Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 Issue 2, 2014

Research Article

SYNTHESIS OF NOVEL *N*-(ARYL) DIAZENYL THIAZOL-2-AMINES AND BEZYLIDENE-THIAZOLIDIN-4-ONES LINKED TO INDOLE NUCLEUS AS ANTIOXIDANT, ANTIMICROBIAL, ANTIMYCOBACTERIAL AND CYTOTOXIC AGENTS

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Received: 27 Dec 2013, Revised and Accepted: 14 Feb 2014

ABSTRACT

Objective: Synthesis of Some of the novel indole derivatives viz., *N*-4-aryl-*N*-{[(aryl)diazenyl](2-phenyl-1*H*-indol-3-yl)methylene}thiazol-2-amines 4a-f and 5-(4-substituted bezylidene)-3-[4-(arylthiazol-2-yl)-2-(2-phenyl-1*H*-indol-3-yl)]thiazolidin-4-ones 6a-i. The structures of all the newly synthesized compounds were characterized by their IR, PMR, CMR and mass spectral studies,

Methods: All these newly synthesized compounds were screened for their *in-vitro* antimicrobial activity by broth micro-dilution 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 cytotoxic activity was assessed by calorimetric method.

Results: Compounds 4b, 4e and 6f exhibited good radical scavenging activity (RSA) (MIC <25 µg/ml), 6a displayed good ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) at a concentration 100 µg/ml, compounds 4a and 6a exhibited good activity against all the screened bacteria and fungi, 4a exhibited excellent anti-mycobacterial activity (MIC 0.2 µg/ml), where as compound 4a, 4d and 6g showed promising cytotoxic activity (MIC <10 µg).

Conclusion: Compounds 4a and 6a exhibited potent anti-microbial activity against all the screened bacteria and fungi, compound 4a shown potent anti-mycobacterial activity.

Keywords: Indole, Thiazolidin-4-one, Antioxidant, Antimicrobial, Anti-mycobacterial, Cytotoxic activities.

INTRODUCTION

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as single oxygen, superoxide, peroxyl radical, hydroxyl radical, and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results have been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases[1]. Exposure of a normal cell to free radical is known to damage structures and consequently interfere with functions of enzymes and critical macromolecules (e.g., lipids, proteins and nucleic acids). The human body possesses innate defense mechanisms to superoxide dismutase, catalase, and glutathione peroxidase. The unbalance between formation and detoxification of free radical species results in the progression of oxidative stress and leads to the development of chronic and degenerative diseases. Therefore, inhibition of oxidative damage by supplementation with antioxidant and/or free radical scavengers might reduce the risk of these diseases[2, 3].

Cancer is one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor. Chemotherapy is a major form of cancer treatment[4]. However, the majority of cancers are either resistant to chemotherapy or acquire resistance during treatment. As a result, the design and discovery of non-traditional, efficient, and safe chemical classes of agents are the prime targets in contemporary medicinal chemistry[5, 6].

Tuberculosis is one of the serious health problems with a wide variety of manifestations caused by *Mycobacterium tuberculosis*, and as per the recent report, it has been estimated that approximately one third of the world's population is infected with this microorganism. The treatment of mycobacterial infections, especially the tuberculosis, has become an important problem due to the emergence of monodrug and multidrug-resistant strains of *M. tuberculosis*[7, 8]. Therefore, to combat the mycobacterium tuberculosis and its resistant strains there is urgent need to develop novel anti-tubercular drugs, which are safe and effective.

Indole is an important substrate used in organic synthesis[9] and its physiological activity attracts much scientific attention as well[10]. Many derivatives of indole were synthesized and their biological activities were demonstrated by *in vitro* or *in vivo* methods[11, 12]. For example, indole-3-carbinol[13], indole-3-acetic acid[14], indole ethyl isothiocyanate[15] and melatonin[16] have been investigated because of their pharmacological and physiological activities. Some of the indole analogues were found to reduce ciplatin-induced reactive oxygen species[17] and scavenge hydroxyl radical directly[18, 19]. Thiazolyl derivatives have been reported to possess anti-inflammatory and antioxidant activities[20, 21].

We have earlier reported the synthesis of indole derivatives containing formazan(**1**)[22] thiazole(**2**)[23] thiazolidinone (**3**)[24] andthiazolidinon-4-one (**4**)[25] which exhibited promising antimicrobial, antioxidant, anti-TB and cytotoxic activities.

In the light of above reports and also in continuation of our research work on the synthesis of bioactive indole derivatives, a strategy has been planned to synthesize new indole derivatives possessing, thiazole, formazan and bezylidenethiazolidin-4-one moieties, with the hope to get molecule with better biological activities.

All the newly synthesized compounds have been screened for their antioxidant, antimicrobial, anti-mycobacterial and cytotoxic activities.

MATERIALS AND METHODS

Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC using silica gel-G coated aluminum plates (Merck) and spots were visualized by exposing the dry plates to iodine vapors. The IR (KBr) spectra were recorded with a Perkin-Elmer spectrum one FT-IR spectrometer. The ^{1}H NMR (DMSO-d_6) spectra recorded on a Bruker NMR (500 MHz) and the chemical shifts were expressed in ppm (δ scale) downfield from TMS. ^{13}C NMR (125 MHz, DMSO) spectra recorded with on Bruker NMR. Mass spectra were obtained on JEOL

GCMATE II GC-MS mass spectrometer. Elemental analysis was carried out using Flash EA 1112 series elemental analyzer.

