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Synthesis of novel indolyl-thiazolidinone derivatives as antioxidant, antimicrobial and atitubercular agents

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ABSTRACT

*The present study envisaged the development of novel antioxidant, antimicrobial and antitubercular candidates using the indole scaffold. Several novel indole derivatives (**3a-i**) were prepared. The structures of these compounds were confirmed by their elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds **3a-i** were screened for their in vitro antioxidant activity by various methods such as 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA), Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) and Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) antimicrobial and antitubercular activities. In vitro antioxidant, antimicrobial and antitubercular activities Compounds **3a** and **3c** exhibited good RSA and FRAP at a concentration 100 µg/mL.Compounds **3a** and **3c** exhibited good metal chelating activity at a concentration 100 µg/mL. Compound **3a** exhibited promising antitubercular activity MIC 3.125 µg/mL. Compound **3a** exhibitted potent antimicrobial activity with MIC 125 µg/mL against K. pneumonia (NCTC-13368) and Aspergillus terreus (MTCC-1782). Whereas, the compound **3a** exhibited promising antitubrcular activity against mycobacterium tuberculosis (ATCC-2794) with MIC 3.125 µg/mL.*

Keyword: Indole, thiazolidinone, azetidinone, antioxidant, antimicrobial**,** antitubercular

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INTRODUCTION

There are numerous biologically active molecules which contain various heteroatoms such as nitrogen, sulphur and oxygen, always drawn the attention of chemist over the years mainly because of their biological importance. Thiazolidinones are thiazolidine derivatives and have an atom of sulfur at position 1, an atom of nitrogen at position 3 and a carbonyl group at position 2, 4, or 5. However, its derivatives belong to the most frequently studied moieties and its presence in penicillin was the first recognition of its occurrence in nature.1,2 Similarly 1,3-thiazolidin-4-ones are heterocyclic nucleus that have an atom of sulfur and nitrogen at position 1 and 3, respectively. The importance of heterocyclic compounds has long been recognized in the field of synthetic organic chemistry. It is well known that number of heterocyclic compounds containing nitrogen and sulphur exhibit a wide variety of biological activities. The review of literature data shows that 4- thiazolidinone scaffold is very versatile and has featured in a number of clinically used drugs. They have found uses as Antibacterial, antifungal and antimycobacterial activity [1], antithyroid [2], amoebicidal [4, 5], mollusicicidal [6], anti- anticancer [7] and antidiabatic [8] activities.

Multiple antibiotic resistant bacteria represent a challenge in the treatment of infections. It is imperative, therefore, that new substances with antimicrobial properties be found to fight these microorganisms [9]. To be considered a bacterium resistant to a certain antibiotic, the microorganism should be able to grow in vitro when subjected to an

inhibitory concentration equal to that obtained in the blood. However, the concentration of several antibiotics in the bloodstream can be much lower than that achieved by the same antibiotic in other body tissues or fluids. Thus, a bacterium could be "resistant" to a certain antibiotic when it is present in the bloodstream but "sensitive" when it is in the urinary tract or vice versa [10].

Bacteria become resistant to chemotherapeutic agents by three main mechanisms: destruction or inactivation of the drug, prevention of the penetration of the target site within the microbe and alteration of drug target sites. There may be variations in these mechanisms [11]. With increasing bacterial resistance to antibiotics, attention has become focused on the development of new derivatives to be used as antimicrobial therapy in infection control [12]. Antibiotics are substances produced synthetically by bacteria and fungi with the function of suppressing the growth of microorganisms [13]. Currently, new antibiotics are needed for the treatment of multidrug resistant bacteria. The clinical use of new drugs has decreased since the 1980s, due to a reduction in the discovery of new, more efficient and less toxic drugs by pharmaceutical companies around the world. Other research groups are worried about the rise in recurrence of many infectious diseases and the lack of new drugs and development of new antimicrobial products in the face of increasing resistance to existing agents [14]. Indole shows versatile nucleus and a number of clinically used drugs. They have found posses as Antibacterial and antifungal [15, 16], antioxidant [17], anitubercular [18], antiviral [19], etc.

