 124.18, 123.92, 121.43, 120.70, 120.57, 120.56, 116.75, 114.24, 109.65 (Arom. Cs:); MS (EI) m/z: 475 (M^+), 477 (M^++2); Anal.% $C_{30}H_{18}NO_3Cl$: C, 75.71; H, 3.81; N, 2.94; Found: C, 75.79; H, 3.79; N, 2.96.

3-Benzoyl-2-(5-methyl-2-phenyl-1H-indol-3-yl)-4H-Chromen-4-one (3c) Yield: 70.23% m.p. 220-222 °C; Rf 0.69 benzene: ethyl acetate: (3:2) mixture; FTIR (KBr) cm^{-1}: 3,350 (NH), 1,680 (C=O), 1,665 (C=O), 1,030 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.75 (s, 1H, indole NH); 6.95-8.22 (m, 17H, Ar-H); 3.62 (s, 3H, CH_3); Anal.% $C_{31}H_{21}NO_3$: C, 81.74; H, 4.65; N, 3.07; Found: C, 81.75; H,4.62; N, 3.04.

3-Benzoyl-6-hydroxy-7-nitro-2-(2-phenyl-1H-indol-3-yl)-4H-chromen-4-one (3d): Yield: 74.00% m.p. 218-220 °C; Rf 0.75 benzene: ethyl acetate: (3:2) mixture; FTIR (KBr) cm^{-1}: 3,458 (OH), 3,326 (NH), 1,625 (C=O), 1,542 (C=O), 1,456, 1,332 (NO_2), 1,095 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.49 (s, 1H, indole NH); 11.85 (s, 1H, OH) [42, 43]; 6.81-8.42 (m, 16H, Ar-H); Anal.% $C_{30}H_{18}N_2O_6$: C, 71.71; H, 3.61; N, 5.58; Found: C, 71.70; H, 3.58; N, 5.55.

3-Benzoyl-2-(5-chloro-2-phenyl-1H-indol-3-yl)-6- hydroxyl-7-nitro-4H-chromen-4-one (3e): Yield: 70.44% m.p. 230-232 °C; Rf 0.81 benzene: ethyl acetate: (3:2) mixture; FTIR (KBr) cm^{-1}: 3,449 (OH), 3,321 (NH), 1,637 (C=O), 1,552 (C=O), 1,093 (C-O-C), 1,460, 1,328 (NO_2), 791 (C-Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.53 (s, 1H, indole NH); 11.31 (s, 1H, OH); 6.79-8.24 (m,15H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 190.43 (C=O), 180.12 (C=O), 166.68, 158.39, 147.22, 139.16, 135.90, 134.04, 130.82, 130.31, 130.17, 130.09, 129.51, 129.08, 128.54, 127.37, 127.23, 126.82, 124.86, 123.90, 120.57, 120.56, 117.34, 115.08, 114.28, 109.65 (Arom. Cs:); MS (EI) m/z: 536 (M^+), 538 (M^++2); Anal.% $C_{30}H_{17}N_2O_2Cl$: C, 67.11; H, 3.19; N, 5.22; Found: C, 67.16; H, 3.17; N, 5.20.

3-Benzoyl-6-hydroxy-2-(5-methyl-2-phenyl-1H-indol-3-yl)-7-nitro-4H-chromen-4-one (3f): Yield: 62.32%, m.p. 285-287 °C; Rf 0.65 benzene: ethyl acetate: (3:2) mixture; FTIR (KBr) cm^{-1}: 3,432 (OH), 3,290 (NH), 1,637 (C=O), 1,548 (C=O), 1,458, 1,332 (NO_2), 1,095 (C-O-C), 800 (C-Cl); ^{1}H NMR

(DMSO-d_6, δ, ppm) 12.49 (s, 1H, indole NH); 11.77 (s, 1H, OH); 6.99-8.28 (m, 15H, Ar-H); 3.51 (s, 3H, CH_3). Anal.% $C_{31}H_{20}N_2O_6$: C, 72.09; H, 3.90; N, 5.42. Found: C, 72.08; H, 3.87; N, 5.41.