General procedure for the synthesis of 2-N-(2-phenyl-1H-indol-3-yl) imino-4-arylthiazoles (3a-c) was prepared by following the literature method[25]

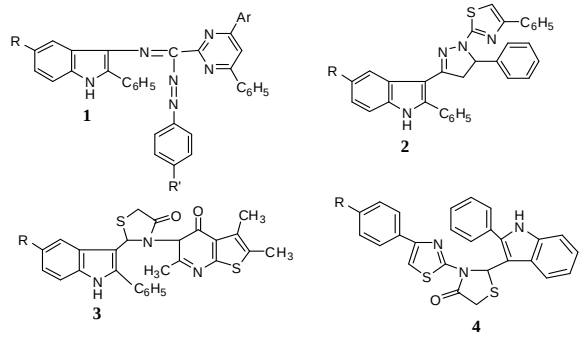


Fig.1: Motivation for the synthesis of bioactive active compounds

General procedure for the synthesis of N-4-(aryl)-N-{[(aryl) diazenyl](2-phenyl-1H-indol-3-yl)methylene}thiazol-2-amines (4a-f) was prepared by following the literature method[26]

4-Substituted aniline (0.01 mol) dissolved glacial acetic acid (5 mL) was added conc. HCl (3 mL) at 0-5 ^{0}C. Then sodium nitrite solution (1 g in 5 mL of water) was added drop wise. Prepared diazonium salt solution was added in solution of compounds (**3a-c**) (0.01 mol) in ethanol drop wise with string in pyridine (50 mL) below 0 ^{0}C. The reaction mixtures were kept at room temperature for about 4 hr, and then poured into ice-old water (250 mL). Then the resulting solid was filtered, washed with water till free from pyridine, dried and recrystalized from ethanol to afford pure (**4a-f**).

4-(4-Chlorophenyl)-*N*-{[(4-chlorophenyl) diazenyl](2-phenyl-1*H*-indol-3-yl)methylene}thiazol-2-amine(4a) Dark reddish crystals, yield 64 %, mp 138-39 ^{0}C, Rf, 0.68 ethyl acetate: toluene (1:1) mixture; FTIR (KBr)ν_{max}= 3137 (NH), 1621 (C=N), 1435 (N=N), 770 (C-Cl), 693 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.38 (s, 1H, indole NH), 6.98-8.70 (m, 17H, Ar-H), 6.18 (s, 1H, thiazole-CH); ^{13}C NMR (125 MHz, DMSO): δ = 160.59, 160.01, 140.13, 137.03, 137.61, 136.43, 135.31, 135.63, 134.02, 134.12, 131.08, 130.87, 130.62, 130.21, 126.83, 124.72, 123.43, 120.20, 119.50, 117.99, 114.35, 114.27, 112.77 & 112.5; EI-MS (70 eV) *m/z* 551 (M$^+$), 553 (M$^+$+2), 555 (M$^+$+4). Anal.Calcd for $C_{30}H_{19}N_5SCl_2$ (551.07): C, 65.22; H, 3.47; N, 12.68. Found: C, 65.30; H, 3.50; N, 12.74.

4-(4-Chlorophenyl)-*N*-{[(4-chlorophenyl) diazenyl](2-phenyl-1*H*-indol-3-yl)methylene} thiazol-2-amine(4b) Reddish crystals, yield 63 %, mp 174-75 ^{0}C, TLC: R$_f$ 0.55 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) ν_{max}= 3143 (NH), 1621 (C=N), 1436 (N=N), 775 (C-Cl), 694 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.42 (s, 1H, indole NH), 6.96-8.73 (m, 17H, Ar-H), 6.17 (s, 1H, thiazole-CH), 2.71 (s, 3H, CH$_3$); ^{13}C NMR (125 MHz, DMSO): δ = 161.09, 161.02, 148.01, 140.12, 137.11, 137.26, 136.21, 135.71, 135.63, 134.29, 134.18, 131.88, 130.87, 130.21, 130.28, 126.88, 124.62, 123.43, 120.50, 119.30, 117.79, 114.25, 114.17, 112.27 112.34 & 13.85. Anal.Calcd for $C_{31}H_{22}N_5SCl$ (531.13): C, 69.98; H, 4.17; N, 13.16. Found: C, 70.03; H, 4.20; N, 13.21.

4-(4-Chlorophenyl)-*N*-{((4-methoxyphenyl) diazenyl](2-phenyl-1*H*-indol-3-yl)methylene}thiazol-2-amine(4c) Brown crystals, yield 64 %, mp 167-68 ^{0}C, TLC: R$_f$ 0.55 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) ν_{max}= 3130 (NH); 1625 (C=N); 1439 (N=N); 1144 (C-O-C); 779 (C-Cl); 693 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.41 (s, 1H, indole NH); 7.01-8.75 (m, 17H, Ar-H); 6.18 (s, 1H, thiazole-CH); 3.74 (s, 3H, OCH$_3$); ^{13}C NMR (125 MHz, DMSO): δ = 161.00; 161.12; 148.10, 140.01; 137.20; 137.29; 136.27; 135.61; 135.33; 134.19; 134.03; 131.86; 130.65; 130.21; 130.17; 126.65; 124.53; 123.68; 120.23; 119.42; 117.33; 114.34; 114.77; 112.41, 112.12 & 55.98. Anal. Calcd for $C_{31}H_{22}N_5SOCl$ (547.12): C, 67.94; H, 4.05; N, 12.78. Found: C, 67.99; H, 4.13; N, 12.85.