In the view of above mentioned facts and in continuation of our research work on the synthesis of biologically important heterocyclic compounds [20-23] we describe herein the synthesis and characterization of some substituted aryl thiazolidin-4-one derivatives and evaluation of their antioxidant, antimicrobial and antitubercular activities.

MATERIALS AND METHODS

Chemistry

Materials and methods

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 2-phenyl-1H-indol-3-carboxaldehydes (**2a-c**) were prepared by literature method [27].

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 (**3a-c**)* were prepared by literature method [28].

General procedure for the synthesis of *2-(2-phenyl-1H-indol-3-yl)-3-(5-substituted 3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(5-substituted 2-phenyl-1H-indol-3-yl) methylene)thiazolidines (**3a-i**).* Metallic sodium (0.01 mol) was added to ethanol (99%, 20 mL) with external cooling. After 30 min, the compounds **5** (0.01 mol) was added and the reaction mixture was refluxed for 5 min, followed by addition of compound **2** (0.01 mol) in ethanol (30 mL). Further contents were refluxed for 45 min. At the end of the reaction, the contents were cooled, poured into ice-cold water, and acidified with glacial acetic acid. The separated product was filtered, washed with cold water, dried and recrystallized from ethanol.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-chloro-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-(5-chloro-2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3a**)* Yield: 55 %, m.p. 159-60 °C; Rf 0.83 ethyl acetate: toluene (1:4) mixture; FTIR (KBr) cm^{-1} : 3,407 (NH), 3,164 (NH), 3,068 (NH), 1,718 (C=O), 1,622 (C=O), 1,485 (CH=C), 697 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.03 (s, 1H, indole NH); 11.13 (s, 1H, indole NH); 9.73 (s, 1H, indole NH); 8.33 (s, 1H, CONH); 7.11-8.29 (m, 25H, Ar-H); 5.33 (s, 1H, CH=C); 3.32 (s, 1H, -N-CH-S-);^{13}C NMR (DMSO-d_6, δ, ppm) 165.94 (C=O), 161.19 (C=O), 149.59, 137.43, 137.16, 136.77, 135.93, 135.63, 134.39, 131.08, 130.69, 130.82, 129.47, 128.21, 128.16, 127.10, 126.17, 125.02 (CH=C), 124.72, 123.66, 123.51, 123.43, 120.29, 117.63, 114.16, 112.77, 112.58, 101.08, 128.50, 49.82 (-N-CH-); MS (EI) m/z 799 (M^+), 801 (M^++2), 803 (M^++4); Anal.% $C_{47}H_{31}N_5O_2SCl_2$: C, 70.50; H, 3.90; N, 8.75. Found: C, 70.52; H, 3.77; N, 8.78.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-chloro-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(5-methyl-2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3b**)* Yield: 59 %, m.p. 230-31 °C; Rf 0.86 ethyl acetate: benzene (1:3) mixture; FTIR (KBr) cm^{-1}: 3,409 (NH), 3,165 (NH), 3,068 (NH), 1720 (C=O), 1,625 (C=O), 1,480 (-CH=C), 699 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.07 (s, 1H, indole NH), 11.11 (s, 1H, indole NH); 9.73 (s, 1H, indole NH), 8.29 (s, 1H, CONH), 7.13-8.22 (m, 25H, Ar-H), 5.38 (s, 1H, CH=C), 3.30 (s, 1H, -N-CH-S-), 2.71 (s, 3H, CH_3); MS (EI) m/z 779 (M^+), 781 (M^++2); Anal.% $C_{48}H_{34}N_5O_2SCl$: C, 73.88; H, 4.39; N, 8.97. Found: C, 73.92; H, 4.33; N, 8.99.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-chloro-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3c**)* Yield: 50 %, m.p. 219-20 °C; Rf 0.81 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) cm^{-1} : 3,409 (NH), 3,168 (NH), 3,061 (NH), 1,720 (C=O), 1,628 (C=O), 1,480 (-CH=C), 703 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.03 (s, 1H, indole NH); 11.09 (s, 1H, indole NH); 9.73 (s, 1H, indole NH); 8.30 (s, 1H, CONH); 7.03-8.20 (m, 26H, Ar-H); 5.40 (s, 1H, CH=C); 3.31 (s, 1H, -N-CH-S-); MS (EI) m/z 765 (M^+), 767 (M^++2); Anal.% $C_{47}H_{32}N_5O_2SCl$: C, 73.67; H, 4.21; N, 9.14. Found: C, 73.68; H, 4.18; N, 9.21.

