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Synthesis of novel indolyl-azetidinone and thiazolidinone derivatives as a potent antioxidant, antimicrobial and antitubercular agents

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ABSTRACT

*The present study envisaged the development of novel antioxidant, antimicrobial and antimycobacterial candidates using the indole scaffold. Several novel indole derivatives, viz., 5-substituted 3-phenyl-N-[(2-phenyl-1H-indol-3-yl) methylene]-1H-indol-2-carboxamides(**3a-c**),1-(5-substituted-3-phenyl-1H-indol-2-carboxamidoyl)-2-oxo-3-chloro-4-(2-phenyl-1H-indol-3-yl)azetidines (**4a-c**) and 2-(2-phenyl-1H-indol-3-yl)-3-(5-substituted 3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-thiazolidines(**5a-c**) were prepared. The structures of these compounds were confirmed on the basis of their elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All these newly synthesized compounds were screened for their in-vitro antimicrobial activity by cup-plate method, anti-TB activity by alamar blue dye method, Antioxidant activities: like, 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA), Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP), Ferrous (Fe^{2+}) metal ion chelating activity and in-vitro growth effect of tuberculosis activity using micro plate alamar blue dye assay (MABA). Compound **3a** exhibited good (68.14 %, IC_{50} value 50.67 µg/ml) RSA at a concentration 100 µg/ml, compound **3a** higest bsorbance of FRAP 1.071 nm at a concentration 100 µg/ml, compound **3a** showed good (57.96 %, IC_{50} value $<$25µg/ml) chelating activity at a concentration 100 µg/ml, compounds **3a, 4a** and **5a** showed highest growth inhibitory effect against all tested of bacteria E. coli, S. aureus, K. pneumonia and P. aeuroginosa. Compound **3a** exhibited potent growth inhibitory effect against M. tuberculosis H37Rv (MIC= 0.2 µg/mL). Screened in-vitro result of antioxidant, antimicrobial and antitubercular activities proved that the presence of electron-withdrawing group chlorine on the phenyl ring mostly favors the activity.*

Keywords: Indole, thiazolidinone, azetidinone, antioxidant, antimicrobial**,** antitubercular.

INTRODUCTION

Antioxidants are of great interest because of their involvement in biological and industrial processes. In general, compounds with antioxidant activity have been found to posses anticancer, anti-cardiovascular, anti-inflammatory and many other activities [1-3]. Reactive oxygen species (ROS) and free radicals are considered to be implicated in a variety of pathological events, such as cancer and aging [4-6]. An antioxidant is a molecule capable of showing or preventing the oxidation of other molecules. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even in living systems, and also participates in numerous pathological processes. A balance between concentration of ROS and natural antioxidative mechanisms in tissue is disturbed under such conditions. This may either result from an increased local production of ROS or from exhaustion of the antioxidant capacity of the tissue. In cellular oxidation reactions, super oxide radical normally is formed first, and its effect can

be magnified because it produces other kinds of cell damaging free radicals and oxidizing agents. The damaging action of the hydroxyl radical is the strongest among free radicals [7].

Indole derivatives constitute an important class of therapeutic agents in medicinal chemistry such as antimicrobial [8], antioxidant [9], antiviral [10], anti-HIV and antimalarial [11] and antituberculosis [12]. Literature survey reveals that, most of the synthesized compounds possessing -NHN=CH- group constitutes an important class of active pharmacophore, exhibits wide range of biological activities such as anticonvulsant [13], antimalarial [14], antimicrobial [15], anti-tubercular [16], antitumor [17] and antiviral [18], etc.

2-Azetidinone commonly known as β-lactam is well known heterocycle among organic and medicinal chemists [19]. Azetidinone analogues are found to exhibit a wide range of biological activities such as, antimicrobial [20], anticancer [21], antiviral [22], etc. There has been substantial interest in the chemistry of thiazolidin-4-one ring system, which is the core structure in a variety of synthetic pharmaceuticals with a broad spectrum of biological activities [23], such as antimycobacterial [24], antifungal [25], anticancer [26], antituberculosis [27] and anticonvulsant [28].