***N*-{[(4-chlorophenyl)diazenyl](2-phenyl-1H-indol-3-yl)methylene}-4-p-tolylthiazol-2-amine(4d)** Dark red crystals, yield 55%, mp 143-44 ^{0}C, TLC: R$_f$ 0.49 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3142 (NH), 1622 (C=N), 1438 (N=N), 771 (C-Cl), 694 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.43 (s, 1H, indole NH), 6.99-8.75 (m, 17H, Ar-H), 6.19 (s, 1H, thiazole-CH), 2.75 (s, 3H, CH$_3$); EI-MS (70 eV) *m/z* 531 (M$^+$), 533 (M$^+$+2). Anal.Calcd for $C_{30}H_{19}N_5SCl_2$ (531.13): C, 69.98; H, 4.16; N, 13.16. Found: C, 70.06; H, 4.25; N, 13.22.

***N*-[(2-phenyl-1*H*-indol-3-yl)(p-tolyldiazenyl]methylene]-4-p-tolylthiazol-2-amine (4e)** Dark red crystals, yield 52%, mp 136-37 ^{0}C, TLC: R$_f$ 0.72 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3148 (NH), 1618 (C=N), 1435 (N=N), 669 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.46 (s, 1H, indole NH), 6.90-8.71 (m, 17H, Ar-H), 6.22 (s, 1H, thiazole-CH), 2.35 (s, 3H, CH$_3$), 2.28 (s, 3H, CH$_3$). Anal.Calcd for $C_{32}H_{25}N_5S$ (511.18): C, 75.12; H, 4.93; N, 13.69. Found: C, 75.22; H, 5.04 N, 13.78.

***N*-{[(4-Methoxyphenyl)diazenyl](2-phenyl-1*H*-indol-3-yl)methylene}-4-p-tolylthiazol-2-amine(4f)** Brown crystals, yield 49%, mp 128-29 ^{0}C, TLC: R$_f$ 0.54 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3145 (NH), 1624 (C=N), 1437 (N=N), 1140 (C-O-C), 696 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.46 (s, 1H, indole NH), 6.90-8.73 (m, 17H, Ar-H), 6.20 (s, 1H, thiazole-CH), 3.91 (s, 3H, OCH$_3$), 2.28 (s, 3H, CH$_3$). Anal.Calcd for $C_{32}H_{25}N_5OS$ (527.18): C, 72.84; H, 4.78; N, 13.27. Found: C, 72.93; H, 4.85; N, 13.33.

General procedure for the synthesis of 3-(arylthiazol-2-yl)-2-(2-phenyl-1H-indol-3-yl) thiazolidin-4-ones (5a-c) was prepared by following the literature method[24].

General procedure for the synthesis of 5-(4-substitutedbezylidene)-3-[4-(aryl) thiazol-2-yl]-2-(2-phenyl-1H-indol-3-yl)thiazolidin-4-ones (6a-i)

Metallic sodium (0.01 mol) was added to the ethanol (99 %, 25 mL) with stirring and external cooling. After 30 min, the compounds (**5a-c)** were added and the reaction mixture was refluxed for 5 min. To this reaction mixture aryl aldehyde (0.01 mol) in ethanol (99 %, 30 mL) was added and the contents were further refluxed for 45 min. After cooling the reaction mixture at room temperature poured into ice-cold water and acidified with glacial acetic acid. The precipitated product was filtered, washed with cold water, and recrystallized from ethanol to afford (**6a-i**).

5-(4-Chlorobenzylidene)-3-(4-(4-chlorophenyl) thiazol-2-yl)-2-(2-phenyl-1*H*-indol-3-yl) thiazolidin-4-one (6a) Pale yellow crystals, yield 58 %, mp 244-45 ^{0}C, TLC: R$_f$ 0.47 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3142 (NH), 1771 (C=O), 1617 (C=N), 768 (C-Cl), 700 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.44 (s, 1H, indole NH), 6.91-8.36 (m, 17H, Ar-H), 6.11 (s, 1H, thiazole-CH), 5.13 (s, 1H, C=CHAr), 3.73 (s, 1H, -N-CH-); ^{13}C NMR (125 MHz, DMSO): δ = 166.84, 160.97, 138.65, 137.87, 136.37, 135.15, 133.89, 131.93, 131.87, 130.81, 130.69, 130.36, 129.53, 128.9, 128.86, 128.49, 127.94, 127.63, 127.21, 126.40,124.96, 122.11, 115.96, 115.19, 114.27, 113.86 & 43.48; EI-MS (70 eV) *m/z* 609 (M$^+$), 611 (M$^+$+2), 613 (M$^+$+4). Anal.Calcd for $C_{33}H_{21}N_3OS_2Cl_2$ (609.05): C, 64.91; H, 3.47; N, 6.88. Found: C, 65.02; H, 3.54; N, 6.95.