2-*(2-Phenyl-1H-indol-3-yl)-3-(5-methyl-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(5-chloro-2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3d**)* Yield: 54 %, m.p. 188-89 °C; Rf 0.79 ethyl acetate: toluene (1:2) mixture; FTIR (KBr) cm^{-1} : 3,409 (NH), 3,164 (NH), 3,064 (NH), 1,719 (C=O), 1,620 (C=O), 1,480 (-CH=C), 695 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.01 (s, 1H, indole NH); 11.14 (s, 1H, indole NH); 9.75 (s, 1H, indole NH); 8.27 (s, 1H, CONH); 7.10-8.19 (m, 25H, Ar-H); 5.39 (s, 1H, CH=C); 3.36 (s, 1H, -N-CH-S-); 2.75 (s, 3H, CH_3); Anal.% $C_{48}H_{34}N_5O_2SCl$: C, 73.88; H, 4.39; N, 8.97. Found: C, 73.91; H, 4.44; N, 9.02.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-methyl-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(5-methyl-2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3e**)* Yield: 53 %, m.p. 210-11 °C; Rf 0.73 ethyl acetate: benzene (1:4) mixture; FTIR (KBr) cm^{-1} : 3411 (NH), 3160 (NH), 3,061 (NH), 1719 (C=O), 1621 (C=O), 1480 (-CH=C), 698 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.00 (s, 1H, indole NH); 11.21 (s, 1H, indole NH); 9.78 (s, 1H, indole NH); 8.28 (s, 1H, CONH); 7.01-8.08 (m, 25H, Ar-H); 5.41 (s, 1H, CH=C); 3.37 (s, 1H, -N-CH-S-); 2.77 (s, 3H, CH_3); 2.50 (s, 3H, CH_3); Anal.% $C_{49}H_{37}N_5O_2S$: C, 74.45; H, 4.91; N, 9.22. Found: C, 74.50; H, 4.94; N, 9.25.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-methyl-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3f**)* Yield: 55 %, m.p. 196-97 °C; Rf 0.77 ethyl acetate: toluene (1:3) mixture; FTIR (KBr) cm^{-1}: 3,418 (NH), 3,165 (NH), 3,065 (NH), 1,720 (C=O), 1,622 (C=O), 1,488 (-CH=C), 699 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.03 (s, 1H, indole NH); 11.12 (s, 1H, indole NH); 9.81 (s, 1H, indole NH); 8.40 (s, 1H, CONH); 7.06-8.19 (m, 26H, Ar-H); 5.40 (s, 1H, CH=C); 3.39 (s, 1H, -N-CH-S-); 2.78 (s, 3H, CH_3); Anal.% $C_{48}H_{35}N_5O_2S$: C, 72.29; H, 4.73; N, 9.39. Found: C, 73.34; H, 4.71; N, 9.36.