General procedure for synthesis of [4-benzoyl-3-(2-hydroxy)-5-(2-pheynyl-1H-indol-3-yl)-4,5 dihydro-1H-pyrazol-1-yl(pyridin-4-yl)]methanones (4a-f): A solution of compounds **(3a-f)** (0.01 mol) and isoniazide (0.02 mol) was refluxed for an 8-10 hr in pyridine. After the completion of a reaction the reaction mixture was decomposed in ice cold water and acidified with dilute acetic acid. The precipitated product was filtered, washed with sufficient water, dried and recrystallized from ethanol-acetic acid (6:4) mixture.

[4-Benzoyl-3-(2-hydroxyphenyl)-5-(2-phenyl-1H-indol-3-yl)-1H-pyrazol-1-yl(pyridine-4-yl)] methanone (4a): Yield: 72.15%, m.p. 208-10 °C; Rf 0.55 benzene: ethyl acetate: (4:1) mixture; FTIR (KBr) cm^{-1}: 3,410 (OH), 3,200 (NH), 1,620 (C=O), 1,570 (C=O), 1,540 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.76 (s,1H, indole NH); 11.85 (s, 1H, OH); 6.95-8.44 (m, 22H, Ar-H); Anal.% $C_{36}H_{26}N_4O_3$: C, 76.85; H, 4.66; N 9.96; Found: C, 76.86; H, 4.62; N, 8.54.

[4-Benzoyl-5-(5-chloro-2-phenyl-1H-indol-3-yl)-3-(2-hydroxyphenyl)-1H-pyrazol-1-yl(pyridine-4-yl)] mehtonone (4b): Yield: 69.86%, m.p. 262-64 °C; Rf 0.74 benzene: ethyl acetate: (4:1) mixture; FTIR (KBr) cm^{-1}: 3,439 (OH), 3,304 (NH), 1,662 (C=O), 1,608 (C=O) 770 (C-Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.74 (s, 1H, indole NH); 11.81 (s, 1H, OH); 6.97-8.47 (m, 21H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 189.99 (C=O), 179.72 (C=O), 154.65, 150.52, 145.83, 134.72, 134.44, 132.99, 132.61, 131.41, 131.22, 129.58, 128.53, 127.92, 127.92, 127.61, 127.54, 126.50, 126.22, 123.01, 122.85, 122.50, 121.71, 120.93, 116.91, 116.88, 109.99, 59.98, 46.39 (Arom. Cs:); MS (EI) m/z: 591 (M^+), 593 (M^++2); Anal.% $C_{36}H_{25}ClN_4O_3$: C, 72.42; H, 4.22; N, 9.38; Found: C, 72.48; H, 4.19; N, 9.39.

[4-Benzoyl-3-(2-hydroxyphenyl)-5-(5-methyl-2-phenyl-1H-indol-3-yl)-1H-pyrazol-1-yl(pyridin-4-yl)] methanone (4c): Yield: 68.83%, m.p. 205-07 °C; Rf 0.69 benzene: ethyl acetate (4:1) mixture; FTIR (KBr) cm^{-1}: 3,435 (OH), 3,295 (NH), 1,665 (C=O), 1,610 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.77 (s, 1H, indole NH); 11.75 (s, 1H, OH); 6.99-8.24 (m, 21H, Ar-H); 3.59 (s, 3H, CH_3); Anal.% $C_{37}H_{28}N_4O_3$: C, 77.07; H, 4.89; N, 9.72. Found: C, 77.08; H, 4-86; N, 9.72.

[4-Benzoyl-3-(2,5-dihydroxy-4-nitrophenyl)-5-(2-phenyl-1H-indol-3-yl)-1H-pyrazol-1-yl(pyridin-4-yl)] methanone (4d): Yield 66.52 % m.p. 178-80 °C; Rf 0.72 benzene: ethyl acetate (4:1) mixture; FTIR (KBr) cm^{-1}: 3,400 (OH), 3,218 (NH), 1,637 (C=O), 1,598 (C=O), 1,535 (C=N), 1,450, 1,328 (NO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.21 (s, 1H, indole NH); 11.69 (s, 1H, OH); 11.36 (s, 1H, OH); 6.79-8.01 (m, 20H, Ar-H); Anal.% $C_{36}H_{25}N_5O_6$: C, 69.34; H, 4.04; N, 11.23; Found: C, 69.45; H, 4.02; N, 11.25.

