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Two Step Synthesis, Characterization and *In Vitro* cancer activity of series of 6- substituted-1*H*-indazol-3-ols

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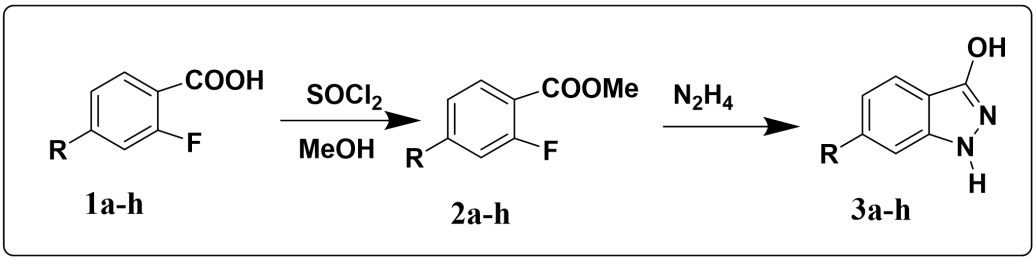
Abstract: Herein, we describe the two step synthesis of different series of6- substituted)-1*H*-indazol-3-ols (3a-h) viz, esterification followed by dehydro halogenation through cyclozation whenever treated with N_2H_4heated at 70 ^{0}C for 24 h to get promising yields shown in Scheme-I and also screened for In vitro anti-cancer evaluation. Among tested indazole compounds **3c** and **3h**are found to be more potent against the HCT-116 cell line than the MDA-MB-231 cell line. The compound **3c** IC_{50} value against the HCT-116 cell line 92.9 ± 6.5 whereas MDA-MB-231 cell line 102.3± 13.2. Compound **3h** IC_{50} value against the HCT-116 cell line 93.6 ± 7.2 whereas MDA-MB-231 cell line 94 ± 6.4. Doxorubicin is used as standard.

Keywords: Indazoles, cyclization, anticancer activity



Scheme I. Synthetic path way for compounds**3a-h**

1. INTRODUCTION

The nitrogen-containing heterocycles are important building blocks for many bioactive natural products and commercially available drugs. Indazole and its derivatives belong to an enormously important family of nitrogen-containing heterocyclic systems

(Figure 1), often allied with a wide range of biological activities [1–5]. Many of indazole derivatives have been reportedly found to possess potent pharmacological activity such as anti-tumor [6,7], anti-platelet [8], antiviral [9], antioxidant [10], anti-spermatogenic activity [11], anti-tubercular [12,13], anti-inflammatory and anti-microbial [14], neuroprotection [15], and COX inhibition activity [16]. Moreover, some indazoles were reported as protein kinase C-B/AKt inhibitors [17], potent IDO1/TDO dual inhibitors [18], and also as 5-HT_2 receptor antagonists [19, 20].

