Parasuraman Amutha and Samuthira Nagarajan*

Department of Chemistry, Annamalai University, Annamalainagar 608 002, India
*E-mail: nagarajan.au@gmail.com
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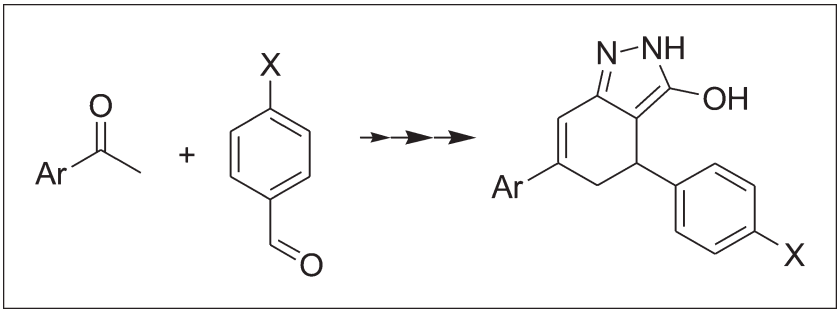

A series of new 4,6-diaryl-4,5-dihydro-3-hydroxy-2H-indazoles **5a–5k** were synthesized by the cyclization of ethyl 2-oxo-4,6-diarylcyclohex-3-ene carboxylates **4a–4k**. The compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, and elemental analysis. The synthesized compounds were evaluated for *in vitro* antibacterial and antifungal activities against *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, Aspergillus flavus*, and *Rhizopus* sp. Most of the compounds exhibited good activity against the tested organisms.

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INTRODUCTION

The indazole ring is an important pharmacophore in medicinal chemistry. During the last decade, considerable interest has been paid to chemistry of indazoles. This is undoubtedly due to its broad variety of biological activities [1,2]. Indazole subunits are frequently found motifs in drug substances, for example, Benzdac [3] and benzydamine [4] are commercially available anti-inflammatory drugs. Apart from this, the range of pharmacological effects of indazole derivatives include nitric oxide synthase inhibition [5], analgesic [6], anti-inflammatory [7], antiviral [8], HIV protease inhibitory activity [9], anticancer [10], antitumor [11], YC1 guanylyl cyclase activator [12], antimicrobial activities [13,14], and antiproliferative activity [15], which inspired the development of new synthesis and optimization as well as functionalization of indazole ring. Being involved in study of heterocyclic compounds, with the purpose to perform a large program of biological screening, we focused our attention on synthesis of some pyrimidine derivatives with antimicrobial activity [16]. In continuation of our research work, we herein report the synthesis and antimicrobial studies of series of 4,6-diaryl-4,5-dihydro-3-hydroxy-*2H*-indazoles.

