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Synthesis of novel (2*H*) indazole scaffold as an antimicrobial and anti-tubercular agent

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Abstract: In present development of drug research, indazole and its derivatives are being considered as useful and promising leads due to their potentials to exhibit biological activities such as antibacterial and antitubercular activities. Different indazole derivatives **(5a-o)**. Were synthesized by cyclization of substituted chalcones with methyl acetoacetate in acidic conditions to form cyclohexanone derivatives **(4a-o)** they react with hydrazine in acidic condition to form. The reactions were performed under conventional heating. All the synthesized compounds were characterized by their spectral study (IR, MS, ^{1}H and ^{13}C NMR) and were tested for their antibacterial, anti-fungal and anti-tubercular activity.

Keywords: chalcones, indazole, antimicrobial activity, antitubercular activity

1. INTRODUCTION

Indazole is nitrogen containing five-member heterocycles that forms a structurally various indazole nucleus have aroused great interest due to their wide range of biological importance. A large number of these compounds and their derivatives have been described in the chemistry and biological literature. Indazole belongs to the azoles family containing two nitrogen atoms. Indazoles are also called as isoindazolone heterocyclic organic compounds. The indazole ring has two nitrogen atoms and can be functionalized with high selectivity at different positions. Indazole nucleus is present in naturally occurring alkaloids. Indazole based heterocycles like indazolo pyrimidines and their derivatives are found to have a wide range of biological activities. Previous research on indazole derivatives are known to be active as cancer cell proliferative disorders, alzheimers disease, protein kinase inhibitors and viral infections. In recent years, some of the indazole ring systems are being evaluated as potential drugs for variety of physiological activities[1]. Indazole possess similar general properties in a wide spectrum of biological activities such as antibacterial[2, 3, 4], anti-fungal[5], anti-tubercular[6, 7], antiviral[8], anti-hypertensive[9], antioxidant[2, 10],

anti-depressant[11], neuroprotective[12], anti-diabetic[13], anti-inflammatory[14] and anticancer[15].

Chalcone is a generic term for the compounds bearing the 1, 3- diphenyl-prop-2-en-1-one framework [16]. Under homogeneous conditions, these compounds are usually prepared by base or acid catalyzed aldol condensation between aromatic aldehydes and ketones[17]. Chalcones represent an important class of compounds due to their chemical flexibility, as synthons for the production of five and six-member ring systems[18] for example Pyrazolines[19], Pyrazoles[20], isoxazolines[21], pyrimidine[22,23,24], di-aryl cyclohexenones [25]. Indazole derivatives From a chemical point of view, an important characteristic of chalcones and their hetero-analogs is the ability to act as activated unsaturated systems in conjugated addition reactions of carbanions in the presence of basic catalysts[26]. Fused indazole synthesized using low-valent titanium [27]. This inspired the development of new synthesis and optimization as well as functionalization of indazole ring. Study of heterocyclic compounds, with the function to perform a large series of biological screening.

Present study, the synthetic approach for the preparation of fused indazole derivatives involves three steps, which is described as follows. Condensation of the 1-(2-hydroxy-4-isobutoxyphenyl) ethanone and the substitute benzaldehyde in the presence of sodium hydroxide yields the respective 3-(substituted-phenyl)-1-(2-hydroxy-4-isobutoxy phenyl) prop-2-en-1-one. Their reaction with ethyl acetoacetate in the presence of anhydrous K_2CO_3 gives methyl 6-(4-substituted phenyl)-4-(2-hydroxy-4-isobutoxyphenyl)-2-oxocyclohex-3-enecarboxylate (4 a-0). The formed ketones on react with hydrazine hydrate in the presence of catalytic amount of glacial acetic acid in reflux in ethanol yield 6-(2-hydroxy-4-isobutoxyphenyl)-4-(substituted phenyl)-4,5-dihydro-2*H*-indazol-3(3a*H*)-one, respectively (5 a-o) (Table-1). We wish to report the development of indazoles derivatives thus paving the way for a novel class of antimicrobial and anti-tubercular properties of the synthesized compounds are also presented.

2. EXPERIMENTAL SECTION

2.1. *Materials*

Melting points were determined in open capillary on an electro thermal apparatus and were uncorrected. 1-(2-hydroxy-4-isobutoxyphenyl) ethanone was prepared by reported procedure. Other reagent from Spectrochem, India, were of analytical grade and used without further purification. TLC was performed on silica gel PF_{254} plates (Merck).

2.2 Methods

2.2a General procedure conventional synthesis of 3-(substituted-phenyl)-1-(2-hydroxy-4-isobutoxy phenyl) prop-2-en-1-one (3a-o).

A mixture of 10 mmol of (1) and 10 mmol of substituted aldehyde (2a-o) in methanol (40 ml) was stirred at room temperature for 24 hours using 20% NaOH as a catalyst to make the solution alkaline the reaction was monitored by continuous TLC. The reaction mixture was poured in ice cold water. The crude 3-(substituted-phenyl)-1-(2-hydroxy-4-isobutoxy phenyl) prop-2-en-1-one (3a-o) was isolated and recrystallized from hot absolute ethanol. TLC solvent system is MDC : Methanol (9 : 1).

2.2b General procedure conventional synthesis of methyl 6-(4-substituted phenyl)-4-(2-hydroxy-4-isobutoxyphenyl)-2-oxocyclohex-3-enecarboxylate (4a-o).

A mixture of 10 mmol of (3a-o), 15 mmol

methyl acetoacetate and 40 mmol anhydrous K_2CO_3 were taken into dry acetone (50 ml). The reaction mixture was stirred at room temperature for 5 hours and it was left at room temperature overnight. The reaction was monitored by continuous TLC. After reaction complies, the reaction mixture was filtered and the filtrate was evaporated. The residue obtained was recrystallized from ethanol as pale yellow crystals. TLC solvent system is Toluene : Ethyl acetate : Methanol (6 : 5 : 1).