Tuberculosis (TB) remains among the worlds great public health challenges. Worldwide resurgence of TB is due to the two major problems: the acquired immunodeficiency syndrome (AIDS) epidemic, which started in the mid-1980s, and outbreak of multidrug resistant tuberculosis (MDR-TB). Thus, there is an urgent need for new anti-TB drugs with improved properties such as, enhanced activity against MDR strain, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action, the ability to penetrate host cells and exert antimycobacterial effects in the intracellular environment with greater potency and efficacy than the existing drugs [29].

In view of the above findings and in continuation of our research on the synthesis of biologically active molecules [30-34], in present investigation we report the synthesis, antioxidant, antimicrobial and atitubercular activities of novel indole derivatives.

MATERIALS AND METHODS

Chemistry

All the reagents were obtained commercially and used by further purification using standard procedures. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (Spectrum ONE) FT-IR Spectrometer. The ^{1}H and ^{13}C NMR (DMSO-d_6) spectra were recorded with a BRUKER NMR 500 and 125 MHz spectrometers, and the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were 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.

5-Substituted 3-phenyl-1H-indol-2-carboxyhydrazides (**1a-c**) were prepared by literature method [37].

5-Substituted 2-phenyl-1H-indol-3-carboxaldehydes (**2a-c**) were prepared by literature method [38].

General procedure for the synthesis of *5-substituted 3-phenyl-N-[(2-phenyl-1H-indol-3-yl) methylene]-1H-indol-2carboxamides (**3a-c**).* A mixture of the compound **1** (0.01 mol) and 2-phenyl-1*H*- indol-3-carboxaldehyde **2** (0.01 mol) containing 4-5 drops of glacial acetic acid in methanol (30 mL) was refluxed on a water bath for 8 h. Then accesses of solvent were removed under vacuum, cooled at room temperature, and decompose in to ice-cold water. The product thus separated was filtered, washed with cold water, dried and recrystallized from ethanol.

*5-Chloro-3-phenyl-N-[(2-phenyl-1H-indol-3'-yl)methylene]-1H-indol-2-carboxamide (**3a**)*

Yield: 68 %, m.p. 285-86 °C; Rf 0.75 ethyl acetate: benzene (1:3) mixture; FTIR (KBr) cm^{-1} : 3,411 (NH), 3,268 (NH), 3,054 (NH), 1,624 (C=O), 1,553 (CH=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.11 (s, 1H, indole NH); 11.31 (s, 1H, indole NH); 8.97 (s, 1H, CONH); 6.60-7.96 (m, 18H, 17Ar-H+1H, N=CH); ^{13}C NMR (DMSO-d_6, δ, ppm) 161.54, 143.16, 137.63, 136.43, 135.93, 135.69, 134.47, 131.50, 130.63, 130.21, 129.39, 128.82, 128.16, 128.08, 127.10, 126.17, 125.02, 123.51, 123.43, 119.49, 114.27, 112.77, 112.58; MS (EI) m/z 488 (M^+), 490 (M^++2); Anal.% $C_{30}H_{21}N_4OCl$: C, 73.69; H, 4.33; N, 11.46. Found: C, 73.65; H, 4.30; N, 11.48.

*5-Methyl-3-phenyl-N-[(2-phenyl-1H-indol-3'-yl)methylene]-1H-indol-2-carboxamide (**3b**)*
Yield: 63 %, m.p. 284-85 °C; Rf 0.71 ethyl acetate: benzene (1:3) mixture; FTIR (KBr) cm^{-1}: 3,418 (NH), 3,256 (NH), 3,158 (NH), 1,640 (C=O), 1,548 (CH=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.09 (s, 1H, indole NH); 11.30 (s, 1H, indole NH); 8.99 (s, 1H, CONH); 6.61-7.97 (m, 18H, 17Ar-H+1H, N=CH); 2.70 (s, 3H, CH_3); MS (EI) m/z 470 (M^+); Anal.% $C_{31}H_{24}N_4O$: C, 79.46; H, 5.16; N, 11.96. Found: C, 79.40; H, 5.12; N, 12.00.