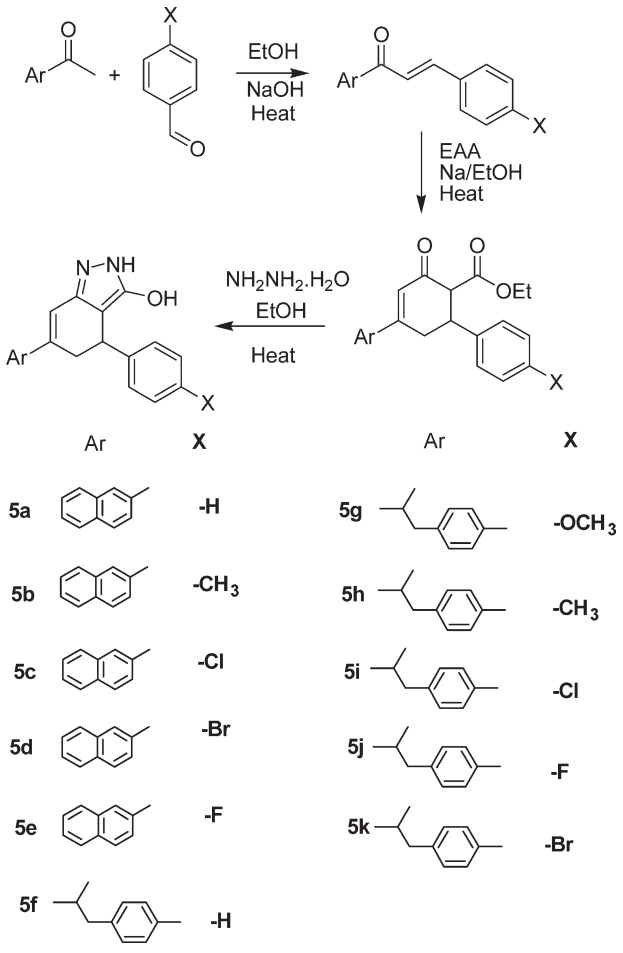
RESULTS AND DISCUSSION

The synthetic procedures adopted to obtain the target compounds are depicted in Scheme 1. The starting compounds 1,3-diarylprop-2-en-1-ones **3a–3k** were prepared by the reaction of 1-(2-naphthyl)ethanone/1-(4-isobutylphenyl)ethanone with respective benzaldehydes in the presence of sodium hydroxide. The precursors of **5a–5k** ethyl 2-oxo-4,6-diarylcyclohex-3-enecarboxylate **4a–4k** were obtained by Knoevenagel reaction of ethyl acetoacetate and 1,3-diarylprop-2-en-1-one. The compounds **4a–4k** on treatment with hydrazine hydrate in ethanol afforded the target compounds 4,6-diaryl-4,5-dihydro-3-hydroxy-2H-indazoles **5a–5k**.

The yield, melting point, molecular formula, and elemental compositions of compounds **5a–5k** are given in Table I. The IR spectra of **5** displayed characteristic absorption bands in the region 3123–3265 cm^{-1} (NH), 3353–3429 cm^{-1} (OH), 1606−1596 (C=N). Thus, the absence of C=O stretching frequency and presence of OH absorptions supports the formation of hydroxyindazoles.

In the ^{1}H NMR spectra of **5** two double doublets were observed in the region 4.2 ppm and 2.8–3.0 ppm for H_4 and H_{5b} protons, respectively. The H_{5a} proton appears as a multiplet in the region 3.1–3.3 ppm instead of expected double doublet. Similarly, H_7 proton appears as a doublet in the region 6.7–6.9 ppm instead of expected singlet this may be due to long-range coupling with H_{5a} proton. In the ^{13}C NMR spectra, the carbon resonance around 36 and 34 ppm was due to C−5 and C−4 carbons, respectively. The signal at around 114 ppm was attributed to C−7 carbon. The individual assignments were further confirmed with the help of

Scheme 1. Synthetic pathway to **5a–5k**.



^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra. In ^{1}H-^{1}H COSY, the signal for H_7 proton has cross-peak with H-5a proton and *vice versa* this confirms that the multiplicity of H_{5a} proton instead of expected double doublet is due to long-range coupling with H_7 proton. In HSQC spectrum of **5g**, the signal at 36.81 ppm has cross-peak with signal for H_{5a} and H_{5b} protons. Similarly, the carbon signal at 33.80 ppm has correlation with signal for H_4 proton. Likewise the carbon resonance at 113.90 ppm has correlation with H_7 proton.

Pharmacology. The antibacterial and antifungal activities of compounds **5a–5k** were assessed in comparison with Ciprofloxacin and Amphotericin B, respectively, against bacterial and fungal strains. The results obtained are summarized in Tables II and III. The antibacterial and antifungal data indicated that the compounds **5a–5k** have good activity against tested bacterial and fungal strains. The naphthyl derivatives **5d** and **5e** with 4-chloro and 4-fluoro substituents showed potent activity than the rest of the compounds. Among the isobutyl derivatives also **5i** and **5j** with 4-fluoro and 4-chloro substituted compounds are more effective than the other compounds. Thus, the electron withdrawing groups play a vital role in antimicrobial activity. The importance of electron withdrawing groups in enhancing the antimicrobial activity is supported by similar results [14,17]. Rest of the compounds showed moderate to good activity. The nature and position of the substituent influence the extent of antibacterial and antifungal activity. From the analysis of the structures of most active compounds **5e** and **5j**, it may be concluded that among the electron withdrawing halo groups, the presence of a 4-fluorophenyl group improved the antimicrobial activity. The presence of isobutyl chain in the phenyl ring not much influenced the antimicrobial activity of the tested organisms.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded on a NICOLET AVATAR (FTIR-330) spectrophotometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 using

Table 1
Physical and analytical data for **5a**–**5k**.

