TWO STEP SYNTHESIS OF INDAZOLE DERIVATIVES AND THEIR ANTI-CANCER EVALUATION

G. Laxman, Jagadeesh Kumar Ega and Kavitha Siddoju✉

Department of Chemistry, Chaitanya Deemed to be University, Kishanpura, Hanamkonda, Warangal Urban 506001, Telangana State, India.

✉Corresponding Author: siddojukavitha81@gmail.com

ABSTRACT

Herein we described the synthesis of indazole derivatives through C-N cyclization and evaluated for their anticancer activities against three human cancer cell lines such as HeLa, MCF-7, and SKOV3 using MTT assay. The synthesized indazole hybrids are characterized based on ¹H NMR, ¹³C NMR spectral data, and elemental analysis. The results indicated that the most potent molecule in this series is compound 3b against MCF-7, compound 3h against HeLa, and compound 3d against SKOV3, which showed the highest activity with an IC₅₀ value of 32.92 µg/ml, 12.08 µg/ml, and 29.06 µg/ml respectively.

Keywords: Indazole, Therapeutic Applications, Cyclization, Anticancer Activity

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INTRODUCTION

Indazoles are heterocyclic moieties rarely found in nature. Indazole is described with a wide variety of medicinal activities. It is a great interest in the development of novel indazole-based therapeutic agents. The coming of single-atom catalysis as another boondocks that incorporates the benefits of both homogeneous and "heterogeneous catalysis"¹⁻² has gathered boundless consideration since its fundamental money in 2011³. Well-characterized active centers, stabilized by judiciously designed support, are highly desirable in heterogeneous catalysis for several reasons, their maximized atom utilization high surface coverage, and uniform structure to excellent selectivity and they have reactive sites wherein the isolated atoms and their local coordination environment can be reliably elucidated through spectroscopy techniques⁴. In the past years, these compounds have been well-established with their various functioning biological activities of antimicrobial^{5,6}, antimalarial⁷, anti-inflammatory⁸, anticancer^{9,10} and antihyperlipidemic¹¹, antihyperglycemic¹², cholesterol esterase inhibitor¹³, 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibitor¹⁴, and bacterial arylamine N-Acetyltransferases (NATs) inhibitor¹⁵. Indazoles and their subsidiaries accomplished extraordinary importance for therapeutic physicists for the explanation that they have different natural exercises. Indazole is a fragrant heterocyclic natural compound found in particular normal items and medication particles. This indazole structure delineates a tremendous scope of natural and pharmacological activities¹⁶ comprehensive of antibacterial¹⁷, calming¹⁸, anticancer¹⁹, antitubercular.²⁰ A portion of the indazole subordinates was portrayed as pharmacologically dynamic specialists as addressed in Figs.-1 to 3.

EXPERIMENTAL

Synthesis of *E*-hydrazone Esters from Diazo Compounds 2a-h

Carboxylic ester **1** (0.1 mmol), *p*-ABSA (0.12 mmol), and CH₃CN (0.3 ml) were added to a glass vial (4 ml). With blending, **1**, 5-diazabicyclo [4,3,0]non-5-ene (DBN) (0.12 mmol) was added dropwise. The response combination was mixed at room temperature (RT) for 60 minutes. Then, at that point, Pt/Ce4+ of 8.5 mg (nano CeO₂) impetuses and two ml of CH₃OH were added straightforwardly and sonicated for 15 min. From that point onward, 0.9 mmol of smelling salts borane (Stomach muscle) was added. It was immediately shut, and the response blend was mixed for an additional 3 hours at RT. 0.1 mmol of the

diazo compound and 0.3 mmol of abdominal muscle were added successively. 4 ml was fixed rapidly, and the response combination was mixed at RT for 40 min. besides conditions to the one-pot blend from aryl acetic acid derivation esters.