[4-Benzoyl-5-(5-chloro-2-phenyl-1H-indol-3-yl)-3-(2,5-dihydroxy-4-nitrophenyl)-1H-pyrazol-1-yl (pyridin-4-yl)]methanone (4e): Yield 63.68 % m.p. 160-62 °C; Rf 0.65 benzene: ethyl acetate (4:1) mixture; FTIR (KBr) cm^{-1}: 3,419 (OH), 3,210 (NH), 1,637 (C=O), 1,600 (C=O) 1,530 (C=N), 1,458, 1,330 (NO_2), 76 (C-Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.14(s, 1H, indole NH); 11.73 (s, 1H, OH); 11.68 (s, 1H, OH); 6.62-7.87 (m, 19H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 191.25 (C=O), 180.02 (C=O), 150.11, 149.04, 144.51, 139.51, 137.82, 134.51, 133.22, 132.05, 131.58, 128.12, 127.99, 127.91, 127.85, 127.56, 126.94, 126.50, 124.91, 122.85, 122.05, 121.32, 119.88, 119.24, 113.61, 113.22, 109.02, 60.11, 49.86 (Arom. Cs); MS (EI) m/z: 652 (M^+), 654 (M^++2); Anal.% $C_{36}H_{24}N_5O_6Cl$: C, 65.7; H, 3.68, N, 10.64, Found: C, 65.75; H, 3.65; N, 10.65.

[4-Benzoyl-3-(2,5-dihydroxy-4-nitrophenyl)-5-(5-methyl-2-phenyl-1H-indol-3-yl)-1H-pyrazol-1-yl (pyridin-4-yl)]methanone (4f): Yield: 62.49 % m.p. 191-92 °C; Rf 0.65 benzene: ethyl acetate (4:1) mixture; FTIR (KBr) cm^{-1}: 3,415 (OH) 3,292 (NH) 1,635 (C=O) 1,598 (C=O) 1,538 (C=N) 1,444, 1,328 (NO_2), ^{1}H NMR (DMSO-d_6, δ, ppm): 12.39 (s, 1H, indole NH); 11.75 (s, 1H, OH); 11.69 (s, 1H, OH) 6.69-

8.12 (m, 194, Ar-H); 3.48(s, 3H, CH_3); Anal.% $C_{37}H_{27}N_5O_6$: C, 69.64; H, 4.12; N, 10.95; Found: C, 69.69; H, 4.27; N, 10.98.

RESULTS AND DISCUSSION

Biological Activities: All the newly synthesized compounds **(3** and **4)** were screened for their antioxidant (free radical scavenging, ferric ion reducing antioxidant power and metal chelating activity), antimicrobial, antitubercular and anticancer activities.

ANTIOXIDANT ACTIVITIES

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA): The synthesized compounds (**3** and **4**) were screened for their free radical scavenging activity using DPPH method [37]. This model of radical scavenging activity by DPPH radical is extensively applied to evaluate the antioxidant activity in shorter time. The odd electron in the DPPH free radical gives a strong absorption band at 517 nm, which is purple in color. This property makes it suitable for spectrometric studies. The free radical scavenging capacity of the synthesized compounds are measured at different concentrations (25, 50, 75 and 100 µg/mL in methanol) in presence of freshly prepared solution of stable free radial DPPH in methanol. The results are compared with the results obtained for reference standards butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and ascorbic acid (AA). The test compounds donates electron or hydrogen atom to a DPPH radical and convert it into 1,1-diphenyl-2-picrylhydrazine a neutral diamagnetic molecule. The extent of decolourization is an indicative potentiality of antioxidant behavior of a particular compound.

The analysis of results (**Fig-1** and **2)** indicated that, compounds **3a, 3b, 4d**, and **4e** exhibited very good radical scavenging activity 65.91, 70.70, 83.66 and 84.50%, respectively at concentration 50 µg/mL. Compounds **4b, 4d, 4e** and **4f** showed 72.39, 67.60, 70.98 and 70.70% radical scavenging ability at concentration 25 µg/mL, respectively. The slightly higher RSA of compounds **4b** and **4e** than compound **4d** may be attributed to the presence of chloro, group in their structure which is missing in **4d.** This indicates that chloro group may be responsible in stabilizing a free radical formed after donating an electron or hydrogen atom to the DPPH radical. Whereas other compounds without these substituents exhibited less RSA than **4b** and **4e**.