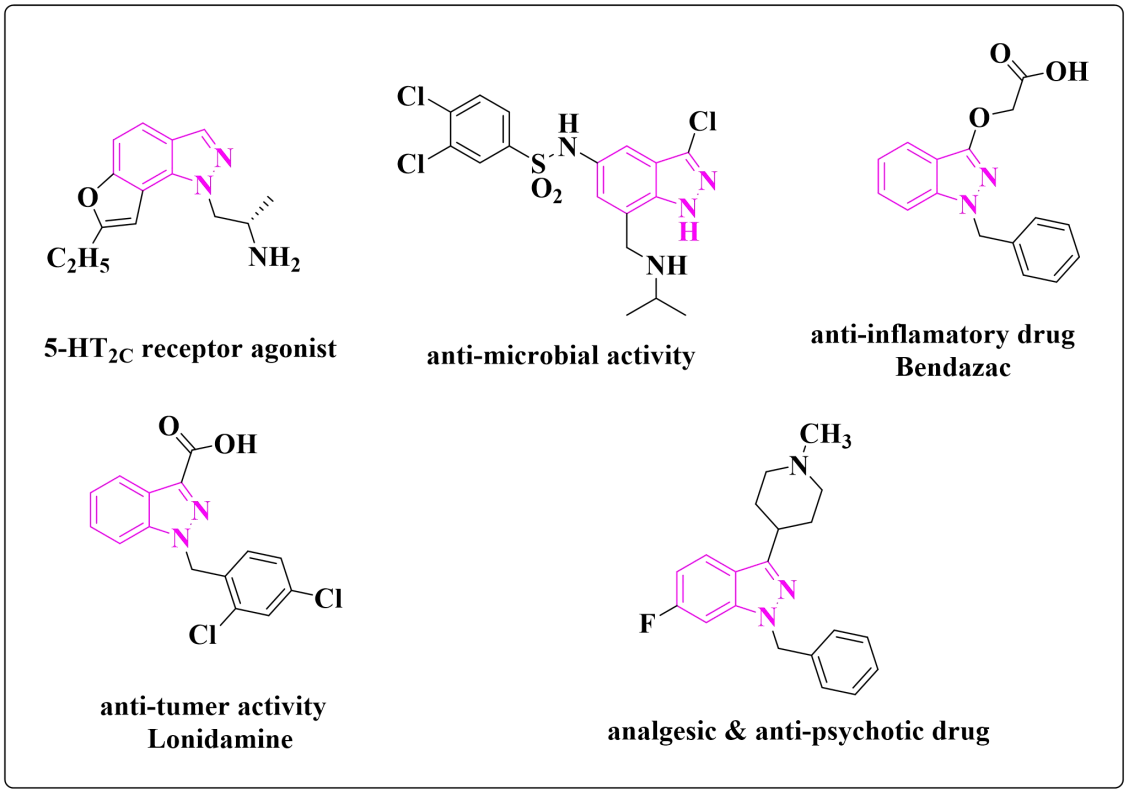


Figure 1. Some biologically active molecules of indazoles.

Niraparib has been widely used as an anticancer drug for the treatment of recurrent epithelial ovarian, fallopian tube, breast, and prostate cancer [21], pazopanib [22] and axitinib [23] are tyrosine kinase inhibitors approved by the FDA for renal cell carcinoma; these are some of the present-day anticancer drugs[24-27] possessing a privileged indazole skeleton.(Figure 2)

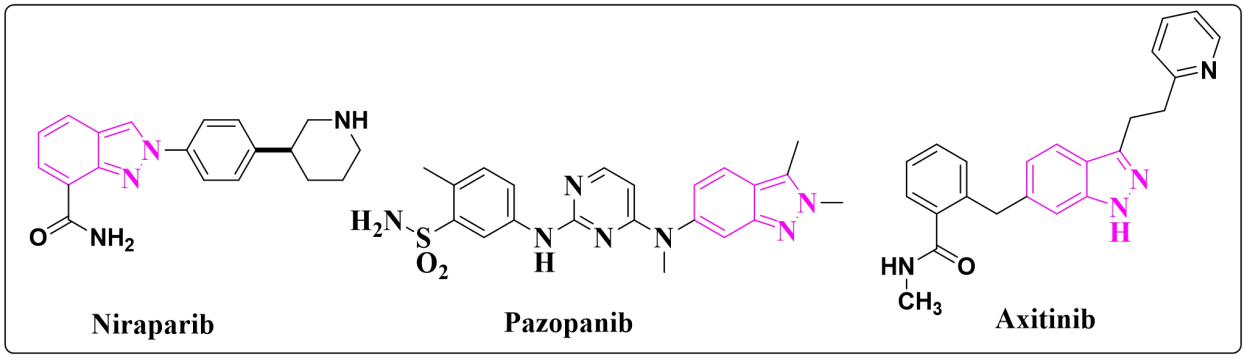


Figure 2. Indazoles containing anticancer drugs.

Herein, we describe the two step synthesis of series of various 6- substituted)-1*H*-indazol-3-ols depicted.**(3a-h)**(Figure 3)

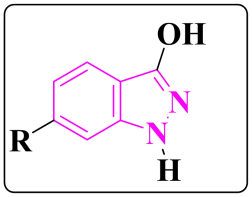


Figure 3. Desired Indazoles**(3a-h)**

2. EXPERIMENTAL SECTION

2.1General information

Electro thermal apparatus was used to record the melting point of synthesized compounds and are uncorrected. Thin-layer chromatography (TLC) was performed by using Merck silica gel 60 F254 precoated plates (0.25 mm) and column chromatography was performed by using Silica gel (particle size 100-200 mesh). ^{1}H NMR spectra were recorded on a Bruker AMX 500 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker AMX 125 MHz spectrometer. Chemical shift values were given in ppm (δ) with TMS as an internal standard. Mass spectra were determined on Agilent LC-1100 (LC-MS) series instrument. Elemental analyses were performed on a Carlo Erba 106 and Perkin Elmer model 240 analyzers.