Fig.-1: Structures of the indazole nucleus

Fig.-2: Some important indazole-containing drugs with pharmacological activity

Fig.-3: Some medicinally potent indazoles

After the response, the blend was centrifuged to eliminate the impetus and washed multiple times with DCM. The supernatant was vapor under decreased pressing factor, and the residuals were exposed to be isolated utilizing a slight layer chromatography plate. The yield was determined by separating the measure of the got wanted product **2a-h** by the great yield. The gram-scale amalgamation of deformity rich CeO₂ nanorods.²¹⁻²²

Synthesis of Indazole Derivatives

E-hydrazone esters **2a-h** were treated with 10% CuI, 0.9 mmol of Cs₂CO₃ under DMSO solvent stirred for 2 hours and followed by KOH, EtOH at 68 °C upon reflux condensation further hydrolysis indazole esters

derivatives are converted into indazole substituted carboxylic acids **3a-h**. All the products are monitored by TLC and isolated by GC. All the derivatives were characterized by ^1H NMR, ^{13}C -NMR spectra and CHN analyzer shown in Table-1 and 2.

Fig.-4: Yield Percentages of Compounds **2 a-h**

RESULTS AND DISCUSSION

Chemistry

We have prepared a series of substituted indazole carboxylic acids **3a-h** two steps (**Scheme-1**). In the first step, the one-pot union of *E*-hydrazone esters from carboxylic esters by treating with Carboxylic ester **1** (0.1 mmol), *p*-ABSA (0.12 mmol), and CH_3CN (0.3 ml) were added to a glass vial (4 ml). With blending, 1,5-diazabicyclo[4,3,0]non-5-ene (DBN) (0.12 mmol) was added dropwise. The response blend was mixed at room temperature (RT) for 60 minutes. Then, Pt/ Ce^{4+} of 8.5 mg (nano CeO_2) catalysts and two ml of CH_3OH were added directly and sonicated for 15 min. *E*-hydrazone esters **2a-h** were treated with 10% CuI, 0.9 mmol of CS_2CO_3 under DMSO solvent stirred for 2 hours leads to cyclization and followed by KOH, EtOH at 68°C upon reflux condensation further hydrolysis indazole esters derivatives to result in the corresponding indazole substituted carboxylic acids **3a-h** in quantitative yields.

Scheme-1: Synthesis of Indazole derivatives **3(a-h)**

(3a. yield% 71; 3b. yield% 73; 3c. yield% 81; 3d. yield% 89; 3e. yield% 69; 3f. Yield% 79; 3g. yield% 80; 3h. yield% 83.)

Anti-Cancer Activity

The orchestrated mixtures were evaluated for in vitro cytotoxic movement utilizing the microculture tetrazolium test, tried against human malignancy lines MCF-7, HeLa, and SKOV3. The different groupings of the engineered compounds (last focuses 1, 10, 20, 30, 40, and 50 $\mu\text{g/ml}$) were applied to ascertain IC_{50} . The IC_{50} focus for each compound was determined concerning a control test, which addresses the fixation of those outcomes in a half reduction in cell development/expansion after 48 h hatching within the sight of the medication. The determined IC_{50} is arranged in Table-1. From Table 1 it is clear that compound **3b** against MCF-7, compound **3i** against HeLa, and compound **3d** against SKOV3, showed the most noteworthy action with an IC_{50} worth of 32.92 $\mu\text{g/ml}$, 12.08 $\mu\text{g/ml}$, and 29.06 $\mu\text{g/ml}$ individually. The cytotoxic exercises of combined mixtures were contrasted and the action showed by the reference drug Doxorubicin.