3-(4-(4-Chlorophenyl) thiazol-2-yl)-5-(4-nitrobenzylidene)-2-(2-phenyl-1*H*-indol-3-yl) thiazolidin-4-one (6b) Pale yellow crystals, yield 50 %, mp 186-87 ^{0}C, TLC: R$_f$ 0.74 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3142 (NH), 1773 (C=O), 1618 (C=N), 1418, 1373 (NO$_2$), 768 (C-Cl), 696 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.43 (s, 1H, indole NH), 6.88-8.30 (m, 17H, Ar-H), 6.14 (s, 1H, thiazole-CH), 5.14 (s, 1H, C=CHAr), 3.73 (s, 1H, -N-CH-);^{13}C NMR (125 MHz, DMSO): δ = 166.41, 160.11, 148.82, 138.16, 136.90, 135.77, 133.19, 131.99, 131.55, 130.94, 130.69, 130.16, 129.35, 128.92, 128.67, 128.15, 127.94, 127.31, 127.01,126.84, 124.16, 122.44, 115.54, 115.75, 114.55, 113.21 & 44.15.Anal.Calcd for $C_{33}H_{21}N_4O_3S_2Cl$ (620.07): C, 63.81; H, 3.41; N, 9.02. Found: C, 63.94; H, 3.55; N, 9.11.

3-(4-(4-Chlorophenyl) thiazol-2-yl)-5-(4-methoxybenzylidene)-2-(2-phenyl-1*H*-indol-3-yl)thiazolidin-4-one (6c)

Brown solid,, yield 53 %, mp 99-100 ^{0}C, TLC: R_f 0.66 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) ν_{max}= 3192 (NH) 1781 (C=O), 1626 (C=N), 1141 (C-O-C), 772 (C-Cl), 743 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.46 (s, 1H, indole NH), 6.89-8.25 (m, 17H, Ar-H), 6.17 (s, 1H, thiazole-CH), 5.17 (s, 1H, C=CHAr), 3.89 (s, 3H, OCH_3), 3.69 (s, 1H, -N-CH-); ^{13}C NMR (125 MHz, DMSO): δ = 166.14, 160.93, 138.34, 137.81, 136.33, 135.25, 133.71, 131.93, 131.17, 130.40, 130.47, 130.21, 129.31, 128.13, 128.18, 128.57, 127.22, 127.14, 127.01, 126.48, 124.96, 122.77, 116.06, 115.09, 114.70, 113.63, 56. 96 & 43.17. Anal.Calcd for $C_{34}H_{24}N_3O_2S_2Cl$ (605.10): C, 67.37; H, 3.99; N, 6.93. Found: C, 67.45; H, 4.10; N, 7.05.

5-(4-Chlorobenzylidene)-2-(2-phenyl-1*H*-indol-3-yl)-3-(4-p-tolylthiazol-2-yl)thiazolidin-4-one (6d) Brown solid, Yield 55 %, mp 160-61 ^{0}C, TLC: R_f 0.57 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3143 (NH); 1765 (C=O), 1618 (C=N), 698 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.39 (s, 1H, indole NH), 6.89-8.33 (m, 17H, Ar-H), 6.14 (s, 1H, thiazole-CH), 5.17 (s, 1H, C=CHAr), 3.75 (s, 1H, -N-CH-), 2.31 (s, 3H, CH_3);EI-MS (70 eV) *m/z* 589 (M^+), 591 (M^++2). Anal.Calcd for $C_{34}H_{24}N_3OS_3$ (590.10): C, 69.20; H, 4.10; N, 7.12. Found: C, 69.31; H, 4.13; N, 7.17.

4.5.5.5-(4-Nitrobenzylidene)-2-(2-phenyl-1*H*-indol-3-yl)-3-(4-p-tolylthiazol-2-yl) thiazolidin-4-one (6e)

Pale yellow crystals, yield 51 %, mp 139-40 ^{0}C, TLC: R_f 0.66 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3148 (NH), 1768 (C=O), 1619 (C=N), 1417, 1379 (NO_2), 693 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.46 (s, 1H, indole NH), 6.85-8.28 (m, 17H, Ar-H), 6.16 (s, 1H, thiazole-CH), 5.11 (s, 1H, C=CHAr), 3.73 (s, 1H, NCH), 2.35 (s, 3H, CH_3); Anal. Calcd for $C_{34}H_{24}N_4O_3S_2$ (569.16): C, 67.98; H, 4.03; N, 9.33. Found: C, 68.05; H, 4.12; N, 9.38.

5-(4-Methoxybenzylidene)-2-(2-phenyl-1*H*-indol-3-yl)-3-(4-p-tolylthiazol-2-yl) thiazolidin-4-one (6f)

Brown solid, yield 53 %, mp 178-79 ^{0}C, TLC: R_f 0.50 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) ν_{max}= 3149 (NH), 1764 (C=O), 1622 (C=N), 1140 (C-O-C), 697 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.41 (s, 1H, indole NH), 6.83-8.21 (m, 17H, Ar-H), 6.15 (s, 1H, thiazole-CH), 5.14 (s, 1H, C=CHAr), 3.90 (s, 3H, OCH_3), 3.71 (s, 1H, -N-CH-), 2.31 (s, 3H, CH_3). Anal.Calcd for $C_{35}H_{27}N_3O_2S_2$ (585.15): C, 71.77; H, 4.65; N, 7.17. Found: C, 71.80; H, 4.73; N, 7.25.