Compound	m.p. (°C)	Yield (%)	Molecular formula	Elemental analysis (%)		
				C found (calculated)	H found (calculated)	N found (calculated)
5a	144–149	71	$C_{23}H_{18}N_2O$	81.37 (81.63)	5.09 (5.36)	8.02 (8.28)
5b	146–150	65	$C_{24}H_{20}N_2O$	81.61 (81.79)	5.55 (5.72)	7.83 (7.95)
5c	148–153	68	$C_{23}H_{17}ClN_2O$	73.90 (74.09)	4.36 (4.60)	7.39 (7.51)
5d	163–167	73	$C_{23}H_{17}BrN_2O$	65.98 (66.20)	3.99 (4.11)	6.58 (6.71)
5e	149–152	70	$C_{23}H_{17}FN_2O$	77.36 (77.51)	4.69 (4.81)	7.67 (7.86)
5f	167–169	67	$C_{23}H_{24}N_2O$	80.01 (80.20)	6.90 (7.02)	7.94 (8.13)
5g	146–149	71	$C_{24}H_{26}N_2O_2$	76.72 (76.98)	6.82 (7.00)	7.21 (7.48)
5h	152–155	68	$C_{24}H_{26}N_2O$	80.31 (80.41)	7.04 (7.31)	7.57 (7.81)
5i	158–161	72	$C_{23}H_{23}ClN_2O$	72.62 (72.91)	5.66 (6.12)	7.20 (7.39)
5j	143–146	74	$C_{23}H_{23}FN_2O$	75.97 (76.22)	6.20 (6.40)	7.62 (7.73)
5k	151–153	69	$C_{23}H_{23}BrN_2O$	65.11 (65.25)	5.25 (5.48)	6.47 (6.62)

Table 2

In vitro antimicrobial activity (zone of inhibition) values for **5a**–**5k**.

	Diameter of zone of inhibition (mm)								
	Bacterial strains					Fungal strains			
Compound	*S. aureus*	*E. coli*	*K. pneumoniae*	*S. typhimurium*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. flavus*	*Rhizopus* sp.
5a	07	07	10	08	11	07	10	08	11
5b	07	05	08	10	08	07	10	06	09
5c	10	12	09	12	13	09	11	10	07
5d	12	14	10	10	12	14	15	12	12
5e	15	10	12	14	12	12	14	13	10
5f	08	12	10	07	09	09	10	06	07
5g	09	10	08	11	06	10	08	08	12
5h	11	10	07	13	10	07	10	10	07
5i	15	14	12	12	16	13	14	14	15
5j	14	13	12	15	15	16	12	15	14
5k	13	10	07	12	11	13	10	14	11
Cip.	12	15	16	16	11	–	–	–	–
Amp. B	–	–	–	–	–	13	12	14	11

Cip., Ciprofloxacin; Amp. B, Amphotericin B.

Bruker AMX 500-MHz spectrometer. ^{13}C NMR spectra were recorded at an operating frequency of 125 MHz. The 2D NMR spectra were recorded on DRX 500 NMR spectrometer. Chemical shifts are expressed in parts per million using residual solvent proton and carbon as internal standards.

Synthesis of 1,3-diarylprop-2-en-1-ones (3a–3k). A solution of substituted benzaldehyde (10 mmol) and 1-(2-naphthylethanone) (10 mmol) in 65% aqueous ethanol (50 mL) containing NaOH (12.5 mmol, 0.5 g) was heated over water bath for 30 min. The solution was allowed to cool and poured into crushed ice; the separated solid was filtered, washed with water, and recrystallized from ethanol.