2.2c General procedure conventional synthesis of 6-(2-hydroxy-4-isobutoxyphenyl)-4-(substituted phenyl)-4,5-dihydro-2H-indazol-3(3aH)-one (5a-o).

A mixture of 10 mmol of (4a-o), 20 mmol hydrazine hydrate and catalytic amount of glacial acetic acid were refluxed in ethanol (40 ml) for 6 hours. The reaction was monitored by continuous TLC. After reaction complies, the mixture was cooled and settled down for 30 min the residue was obtained filtered and recrystallized from methanol as colorless crystals. TLC solvent system is Hexane : Ethyl acetate : Methanol (7: 8 : 1). The same reaction was carried out on oil bath. The (5a-o) was also synthesized similarly; the physical data are recorded in Table-1.

2.3 Characterization

^{1}H and ^{13}C NMR spectra were recorded on Mercury plus 300 MHz NMR spectrometer. Chemical shifts are expressed in δ ppm downfield from internal TMS as reference. ^{1}H NMR data are reported in order: multiplicity (bs, broad singlet; s, singlet; d, doublet; t, triplet; m, multiplet). IR spectra were recorded on Parkin-Elmer Spectrophotometer; Frequency range: 4000-500cm^{-1} (KBr disk). Mass spectra were recorded JEOL-AccuToF JMS-T100 LC Mass spectrophotometer. Elemental analysis was performed on a Carlo Erba EA 1108 elemental analyzer. The results of elemental analyses (C, H, and N) were within ± 0.4% of the theoretical values.

2.3a 6-(2-hydroxy-4-isobutoxyphenyl)-4-phenyl-4,5-dihydro-2H-indazol-3(3aH)-one(5a). Yield: 70%; MP: 215-218^{0}C; IR (ν_{max}, cm^{-1}): 3370 (−OH str. of Phenolic OH), 3293 (N−H str. of Sec. Amine), 3040 (C=C−H str.), 2958 (C−H str.), 1650 (C=O str.), 1590 (C=C− str.), 1231 (C−O−C str. of ether), 880 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.90 (6H, d, *J* = 7.5 Hz), 1.87 (1H, m), 2.64(1H, dd *J* = 2.9, 14.6 Hz), 2.91 (1H, dd, *J* = 3.4, 11.2 Hz), 3.65 (2H, d, *J* = 7.2 Hz), 3.63 (1H, d, *J* = 10.1Hz), 3.99 (1H, dd, *J* = 3.1, 8.2 Hz), 6.40-6.52 (2H, m), 6.62 (1H, s), 6.99-7.1 (1H, m), 7.28- 7.41 (5H, m), 7.99 (1H, s), 9.9 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.42, 28.10, 34.12, 51.40, 74.89, 103.27, 106.70, 110.41, 125.08, 126.41, 128.28, 147.06, 155.90, 160.94, 178.03; MS, m/z (M + 1) : 377; Elemental Analysis $C_{24}H_{26}N_2O_4$: Calculated (%): C, 73.38; H, 6.43; N, 7.44, Found (%): C, 73.35; H, 6.45; N, 7.40.

2.3b 4-(2-chlorophenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5b).

Yield: 69%; MP: 165167^{0}C; IR (ν_{max}, cm^{-1}): 3364 (−OH str. of Phenolic OH), 3298 (N−H str. of Sec. Amine), 3032 (C=C−H str.), 2935 (C−H str.), 1642 (C=O str.), 1593 (C=C− str.), 1228 (C−O−C str. of ether), 887 (C−H .mul. def.), 730 (C-Cl str.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.91 (6H, d, *J* = 6.8 Hz), 1.92 (m, 1H), 2.70 (1H, dd *J* = 2.6, 12.3 Hz), 2.98 (1H, dd, *J* = 5.3, 14.4 Hz), 3.62 (2H, d, *J* = 8.2 Hz), 3.64 (1H, d, *J* = 9.1 Hz), 4.08 (1H, dd, *J* = 2.4, 7.7 Hz), 6.19-6.27 (2H, m), 6.62 (1H, s), 6.70-6.83 (2H, m), 6.99- 7.10 (3H, m), 7.89 (1H, s), 9.3 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.28, 28.25, 31.81, 37.70, 47.71, 74.48, 103.90, 107.01, 111.57, 114.17, 124.29, 127.81, 128.24, 128.89, 129.81, 133.34, 145.92, 148.31, 156.31, 158.92, 162.59, 179.12; MS, m/z (M + 1) : 312; Elemental Analysis $C_{23}H_{23}ClN_2O_3$: Calculated

(%): C, 67.23; H, 5.65; N, 6.82; Cl, 8.63, Found (%): C, 67.21; H, 5.62; N, 6.80; Cl, 8.64.

2.3c 4-(4-chlorophenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5c).

Yield: 68%; MP: 185-188^{0}C; IR (ν_{max}, cm^{-1}): 3374 (−OH str. of Phenolic OH), 3288 (N−H str. of Sec. Amine), 3033 (C=C−H str.), 2938 (C−H str.), 1648 (C=O str.), 1590 (C=C− str.), 1230 (C−O−C str. of ether), 881 (C−H .mul. def.), 729 (C-Cl str.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.93 (6H, d, *J* = 6.9 Hz), 1.90 (m, 1H), 2.68(1H, dd *J* = 2.5, 12.7 Hz), 2.92 (1H, dd, *J* = 5.4, 14.3 Hz), 3.64 (2H, d, *J* = 8.8 Hz), 3.68 (1H, d, *J* = 9.5 Hz), 4.02 (1H, dd, *J* = 2.3, 7.9 Hz), 6.35-6.40 (2H, m), 6.62 (1H, s), 6.96-7.14 (2H, m), 7.20- 7.32 (3H, m), 7.99 (1H, s), 9.9 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.18, 28.22, 32.10, 36. 04, 48.51, 73.89, 104.67, 106.80, 112.45, 114.10, 125.20, 128.10, 128.24, 128.89, 130.80, 133.74, 142.90, 147.81, 154.90, 159.05, 163.02, 180.03; MS, m/z (M + 1) : 312; Elemental Analysis $C_{23}H_{23}ClN_2O_3$: Calculated (%): C, 67.23; H, 5.65; N, 6.82; Cl, 8.63, Found (%): C, 67.25; H, 5.66; N, 6.78; Cl, 8.61.