5-(4-Chlorobenzylidene)-2-(2-phenyl-1*H*-indol-3-yl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (6g)

Yellow crystals, yield 63 %, mp 236-64 ^{0}C, TLC: R_f 0.73 ethyl acetate: toluene (1:1) mixture; FTIR (KBr) ν_{max}= 3147 (NH), 1768 (C=O), 1621 (C=N), 746 (C-Cl), 695 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.33 (s, 1H, indole NH), 6.93-8.28 (m, 18H, Ar-H), 6.18 (s, 1H, thiazole-CH), 5.19 (s, 1H, C=CHAr), 3.78 (s, 1H, -N-CH-); EI-MS (70 eV) *m/z* 575 (M^+), 577 (M^++2). Anal.Calcd for $C_{33}H_{22}N_3OS_2Cl_2$ (575.09): C, 68.80; H, 3.85; N, 7.29. Found: C, 68.83; H, 3.92; N, 7.38.

5-(4-Nitrobenzylidene)-2-(2-phenyl-1*H*-indol-3-yl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one(6h)

Yellow crystals, yield 59 %, mp 180-81^{0}C, TLC: R_f 0.55 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) ν_{max}= 3143 (NH), 1765 (C=O), 1623 (C=N), 1421, 1382 (NO_2), 693 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.37 (s, 1H, indole NH), 6.93-8.22 (m, 18H, Ar-H), 6.20 (s, 1H, thiazole-CH), 5.20 (s, 1H, C=CHAr), 3.73 (s, 1H, -N-CH-); Anal. Calcd for $C_{33}H_{22}N_4O_3S_2$ (586.11): C, 67.56; H, 3.78; N, 9.55. Found: C, 67.61; H, 3.84; N, 10.06.

5-(4-Methoxybenzylidene)-2-(2-phenyl-1*H*-indol-3-yl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (6i)

Yellow crystals, yield 61 %, mp 137-38 ^{0}C, TLC: R_f 0.63 ethyl acetate: benzene (1:1) mixture; FTIR (KBr) ν_{max}= 3146 (NH), 1769 (C=O), 1625 (C=N), 1571 (C=CHAr), 1147 (C-O-C), 699 (C-S-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.39 (s, 1H, indole NH), 6.98-8.21 (m, 18H, Ar-H), 6.18 (s, 1H, thiazole-CH), 5.19 (s, 1H, C=CHAr), 3.90 (s, 3H, OCH_3), 3.80 (s, 1H, -N-CH-). Anal.Calcd for $C_{34}H_{25}N_3O_2S_2$ (571.14): C, 71.43; H, 4.41; N, 7.35. Found: C, 71.48; H, 4.49; N, 7.3.

BIOLOGY

***In vitro* antimicrobial activity**

The *in-vitro* antimicrobial activity of all the synthesized compounds (**4** and **6**) was carried out by broth micro dilution method[27] in DMF at concentrations 500, 250, 125 and 62.5 µg/mL. Muller Hinton broth was used as nutrient medium to growth and diluted the compound suspension for the test bacteria and Saboured Dextrose broth used for fungal nutrition. Inoculums size for test strain was adjusted to 10^8 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strain employed for the activity was procured from Department of Microbiology, Gulbarga University, Gulbarga.

The compounds **4** and **6** were screened for their antibacterial activity against *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368) and *Pseudomonas aeruginosa* (MTCC-1688), as well antifungal activity against *Aspergillusoryzae*(MTCC-3567^T), *Aspergillusniger*(MTCC-281), *Aspergillusflavus*(MTCC-1973) and *Aspergillusterreus*(MTCC-1782). DMSO isused as a vehicle to get desired concentration of compounds to test upon microbial strains. The lowest concentration which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotics used for comparison in present study were gentamycin for evaluating for antibacterial activity and fluconazole for antifungal activity. The protocols are summarized in (Table-1)

Anti-TB activity using Alamar Blue Dye

The anti-mycobacterial activity of compounds **(4** & **6)** was assessed against *M. tuberculosis* $H37R_V$ strain using micro plate alamar blue dye assay (MABA)[28]. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of the middle brook 7H9 broth 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 ^{0}C for five days.

After this time, 25 µL freshly prepared 1:1 mixture of almar blue reagent and 10 % tween-80 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 Table-2.

Antioxidant activity assay

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging Activity (RSA)

The free radical scavenging activity (RSA) of compounds **(4** & **6)** 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 Hatano's method[29] using 2-tert-butyl-4-methoxyphenol (butylatedhydroxy 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 in the presence test compounds and absorption of DPPH in the absence of test compounds at λ 517 nm on ELICO SL 171 Mini Spec spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation:

$$\% \text{ ofDPPH RSA} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The results are shown in Table-3.

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

The Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) of the synthesized compounds **(4 & 6)** 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 ^{0}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 λ 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The results are shown in Table-4.

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

The chelating activity of ferrous ion by synthesized compounds **(4** & **6)** 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 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 λ 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 following formula:

$$\% \text{ of Ferrous ion Chelating} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The control contains $FeCl_2$ and ferrozine, complex formation molecule. The results are shown in Table-5.

MTT Assay

1. MTT solution preparation: 10 mg MTT in 10 ml of Hanks balanced solution.

2. *Cell culture*: The cell line were maintained in 96 wells micro titer plate containing MEM media supplemented with 10 % heat inactivated fetal calf serum (FCS), containing 5 % of mixture of gentamycin, Penicillin (100 Units/ml) and streptomycin (100 µg/ml) in the presence of 5 % CO_2at 37 ^{0}C for 3-4 days. After 3-4 days the supernatant was removed, MEM media was replaced with Hanks balanced solution supplemented with gentamycin, penicillin and streptomycin and incubated overnight.