Synthesis of ethyl 2-oxo-4,6-diarylcyclohex-3-enecarboxylates (4a–4k). A mixture of sodium (2-g sodium in 60-mL distilled ethanol), distilled ethyl acetoacetate (0.01 mol), and 1,3-diarylprop-2-en-1-ones (0.01 mol) in absolute ethanol (20 mL) was refluxed for 2 h on steam bath and then cooled. The separated solid was filtered, washed with water, and recrystallized from ethanol.

Synthesis of 4,6-diaryl-4,5-dihydro-3-hydroxy-2H-indazoles (5a–5k). The ethyl 2-oxo-4,6-diarylcyclohex-3-enecarboxylate (0.1 mol) was dissolved in hot ethanol (25 mL), and after addition of hydrazine hydrate (0.15 mol), the reaction mixture was refluxed for 2–4 h. The hot solution was poured into ice, and the separated solid was filtered and washed with water. The crude product was recrystallized from ethanol. Further the product was purified using column chromatography (silica gel), eluent, benzene:ethylacetate (3:2).

4,5-Dihydro-6-(naphthalen-2-yl)-4-phenyl-2H-indazol-3-ol (5a). IR (KBr) (cm^{-1}): 3402, 3205, 3055, 3019, 2924, 2854,

Table 3

In vitro antimicrobial activity (MIC) values for **5a**–**5k**.

	Minimum inhibitory concentration (μg/mL)								
	Bacterial strains					Fungal strains			
Compound	*S. aureus*	*E. coli*	*K. pneumoniae*	*S. typhimurium*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. flavus*	*Rhizopus* sp.
5a	100	200	50	100	50	200	50	100	50
5b	200	50	100	50	100	100	200	50	50
5c	100	50	100	50	50	100	50	50	200
5d	25	12.5	100	100	50	25	12.5	50	50
5e	12.5	100	100	25	50	50	12.5	100	100
5f	100	50	50	200	100	50	100	100	25
5g	200	50	100	50	200	200	50	50	100
5h	25	50	100	25	100	25	100	50	25
5i	12.5	100	100	50	25	25	100	50	50
5j	12.5	50	50	25	25	12.5	25	12.5	50
5k	50	100	100	50	50	25	100	50	50
Cip.	25	25	12.5	50	12.5	–	–	–	–
Amp. B	–	–	–	–	–	25	25	50	50

Cip., Ciprofloxacin; Amp. B, Amphotericin-B.

1598, 1508, 746, 697; ^{1}H NMR (δ ppm): 3.09 (dd, 1H, H_{5b}), 3.27–3.32 (m, 1H, H_{5a}), 4.25 (dd, 1H, H_4, J = 8, 3.5 Hz), 6.97 (d, 1H, H_7, J = 2.0 Hz), 7.10–7.99 (m, 12H, Ar−H); ^{13}C NMR (δ ppm): 34.76 (C−4), 36.43 (C−5), 109.73 (C−9), 114.74 (C−7), 123.85–128.62 (Ar−C), 132.77, 133.55, 136.27, 137.68, 145.82 (*ipso* carbons).

4,5-Dihydro-4-(4-methylphenyl)-6-(naphthalen-2-yl)-2H-indazol-3-ol (5b). IR (KBr) (cm^{-1}): 3402, 3216, 3052, 3019, 2922, 2860, 1596, 1509, 814, 745; ^{1}H NMR (δ ppm): 2.19 (s, 3H, CH_3), 3.05 (dd, 1H, H_{5b}, J = 16.75, 4.25 Hz), 3.24–3.27 (m, 1H, H_{5a}), 4.20 (dd, 1H, H_4, J = 9.5, 4.5 Hz), 6.95 (d, 1H, H_7, J = 1.5 Hz), 7.00–7.98 (m, 11H, Ar−H), 9.67 (s, 1H, NH), 11.56 (s, 1H, OH); ^{13}C NMR (δ ppm): 20.99 (CH_3), 34.41 (C−4), 36.63 (C−5), 109.82 (C−9), 114.14 (C−7), 123.87–129.08 (Ar−C), 132.78, 133.56, 135.30, 136.24, 137.84, 142.77 (*ipso* carbons).