2.3d 4-(3-chlorophenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5d).

Yield: 70%; MP: 150-152^{0}C; IR (ν_{max}, cm^{-1}): 3372 (−OH str. of Phenolic OH), 3310 (N−H str. of Sec. Amine), 3050 (C=C−H str.), 2948 (C−H str.), 1633 (C=O str.), 1576 (C=C− str.), 1235 (C−O−C str. of ether), 882 (C−H .mul. def.), 710 (C-Cl str.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.92 (6H, d, *J* = 6.6 Hz), 1.90 (1H, m), 2.690 (1H, dd *J* = 3.3, 14.6 Hz), 2.95(1H, dd, *J* = 4.9, 13.8 Hz), 3.57 (2H, d, *J* = 7.1 Hz), 3.66 (1H, d, *J* = 10.3 Hz), 3.96 (1H, dd, *J* = 3.2, 8.7 Hz), 6.19-6.27 (2H, m), 6.62 (1H, s), 6.60-6.76 (2H, m), 7.21- 7.40 (3H, m), 7.03 (1H, s), 10.2 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.13, 28.23, 31.80, 36.3, 46.89, 73.90, 104.30, 105.84, 110.97, 115.12, 125.39, 126.41, 128.14, 128.89, 129.81, 136.32, 146.42, 149.24, 156.31, 158.42, 163.09, 178.03; MS, m/z (M + 1) : 312; Elemental Analysis $C_{23}H_{23}ClN_2O_3$: Calculated (%): C, 67.23; H, 5.65; N, 6.82; Cl, 8.63, Found (%): C, 67.24; H, 5.63; N, 6.80; Cl, 8.66.

2.3e 4-(3-bromophenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5e).

Yield: 67%; MP: 186-189^{0}C; IR (ν_{max}, cm^{-1}): 3382 (−OH str. of Phenolic OH), 3305 (N−H str. of Sec. Amine), 3034 (C=C−H str.), 2938 (C−H str.), 1652 (C=O str.), 1590 (C=C− str.), 1222 (C−O−C str. of ether), 882 (C−H .mul. def.), 554 (C-Br str.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.94 (6H, d, *J* = 6.4 Hz), 1.89 (1H, m), 2.73 (1H, dd *J* = 3.2, 11.8 Hz), 2.94 (1H, dd, *J* = 4.6, 12.9 Hz), 3.56 (2H, d, *J* = 7.9 Hz), 3.61 (1H, d *J* = 9.1 Hz), 4.01 (1H, dd, *J* = 2.1, 8.1 Hz), 6.20-6.29 (2H, m), 6.63 (1H, s), 6.69-6.76 (2H, m), 7.04- 7.14 (3H, m), 7.90 (1H, s), 9.9 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.38, 28.21, 32.45, 36.93, 48.74, 73.48, 102.89, 106.13, 110.89, 115.23,122.7, 124.18, 126.14, 127.23, 128.81, 131.39, 147.94, 149.31, 154.39, 157.30, 164.12, 176.89; MS, m/z (M + 1) : 456; Elemental Analysis $C_{23}H_{23}BrN_2O_3$: Calculated (%): C, 60.67; H, 5.09; N, 6.15; Br, 17.55, Found (%): C, 60.64; H, 5.07; N, 6.10; Br, 17.69.

2.3f 6-(2-hydroxy-4-isobutoxyphenyl)-4-(3-nitrophenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5f).

Yield: 61%; MP: 220-223^{0}C; IR (ν_{max}, cm^{-1}): 3373 (−OH str. of Phenolic OH), 3290 (N−H str. of Sec. Amine), 3045 (C=C−H str.), 2930 (C−H str.), 1648 (C=O str.), 1543 (N=O Str.) 1590 (C=C str.), 1224 (C−O−C str. of ether), 881 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.92 (6H, d, *J* = 7.2 Hz), 1.89 (1H, m), 2.74 (1H, dd *J* = 3.2, 13.4 Hz), 2.94 (1H, dd, *J* = 4.9, 15.2 Hz), 3.60 (2H, d, *J* = 7.9 Hz), 3.63 (1H, d, *J* = 9.8 Hz), 4.01 (1H, dd, *J* = 3.4, 7.9 Hz), 6.39-6.45 (2H, m), 6.58 (1H, s), 7.52-7.69 (3H, m), 8.01- 8.10 (2H, m), 7.03 (1H, s), 9.0 (1H, s);^{13}C

NMR (75 MHz, $CDCl_3$), 19.48, 27.26, 32.30, 50.01, 75.07, 104.06, 105.71,108.92, 120.72, 127.02, 129.08, 131.34, 147.92, 149.06, 153.30, 157.09, 162.39, 176.42; MS, m/z (M + 1) : 447; Elemental Analysis $C_{23}H_{23}N_3O_5$: Calculated (%): C, 65.55; H, 5.55; N, 9.97, Found (%): C, 65.52; H, 5.52; N, 9.94.

2.3g 6-(2-hydroxy-4-isobutoxyphenyl)-4-(p-tolyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5g).