Cytotoxic Assay

In vitro growth effect of test compound was assessed by calorimetric method[32]. 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^{0}C in a humidified atmosphere of 5 % CO_2, 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 are shown in Table-6.

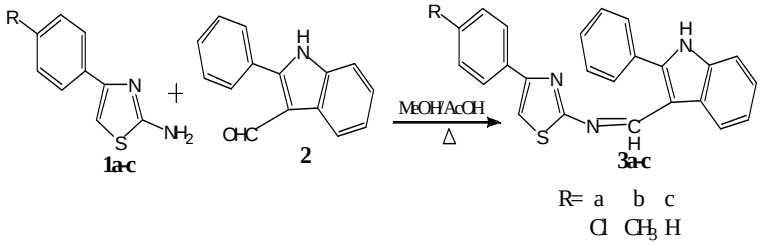
The results were represents the mean of three readings. The concentration at which the absorbance of treated cells was reduced by 50 % with respect to the untreated control was calculated using the following formula:

$$\text{Surviving Cells (\%)} = \frac{\text{Mean Optical Density of test Sample}}{\text{Mean Optical Density of Control}} \times 100$$

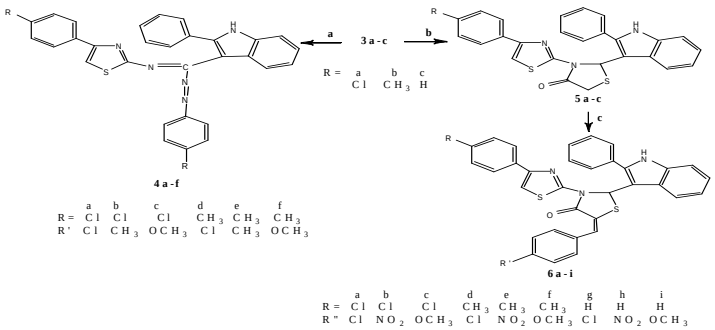
RESULTS AND DISCUSSION

Chemistry

In the present investigation, 2-*N*-(2-phenyl-1*H*-indol-3-yl)imino-4-arylthiazoles **3a-c** were prepared by condensation 4-arylthiazol-2-amines **1a-c** with 2-phenyl indol-3-carboxaldehyde **2c** by following literature method[24]



Scheme 1: Synthetic pathway of compounds 3a-c



Scheme 2: Reagents and conditions: (a) diazoniun chloride /pyridine 0 ˚C, (b) $HSCH_2COOH$/ 1,4-dioxane, reflux, 5h, (c) Sodium ethoxide/arylaldehyde, reflux, 5h

Table 1: *In-vitro* antimicrobial activity of compounds (4 & 6)

Comp. No.	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]
4a	62.5	125	125	125	125	62.5	125	250
4b	250	250	125	125	125	500	250	250
4c	100	125	500	125	500	250	250	250
4d	100	250	250	500	125	125	250	500
4e	500	250	500	250	250	125	250	500
4f	125	500	250	250	250	500	250	500
6a	62.5	125	125	125	125	62.5	125	250
6b	500	250	500	250	500	125	500	500
6c	125	500	250	250	250	125	250	250
6d	125	250	500	500	500	125	250	500
6e	250	250	500	500	250	100	250	500
6f	100	250	250	250	500	100	500	500
6g	100	250	500	500	250	250	250	500
6h	500	250	500	250	250	100	250	500
6i	500	125	500	250	500	500	500	250
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-*Aspergillusoryzae*(MTCC-3567[T]), [f]AN-*Aspergillusniger* (MTCC-281),

[g]AF-*Aspergillusflavus*(MTCC-1973), [h]AT-*Aspergillusterreus*(MTCC-1782).

Compounds **3a-c** on condensation with aryl diazonium chloride at 0-5 ^{0}C yielded *N*-4-aryl-*N*-{[(aryl)diazenyl](2-phenyl-1*H*-indol-3-yl)methylene}thiazol-2-amines **4a-f.** Compounds **3a-c** when subjected to cyclocondensation with mercaptoacetic acid in 1, 4-dioxane under reflux temperature gave 3-(4-arylthiazol-2-yl)-2-(2-phenyl-1*H*-indol-3-yl)thiazolidin-4-ones **5a-c.** Further, compounds **5a-c** on reaction with aryl aldehydes in presence of sodium ethoxide under reflux temperature in ethanol afforded 5-(4-substituted bezylidene)-3-(4-arylthiazol-2-yl)-2-(2-phenyl-1*H*-indol-3-yl)thiazolidin-4-ones **6a-i**. The detailed synthetic pathway is given in Scheme-2. The synthesized compounds were characterized by elemental analyses and spectral studies (see experimental section).