2.2 General procedure for the synthesis of series of 4- substituted methyl 2-fluorobenzoates via esterification from (1a-h):

A solution of series of para substituted 2-fluorobenzoic acid(1a-h) (0.3 g, 1.7 mmol) in dry methanol (10 mL) was prepared in a three-necked, round-bottom flask fitted witha reflux

condenser and a calcium chloride tube. The flask was placed in an ice/water bath, thionyl chloride (1.4 mL) was then added drop wise, and the mixture was heated at 60-65 ^{0}C for three days under argon. After removal of solvent, the resulting residue was purified by extraction with pentane and using column chromatography on silica gel (hexane/diethyl ether 6:1) for desired products of 4- substituted methyl 2-fluorobenzoates**(2a-h)**.

2.3 General Procedure for Synthesis of 6-substituted -1*H*-indazoles (3a–h):

In a three-neck round-bottom flask equipped with a reflux condenser,4- substituted methyl 2-fluorobenzoate**(2a-h)** (0.20 g, 1.1mmol) was dissolved in tetrahydrofuran (20 mL); then, a solution of 98% hydrazine monohydrate (0.15 g, 3.0 mmol) in ethanol (10 mL) was added drop wise. The mixture was heated at 70 ^{0}C for 24 h. After cooling at room temperature, the solution was decanted and the organic solvent evaporated under vacuum. The solid residue was washed with dichloromethane and dried to afford desired indazoles (**3a–h**) (0.13 g,) as a white solid with 57-72% yields.

2.4 Procedure for Anti-cancer Activity:

The MTT cell proliferation assay method was used to analyze the cell growth on a protocol

of 48 h [28]. Human colorectal cancer cell lines (HCT-116 and MDA-MB-231) were procured fromthe National Centre for Cell Sciences (NCCS), Pune, India, and maintained in DMEM. The cell lineswere cultured with DMEM supplemented with 10% FBS, L-glutamine, $NaHCO_3$, and an antibioticsolution containing penicillin (100 U/mL) and streptomycin (100 µg/mL). The exponentially growingcells were seeded at 5×10^3 cells per well into 96-well plates. The culture medium was removed after24 h incubation at 37 ^{0}C and restored with fresh medium containing the candidate compounds indifferent concentrations.

Furthermore, the cells were incubated for another 72 h. Then, 20 mL of MTT (3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium

bromide) solution (5 mg/mL) was added to all wellsand incubated for 4 h at 37 ^{0}C. The medium containing MTT was discarded, 150 mL of DMSO was added to each well and the plates agitated until the dark blue crystals (formazan)had completely dissolved; the absorbance was measured using a microplate reader at a wavelength of570 nm. Each concentration was analyzed in triplicate, and the experiment is repeated three times.The average 50% inhibitory concentration (IC_{50}) is determined from the concentration-response curvesaccording to the inhibition ratio for each concentration.

2.5 Cytotoxic Study:

Initially, the prepared compounds **3a–3h**are screened for their in-vitro anticancer activity againstthe human colon carcinoma cell line (HCT-116) and the human breast cancer cell line (MDA-MB-231). The cell lines are cultured with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and an antibiotic solution containing penicillin (100 U/mL) and streptomycin (100 µg/mL). All cell lines are maintained in culture at 37 ^{0}C in an atmosphere of 5% carbon dioxide. The synthesized indazoles **3a–3h**are screened for in vitro cytotoxic activity against HCT-116 and MBA-MB-231 cell lines. The anticancer properties of these analogs, i.e., **3a–3h**, are compared with the standard doxorubicin. IC_{50} values of the test compound for 24 h on each cell line are calculated and presented in Table 3.

It is evident from the results that the tested indazole compounds **3c**and **3h** are found to be more potent activity. The compound**3c**IC_{50}value against the HCT-116 cell line92.9 ± 6.5 whereas MDA-MB-231cell line102.3± 13.2. Compound **3h**IC_{50}value against the HCT-116 cell line93.6 ± 7.2 whereas MDA-MB-231cell line94 ± 6.4.The compounds **3a**, **3b,** and **3e** are found to be inactive against the tested two cell lines.