4-(4-Chlorophenyl)-4,5-dihydro-6-(naphthalen-2-yl)-2H-indazol-3-ol (5c). IR (KBr) (cm^{-1}): 3397, 3200, 3054, 2923, 2843, 1598, 1491, 817, 746; ^{1}H NMR (δ ppm): 3.06 (dd, 1H, H_{5b}, J = 17.0, 4.0 Hz), 3.31–3.27 (m, 1H, H_{5a}), 4.26 (dd, 1H, H_4, J = 8.5, 4.0 Hz), 6.97 (d, 1H, H_7, J = 2Hz), 7.20–7.99 (m, 11H, Ar−H), 9.85 (s, 1H, NH), 11.49 (s, 1H, OH); ^{13}C NMR (δ ppm): 34.34 (C−4), 36.40 (C−5), 114.00 (C−7), 123.86–131.00 (Ar−C), 132.82, 133.53, 136.22, 137.62, 144.72 (*ipso* carbons).

4-(4-Bromophenyl)-4,5-dihydro-6-(naphthelen-2-yl)-2H-indazol-3-ol (5d). IR (KBr) (cm^{-1}): 3422, 3265, 3052, 2923, 2853, 1605, 1534, 674; ^{1}H NMR (δ ppm): 3.05 (dd, 1H, H_{5b}, J = 16.75, 5.25 Hz), 3.25–3.31 (m, 1H, H_{5a}), 4.24 (dd, 1H, H−4, J = 8.5, 4.0 Hz), 6.97 (d, 1H, H_7, J = 2.5 Hz), 7.14–7.98 (m, 11H, Ar−H), 9.88 (s, 1H, NH), 11.55 (s, 1H, OH); ^{13}C NMR (δ ppm): 34.38 (C−4), 36.34 (C−5), 109.63 (C−9), 113.96 (C−7), 123.85–129.75, 131.41, 132.81, 133.54, 136.23, 137.60, 145.17 (*ipso* carbons).

4,5-Dihydro-4-(4-fluorophenyl)-6-(naphthalen-2-yl)-2H-indazol-3-ol (5e). IR (KBr) (cm^{-1}): 3424, 3221, 3056, 2924, 2843, 1600, 1507, 817, 746; ^{1}H NMR (δ ppm): 3.06 (dd, 1H, H_{5b}, J = 17.0, 4.0 Hz), 3.26–3.31 (m, 1H, H_{5a}), 4.27 (dd, 1H, H_4, J = 8.0, 4.0 Hz), 6.97 (d, 1H, H_7, J = 2.5 Hz), 7.02–7.99 (m, 11H, Ar−H), 9.94 (s, 1H, NH), 11.16 (s, 1H, OH); ^{13}C NMR (δ ppm): 34.17 (C−4), 36.57 (C−5), 108.36 (C−9), 115.26 (C−7), 123.87–129.19 (Ar−C), 132.81, 133.56, 136.25, 137.67, 141.85 (*ipso* carbons).

4,5-Dihydro-6-(4-isobutylphenyl)-4-phenyl-2H-indazol-3-ol (5f). IR (KBr) (cm^{-1}): 3402, 3194, 3112, 3084, 2954, 2922, 2867, 1599, 1507, 795, 751; ^{1}H NMR (δ ppm): 0.84 (d, 6H, $(CH_3)_2$, J = 8.5 Hz), 1.76–1.84 (m, 1H, CH), 2.41 (d, 2H, CH_2, J = 7.0 Hz), 2.90 (dd, 1H, H_{5b}, J = 17.0, 3.5 Hz), 3.13–3.18 (m, 1H, H_{5a}), 4.18 (dd, 1H, H_4, J = 9.0, 3.5 Hz), 6.73 (s, 1H, H_7, J = 2.0 Hz), 7.11–7.39 (m, 9H, Ar−H); ^{13}C NMR (δ ppm): 22.61 [$(CH_3)_2$], 30.04 (CH), 36.59 (C−5), 34.63 (C−4), 44.68 (CH_2), 99.03 (C−9), 112.93 (C−7), 125.29–129.62 (Ar−C), 136.61, 137.97, 141.10, 145.83 (*ipso* carbons).