Yield: 68%; MP: 195197^{0}C; IR (ν_{max}, cm^{-1}): 3380 (−OH str. of Phenolic OH), 3278 (N−H str. of Sec. Amine), 3030 (C=C−H str.), 2938 (C−H str.), 1642 (C=O str.), 1593 (C=C str.), 1220 (C−O−C str. of ether), 887 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.90 (6H, d, *J* = 7.1 Hz), 1.91 (1H, m), 2.29 (3H, s), 2.70 (1H, dd *J* = 2.9, 12.2 Hz), 2.91 (1H, dd, *J* = 3.8, 14.1 Hz), 3.50 (2H, d, *J* = 8.2 Hz), 3.66 (1H, d *J* = 8.8 Hz), 3.97 (1H, dd, *J* = 2.5, 9.2 Hz), 6.32-6.41 (2H, m), 6.63 (1H, s), 7.02-7.15 (5H, m), 7.71 (1H, s), 9.43 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.15,20.22, 26.42, 27.43, 32.23, 51.31, 72.52, 104.21, 105.06, 109.29, 127.32, 129.25, 134.39, 143.69, 154.38, 155.92, 159.74, 164.12, 179.53; MS, m/z (M + 1) : 391; Elemental Analysis $C_{23}H_{24}N_2O_3$: Calculated (%): C, 73.82; H, 6.71; N, 7.17, Found (%): C, 73.80; H, 6.70; N, 7.21.

2.3h 6-(2-hydroxy-4-isobutoxyphenyl)-4-(4-methoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5h)

Yield: 67%; MP: 127-129^{0}C; IR (ν_{max}, cm^{-1}): 3369 (−OH str. of Phenolic OH), 3302 (N−H str. of Sec. Amine), 3030 (C=C−H str.), 2938 (C−H str.), 1647 (C=O str.), 1598 (C=C− str.), 1221 (C−O−C str. of ether), 876 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.93 (6H, d, *J* = 6.8 Hz), 1.96 (m, 1H), 2.75 (1H, dd *J* = 2.4, 12.6 Hz), 3.08 (1H, dd, *J* = 6.3, 13.8 Hz), 3.62 (2H, d, *J* = 7.5 Hz), 3.66 (3H, s), 3.69 (1H, d, *J* = 9.9 Hz), 4.02 (1H, dd, *J* = 2.4, 5.7 Hz), 6.29-6.37 (2H, m), 6.62 (1H, s), 6.73-6.81 (2H, m), 6.99- 7.10 (3H, m), 7.91 (1H, s), 9.58 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$), 19.21, 27.92, 28.85, 33.36, 53.06, 56.07, 73.81, 102.09, 105.46, 108.81, 113.92, 125.4, 128.3, 142.4, 149.92, 156.43, 156.79, 157.24, 162.87, 177.79; MS, m/z (M + 1) : 407; Elemental Analysis $C_{24}H_{26}N_2O_4$: Calculated (%): C, 70.92; H, 6.45; N, 6.89, Found (%): C, 70.94; H, 6.47; N, 6.85.

2.3i 6-(2-hydroxy-4-isobutoxyphenyl)-4-(4-phenoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5i).

Yield: 62%; MP: 130-132^{0}C; IR (ν_{max}, cm^{-1}): 3380 (−OH str. of Phenolic OH), 3290 (N−H str. of Sec. Amine), 3039 (C=C−H str .), 2947 (C−H str.), 1654 (C=O str.), 1588 (C=C str.), 1230 (C−O−C str. of ether), 877 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.89 (6H, d, *J* = 7.2 Hz), 1.87 (1H, m), 2.66(1H, dd *J* = 3.2, 13.1 Hz), 2.89 (1H, dd, *J* = 5.1, 12.4 Hz), 3.63 (2H, d, *J* = 9.1 Hz), 3.67 (1H, d, *J* = 8.9 Hz), 4.12 (1H, dd, *J* = 3.1, 8.2 Hz), 6.39-6.41 (2H, m), 6.62 (1H, s), 6.98-7.13 (4H, m), 7.12-7.17 (2H, m), 7.23-7.38 (4H, m), 7.03 (1H, s), 9.1 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.12, 27.23, 28.92, 32.95, 52.81, 76.65, 104.24, 105.93, 108.83, 117.41, 120.24, 121.32, 124.95, 125.82, 129.72, 143.46, 149.25, 155.61, 156.73, 157.42, 157.98, 161.94, 178.43; MS, m/z (M + 1) : 483; Elemental Analysis $C_{30}H_{30}N_2O_4$: Calculated (%): C, 74.67; H, 6.27; N, 5.81, Found (%): C, 74.63; H, 6.26; N, 5.85.

2.3j 4-(3,4-dimethoxyphenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5j).

Yield: 67%; MP: 205-207^{0}C; IR (ν_{max}, cm^{-1}): 3367 (−OH str. of Phenolic OH), 3296 (N−H str. of Sec. Amine), 3029 (C=C−H str.), 2938 (C−H str.), 1647 (C=O str.), 1591 (C=C− str.), 1224 (C−O−C str. of ether), 892 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.91 (6H, d, *J* = 6.6 Hz), 1.92 (1H, m), 2.71 (1H, dd *J* = 2.8, 12.3 Hz), 2.91 (1H, dd, *J* = 3.9, 14.7 Hz), 3.63 (2H, d, *J* = 7.4 Hz), 3.67 (1H, d, *J* = 8.7 Hz), 3.72

(6H, s), 3.99 (1H, dd, *J* = 5.2, 9.3 Hz), 6.38-6.45 (2H, m), 6.51 (1H, s), 6.72-6.83 (2H, m), 6.99-7.12 (2H, m), 7.53 (1H, s), 8.7 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.21, 27.81, 29.91, 33.61, 51.91, 55.41, 73.24, 102.87, 105.61,108.20, 110.44, 111.65, 120.86, 125.45, 139.41, 145.62, 147.62, 148.87, 153.72, 155.09, 160.20, 176.12; MS, m/z (M + 1) : 437; Elemental Analysis $C_{25}H_{28}N_2O_5$: Calculated (%): C, 68.79; H, 6.47; N, 6.42, Found (%): C, 68.81; H, 6.49; N, 6.38.

2.3k 6-(2-hydroxy-4-isobutoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5k).