*5-Methoxy-3-phenyl-N-[(2-phenyl-1H-indol-3'-yl)methylene]-1H-indol-2-carboxamide (**3c**)*
Yield: 65 %, m.p. 263-64 °C; Rf 0.82 ethyl acetate: benzene (1:4) mixture; FTIR (KBr) cm^{-1} : 3,420 (NH), 3,256 (NH), 3,061 (NH), 1,643 (C=O), 1,550 (CH=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.10 (s, 1H, indole NH); 11.31 (s, 1H, indole NH); 9.01 (s, 1H, CONH); 6.62-7.99 (m, 18H, 17Ar-H+1H, N=CH); 3.81 (s, 3H, OCH_3); Anal.% $C_{31}H_{24}N_4O_2$: C, 76.93; H, 5.09; N, 12.08. Found: C, 77.00; H, 5.05; N, 12.00.

General procedure for the synthesis of *1-(5-substituted 3-phenyl-1H-indol-2-carboxamidoyl)-2-oxo-3-chloro-4-(2-phenyl-1H-indol-3-yl)azetidines (**4a-c**).* To a solution of schiff's base **3** (0.02 mol) in dry benzene (30 mL) containing few drops of trietheyl amine, chloroacetyl chloride (0.02 mol) was added dropwise with stirring during 15 min at room temperature. The mixture was then refluxed for 1h. Triethylamine hydrochloride formed was filtered off and washed several times with benzene. The combined filtrate was concentrated under reduced pressure to its one fourth volumes and cooled to room temperature. The products thus separated on cooling was filtered off, dried and recrystallized from 1,4-dioxane.

1-(5-Chloro-3-phenyl-1H-indol-2-carboxamidoyl)-2-oxo-3-chloro-4-(2-phenyl-1H-indol-3-yl) azetidine (**4a**) Yield: 70 %, m.p. 195-96 °C; Rf 0.78 ethyl acetate: toluene (1:4) mixture; FTIR (KBr) cm^{-1} : 3,413 (NH), 3,312 (NH), 3,068 (NH), 1,718 (C=O), 1,640 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.99 (s, 1H, indole NH), 11.45 (s, 1H, indole NH), 9.20 (s, 1H, CONH), 7.09-8.31 (m, 17H, Ar-H), 5.53 (d, 1H, CHCl, J= 5.31 Hz), 3.53 (d, 1H, -N-CH-, J= 5.31 Hz); ^{13}C NMR (DMSO-d_6, δ, ppm) 166.96, 161.11, 136.21, 134.78, 134.27, 131.89, 131.59, 130.78, 130.65, 130.56, 129.96, 129.89, 129.11, 128.25, 127.83, 126.77, 123.43, 122.45, 122.26, 118.21, 117.43, 112.78, 112.59, 65.17, 50.11; MS (EI) m/z 564 (M^+), 566 (M^++2), 568 (M^++4); Anal.% $C_{32}H_{22}N_4O_2Cl_2$: C, 67.97; H, 3.92; N, 9.94. Found: C, 67.94; H, 4.00; N, 10.00.

1-(5-Methyl-3-phenyl-1H-indol-2-carboxamidoyl)-2-oxo-3-chloro-4-(2-phenyl-1H-indol-3-yl) azetidine (**4b**) Yield: 67 %, m.p. 213-14 °C; Rf 0.79 ethyl acetate: toluene (1:3) mixture; FTIR (KBr) cm^{-1}: 3,411 (NH), 3,298 (NH), 3,062 (NH), 1,719 (C=O); 1,638 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.01 (s, 1H, indole NH), 11.50 (s, 1H, indole NH), 9.22 (s, 1H, CONH), 7.01-8.25 (m, 17H, Ar-H), 5.58 (d, 1H, CHCl, J= 5.29 Hz), 3.58 (d, 1H, -N-CH-, J= 5.29 Hz), 2.73 (s, 3H, CH_3); MS (EI) m/z 544 (M^+), 546 (M^++2); Anal.% $C_{32}H_{25}N_4O_2Cl_2$: C, 72.72; H, 4.12; N, 9.95. Found: C, 72.77; H, 4.00; N, 10.00.