2-(2-Phenyl-1H-indol-3-yl)-3-(5-methoxy-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(5-chloro-2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3g**) Yield: 48 %, m.p. 199-200 °C; Rf 0.85 ethyl acetate: benzene (1:5) mixture; FTIR (KBr) cm^{-1} : 3,414 (NH), 3,168 (NH), 3,067 (NH), 1,715 (C=O), 1,622 (C=O), 1,479 (-CH=C), 698 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.05 (s, 1H, indole NH); 11.21 (s, 1H, indole NH); 9.73 (s, 1H, indole NH); 8.58 (s, 1H, CONH); 7.08-8.17 (m, 25H, Ar-H); 5.43 (s, 1H, CH=C); 3.89 (s, 3H, OCH_3); 3.36 (s, 1H, -N-CH-S-); Anal.% $C_{48}H_{34}N_5O_3SCl$: C, 72.40; H, 4.30; N, 8.79. Found: C, 72.46; H, 4.33; N, 8.76.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-methoxy-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(5-methyl-2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3h**)* Yield: 57 %, m.p. 213-14 °C; Rf 0.78 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) cm^{-1} : 3,416 (NH), 3,168 (NH), 3,061 (NH), 1,715 (C=O), 1,623 (C=O), 1,473 (-CH=C), 699 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.12 (s, 1H, indole NH); 11.11 (s, 1H, indole NH); 9.72 (s, 1H, indole NH); 8.51 (s, 1H, CONH); 7.09-8.21 (m, 25H, Ar-H); 5.45 (s, 1H, CH=C); 3.88 (s, 3H, OCH_3); 3.37 (s, 1H, -N-CH-S-); 2.70 (s, 3H, CH_3); Anal.% $C_{49}H_{37}N_5O_3S$: C, 75.85; H, 4.81; N, 9.03. Found: C, 75.88; H, 4.80; N, 9.04.

*2-(2-Phenyl-1H-indol-3-yl)-3-(5-methoxy-3-phenyl-1H-indol-2-carboxamidoyl)-4-oxo-5-(2-phenyl-1H-indol-3-yl)methylene)thiazolidine (**3i**)* Yield: 54 %, m.p. 194-95 °C, Rf 0.82 ethyl acetate: toluene (1:2) mixture; FTIR (KBr) cm^{-1}: 3,414 (NH), 3,165 (NH), 3,060 (NH), 1,721 (C=O), 1,621 (C=O), 1,472 (-CH=C), 695 (C-S-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.10 (s, 1H, indole NH); 11.14 (s, 1H, indole NH); 9.74 (s, 1H, indole NH); 8.51 (s, 1H, CONH); 7.08-8.22 (m, 26H, Ar-H); 5.46 (s, 1H, CH=C); 3.89 (s, 3H, OCH_3); 3.35 (s, 1H, -N-CH-S-); Anal.% $C_{48}H_{35}N_5O_3S$: C, 75.67; H, 4.63; N, 9.19. Found: C, 75.70; H, 4.65; N, 9.22.

Antioxidant activity assay

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

The free radical scavenging activity (RSA) of compounds **(3a-i)** 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 [29]. 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:

$$\% \text{ DPPH Radical Scavenging Activity} = \frac{\text{AC-AS}}{\text{AC}} \text{ x } 100$$

Where, AC- Absorbance of control, AS- Absorbance of test sample.

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

The reducing power of the synthesized compounds (**3a-i**) was determined according to the literature method [30]. 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.

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

The chelating activity of ferrous ions by synthesized compounds (**3a-i**) was estimated by following reported method [31]. 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:

$$\text{Ferrous ion chelating effect (\%)} = \frac{\text{AC-AS}}{\text{AC}} \text{ x } 100$$

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 **(3a-i)** 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 [32] 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.