Yield: 65%; MP: 158-160^{0}C; IR (ν_{max}, cm^{-1}): 3367 (−OH str. of Phenolic OH), 3298 (N−H str. of Sec. Amine), 3042 (C=C−H str.), 2965 (C−H str.), 1652 (C=O str.), 1595 (C=C− str.), 1239 (C−O−C str. of ether), 889 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.90 (6H, d, *J* = 6.5 Hz), 1.91 (1H, m), 2.70 (1H, dd *J* = 2.7, 12.1 Hz), 2.91 (1H, dd, *J* = 4.1, 15.1Hz), 3.61 (2H, d, *J* = 7.3 Hz), 3.62 (1H, d, *J* = 8.2 Hz), 3.78 (9H, s), 3.97 (1H, dd, *J* = 5.1, 9.5 Hz), 6.38-6.45 (2H, m), 6.58 (1H, s), 6.78 (2H, m), 7.12 (1H, m), 7.9 (1H, s), 9.7 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.12, 27.61, 29.72, 33.41, 51.90, 55.44, 59.24, 75.43, 102.74, 105.23,107.29, 125.32, 135.41, 141.96, 147.61, 151.27, 154.45, 155.91, 162.21, 176.91; MS, m/z (M + 1) : 467; Elemental Analysis $C_{26}H_{30}N_2O_6$: Calculated (%): C, 66.94; H, 6.48; N, 6.00, Found (%): C, 66.92; H, 6.47; N, 6.03.

2.3l 4-(2-bromo-4,5-dimethoxyphenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5l).

Yield: 69%; MP: 209-213^{0}C; IR (ν_{max}, cm^{-1}): 3375 (−OH str. of Phenolic OH), 3288 (N−H str. of Sec. Amine), 3040 (C=C−H str.), 2957 (C−H str.), 1650 (C=O str.), 1593 (C=C str.), 1234 (C−O−C str. of ether), 883 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.90 (6H, d, *J* = 6.9 Hz), 1.90 (1H, m), 2.73 (1H, dd *J* = 3.1, 13.4 Hz), 2.90 (1H, dd, *J* = 4.2, 15.2 Hz), 3.61 (2H, d, *J* = 6.9 Hz), 3.64 (1H, d, *J* = 8.1 Hz), 3.78 (6H, s), 4.09 (1H, dd, *J* = 4.6, 9.2 Hz), 6.43-6.49 (2H, m), 6.57 (1H, s), 6.9 (1H, s), 7.12 (2H, m), 7.93 (1H, s), 9.7 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 19.38, 25.52, 27.24, 35.43, 52.23, 55.81, 75.54, 104.47, 105.42,110.62, 112.23, 114.24, 120.58, 125.61, 138.34, 146.91, 147.42, 154.52, 155.91, 161.40, 176.78; MS, m/z (M + 1) : 530; Elemental Analysis $C_{26}H_{29}BrN_2O_5$: Calculated (%): C, 58.26; H, 5.28; N, 5.54; Br, 15.50, Found (%): C, 58.29; H, 5.27; N, 5.50; Br, 15.52.

2.3m 4-(3-ethoxy-4-methoxyphenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5m).

Yield: 70%; MP: 165-169^{0}C; IR (ν_{max}, cm^{-1}): 3377 (−OH str. of Phenolic OH), 3291 (N−H str. of Sec. Amine), 3047 (C=C−H str.), 2956 (C−H str.), 1649 (C=O str.), 1597 (C=C str.), 1231 (C−O−C str. of ether), 886 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.93 (6H, d, *J* = 7.2 Hz), 1.28 (3H, t, *J* = 15.2 Hz), 1.90 (1H, m), 2.68 (1H, dd *J* = 3.2, 14.1 Hz), 2.87 (1H, dd, *J* = 2.7, 13.9 Hz), 3.61 (2H, d, *J* = 9.5 Hz), 3.69 (1H, d, *J* = 7.9 Hz), 3.79 (3H, s), 3.99 (3H, m), 4.2 (2H, q, *J* = 16.4 Hz), 6.40-6.45 (2H, m), 6.98 (2H, m), 6.99-7.12 (2H, m), 7.86 (1H, s), 9.1 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 13.71, 19.11, 27.63, 31.23, 35.47, 51.20, 56.82, 63.43, 72.62, 102.81, 105.68,108.91, 110.24, 117.25, 125.08, 140.63, 146.88, 147.94, 154.62, 156.82, 160.78, 178.32; MS, m/z (M + 1) : 451; Elemental Analysis $C_{26}H_{30}N_2O_5$: Calculated (%): C, 69.31; H, 6.71; N, 6.22, Found (%): C, 69.35; H, 6.70; N, 6.24.

2.3n 6-(2-hydroxy-4-isobutoxyphenyl)-4-(3-methoxy-4-phenoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5n).

Yield: 66%; MP: 148-151^{0}C; IR (ν_{max}, cm^{-1}): 3378 (−OH str. of Phenolic OH), 3300 (N−H str. of Sec. Amine), 3037 (C=C−H str.), 2950 (C−H str.), 1650 (C=O str.), 1593 (C=C str.), 1221 (C−O−C str. of ether), 887 (C−H .mul.

def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.92(6H, d, *J* = 7.1 Hz), 1.90 (1H, m), 2.70 (1H, dd *J* = 3.1, 13.1 Hz), 3.01 (1H, dd, *J* = 4.8, 14.1 Hz), 3.60 (2H, d, *J* = 7.8 Hz), 3.61 (1H, d, *J* = 8.9 Hz), 3.88 (3H, s), 4.01 (1H, dd, *J* = 3.1, 7.4 Hz), 6.39-6.47 (2H, m), 6.58 (1H, s), 6.93 (1H, m), 7.01-7.17 (5H, m), 7.3(2H, m), 7.95 (1H, s), 9.92 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 18.91, 27.25, 29.21, 32.50, 51.30, 57.32, 73.48, 104.23, 107.34, 110.35, 117.47, 119.34, 121.43, 125.39, 127.65, 141.42, 142.26, 147.48, 150.28, 157.42, 157.88, 159.87, 176.23; MS, m/z (M + 1) : 513; Elemental Analysis $C_{31}H_{32}N_2O_5$: Calculated (%): C, 72.64; H, 6.29; N, 4.47, Found (%): C, 72.65; H, 6.26; N, 5.49.