1-(5-Methoxy-3-phenyl-1H-indol-2-carboxamidoyl)-2-oxo-3-chloro-4-(2-phenyl-1H-indol-3-yl) azetidine (**4c**) Yield: 69 %, m.p. 179-80 °C; Rf 0.75 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) cm^{-1} : 3,418 (NH), 3,297 (NH), 3,065 (NH), 1,724 (C=O), 1,630 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.03 (s, 1H, indole NH), 11.48 (s, 1H, indole NH), 9.22 (s, 1H, CONH), 7.01-8.20 (m, 17H, Ar-H), 5.53 (d, 1H, CHCl, J= 5.28 Hz), 3.83 (s, 3H, OCH_3), 3.58 (d, 1H, -N-CH-,J= 5.28 Hz); Anal.% $C_{32}H_{25}N_4O_3Cl$: C, 70.65; H, 4.49; N, 9.99. Found: C, 70.70; H, 4.52; N, 10.00.

General procedure for the synthesis of *2-(2-phenyl-1H-indol-3-yl)-3-(5-substituted 3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-thiazolidines (**5a-c**).* A mixture of compounds (**3a-c)** (0.01 mol), thioglycolic acid (0.01 mol) and a pinch of anhydrous zinc chloride in dry 1,4-dioxane was refluxed for 12-14 h. The reaction mixture was then cooled and poured into ice-cold water and neutralized with sodium bicarbonate solution (10 %). The product thus separated was filtered, washed with water, dried and recrystalized from ethanol.

2-(2-Phenyl-1H-indol-3-yl)-3-(5-chloro-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-thiazolidine (**5a**) Yield: 67 %, m.p. 223-24 °C; Rf 0.88 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) cm^{-1} : 3,287 (NH), 3,058 (NH), 3,057 (NH), 1,710 (C=O), 1,621 (C=O), 697 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.98 (s, 1H, indole NH), 9.23 (s, 1H, indole NH), 8.39 (s, 1H, CONH), 7.00-8.28 (m, 17H, Ar-H), 4.08 (s, 2H, SCH_2), 3.18 (s, 1H, -N-CH-S-); ^{13}C NMR (DMSO-d_6, δ, ppm) 166.84, 161.61, 137.87, 135.15, 131.93, 131.48, 131.42, 130.81, 130.69, 130.36, 129.53, 128.97, 128.86, 128.49, 128.24, 127.94, 127.63, 127.21, 124.96, 120.75, 115.94, 115.19, 113.86, 45.50, 37.87; MS (EI) m/z 562 (M^+), 564 (M^++2); Anal.% $C_{32}H_{23}N_4O_2SCl$: C, 68.26; H, 4.12; N, 9.95. Found: C, 68.20; H, 4.15; N, 10.00.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-methyl-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-thiazolidine (**5b**)* Yield: 62 %, m.p. 240-41 °C, R_f 0.71 ethyl acetate: benzene (1:2) mixture; FTIR (KBr) cm^{-1} : 3,291 (NH), 3,065 (NH), 3,059 (NH), 1,711 (C=O), 1,616 (C=O), 695 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.97 (s, 1H, indole NH), 9.20 (s, 1H, indole NH), 8.36 (s, 1H, CONH), 7.05-8.18 (m, 17H, Ar-H), 4.08 (s, 2H, SCH_2), 3.19 (s, 1H, -N-CH-S-), 2.69 (s, 3H, CH_3); Anal.% $C_{33}H_{26}N_4O_2S$: C, 73.04; H, 4.83; N, 10.32. Found: C, 73.00; H, 4.90; N, 10.35.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-methoxy-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-thiazolidine (**5c**)* Yield 60 %, m.p. 239-40 °C, R_f 0.74 ethyl acetate: benzene (1:3) mixture; FTIR (KBr) cm^{-1} : 3,288 (NH), 3,064 (NH), 3,061 (NH), 1,718 (C=O), 1,615 (C=O), 689 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.03 (s, 1H, indole NH), 9.22 (s, 1H, indole NH), 8.34 (s, 1H, CONH), 7.01-8.24 (m, 17H, Ar-H), 4.12 (s, 2H, SCH_2), 3.80 (s, 3H, OCH_3), 3.20 (s, 1H, -N-CH-S-); Anal.% $C_{32}H_{26}N_4O_3S$: C, 70.95; H, 4.69; N, 10.03. Found: C, 71.00; H, 4.70; N, 10.00.