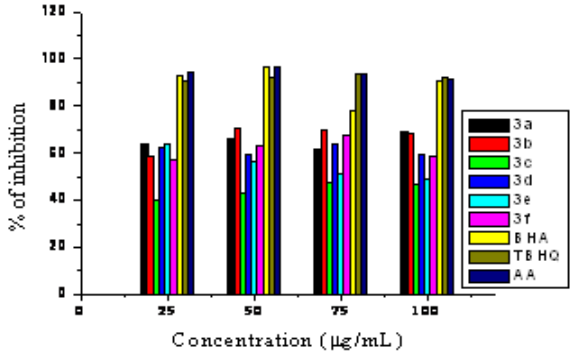


Fig 1: RSA of compounds **(3a-f)**

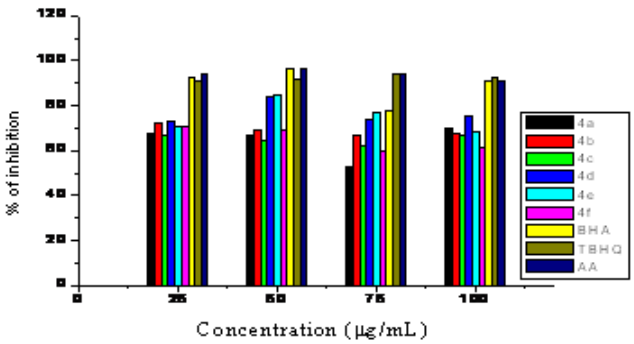


Fig 2: RSA of compounds **(4a-f)**

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP): The ferric ion (Fe^{3+}) is relatively biologically inactive form of iron. However, it can be reduced to the active ferrous ion (Fe^{2+}) depending on conditions, particularly pH [38] and oxidizing back through Fenton type reaction with the production of hydroxyl radical or Haber-Weiss reaction with superoxide anions. Reducing power is to measure the reductive ability of an antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} by donation of an electron in the presence of test compounds. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

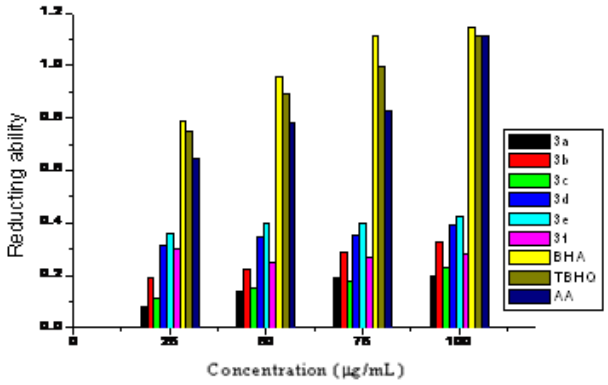


Fig 3: FRAP of compounds **(3a-f)**

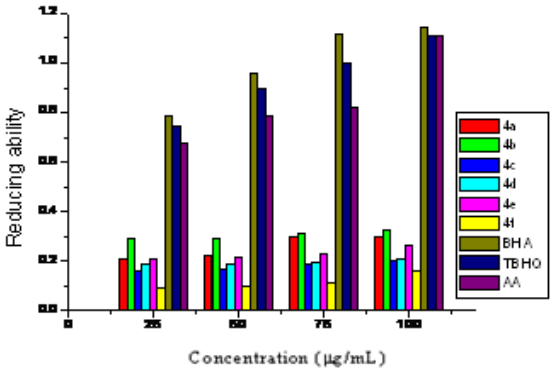


Fig 4: FRAP of compounds **(4a-f)**

The FRAP of synthesized compounds (**3** and **4**) was determined at different concentrations (25, 50, 75 and 100 µg/mL in methanol) at pH 6.6 using literature method [39]. The increase in absorbance at 700 nm indicates the increase in reducing ability of a compound. The results shown in **fig**. **3** and **4** indicated that, the compounds **3b, 3e, 4b**, and **4f** exhibited good reducing power activity.