Table 1. Optimization conditions & m/z for synthesized compounds 3a-3h

Entry	Name/ Structure	MF	Time (hr)	Yield (%)	M.P (ºC)	m/z (ESI-MS) $(M + H)^+$.
3a	6-(piperidin-4-yl)-1H-indazol-3-ol	$C_{12}H_{15}N_3O$	24	60	293	218.12

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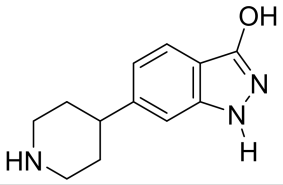
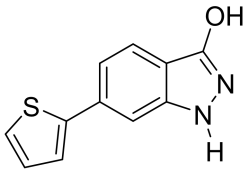
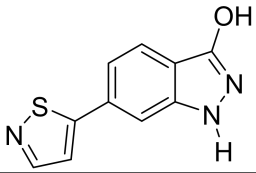
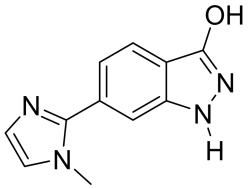
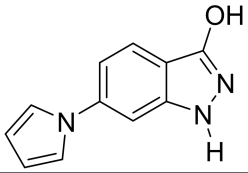
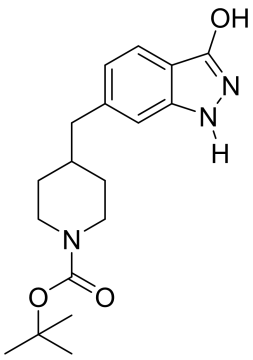
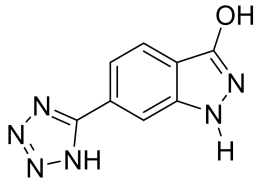
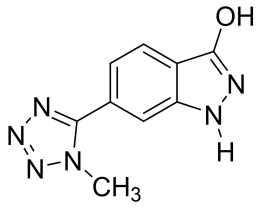
						
3b	6-(thiophen-2-yl)-1H-indazol-3-ol 	$C_{11}H_8N_2OS$	24	58	234	217.04
3c	6-(isothiazol-5-yl)-1*H*-indazol-3-ol 	$C_{10}H_7N_3OS$	24	64	216	218.03
3d	6-(1-methyl-1H-imidazol-2-yl)-1H-indazol-3-ol 	$C_{11}H_{10}N_4O$	24	71	228	215.09
3e	6-(1H-pyrrol-1-yl)-1H-indazol-3-ol 	$C_{11}H_9N_3O$	24	62	206	199.07
3f	tert-butyl 4-((3-hydroxy-1H-indazol-6-yl)methyl)piperidine-1-carboxylate 	$C_{18}H_{25}N_3O_3$	24	57	268	332.19
3g	6-(1H-tetrazol-5-yl)-1H-indazol-3-ol	$C_8H_6N_6O$	24	68	217	203.06

						
3h	6-(1-methyl-1H-tetrazol-5-yl)-1H-indazol-3-ol 	$C_9H_8N_6O$	24	72	209	217.08

3. RESULTS AND DISCUSSIONS

A different series of6- substituted)-1*H*-indazol-3-ols (3a-h) viz, esterification followed by dehydro halogenation through cyclozation whenever treated with N_2H_4heated at 70 ^{0}C for 24 h to get promising yields described. It is evident from the results that the tested indazole compounds **3c**and **3h**are found to be morepotent against the HCT-116 cell line than the MDA-MB-231 cell line. The test compounds **3c** and **3h** exhibited almost similar activity against the HCT-116 cell linewith IC50 < 94 µg/mL. The compounds **3h** and **3c** are found to be moderately active against the cell line MDA-MB-231 with IC50 < 96 and IC50 < 103 µg/mL, respectively. Unfortunately, the compounds **3a**,**3b,** and **3e** are found to be inactive against the tested two cell lines.**(Table 1, 2 & 3)**