Antioxidant activity assay

1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA)

The free radical scavenging activity (RSA) of compounds **(3-5)** at concentrations (25, 50, 75 and 100 µg/ml) was carried out in the presence of freshly prepared solution of stable free radical DPPH (0.04 % w/v) following reported method [39]. Using 2-tert-butyl-4-methoxyphenol (butylated hydroxy anisole, BHA), 2-(1,1-dimethylethyl)-1,4-benzenediol(2-tert. butyl hydroquinone, TBHQ) and Ascorbic acid (AA) as standards. All the test analyses were performed on three replicates and results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH radical in the presence test compounds and absorption of DPPH radical in the absence of test compounds at 517 nm on ELICO SL 171 Mini Spec spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation:

$$\% \text{ DPPH Radical Scavenging} = \frac{(\text{Absorbance of control- Absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

The results are shown in the Table-1.

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP)

The reducing power of the synthesized compounds **(3-5)** was determined according to the literature method [40]. Different concentration of samples (25, 50, 75 and 100 µg/mL) in DMSO (1 mL) were 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 Xg. 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. The results are shown in the Table-2.

Ferrous (Fe^{2+}) metal ion chelating activity

The chelating activity of ferrous ions by synthesized compounds **(3-5)** was estimated by following reported method [41]. The test samples (25, 50, 75 and 100 µg/mL) in ethanolic solution (0.4 mL) were added to a solution of $FeCl_2$ (0.05 mL, 2 mM). The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM) and the total volume was adjusted to 4 mL with ethanol. Ferozine reacted with the divalent iron to form 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 562 nm. All test analyses were run in triplicate and averaged. The percentage of inhibition of the ferrozine/Fe^{2+} complex formations was calculated using the formula:

The control contains $FeCl_2$ and ferrozine, complex formation molecule. The results are shown in the Table-3.

$$\text{Ferrous ion chelating effect (\%)} = \frac{\text{Absorbance of control- Absorbance of test sample}}{\text{Absorbance of control}} \text{ x } 100$$

Antimicrobial activity

The *in-vitro* antimicrobial activity of the synthesized compounds **(3-5)** 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 cup-plate method [42] using nutrient agar and PDA as medium, respectively. The holes of 6 mm diameter were punched carefully using a sterile cork borer and these were filled with test solution (1000 µg/ml in DMF) and DMF used as control. The plates were incubated at 37 °C for 24 and 72 h in case antibacterial and antifungal activity, respectively. The zones of inhibition around the wells were determined and the averages based on triplicate measurements were recorded. The results are tabulated in the Table-4.

Antitubercular activity

The antimycobacterial activity of compounds **(3-5)** was assessed against *M. tuberculosis* H37R$_V$ strain using micro plate alamar blue dye assay (MABA) [43]. 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. The results are shown in the Table 2.