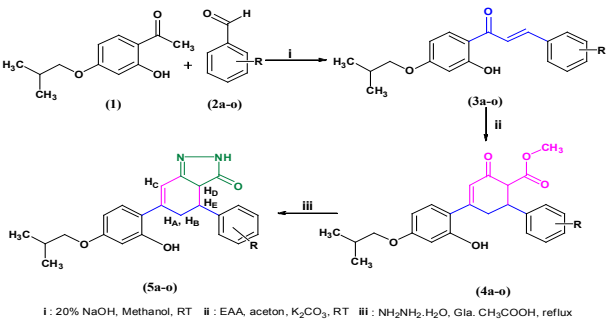
2.3o 4-(4-ethyl-2,5-dimethylphenyl)-6-(2-hydroxy-4-isobutoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one(5o).

Yield: 61%; MP: 178-180^{0}C; IR (ν_{max}, cm^{-1}): 3371 (−OH str. of Phenolic OH), 3296 (N−H str. of Sec. Amine), 3044 (C=C−H str.), 2960 (C−H str.), 1651 (C=O str.), 1594 (C=C str.), 1236 (C−O−C str. of ether), 880 (C−H .mul. def.); ^{1}H NMR (300 MHz, $CDCl_3$), 0.91 (6H, d, *J* = 7.1 Hz), 1.18 (3H, t, *J* = 14.3 Hz), 1.91 (1H, m), 2.57 (2H, q, *J* = 16.3 Hz), 2.72 (1H, dd *J* = 2.8, 13.1 Hz), 2.99 (1H, dd, *J* = 4.9, 13.8 Hz), 3.63 (2H, d, *J* = 8.9 Hz), 3.67 (1H, d, *J* = 8.7 Hz), 4.07 (1H, dd, *J* = 2.6, 7.9 Hz), 6.29-6.37 (2H, m), 6.60 (1H, s), 6.80 (1H, s), 6.92- 7.0 (2H, m), 7.93 (1H, s), 9.8 (1H, s);^{13}C NMR (75 MHz, $CDCl_3$), 14.2, 19.45, 25.81, 27.62, 34.72, 52.44, 73.26, 104.20, 108.25, 125.71, 127.64, 129.92, 131.98, 133.42, 142.20, 147.90, 154.82, 156.30, 160.71, 178.03; MS, m/z (M + 1) : 465; Elemental Analysis $C_{27}H_{32}N_2O_5$: Calculated (%): C, 69.81; H, 6.94; N, 6.03, Found (%): C, 69.79; H, 6.91; N, 6.08.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic procedures adopted to obtain the target compounds from synthesis of cyclohexenone derivatives (4a-o) were carried out by reacting chalcones (3a-o) with ethyl acetoacetate in the presence of base as described in literature [10]. The chalcones (3a-o), in turn prepared by the Claisen-Schmidt condensation of 1-(2-hydroxy-4-isobutoxyphenyl) ethanone and the substitute benzaldehyde. The cyclo condensation of acetoacetic ester with chalcones led to the generation of two chiral centes in cyclohexenones. The diastereomeric cyclohexenones were characterized [28] and utilized as such in the preparation of target compounds. No effort was undertaken to separate the diastereomeric cyclohexenones and was used as such for further reaction. The cyclohexenone derivatives, methyl 2-hydroxy-4-isobutoxy-5'-oxo-2',3',4',5'-tetrahydro-[1,1':3',1''-terphenyl]-4'- carboxylate(4a) which contain 1,3-dicarbonyl system, reacted with hydrazine hydrate in the presence of catalytic amount of glacial acetic acid medium resulted in the formation of indazole derivatives, 6-(2-hydroxy-4-isobutoxyphenyl)-4-phenyl-4,5-dihydro-2H-indazol-3(3aH)-one (5a-o), respectively (Scheme 1). The yield, melting point, molecular formula, and R_f value of compounds (5a–o) are given in (Table 1).



Reaction scheme 1: synthesis of(2*H*) indazole derivatives (5a-o).

The IR spectra of 6-(2-hydroxy-4-isobutoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-4,5-dihydro-2H-indazol-3(3aH)-one (5k

supplementary fig-S1) displayed absorption band at 1547 cm^{-1}, which is a characteristics of -C=N. It was also observed that -NH of ring showed stretching band at 3298 cm^{-1} and carbonyl group of indazole ring showed characteristic absorption band at 1640 cm^{-1}. The strong broad band observed at 3367 cm^{-1} recognized the Ar -OH and the band due to C-O stretching appeared at 1177 cm^{-1}. Due to ether linkage (C-O-C) two bands appeared in the range of 1275-1200 cm^{-1}(symmetric) and 1019-1020cm^{-1} (asymmetric) as stretching band.

In the ^{1}H NMR spectra of the compound (5g supplementary fig-S2, 5h, supplementary fig-S3), the two methylene proton H_A and H_B appeared as doublet of doublet at 2.71 - 2.75 δppm and 3.01 - 3.10 δppm respectively. The signal of proton H_C is merged with doublet of $-OCH_2CH-$ and appeared in the form of multiplet at around 3.63-3.70 δppm.

Table-1: physical properties of synthesized indazole derivatives (5a-o).