Table 2. ^{1}H NMR &^{13} C NMR data for compounds 3a-h

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Entry	^{1}H NMR (500 MHz, $CDCl_3$)	^{13}C NMR (125 MHz, $CDCl_3$)
3a	δ 9.14 (s, 1H), 7.46 (d, *J* = 92.0 Hz, 2H), 7.06 (d, *J* = 8.9 Hz, 1H), 3.06 – 2.60 (m, 5H), 2.22 – 1.71 (m, 4H), 1.32 (s, 1H).	δ 157.64, 146.52, 141.22, 119.29, 117.71, 113.76, 108.51, 45.43, 43.14, 33.20.
3b	δ 9.23 (s, 1H), 8.30 (s, 1H), 7.90 (dd, *J* = 66.1, 8.2 Hz, 2H), 7.51 (ddd, *J* = 33.7, 7.4, 1.5 Hz, 2H), 7.21 (t, *J* = 7.5 Hz, 1H).	δ 157.64, 141.15, 140.79, 139.11, 132.02, 128.79, 120.87, 114.22, 114.04, 107.43.
3c	δ 8.56 (d, *J* = 7.5 Hz, 1H), 8.05 (s, 1H), 7.92 – 7.66 (m, 2H), 7.49 (d, *J* = 7.5 Hz, 1H), -0.21 (s, 1H).	δ 161.83, 157.64, 156.90, 138.99, 119.23, 118.61, 116.66, 114.07, 108.51.
3d	δ 9.21 (s, 1H), 7.92 (s, 1H), 7.79 (d, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 8.9 Hz, 1H), 6.90 (d, *J* = 7.5 Hz, 1H), 6.58 (s, 1H), 3.62 (s, 3H).	δ 157.64, 146.09, 141.20, 133.06, 131.58, 124.81, 122.44, 120.65, 115.41, 109.35, 33.21.
3e	δ 9.19 (s, 1H), 8.17 (s, 1H), 7.87 (dt, *J* = 24.6, 4.4 Hz, 2H), 7.30 (dd, *J* = 5.5, 3.4 Hz, 2H), 6.27 (dd, *J* = 5.6, 3.4 Hz, 2H).	δ 157.64, 145.11, 136.06, 118.20, 117.28, 112.43, 111.36, 107.00.
3f	δ 7.63 (d, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 1.6 Hz, 1H), 7.11 (d, *J* = 7.5 Hz, 1H), 3.95 – 3.78 (m, 2H), 3.51 – 3.34 (m, 2H), 3.17 (d, *J* = 7.0 Hz, 2H), 2.01 (dt, *J* = 19.7, 6.5 Hz, 2H), 1.84 – 1.57 (m, 3H), 1.48 (s, 9H), -0.14 (s, 1H).	δ 157.64, 155.11, 142.59, 142.04, 122.23, 118.02, 111.86, 110.61, 80.96, 44.87, 43.59, 36.54, 30.60, 28.41
3g	δ 9.45 (s, 1H), 9.18 (s, 1H), 8.14 – 7.92 (m, 2H), 7.85 (d, *J* = 7.5 Hz, 1H).	δ 157.64, 150.64, 143.09, 129.65, 125.60, 122.60, 114.70, 111.76.
3h	δ 9.18 (s, 1H), 8.12 (s, 1H), 7.87 (dt, *J* = 14.3, 4.4 Hz, 2H), 3.92 (s, 3H).	δ 157.64, 156.08, 141.87, 127.47, 125.32, 119.86, 108.98, 33.46.

^{1}HNMR N-H proton signal disappears in the presence of $CDCl_3$ solvent.

Table 3 Anticancer activity of compounds 3a-3h.

Entry	IC_{50} (µg/mL)	
	HCT-116	MDA-MB-231
3a	-	-
3b	-	-
3c	**92.9 ± 6.5**	**102.3± 13.2**
3d	124.6 ± 3.8	114.5 ± 15.0
3e	-	-
3f	109.6 ± 12.3	107.4 ± 10.0
3g	106.6 ± 14	112.2 ± 17.9
3h	**93.6 ± 7.2**	**94 ± 6.4**
Standard *	**1.2 ± 0.3**	**0.3 ± 0.1**

"–" indicates IC_{50} value >200 _g/mL; IC_{50} values are reported in micromolar concentrations of the compound to affect 50% inhibition of the tumor cell growth; * doxorubicin is employed as standard.

4. CONCLUSION

In summary, a novel, cost-effective, eco-benign and practical method was developed to synthesize the series of 6- substituted-1*H*-indazol-3-olsas anticancer potent. The advantages of this method include a simple reaction set-up not requiring specialized equipment's, low-toxicity of the reagent, less reaction times, and high product yields with excellent purity.Among them **3c**and **3h** are found to be more potent.Doxorubicin is used for the positive control.

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