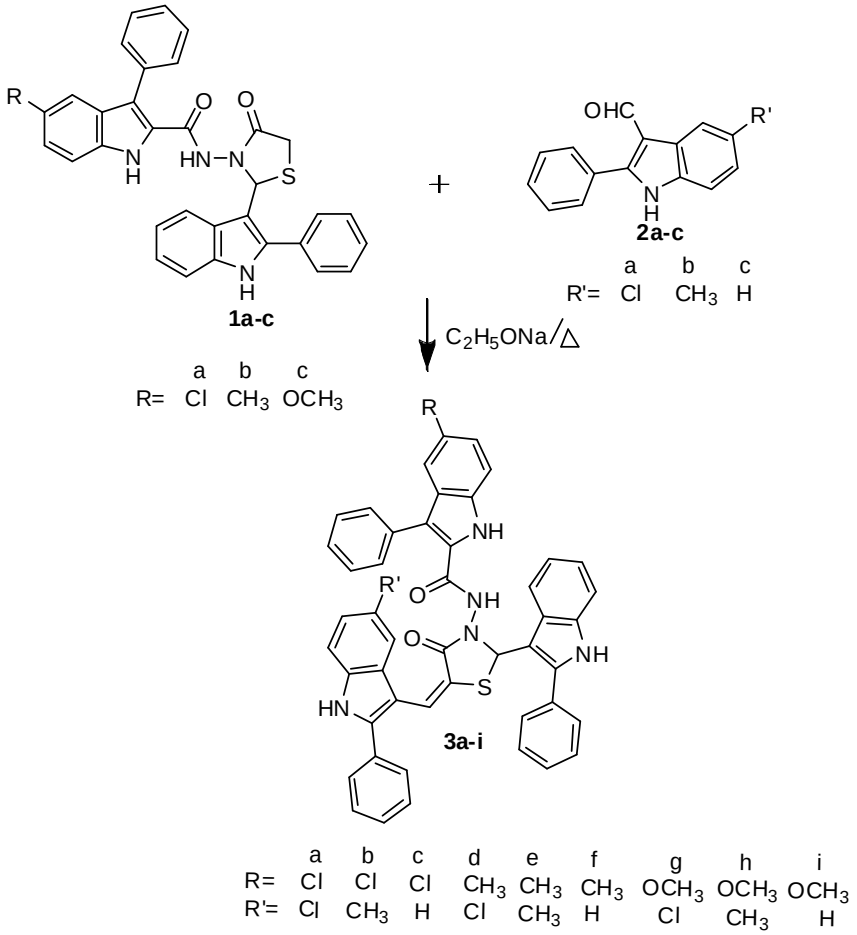
Antiubercular activity

The antitubercular activity of compounds (**3a-i**) was assessed against *M. tuberculosis* $H37R_V$ strain using micro plate alamar blue dye assay (MABA) [33]. 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.

RESULTS AND DISCUSSION

In the present work, the key intermediate 2-(2-phenyl-*1H*-indol-3-yl)-3-(5-substituted 3-phenyl-*1H*-indol-2-carboxmidoyl)-4-oxo-thiazolidines (**1a-c)** were pepared by litrature method. Compounds **1a-c** on reaction with 5-substituted 2-phenyl-*1H*-indol-3-carboxaldehydes (**2a-c)** in the presence of sodium ethoxide in ethanol at reflux temperature gave 2-(2-phenyl-1*H*-indol-3-yl)-3-(5-substituted 3-phenyl-1*H*-indol-2-carboxamidoyl)-4-oxo-5-[(5-substituted 2phenyl-1*H*- indol-3-yl) methylene]thiazolidines (**3a-i)**. Structure of all the newly synthesized compounds have been accomplished on the basis of elemental analyses and spectral techniques like IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. The detailed synthetic strategy is outlined in Scheme 1. Analytical and spectral data of the synthesized compounds are given in the experimental section.



Scheme 1 Schematic pathway for the synthesis of compounds 3a-i

RESULTS AND DISCUSSION

Biological Activities

All the synthesized compounds **3a-i** were screened for their *in vitro* antioxidant activity by various methods such as 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA), Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) and Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) antimicrobial and antitubercular activities. *In vitro* antioxidant, antimicrobial and antitubercular activities of synthesized compound are summarized in Table 1-5.