4,5-Dihydro-6-(4-isobutylphenyl)-4-(4-methoxyphenyl)-2H-indazol-3-ol (5g). IR (KBr) (cm^{-1}): 3194, 3024, 2997, 2953, 2931, 2867, 1606, 1509, 1246, 831, 795; ^{1}H NMR (δ ppm): 0.84 (d, 6H, $(CH_3)_2$, J = 7.0 Hz), 1.76–1.84 (m, 1H, CH), 2.42 (d, 2H, CH_2, J = 7.0 Hz), 2.86 (dd, 1H, H_{5b}, J = 16.75, 3.25), 3.09–3.14 (m, 1H, H_{5a}), 3.66 (s, 3H, OCH_3), 4.12 (dd, 1H, H_4, J = 8.5, 3.5 Hz), 6.72 (d, 1H, H_7, J = 3 Hz), 6.75–7.39 (m, 8H, Ar−H), 9.87 (s, 1H, NH), 11.37 (s, 1H, OH); ^{13}C NMR (δ ppm): 22.62 [$(CH_3)_2$], 30.05 (CH), 33.80 (C−4), 36.81 (C−5), 44.68 (CH_2), 55.37 (OCH_3), 101.04 (C−9), 113.90 (C−7), 125.28–129.62 (Ar−C), 136.57, 137.75, 141.06, 157.97 (*ipso* carbons).

4,5-Dihydro-6-(4-isobutylphenyl)-4-(4-methylphenyl)-2H-indazol-3-ol (5h). IR (KBr) (cm^{-1}): 3353, 3205, 3013, 2954, 2922, 2865, 1598, 1511, 1460, 1020, 794; ^{1}H NMR (δ ppm): 0.84 (d, 6H, $(CH_3)_2$, J = 8.5 Hz), 1.78–1.83 (m, 8H, Ar−H), 2.20 (s, 3H, CH_3), 2.41 (d, 2H, CH_2, J = 7.5 Hz), 2.86 (dd, 1H, H_{5b}, J = 16.75, 3.25), 3.10–3.15 (m, 1H, H_{5a}), 4.12 (dd, 1H, H_4, J = 8.5, 3.5 Hz), 6.71 (d, 1H, H_7, J = 2.5 Hz), 6.99–7.38 (m, 8H, Ar−H); ^{13}C NMR (δ ppm): 21.61 (CH_3), 22.62 [$(CH_{-3})_2$], 30.05 (CH), 34.23 (C−4), 36.74 (C−5), 44.67 (CH_2), 102.23 (C−9), 113.38 (C−7), 125.27–129.61 (Ar−C), 135.26, 136.56, 138.02, 141.06, 142.75 (*ipso* carbons).

4-(4-Chlorophenyl)-4,5-dihydro-6-(4-isobutylphenyl)-2H-indazol-3-ol (5i). IR (KBr) (cm^{-1}): 3408, 3123, 3029, 2954, 2921, 2867, 1608, 1574, 1490, 1090, 794, 682; ^{1}H NMR (δ ppm): 0.84 (d, 6H, $(CH_3)_2$, J = 6.5 Hz), 1.76–1.86 (m, 1H, CH), 2.42 (d, 2H, CH_2, J = 7.0 Hz), 2.86 (dd, 1H, H_{5b}, J = 16.75, 3.25 Hz), 3.11–3.17 (m, 1H, H_{5a}), 4.18 (dd, 1H, H_4, J = 8.0, 3.5 Hz), 6.73 (d, 1H, H_7, J = 2.0 Hz), 7.13–7.39 (m, 8H, Ar−H), 9.69 (s, 1H, NH), 11.65 (s, 1H, OH); ^{13}C NMR (δ ppm): 22.61 [$(CH_3)_2$], 30.05 (CH), 34.15 (C−4), 36.52 (C−5), 44.67 (CH_2), 100.79 (C−9), 113.12 (C−7), 125.32–129.64 (Ar−C), 130.95, 136.48, 137.86, 141.17, 144.74 (*ipso* carbons).