RESULTS AND DISCUSSION

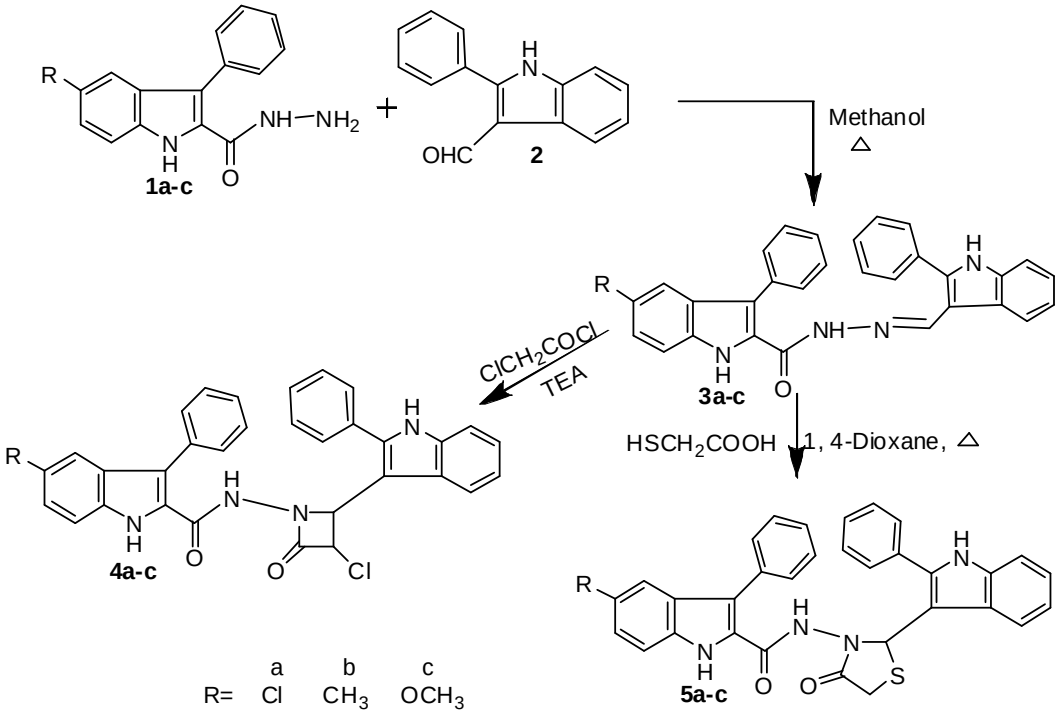
In the present work, the key intermediate 5-substituted 3-phenyl-N-[(2-phenyl-1*H*-indol-3-yl)methylene]-1*H*-indol-2-carboxamides (**3a-c)** were prepared by condensation of 5-substituted 3-phenyl-1*H*-indol-2-caboxyhydrazides (**1a-c)** with 2-phenyl-1*H*-indol-3-carboxaldehyde (**2c)** using catalytic amount of acetic acid in methanol under reflux condition. Compounds **3a-c** on cyclocondensation with chloroacetyl chloride containing catalytic amount of triethyl amine in dry benzene under reflux condition afforded 1-(5-substituted 3-phenyl-*1H*-indol-2-carboxamidoyl)-2-oxo-3-chloro-4-(2-phenyl-*1H*-indol-3-yl)azetidines (**4a-c)**. Compounds **3a-c** when subjected to cyclocondensation with mercaptoacetic acid in 1,4-dioxane under reflux temperature yielded 2-(2-phenyl-*1H*-indol-3-yl)-3-(5-substituted 3-phenyl-*1H*-indol-2-carboxmidoyl)-4-oxo-thiazolidines (**5a-c)**. Structure of the newly synthesized compounds has been accomplished on the basis of elemental analysis and spectral techniques like IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. The detailed synthetic strategy is outlined in Scheme 1. Analytical and spectral data of the synthesized compounds are given in the experimental section.

Biological activities

Antioxidant activities

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

Numbers of methods are available for the determination of free radical scavenging activity (RSA) but the assay employing the stable DPPH· has received much attention owing to its ease of use and convenience. This assay is the most widely used *in vitro* test to asses free radical scavenging capacities of test compounds. The RSA of synthesized compounds was carried out at 25, 50, 75 and 100 µg/mL concentrations in methanol using DPPH method. All the tests analyses were performed on three replicate and the results are averaged. Results are expressed as percentage decrease with respect to the control values. The results are illustrated in the table-. Compounds **3a**, **3c**, **4a**, **4c**, **5a**, **5c**, showed good RSA 68.14, 64.89, 57.22, 54.57, 59.29, 53.98 %) at concentration 100 µg/mL. The best result was obtained by compound **3a** (68.14 %, IC$_{50}$ value 50.67 µg/ml**)** when compared to standards BHA (72.28 %), TBHQ (91.46 %) and AA (94.96 %). This higher RSA may be attributed to the presence of amide and azomethine groups present in it, which may be responsible for stabilization of free radical formed after donating a hydrogen atom to DPPH free radical. However, none of the compounds exhibited better activity than the standards.