Ferrous (Fe^{2+}) metal ion chelating activity: Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The effective ferrous ions chelators may also afford protection against oxidative damage by removing iron (Fe^{2+}) that may otherwise participate in hydroxyl radical generating Fenton type reactions [40].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

Ferric (Fe^{3+}) ions also produce radicals from peroxides although the rate is tenfold less than that of ferrous (Fe^{2+}) ions [41]. Ferrous ion is the pro-oxidant among the various species of metal ions [42]. Minimizing ferrous (Fe^{2+}) ion may afford protection against oxidative damage by inhibiting production of reactive oxygen species (ROS) and lipid production. All the newly synthesized indole derivatives **(3** and **4)** are screened for their metal chelating activity at concentration 25, 50, 75, and 100 µg/mL in methanol using reported method [43]. Ferrozine can quantitatively form a complex with ferrous ions in this method. In the presence of chelating agents the complex formation is disrupted resulting in a decrease in red color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity of the co-existing chelators. Lower absorbance indicates higher metal chelating activity.

The results (**fig-5** and **6)** are compared with the results obtained for standards BHA, TBHQ and AA. Compounds **3b, 3d, 3f, 4b,** and **4d** exhibited good metal chelating activity, whereas other compounds exhibited either moderate or poor chelating activity. These results suggested that the compounds which exhibited good chelating activity interfered with the formation of ferrous and ferrozine complex.

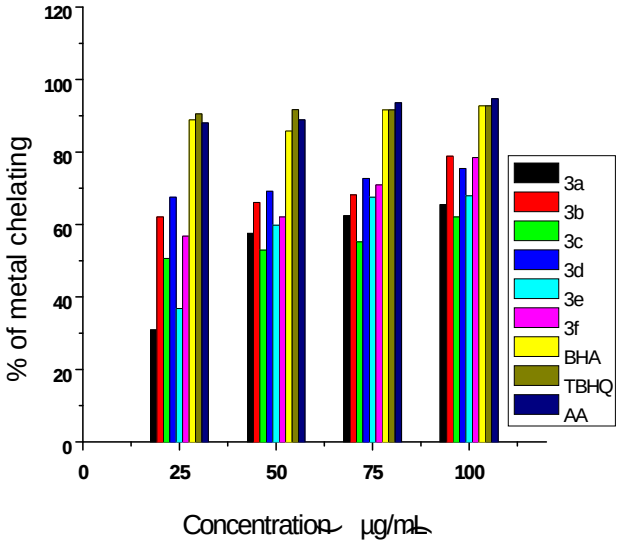


Fig 5: Metal chelating activity of compounds **(3a-f)**

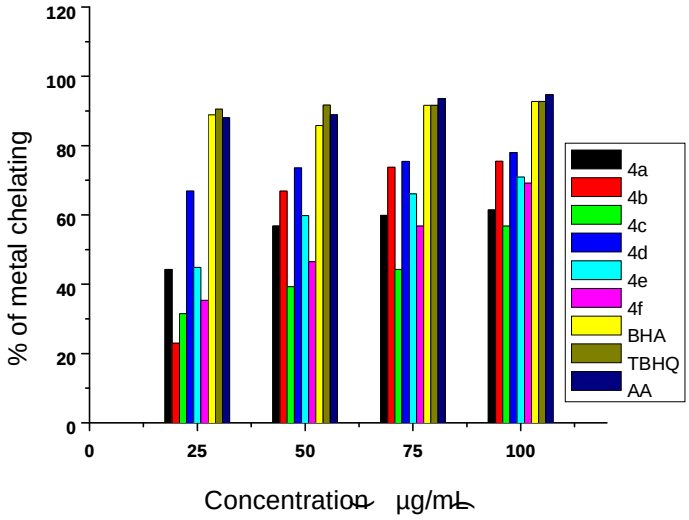


Fig 6: Metal chelating activity of compounds **(4a-f)**.