Antimicrobial activity

Antibacterial screening data (Table-1) revealed that, compounds **4a** and **6a** exhibited good activity against gram negative bacteria *E. coli* (MTCC-723) with MIC 62.5 µg/ml, compounds **6c** and **6d** exhibited good activity against gram negative bacteria *E. coli* (MTCC-723) with MIC 125 µg/ml. Compounds **4a, 4c, 6a** and **6i** showed good activity against gram positive bacteria *S. aureus* (ATCC-29513) with MIC 125 µg/ml. Compounds **4a, 4b, 6a** exhibited good activity against gram negative bacteria *K. pneumonia* (NCTC-13368) with MI*C* 125 µg/ml, compounds **6c** and **6f** showed good activity against gram negative bacteria *K. pneumonia* (NCTC-13368) with MI*C* 250 µg/ml respectively. Whereas, compounds **4a, 4b, 4c** and **6a** exhibited good activity against gram negative bacteria *P. aeruginosa* (MTCC-1688) with MIC 125 µg/ml.

Table 2: Anti-mycobacterial activity of compounds (4&6)

Comp. No.	MIC[a] Value (µg/ml)
4a	0.2
4d	12.5
4e	50
6a	12.5
6b	50
6c	50
6g	25
6i	50
Pyrazinamide	3.125
Sreptomycin	6.25

[a]MIC-Minimum inhibitory concentrations

Antifungal activity result revealed that compounds **4a, 4b, 4d** and **6a** showed good activity against *A. oryzae*(MTCC-3567[T]) with MIC 125 µg/ml respectively. Compounds **4a** and **6a** exhibited excellent activity against *A. niger* (MTCC-281) MIC 62.5 µg/ml, compounds **4a** and **6a** exhibited good activity against *A. flavus* (MTCC-1973), MIC 125 µg/ml. Whereas, the compounds **4a, 4b, 4c, 6a, 6c** and **6i** exhibited good activity against *A. flavus* (MTCC-1782) with MIC 250 µg/ml. The obtained results support that the tested compounds **4a** and **6a** exhibited excellent activity against all against gram negative bacteria *E. coli* and *K. pneumonia* this excellent activity may be due to the presence lipid solubility with hydrophilicity of chlorine atoms.

Anti-mycobacterial activity

Based on antibacterial activity results, we have selected few compounds randomly for anti-mycobacterial activity which have exhibited better antibacterial activity. Compounds **4a**, **4d**, **4e**, **6a, 6b, 6c, 6g** and **6i** were screened for their anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC2794) and results of the *in vitro* anti-mycobacterial activity screening of the test compounds are summarized in Table-2. Compound **4a**exhibited excellent anti-mycobacterial activity with MIC 0.2 µg/ml when compared with reference drugs pyrazinamide and streptomycin with (MIC 3.125 and 6.25 µg/ml), respectively. Compound **4d** and **6a** exhibited good anti-mycobacterial activity with minimum inhibition concentration 12.5 µg/ml, respectively. Compound **6e** exhibited anti-mycobacterial activity with MIC 25 µg/ml, Whereas, the compounds **6b, 6c, 6g** and **6i** exhibited moderate anti-mycobacterial activity with MIC 50 µg/ml, respectively. Compound **4a** exhibited excellent anti-mycobacterial activity with MIC 0.2 µg/ml, this may be due to the presence of chlorine atoms at 4-position thiazole phenyl and formazan phenyl ring.

Antioxidant activities

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

This *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 activity of synthesized compounds by DPPH method was shown in Table-3.

Table 3: DPPH radical scavenging activity of compounds (4 & 6)

Comp. No.	Radical scavenging activity (%) Concentration µg/ml			
	25	**50**	**75**	**100**
4a	23.00	49.49	50.44	68.14
4b	20.64	32.44	56.04	64.89
4c	31.85	43.06	59.88	61.06
4d	25.07	35.39	43.65	57.22
4e	17.66	25.00	39.82	46.31
4f	28.9	30.97	42.47	54.57
6a	14.15	30.97	41.59	53.98
6b	11.79	27.79	44.24	59.29
6c	10.02	15.92	23.89	50.73
6d	13.56	29.2	42.18	46.01
6e	25.36	32.44	43.65	52.8
6f	35.69	48.96	60.76	66.96
6g	18.28	35.98	48.08	65.78
6h	18.55	25.04	38.93	63.12
				Contd…
6i	13.27	18.18	23.89	37.16
BHA	74.92	79.64	84.66	86.92
TBHQ	74.04	76.69	81.71	84.95
AA	75.22	79.64	81.12	85.54

The RSA results suggested that compounds **4a, 4b, 4c, 6f, 6g** and **6h** exhibited good radical scavenging activity (68.14, 64.89, 61.06, 66.96, 65.78 and 63.12%) at concentration 100 µg/ml. The RSA observed in the rest of the compounds are in the following order **4a>6f>6g>4b>6h>4c** at concentration 100 µg/ml. Among the **4** series compound **4a** exhibited highest 68.14% RSA at concentration 100 µg/ml, This highest activity of compound **4a** may be due to the presence of chlorine substituent at both the phenyl rings which may donate hydrogen atom or electron to DPPH radical and so that it become stable diamagnetic molecule. The formed radical can be stabilized by delocalization. This fact confirm its good hydrogen or electron donating ability and to act as a radical scavenger. Rest of the compounds showed moderate to less activity when compared with the standard drugs. However, none of the compounds exhibited better RSA than the standards.