Code	R	Molecular Formula	Molecular weight (g/mol)	% of Yield	M.P (^{0}C)	R_f Value
5a	**H**	$C_{24}H_{26}N_2O_4$	376	70	215-218	0.34
5b	2-Cl	$C_{23}H_{23}ClN_2O_3$	411	69	165-167	0.35
5c	4-Cl	$\mathbf{C_{23}H_{23}ClN_2O_3}$	411	68	185-188	0.32
5d	**3-Cl**	$C_{23}H_{23}ClN_2O_3$	411	70	150-152	0.30
5e	3-Br	$C_{23}H_{23}BrN_2O_3$	455	67	186-189	0.30
5f	3-NO_2	$C_{23}H_{23}N_3O_5$	446	61	220-223	0.28
5g	4-CH_3	$C_{23}H_{24}N_2O_3$	39	68	195-197	0.29
5h	**4-OCH_3**	$C_{24}H_{26}N_2O_4$	406	67	127-129	0.30
5i	**4-OC_6H_5**	$C_{30}H_{30}N_2O_4$	482	62	130-132	0.27
5j	**3, 4-di-OCH_3**	$C_{25}H_{28}N_2O_5$	436	67	205-207	0.39
5k	**3, 4, 5-tri-OCH_3**	$C_{26}H_{30}N_2O_6$	466	65	158-160	0.37
5l	**2-Br-4, 5-di-OCH_3**	$C_{26}H_{29}BrN_2O_5$	529	69	209-213	0.27
5m	**4-OCH_3-3-OC_2H_5**	$C_{26}H_{30}N_2O_5$	450	70	165-169	0.38
5n	**3-OCH_3-4-OC_6H_5**	$C_{31}H_{32}N_2O_5$	512	66	148-151	0.35
5o	**2, 5-di-OCH_3-4-C_2H_5**	$C_{27}H_{32}N_2O_5$	464	61	178-180	0.35

The H_D showed as doublet at 4.01-4.03 δppm, where as the H_E appeared as a singlet at 6.60 δppm confirmed the presence of cyclohexenone nucleus. The proton of -NH exhibited as singlet at 7.89 δppm while the singlet due to Ar-OH group appeared at 9.58 δppm. The presence of isobutoxy group was confirmed by the doublet of six protons of $-CH(CH_3)_2$ exhibited at 0.92-0.94 δppm and a multiplet of $-CH(CH_3)_2$ in the range of 1.94-1.97 ppm. Protons of $-CH_3$ showed as singlet at 2.19 δppm. In the aromatic region, the double doublet is merged with the signal, and appeared in the range of 6.32- 6.36 ppm. Other aromatic proton showed as multiplet in the range of 6.97-7.05 δppm. In ^{13}C NMR spectra of the compound (5b, supplementary fig-S4), carbons of the indazole nucleus resonated at δ 156.31, 114.17, 148.31, 37.70, 28.25, 47.71 ppm. The presence of carbonyl group (-C=O) of indazole nucleus was confirmed by the signal observed at δ 179.12 ppm. The presence of isobutoxy group is confirmed by the signal appeared at δ 19.28 ppm attributed to two methyl carbons and the signals of carbons were detected at δ 31.81 and δ 74.48 ppm, respectively. The aromatic carbon showed signals at 103.90, 107.01, 111.57, 124.29, 127.81, 128.24, 128.89, 129.81, 133.34, 145.92, 158.92 and 162.59 ppm. Mass spectra supported the formation of indazole derivatives (5h supplementary fig-S5). Elemental analysis also gave satisfactory results for all the compounds. Similarly, the structures 2H indazole derivatives (5a–o) were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The detailed spectral data are given in the experimental section.

3.2 Biological Activity

Lack of development of new antimicrobial agents is a serious problem these days because the microorganisms are getting more and more 'use to' to the drugs available in the market [29]. The activity of compounds was determined as per National Committee for Clinical Laboratory

Standards (NCCLS) protocol using Mueller Hinton Broth [30, 31]. The antibacterial and antifungal activity was determined applying the Broth dilution method and antitubercular activity was determined applying the L.J Slope method [32, 33].

Results were obtained in duplicate, and results with differences higher than 5% were discarded and the measurement repeated. The wide range of activity profile of indazole derivatives to test and study the biological activities of some of the synthesized novel analogues. The newly synthesized compounds 5a−o to be tested in vitro for their antibacterial activity against two gram positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC-442 and two gram negative bacteria *Escherichia coli* MTCC-443, *Pseudomonas aeruginosa* MTCC-1688 by the Broth dilution method, antifungal activity against *Candida albicans* MTCC-227, *Aspergillus niger* MTCC-28 and antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ MTCC-200.

Table-2: Antimicrobial activity of synthesized indazole derivatives (5a-o).

Code	R	Bacterial Activity (MIC) in µg/ml				Fungal Activity (MIC) in µg/ml	
		S. a MTCC 96	S. p MTCC 442	E. c MTCC 443	P. a MTCC 1688	C. a MTCC 227	A.n MTCC 282
5a	**H**	50	12.5	25	25	200	200
5b	2-Cl	25	50	-	-	125	-
5c	4-Cl	-	12.5	12.5	-	-	100
5d	**3-Cl**	-	50	12.5	50	-	100
5e	3-Br	-	-	25	25	125	200
5f	3-NO_2	50	25	12.5	25	-	-
5g	4-CH_3	25	50	50	6.25	125	250
5h	**4-OCH_3**	-	-	50	50	125	-
5i	**4-OC_6H_5**	50	-	-	-	125	200
5j	**3, 4-di-OCH_3**	50	-	50	-	125	100
5k	**3, 4, 5-tri-OCH_3**	-	12.5	25	50	-	-
5l	**2-Br-4, 5-di-OCH_3**	-	-	12.5	-	100	-
5m	**4-OCH_3-3-OC_2H_5**	50	50	50	50	-	-
5n	**3-OCH_3-4-OC_6H_5**	-	12.5	50	25	250	100
5o	**2, 5-di-CH_3-4-C_2H_5**	-	-	50	25	200	100
Sdt. Drug	Gentamycin	**0.5**	**1.0**	**0.25**	**0.5**	-	-
	K.Nystatin	-	-	-	-	**100**	**100**

DMSO was used as a control solvent Gentamycin, K. Nystatin and Rifampicin as standard drugs.