Antimicrobial Activity: All the newly synthesized compounds **(3** and **4)** are screened for their antimicrobial activity by liquid broth microdilution method [44, 45] against the bacteria *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368), *Pseudomonas aeruginosa* (MTCC-1688), and fungi *Aspergillus oryzae* (MTCC-3567^{T}), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973) and *Aspergillus terreus* (MTCC-1782) using gentamycin and fluconazole as reference standards for antibacterial and antifungal activities, respectively.

The minimal inhibitory concentration (MIC) values are obtained by the broth microdilution method (table-1). Synthesized compounds have comparable and similar inhibitory effects (low to moderate MIC values 64 and 512 µg/mL). The antibacterial activity results revealed that, compounds **3b, 4b** and **4e** showed moderate activity with MIC 128 µg/mL against *E. coli* and *S. aureus*. Compounds **3b, 3e** and **4e** showed good activity with MIC 64 µg/mL against *K. pneumonia*, compounds **3b, 3c, 3e, 4b** and **4e** exhibited moderate activity with MIC 128 µg/mL against *P. aeruginosa.* On the other hand the antifungal activity results revealed that, compounds **4b, 4c** and **4e** exhibited good activity with MIC 64, 128 and 64µg/mL against *A. oryzae* respectively. **3b, 3e, 4b, 4c,** and **4e** showed moderate activity with MIC 128 µg/mL against *A. niger* and *A. flavus*, whereas compounds **3b** and **4b** exhibited good activity with MIC 64 µg/ mL against *A. terreus.*

Table-1: *In-vitro* antimicrobial activity of compounds (**3** & **4)**

Comp. Code	Antibacterial activity (MIC µg/mL)				Antifungal activity (MIC µg/mL)			
	EC[a]	*SA*[b]	*KP*[c]	*PA*[d]	*AO*[e]	*AN*[f]	*AF*[g]	*AT*[h]
3a	512	1024	512	1024	512	256	256	256
3b	128	128	64	128	512	128	64	64
3c	512	512	256	128	1024	256	512	512
3d	256	512	1024	512	1024	512	256	256
3e	256	512	64	128	512	128	64	512
3f	512	512	256	512	256	256	256	512
4a	1024	256	512	512	256	256	256	256
4b	128	128	256	64	64	128	128	64
4c	512	512	256	256	128	128	128	256
4d	256	512	256	256	256	256	512	256

4e	128	256	64	64	64	128	128	512
4f	512	256	512	512	256	256	256	256
Gentamycin	16	08	08	08	--	--	--	--
Fluconazole	--	--	--	--	16	16	16	16

[a]EC- *Escherichia coli* (MTCC-723), [b]SA- *Staphylococcus aureus*(ATCC-29513),[c]KP-*Klebsiella pneumonia* (NCTC-13368), [d]PA- *Pseudomonas aeruginosa*(MTCC-1688)[e]AO- *Aspergillusoryzae*(MTCC-3567[T]),[f]AN-*Aspergillusniger*(MTCC-281), [g]AF-*Aspergillusflavus*(MTCC-1973), [h]AT-*Aspergillusterreus*(MTCC-1782).

Antitubercular Activity: The antitubercular activity of compounds **(3** and **4)** is assessed against *M. tuberculosis* (ATTC-27294) using the micro plate almar blue dye assay (MABA) [46]. The final drug concentrations tested are 100 to 0.2 µg/mL and the results are compared with standards pyrazinamide (MIC 3.125 µg/mL) and streptomycin (MIC 6.25 µg/mL). The MIC was defined as the lowest drug concentration which prevented a color change from blue to pink. The results are shown in table 2. Compounds **4b, 4c, 4d, 4e** and **4f** exhibited promising activity with MIC 25 µg/mL. Compound **3c, 3d, 3e and 3f** showed moderate activity with MIC 50 µg/mL. Compound **4** showed good activity than compound **3,** this may be due to the presence of isoniazide moiety in compound **4**.