Table 4: Reducing Power activity of compounds (4 & 6)

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

Comp. No.	Ferric ions (Fe^{3+}) reducing antioxidant power in nm Concentration µg/ml			
	25	**50**	**75**	**100**
4a	0.151	0.178	0.221	0.279
4b	0.127	0.158	0.188	0.221
4c	0.098	0.142	0.185	0.218
4d	0.115	0.152	0.193	0.223
4e	0.085	0.135	0.171	0.234
4f	0.113	0.166	0.199	0.309
6a	0.119	0.228	0.295	0.375
6b	0.103	0.205	0.265	0.290
6c	0.119	0.245	0.301	0.364
6d	0.108	0.173	0.194	0.268
6e	0.119	0.165	0.198	0.321
6f	0.118	0.214	0.306	0.351
6g	0.099	0.143	0.186	0.242
6h	0.116	0.174	0.235	0.302
6i	0.095	0.202	0.294	0.368
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 FRAP results (Table-4) suggested that, the compounds **6a, 6c**, **6f** and **6i** showed good absorbance 0.375, 0.364, 0.351 and 0.368 nm at concentration 100 µg/ml, indicating that these compounds have good ferric ions (Fe^{3+}) reducing antioxidant power at concentration 100 µg/ml. In other words, these compounds showed the ability of electron donor to scavenge free radicals. Among the **6** series compound **6i** exhibited highest ferric ions (Fe^{3+}) reducing antioxidant power at concentration 100 µg/ml. This may be due to the presence of methoxy substituent at 4-position of bezylidine ring. The rest of the compounds showed lower absorbance as compared to the standards. The higher the absorbance of the compounds indicated greater reducing power. Ferrous (Fe^{2+}) metal ion chelating activity results (Table-5) revealed that, synthesized compounds interfered with the formation of ferrous and ferrozine complex. Compounds **4f, 6c, 6e, 6f** and **6i** exhibited (51.27, 57.96, 51.59, 54.45 and 54.55 %) good metal chelating activity at concentration 100 µg/ml. Compound **6c** showed highest (57.96 %) metal chelating activity at concentration 100 µg/ml, this may be due to the presence of chlorine atom at thiazole phenyl ring and methoxy substituent 4-position of bezylidine ring. Highest metal chelating activity of these compounds indicates that these compounds are able to capture ferrous ion before ferrozine. This might be reason for the highest the metal chelating activity. Rest of the compounds showed moderate to less activity when compared with the standard drugs.

Table 5: Metal chelating activity of compounds (4 & 6)

Comp. No.	Metal chelating activity (%) Concentrations µg/ml			
	25	**50**	**75**	**100**
4a	31.84	38.32	39.45	41.91
4b	24.52	32.80	40.44	48.41
4c	13.69	19.20	26.75	32.16
4d	19.10	29.93	35.66	41.08
4e	26.75	34.90	42.67	44.9
4f	37.57	32.10	48.72	51.27
6a	27.07	32.16	35.98	39.8
6b	24.51	33.12	41.71	35.95
6c	41.08	48.08	54.14	57.96
6d	29.29	32.16	35.98	41.08
6e	41.71	42.99	47.13	51.59
6f	38.21	46.49	51.91	54.45
6g	23.88	29.93	35.98	40.76
6h	34.39	42.03	45.85	50.63
6i	41.08	46.81	51.27	54.55
BHA	51.59	53.82	56.05	61.11
TBHQ	60.5	62.14	68.47	71.01
AA	61.14	64.64	68.47	73.52

Table 6: Cytotoxic activity of compounds (4 & 6)

Comp. No.	Concentration (µg)	O.D at 492 nm	% of cell lysis	IC_{50}
4a	10	0.735	100%	Very <10
	20	1.092	100%	µg
	30	0.693	100%	
4d	10	0.998	100%	Very <10
	20	1.528	100%	µg
	30	2.303	100%	
4e	10	0.693	50%	10 µg
	20	0.721	75%	
	30	0.967	>75%	
6b	10	0.431	75%	10 µg
	20	0.433	>75%	
	30	0.451	100%	
6c	10	0.830	75%	10 µg
	20	1.213	75%	
	30	1.701	100%	
6g	10	0.940	100%	Very <10
	20	2.168	100%	µg
	30	2.604	100%	
6i	10	1.475	50%	
	20	1.797	75%	10 µg
	30	1.973	100%	

Cytotoxic activity

Cytotoxic evaluation results are given in Table 6. In general it has been observed that, the compounds having antioxidant activity are

found to exhibit anticancer activity[32], based on antioxidant activity results, compounds **4a**, **4d**, **4e**, **6b**, **6c**, **6g** and **6i** were evaluated for anticancer activity against A549 (Human Lung Adenocarcinoma) cell lines. The compounds **4a**, **4d** and **6g** were found MIC Very <10 µg. These compounds exhibited good cytotoxicity due to the presence of chloro substitution at phenyl ring. Rest of the test compounds exhibited moderate to less cytotoxicity when compared with the standard drug doxorubicin.

CONCLUSION

In summary, series of novel indole derivatives were prepared, in moderate to high yields. Thus, the preliminary results showed that, majority of compounds having lipophilic nature of chlorine atoms plays an important role for better activities.

ACKNOWLEDGEMENT

The authors are thankful to the Chairman, Department of Chemistry and Microbiology, Gulbarga University, Gulbarga, for providing laboratory facilities, to the Principal, M. M. N. G. H. Institute, Belgaum-10, Karnataka, for carrying out anti-mycobacterial and cytotoxic activities, and to the Director of IIT, Chennai for providing ^{1}H, $^{13}CNMR$ and Mass spectra. One of us (P.W.) is thankful to CSIR, New Delhi for providing the financial support as a CSIR-SRF.

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