3.2a Antimicrobial activity

Compounds 5a, 5c, 5e, 5g, 5k and 5n shows remarkable antibacterial activity against gram positive bacteria. 5g with –CH_3 substituent to phenyl nucleus exhibited excellent activity against *Staphylococcus aureus* and 5a phenyl nucleus, 5c (-Cl), 5k, 5n (-OCH_3) substituent shows excellent activity against *Streptococcus pyogenes*. Compounds 5c, 5d, 5e, 5l and 5g displayed excellent antibacterial activity against gram negative bacteria. 5c, 5d (-Cl), 5e (-NO_2) and 5l (-Br, -OCH_3) substituent exhibited excellent activity against *Escherichia coli* and 5g (-CH_3) shows good activity against *Pseudomonas aeruginosa*. No significant or comparable activities have been observed for rest of the compounds up to concentration of 100 µg/ml. The results of antibacterial activity are mentioned in Table-1 and fig-1.

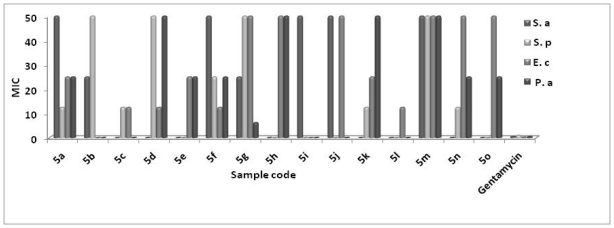


Fig-1. Antibacterial activity of indazole derivatives (5a-o).

Compounds (5a-o) were screened for their antifungal activity. The MIC values of screened compounds suggest that the test compounds 5b, 5e, 5g, 5h, 5i and 5j shows good activity while

compound 5l (-Br, -OCH_3) exhibited excellent antifungal activity against *Candida albicans* comparable to reference agents K Nystatin. Compounds 5c, 5d, 5j, 5n and 5o exhibited excellent antifungal activity against *Aspergillus Niger*.

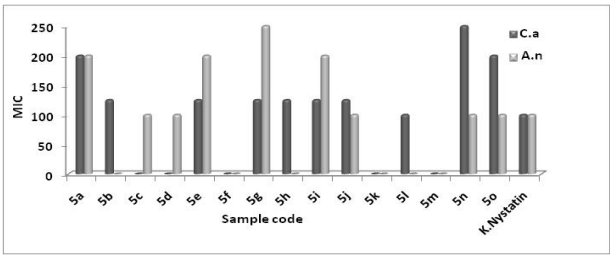


Fig-2. Antifungal activity of indazole derivatives (5a-o).

Rest of the compounds showed poor or no activity even at concentration of 200µg/ml. The overall results of antifungal activity are mentioned in Table-1 and fig-2.

3.2b Antitubercular activity

Compounds 5c (-Cl), 5k (-OCH_3), 5l (-Br, -OCH_3), and 5o (-CH_3, -C_2H_5) shows excellent activity against *Mycobacterium tuberculosis* H_{37}Rv and 5b, 5d, 5f, 5h, 5j and 5m () shows good activity against *Mycobacterium tuberculosis* H_{37}Rv. group at phenyl nucleus respectively exhibited excellent activity at a concentration of 25µg/ml compared to Rifampicin. Rest of the compounds showed poor activity with 100 µg/ml. The results of antitubercular screening are mentioned in Table-3 and fig-3.

Table-3: Antituberculosis activity of synthesized indazole derivatives (5a-o).

Minimal Inhibition Concentrations of H_{37}Rv strain of *M. tuberculosis* (MIC) in Mg/ml		
Code	**R**	**H_{37}Rv strain of *M. tuberculosis***
5a	**H**	100
5b	2-Cl	50
5c	4-Cl	25
5d	**3-Cl**	50
5e	3-Br	100
5f	3-NO_2	50
5g	4-CH_3	100
5h	**4-OCH_3**	50
5i	**4-OC_6H_5**	100
5j	**3,4-di-OCH_3**	50
5k	**3, 4, 5-tri-OCH_3**	25
5l	**2-Br-4,5-di-OCH_3**	25
5m	**4-OCH_3-3-OC_2H_5**	50
5n	3-OCH_3-4-OC_6H_5	100
5o	2,5-di-OCH_3-4-C_2H_5	25
Std. Drug	Rifampicin	**12.5**

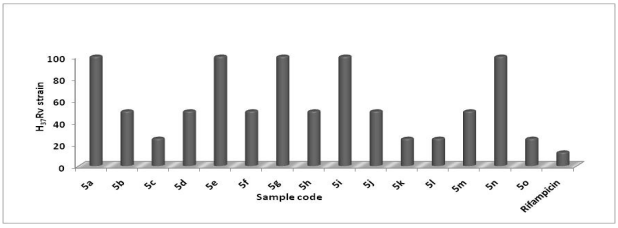


Fig-3. Antituberculosis activity of indazole derivatives (5a-o).

CONCLUSION

In the present article, we report the synthesis, spectral studies, antibacterial, antifungal and antitubercular activities of a novel series of substituted indazole. These were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry study and elemental analyses. The substrates were synthesized by acidic catalyst to obtained good yield in conventional methods. The compounds 5a, 5c, 5k and 5n exhibited significant (maximum) antibacterial activity

against gram positive bacterial species, 5c, 5d, 5e, 5g and 5l against gram negative bacterial species, 5l, 5c, 5d, 5k, 5n and 5o exhibited significant (maximum) antifungal activities and 5c, 5k, 5l and 5o exhibited significant (maximum) antitubercular activity against Mycobacterium tuberculosis. Compound 5c exhibited excellent activity against all bacterial species. This may develop into the potential class of antimicrobial agents.

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