Table-2: Anti-tubercular activity of compounds (**3** & **4**)

Comp. Code.	MIC[a] values (µg/mL)
3a	100
3b	100
3c	50
3d	50
3e	50
3f	50
4a	50
4b	25
4c	25
4d	25
4e	25
4f	25
Pyrazinamide	3.125
Streptomycin	6.25

Anti-Cancer Activity: Compounds (**3** and **4**) are evaluated for anticancer activity against MDA-MB-231 (Human adenocarcinoma mammary gland) cell lines using standard drugs, the results are shown in table.3. *In-vitro* growth effect of test compounds revealed that compounds **3d** and **3f** exhibited 100 % cell lysis at concentration 10 µg/mL. Whereas, the compounds **3e** and **4d** exhibited 100 % cell lysis at concentration 20 µg/mL, **3a, 4b, 4e** and **4f** exhibited 100 % cell lysis against MDA-MB-231 cell lines at concentration of 30 µg/mL.

Table-3: Anti-cancer activity of compounds (**3** & **4**)

Comp. Code	Concentration (µg/mL)	O.D. at 492nm	%of cell lysis	IC50 (µg/mL)
3a	10	0.831	75%	<10µG
3a	20	0.955	>75%	
3a	30	1.667	100%	
3b	10	0.710	>50%	<10 µG
3b	20	0.913	75%	
3b	30	0.990	>75%	
3c	10	0.435	<50%	

3c	20	0.542	50%	20 µG
3c	30	0.805	75%	
3d	10	1.333	100%	Very <10 µG
3d	20	1.548	100%	
3d	30	1.873	100%	
3e	10	1.143	>75%	<10 µG
3e	20	1.734	100%	
3e	30	1.822	100%	
3f	10	0.956	100%	Very <10 µG
3f	20	1.392	100%	
3f	30	1.752	100%	
4a	10	0.377	No lysis	20 µG
4a	20	0.606	50%	
4a	30	0.700	>50%	
4b	10	0.772	75%	
4b	20	0.965	>75%	<10 µG
4b	30	1.889	100%	
4c	10	0.546	<50%	
4c	20	0.578	50%	
4c	30	0.592	>50%	20 µG
4d	10	0.883	>75%	
4d	20	1.984	100%	
4d	30	2.047	100%	<10 µG
4e	10	0.663	50%	
4e	20	1.141	>75%	
4e	30	1.194	100%	10 µG
4f	10	0.778	50%	10 µG
4f	20	1.288	>75%	
4f	30	1.315	100%	
Control	-	0.349	No lysis	

Cell line- **MDA-MB -** Human adenocarcinoma, mammary gland.

APPLICATIONS

Antioxidant activity Assay: 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA): The radical scavenging activity (RSA) of test compounds (**3** and **4**) in methanol at different concentrations (25, 50, 75, 100 µg/mL) containing freshly prepared DPPH in methanol (0.004 % w/v) was carried out using Hatano's method [37]. The results are compared with the results obtained for standards (BHA, TBHQ and AA) All analyses are performed in triplicates and results were reported as average of triplicates. The results in percentage are expressed as the ratio of absorbance of DPPH solutions measured at 517 nm in the presence and the absence of test compounds by using ELICO SL171 mini spec spectrometer. The percentages of DPPH free radical scavenging activity of the samples is determined using the following equation:

$$\% \text{ DPPH radical scavenging} = \frac{\text{Ac-As}}{\text{Ac}} \times 100$$

Where, Ac = Absorbance of control; As = Absorbance of test sample.

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP): The reducing power of the compounds (**3** and **4**) was determined according to the literature method [39]. Different concentration of samples (25, 50, 75 and 100 µg/mL) in DMSO (1 mL) are mixed with phosphate buffer (2.5 mL, 0.2 M, pH=6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. After which a portion of trichloroacetic acid (2.5 mL, 10%) was added to the mixture and centrifuged for 10 min at 1000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1

%). Then absorbance at 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Ferrous (Fe^{2+}) metal ion chelating activity: The ferrous ion chelating activities of compounds (**3** and **4**) and standards are estimated using the method reported by Dinis and colleagues [43]. The test samples (25, 50, 75 and 100 µg/mL) in ethanol (0.4 mL) were added to ferrous chloride (0.05 mL, 2 mM) prepared in ethanol. The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM) and the volume was adjusted to 3.5 mL with ethanol and water (0.5 mL) so as to make the final total volume 4.0 mL. Ferrozine reacts with the divalent iron to forms stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then the absorbance of the solution was measured spectrophotometrically at 592 nm. All analyses were run in triplicates and results are reported as the averages of three. The percent inhibition of the ferrozine-Fe^{2+} complex formation was calculated using the formula:

$$\% \text{ Ferrous ion chelating effect} = \frac{\text{Ac-As}}{\text{Ac}} \text{ x } 100$$

Where, Ac = Absorbance of control; As = Absorbance of test sample.
The control contains $FeCl_2$ and ferrozine, complex formation molecule.