4,5-Dihydro-4-(4-fluorophenyl)-6-(4-isobutylphenyl)-2H-indazol-3-ol (5j). IR (KBr) (cm^{-1}): 3391, 3206, 3128, 2955, 2923, 2866, 1601, 1507, 1460, 1224, 833; ^{1}H NMR (δ ppm): 0.85 (d, 6H, $(CH_3)_2$, J = 6.5 Hz), 1.76−1.84 (m, 1H, CH), 2.42 (d, 2H, CH_2, J = 7.0 Hz), 2.87 (dd, 1H, H_{5b}, J = 17.0, 4.0 Hz), 3.11−3.16 (m, 1H, H_{5a}), 4.18 (dd, 1H, H_4, J = 8.5, 3.5 Hz), 6.73 (d, 1H, H_7, J = 2.5 Hz), 7.01–7.39 (Ar−H), 9.68 (s, 1H, NH), 11.58 (s, 1H, OH); ^{13}C NMR (δ ppm): 22.62 [$(CH_3)_2$], 30.04 (CH), 33.97 (C−4), 36.69 (C−5), 44.67 (CH_2), 104.45 (C−9), 115.25 (C−7), 125.31–129.64 (Ar−C), 136.51, 137.92, 141.15, 141.84 (*ipso* carbons).

4-(4-Bromophenyl)-4,5-dihydro-6-(4-isobutylphenyl)-2H-indazol-3-ol (5k). IR (KBr) (cm^{-1}): 3397, 3189, 3055, 2986, 2926, 2849, 1604, 1509, 1247, 818; ^{1}H NMR (δ ppm): 0.84 (d, 6H, $(CH_3)_2$, J = 6.5 Hz), 1.78–1.83 (m, 1H, CH), 2.42 (d, 2H, CH_2, J = 7.5 Hz), 2.86 (dd, 1H, H_{5b}, J = 16.75, 3.75), 3.11–3.17 (m, 1H, H_{5a}), 4.17 (dd, 1H, H_4, J = 8.5, 3.5 Hz), 6.73 (d, 1H, H_7, J = 2.0 Hz), 7.09–7.42 (m, 8H, Ar−H); ^{13}C NMR (δ ppm): 22.61 [$(CH_3)_2$], 30.04 (CH), 34.23 (C−4), 36.47 (C−5), 44.68 (CH_2), 104.96 (C−9), 119.42 (C−7), 125.32–129.71 (Ar−C), 131.39, 136.47, 137.86, 141.17, 145.18 (*ipso* carbons).

Antimicrobial activity. All the compounds have been screened for antibacterial and antifungal activities using disc-diffusion and twofold serial dilution method. Ciprofloxacin and Amphotericin B were used as standard drugs for antibacterial and antifungal activities. The compounds were screened for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium*, and *Pseudomonas aeruginosa* in nutrient agar medium and for antifungal activity against *Candida albicans, Aspergillus niger, Aspergillus flavus*, and *Rhizopus* sp. in Sabouraud's dextrose agar medium. The sterilized agar medium was poured into petridishes and allowed to solidify. On the surface of the media, microbial suspensions were spread with the help of sterilized glass spreader. Whatmann paper discs of 5-mm diameter were impregnated in test compounds dissolved in dimethyl sulfoxide (DMSO; 200 μg/mL) for 30 min. Commercially available drug

was used as positive reference standard. The discs were placed on the inoculated agar plates and incubated at 37 ± 1°C for about 18–24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

A twofold serial dilution of the compounds and reference drug were dissolved in DMSO. The test compounds were taken at different concentration ranging from 200, 100, 50, 25, 12.5, 6.25, and 3.13 μg/mL for minimum inhibitory concentration (MIC) by using seeded broth dilution. Similarly, the standard solutions of drugs were prepared at concentration of 200, 100, 50, 25, 12.5, 6.25, and 3.13 μg/mL with sterile distilled water and DMSO was maintained throughout the experiment simultaneously as control. The MIC was the lowest concentration of the tested compound that resulted in no visible growth of the organism. To ensure that the solvent had no effect on bacterial growth, a control test was also performed with test medium supplemented with DMSO at same dilutions as used in the experiment.

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