Scheme 1 Schematic pathway for the synthesis of compounds 3-5

Table-1: DPPH radical scavenging activity of compounds (3-5)

Comp. No.	Concentrations				IC_{50} (µg/ml)
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
3a	23.00	49.49	50.44	68.14	50.67
3b	31.85	43.06	59.88	62.06	<25
3c	20.64	32.44	56.04	64.89	48.73
4a	25.07	35.39	43.65	57.22	133.39
4b	17.66	25.00	39.82	46.31	530
4c	28.90	30.97	42.47	54.57	<25
5a	11.79	27.79	44.24	59.29	91.14
5b	10.02	15.92	23.89	50.73	<25
5c	14.13	30.97	41.59	53.98	109.53
BHA	74.92	79.64	84.16	86.92	<25
TBHQ	74.04	76.69	81.71	84.95	<25
AA	75.21	79.44	81.32	85.54	<25

Table-2: Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) of compounds (3-5)

Comp. No.	Concentrations			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
3a	0.661	0.778	0.821	1.071
3b	0.527	0.558	0.688	0.821
3c	0.098	0.142	0.185	0.918
4a	0.515	0.552	0.593	0.723
4b	0.085	0.135	0.171	0.234
4c	0.413	0.466	0.499	0.609
5a	0.119	0.228	0.295	0.375
5b	0.103	0.205	0.265	0.290
5c	0.619	0.645	0.701	0.864
BHA	0.849	0.901	1.112	1.205
TBHQ	0.821	0.95	1.124	1.294
AA	0.731	0.869	1.106	1.291

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP)

The reductive capacities of synthesized compounds were assessed by the extent of conversion of Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form. The reductive power of the compounds was observed at different concentrations and results were compared with standards BHA, TBHQ and AA. The reducing ability of the synthesized compounds augmented with increasing concentration of test samples with increasing the reductive ability Table-2. Compounds **3a, 3b, 3c, 4a, 4c** and **5c** reduced metal ion complexes to their lower oxidation state or take part in electron transfer reaction. In other words, these compounds showed the ability of electron donor to scavenge free radicals. The rest of the compounds showed lower absorbance as compared to the standards. The best result was obtained by compound **3a** higher bsorbance 1.071 nm, at concentration 100 µg/ml when compared to standards BHA, TBHQ and AA. The higher the absorbance of the compounds indicated greater reducing power.

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 (Calis et al., 1993):

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

Ferric (Fe^{3+}) ions also produce radical from peroxides although the rate is tenfold less than that of ferrous (Fe^{2+}) ion [35]. Ferrous ion is the pro-oxidant among the various species of metal ions [36]. Minimizing ferrous (Fe^{2+}) ion may afford protection against oxidative damage by inhibiting production of reactive oxygen species (ROS) and lipid production. Ferrozine can quantitatively form complexes 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 chelating effects of ferrous ions (Fe^{2+}) with test compounds were determined using standards BHA, TBHQ and AA. In this assay, synthesized compounds interfered with the formation of ferrous and ferrozine complex. From the Table-3 it was concluded that, compounds **3a, 3c, 4a** and **4c** exhibited (57.96 %**,** 48.41 %, 51.27 %, 44.00 % IC_{50} value <25 µg/ml), respectively, these compounds exhibited good chelating activity and are able to capture ferrous ions before ferrozine.

Table-3: Metal Chelating Activity of compounds (3-5)

Comp. No.	Concentrations				IC_{50} (µg/ml)
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
3a	41.08	48.08	54.14	57.96	<25
3b	13.69	19.20	26.75	32.16	2408.122
3c	24.52	32.80	40.44	48.41	<25
4a	37.57	32.10	48.72	51.27	<25
4b	19.10	29.93	35.66	41.08	27.41
4c	26.75	34.90	42.67	44.9	<25
5a	31.84	38.32	39.45	41.91	<25
5b	24.51	33.12	41.71	35.95	<25
5c	27.07	32.16	35.98	39.8	<25
BHA	51.59	53.82	56.05	61.11	<25
TBHQ	60.5	62.14	68.47	71.01	<25
AA	61.14	64.64	68.47	73.52	<25

Antimicrobial activity

The results of the antimicrobial evaluation are given in Table 1. Antibacterial results of the test compounds revealed that, compounds **3a, 4a** and **5a** showed highest growth inhibitory effect against all types of bacteria *E. coli*, *S. aureus*, *K. pneumonia* and *P. aeuroginosa*. This may be due to the electronegative nature of chlorine atom present at C-5 position of indole nucleus and compounds possessing -NHN=CH-, indolylazitidinone and indolylthiazolidone system linked to position-2 of indole through carboxamide bridge. Whereas, Rest of the compounds in the series exhibited moderate to less activity.