Antimicrobial Activity: The *in-vitro* antimicrobial activity of the synthesized compounds **(3** and **4)** was carried out against bacterial strains *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368) and *Pseudomonas aeruginosa (MTCC-1688)* and fungal species, *Aspergillus oryzae* (MTCC-3567^T), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973) and *Aspergillus terreus* (MTCC-1782) by serial dilutions in liquid broth method [44, 45]. The materials used were 96-well plates, suspension of microorganism (0.5 McFarland), Muller-Hinton broth (Himedia) and stock solutions of each substance to be tested (2048 µg/mL in DMSO). The following concentrations of the substances to be tested were obtained in the 96-well plates: 1024, 512, 256, 128, 64, 32, 16, and 8 µg/mL. After incubation at 37 °C for 18-24 h, the MIC for each tested substance was determined by Bio-Rad Elisa reader (micro plate reader S/N 12883).

Antitubercular Activity: The antitubercular activity of compounds (**3** and **4**) was assessed against *M. tuberculosis* $H37R_V$ strain using micro plate alamar blue dye assay (MABA) [46]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods. Briefly, sterile deionzed water (200 µL) was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received the middle brook 7H9 broth (100 µL) and serial dilution of compounds was made directly on plate. The final drug concentrations tested were (100 to 0.2 µg/mL) and compared with standards pyrazinamide (3.125 µg/mL) and streptomycin (6.25 µg/mL). Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, freshly prepared 1:1 mixture of almar blue reagent (25 µL) and tween 80 (10 %) was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC (Minimal inhibition concentration) was defined as lowest drug concentration which prevented the color change from blue to pink.

Anti-Cancer Activity: *MTT solution preparation*: 10 mg MTT in 10 ml of Hanks balanced solution.
Cell culture*:* The cell were maintained in 96-well microtiter plate containing MEM media supplemented with 10 % heat inactivated fetal calf serum (FCS) containing 5 % mixture of gentamycin, penicillin (100 units/mL) and streptomycin (100 µg/mL) in the presence of CO_2 (5%) at 37 °C for 3-4 days. After 3-4 days the supernatant was removed, MEM media was replaced with Hanks balanced solution supplemented with Gentamycine, Pencillin and Streptomycin and incubated overnight.

Cytotoxicity assay: *In vitro* growth effect of test compound was assessd by calorimetric method [47]. Determination of conversion of MTT into 'Formazon blue' by living cells. The supernatant was removed from the plate, then fresh Hanks balanced salt solution was added and treated with different concentrations of compounds diluted with DMSO. Control group contain only DMSO. After 24 h incubation at 37 ^{o}C in a humidified atmosphere of CO_2, (5 %) the medium was replaced with MTT solution (100 µg/mL, 1 mg/mL in sterile Hanks balanced solution) and kept 4 h for incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazon blue' were solubilized by adding DMSO (200 µg/ml) and absorbance was measured at λ 570 nm. The results represent the mean of three readings. The concentration at which the absorbance of treated cells was reduced by 50 % with respect to the untreated control was calculated using the following formula:

$$\text{Surviving cells (\%)} = \frac{\text{Mean absorbance of test compound}}{\text{Mean absorbance of control}} \text{ x } 100$$

CONCLUSIONS

In conclusion, we have synthesized novel indole derivatives **(3** and **4)** in moderate to good yield. The present study revealed that, compounds bearing chlorine atom is essential to exhibit better antioxidant and antimicrobial activities. Whereas, isoniazide moiety and chlorine substituent are essential to exhibit better antitubercular activities. Compounds containing methyl and chlorine attached to phenyl ring showed good anti-cancer activitity.

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