Antioxidant activities

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

The *in vitro* method of the scavenging of the stable DPPH radical is extensively used to evaluate antioxidant activity in less time than other methods. DPPH is a stable free radical that can accept hydrogen radical or an electron and must thus be converted to a stable diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons or hydrogen atoms taken up. The DPPH antioxidant assay measures the hydrogen donating capacity of the molecules under study. When the free radical DPPH is reduced by the sample its color changes from violet to yellow. Antioxidant activities of synthesized compounds by DPPH method were shown in table-1.

The RSA results suggested that compounds **3a, 3b, 3c** and **3d s**howed good radical scavenging activity due to the presence of chlorine and methyl group at 5-possition of indole nucleus when compared with the standards BHA, TBHQ and AA. Compound **3g** exhibited modate activity and compouds **3e, 3f, 3g, 3h and 3i** exhibited less RSA respectively.

Table 1 DPPH radical scavenging activity of compounds (3a-i)

Comp. No.	Concentration			
	25 µg/ml (%)	**50 µg/ml (%)**	**75 µg/ml (%)**	**100 µg/ml (%)**
3a	66.88	67.70	69.28	89.31
3b	58.48	51.23	69.97	88.60
3c	59.90	66.41	69.21	79.32
3d	63.11	67.40	65.48	88.55
3e	62.80	64.43	67.74	79.42
3f	50.14	56.19	61.98	68.31
3g	62.89	59.19	64.88	75.66
3h	59.92	61.70	67.49	71.90
3i	62.25	60.05	66.94	77.96
BHA	85.95	88.98	90.90	92.28
TBHQ	86.50	87.87	89.25	91.46
AA	88.15	90.08	92.56	94.21

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

All the newly synthesized compounds **(3a-i)** were screened for Ferric ions (Fe^{+3}) reducing antioxidant power. BHA, TBHQ and AA used as a standards. From the results of FRAP as shown in **Table 2** compounds with chlorine chlorine or chlorine substituents present at 5-postion of indole nucleus e.g., **3a** and **3c** exhibited higher absorbance 0.821 nm and 0.719 nm suggested that these compounds possesses better FRAP. The rest of the compound showed lower absorbance as compared to the standards. The higher the absorbance of the compound indicated greater reducing power.

Table 2 Ferric ions (Fe^{+3}) reducing antioxidant power activity of compounds (3a-i)

Comp. No.	Concentrations			
	25 µg/ml (nm)	**50 µg/ml (nm)**	**75 µg/ml (nm)**	**100 µg/ml (nm)**
6a	0.298	0.391	0.467	0.821
6b	0.166	0.227	0.293	0.312
6c	0.195	0.355	0.503	0.791
6d	0.212	0.361	0421	0.611
6e	0.119	0.184	0.207	0.306
6f	0.175	0.293	0.385	0.439
6g	0.185	0.267	0.386	0.464
6h	0.112	0.180	0.221	0.281
6i	0.117	0.193	0.232	0.304
BHA	0.860	0.910	1.101	1.289
TBHQ	0.802	0.949	1.101	1.295
AA	0.691	0.851	0.999	1.149

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 [24].

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

Ferric (Fe^{3+}) ions also produce radical from peroxides although the rate is tenfold less than that of ferrous (Fe^{2+}) ion [25]. Ferrous ion is the pro-oxidant among the various species of metal ions [26]. 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.

All the newly synthesized indole derivatives **(3a-i)** were screened for their metal chelating activity at concentration 25, 50, 75, and 100 µg/mL, using reported method [47]. Compounds **3a, 3b, 3c** and **3d** exhibited good metal chelating activity (75.63, 68.91, 66.63, 62.01; 100 µg/mL) **(Table 3),** suggesting that these compounds are able to capture ferrous ions before ferozine. In this assay, these compounds interfered with the formation of ferrous and ferrozine complex.