Further, the antifungal activity results indicated that the compounds **3a, 4c, 5b** and **5c** showed highest growth inhibitory effect against *A. oryzae*, compounds **3c**, **5b** and **5c** displayed highest growth inhibitory effect against *A. niger*, compounds **4a**, showed highest growth inhibitory effect against *A. flavus*, whereas, compounds **4c** and **5a**

displayed highest growth inhibitory effect against *A. terreus*. These results suggest that, compound **5a** exhibited the highest growth inhibitory effect against all tested of fugi. This may be due to the presence of electron donating methoxy group at position-5 of indole nucleus and indolylthiazolidinone system linked at position-2 of indole through carboxamide bridge. Rest of the compounds showed moderate to less activity against all tested fungal strains.

Table-4: Antimicrobial activity of synthesized compounds (3-5)

Comp. No.	Conc. µg/ml	Antibacterial activity (Zone of inhibition in mm)				Antifungal activity (Zone of inhibition in mm)			
		EC[a]	*SA*[b]	*KP*[c]	*PA*[d]	*AO*[e]	*AN*[f]	*AF*[g]	*AT*[h]
3a	1000	22	23	22	21	13	15	14	16
3b	1000	17	13	14	10	11	16	12	17
3c	1000	14	16	13	17	14	11	13	12
4a	1000	23	23	21	24	17	13	12	10
4b	1000	18	13	18	11	11	13	15	14
4c	1000	11	20	14	16	13	12	10	11
5a	1000	22	24	21	23	19	18	18	19
5b	1000	18	15	16	19	13	16	15	12
5c	1000	11	19	15	20	14	10	11	13
Gentamycin	1000	26	25	24	25	--	--	--	--
Fluconazole	1000	--	--	--	--	22	22	21	20

Data represent is mean of three replicates.
[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- Aspergillus oryzae (MTCC-3567[T]), [f]AN- Aspergillus niger (MTCC-281)
[g]AF- Aspergillus flavus (MTCC-1973), [h]AT- Aspergillus terreus (MTCC-1782)

Antimycobacterial activity

The results of the antimycobacterial evaluation are given in Table 5. Newly synthesized compounds **3a**, **3c, 4a**, **4c**, **5a** and **5c** were assayed for inhibitory activity towards *Mycobacterium tuberculosis* H37Rv (ATCC2794). The minimum inhibitory concentration (MIC expressed as µg/mL) was determined for each compound. The compound **3a** especially showed excellent growth of inhibition against *M. tuberculosis* H37Rv (MIC= 0.2 µg/ml). Compound **4a** showed excellent growth of inhibition against *M. tuberculosis* H37Rv (MIC= 6.25 µg/ml). Compounds **3c** and **5a** showed excellent good growth of inhibition against *M. tuberculosis* H37Rv (MIC= 12.5 µg/ml). In general, the brief structure–activity relationship (SAR) studies revealed that the presence of electron withdrawing group chlorine on the phenyl ring of indole moiety at C-5 position and possessing -NHN=CH-, indolylazetidinone system linked to position-2 of indole through carboxamide bridge may be attributed for enhanced antitubercular activity in the series and has emerged as promising antitubercular agents.

Table-5: Antitubercular activity of compounds (3-5)

Comp. No.	MIC[a] Value (µg/ml)
3a	0.2
3c	12.5
4a	6.25
4c	50
5a	12.5
5b	25
Pyrazinamide	3.125
Sreptomycin	6.25

[a]MIC-Minimum inhibitory concentrations.

CONCLUSION

The present study revealed that, novel indolyl-2-azetidinone and indolyl-4-thiazolidinone nucleus were prepared, in moderate to high yields, and screened result of antioxidant, antimicrobial and antitubercular activities results proved that the presence of electron-withdrawing group chlorine on the phenyl ring mostly favors the activity. The novel indole derivatives synthesized and tested in the present study were shown to be of reassuring importance for the development of new drugs. However, these are all the preliminary results and to arise at an exact conclusion further studies are required.

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