Table 3 Metal chelating activity of compounds (3a-i)

Comp. No.	Concentrations			
	25 µg/ml (%)	**50 µg/ml (%)**	**75 µg/ml (%)**	**100 µg/ml (%)**
6a	50.88	57.98	65.10	75.63
6b	48.39	54.27	66.43	68.91
6c	37.15	51.79	59.22	66.63
6d	45.29	53.96	59.53	62.01
6e	46.43	49.22	58.82	63.15
6f	33.43	38.39	47.98	52.32
6g	30.95	34.98	41.17	43.96
6h	30.03	40.24	44.58	48.91
6i	39.31	41.79	47.36	50.15
BHA	63.46	68.11	69.65	72.13
TBHQ	61.60	64.39	69.34	70.58
AA.	63.15	65.63	70.58	72.44

Antimicrobial activity

All the newly synthesized compounds **(3a-i)** were screened for their antimicrobial activity by microdilution metho 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 a reference standards for antibacterial and antifungal activities, respectively.

The minimal inhibitory concentration (MIC) values are obtained by the broth microdilution method [28]**.** The antibacterial activity results **(Table 4)** revealed that, compounds **3a** exhibitted potent activity with MIC 125 µg/mL against *K. pneumonia* NCTC- 13368, compounds **3b** and **3c** exhibited equipotent actiivyt with MIC 125, 125 250 and 125 µg/mL against *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368), *Pseudomonas aeruginosa* (MTCC-1688), *S. aureus* (ATCC-29513). respectively, against *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513).

The antifungal activity results **(Table 4)** revealed that, compounds **3a** exhibited potent activity with MIC 125 µg/mL against *Aspergillus terreus* (MTCC-1782). Compounds **3b** and **3d** showed equipotent activity with MIC 125, 125 and 250 µg/mL against *Aspergillus oryzae* (MTCC-3567^{T}), *Aspergillus flavus (*MTCC-1973), *Aspergillus terreus* (MTCC-1782). Compound 6g exhibited equipotent activity with MIC 250 µg/mL against *Aspergillus terreus* (MTCC-1782).

Table 4 *In vitro* Antimicrobial activities of compounds (3a-i)

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]
6a	125	125	125	125	125	62.5	125	125
6b	125	125	250	125	250	125	125	250
6c	125	125	250	125	125	62.5	500	500
6d	100	125	500	500	250	250	250	500
6e	500	250	500	250	125	125	125	250
6f	500	250	500	250	500	500	500	500
6g	250	500	250	125	250	500	500	250
6h	250	250	500	500	250	500	250	500
6i	250	500	250	250	500	250	250	500
Gentamycin	125	125	250	125	--	--	--	--
Fluconazole	--	--	--	--	125	62.5	125	250

[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).

Antitubercular activity

Compounds which exhibited better antibacterial activity have been selected for antitubercular activity. Compounds **3a, 3b, 3c, 3d, 3e, 3f, 3g, 3h** and **3i** were screened for the antitubercular activity against *mycobacterium tuberculosis* (ATCC-2794) and the results are given in the **Table 5.** Compound **3a** exhibited promising activity with MIC 3.125 µg/mL. Compounds **3b** and **3d** showed good activity with MIC 6.25 µg/mL, respectively. Rest of the compounds exhibited moderate activity MIC 25 µg/mL.

Table 5 Antitubercular activity of compounds (3a-i)

Comp. No.	MIC[a] values (µg/mL)
3a	3.125
3b	6.25
3c	12.5
3d	6.25
3e	25
3f	25
3f	25
3g	25
3i	25
Pyrazinamide	3.125
Streptomycin	6.25

CONCLUSION

In conclusion, we have synthesized novel indole derivatives **(3a-i)** in moderate to good yield. The present study revealed that, compounds bearing chlorine atom is essential to exhibit better antioxidant, antimicrobial and antitubercular activities. Based on these excellent results some of the compounds will be screened for anticancer activity which will be reported in due course.

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