Table-1: Anticancer Activity of Synthesized Compounds

Entry	Anticancer Activity (IC ₅₀ in μ M)		
	MCF-7	HeLa	SKOV3
3a	75.72	17.76	91.78
3b	32.92	42.06	> 100
3c	> 100	33.87	> 100
3d	> 100	57.05	29.06
3e	> 100	32.36	> 100
3f	> 100	62.77	> 100
3g	35.15	22.07	> 100
3h	91.66	12.08	> 100
Doxorubicin	35.29	02.96	11.34

¹H, ¹³C-NMR Spectra of 2a

¹H NMR (400 MHz, Chloroform-*d*) δ [ppm] 7.49 (ddd, J = 7.6, 6.3, 1.3 Hz, 2H), 7.45 – 7.40 (m, 1H), 7.32 – 7.28 (m, 2H), 6.27 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 1.35 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, Chloroform-*d*) δ [ppm] 164.4, 137.4, 129.6, 129.3, 129.2, 128.8, 61.3, 14.3.

¹H, ¹³C-NMR Spectra of 2b

¹H NMR (400 MHz, Acetonitrile-*d*3) δ [ppm] 7.55 – 7.48 (m, 1H), 7.36 – 7.22 (m, 3H), 6.78 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, Acetonitrile-*d*3) δ [ppm] 164.7, 160.3 (d, J IF = 244 Hz), 132.1 (d, J 3F = 8 Hz), 131.6 (d, J 3F = 4 Hz), 130.3, 125.5 (d, J 4F = 3 Hz), 118.9 (d, J 2F = 18 Hz), 116.6 (d, J 2F = 22 Hz), 61.1, 14.2.

¹H, ¹³C-NMR Spectra of 2c

¹H NMR (400 MHz, Chloroform-*d*) δ [ppm] 7.66 – 7.62 (m, 2H), 7.22 – 7.19 (m, 2H), 6.31 (s, 2H), 4.31 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, Chloroform-*d*) δ [ppm] 164.1, 136.1, 132.5, 130.6, 128.3, 123.7, 61.4, 14.3.

¹H, ¹³C-NMR Spectra of 2d

¹H NMR (400 MHz, Acetonitrile-*d*3) δ [ppm] 8.02 (d, J = 8.5 Hz, 1H), 7.98 – 7.93 (m, 2H), 7.82 (s, 1H), 7.63 – 7.56 (m, 2H), 7.36 (dt, J = 8.4, 2.2 Hz, 1H), 6.75 (s, 2H), 4.26 – 4.20 (m, 2H), 1.30 – 1.26 (m, 3H); ¹³C-NMR (100 MHz, Acetonitrile-*d*3) δ [ppm] 165.3, 135.8, 133.9, 133.8, 129.2, 129.1, 128.7, 128.3, 128.3, 127.4, 127.1, 127.0, 61.0, 14.3.

¹H, ¹³C-NMR Spectra of 2e

¹H NMR (400 MHz, Acetonitrile-*d*3) δ [ppm] 7.27 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, Acetonitrile-*d*3) δ [ppm] 163.1, 145.0 (m), 143.1 (m), 141.1 (m), 139.1 (m), 137.1 (m), 119.4, 105.6 (m), 61.0, 13.5.

¹H, ¹³C-NMR Spectra of 2f

¹H NMR (400 MHz, Chloroform-*d*) δ [ppm] 7.75 – 7.70 (m, 2H), 7.42 – 7.36 (m, 2H), 6.26 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 1.28 (d, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, Chloroform-*d*) δ [ppm] 163.7, 135.0, 132.9, 131.6, 130.0, 118.2, 113.3, 61.7, 14.3.

¹H, ¹³C-NMR Spectra of 2g

¹H NMR (400 MHz, Chloroform-*d*) δ [ppm] 7.75 (d, J = 8.4 Hz, 1H), 7.40 – 7.38 (m, 1H), 7.34 (s, 1H), 7.30 – 7.27 (m, 1H), 6.23 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, Chloroform-*d*) δ [ppm] 163.6, 146.9, 145.5, 131.0, 129.8, 128.4, 127.7, 123.3, 61.2, 14.2.

¹H, ¹³C-NMR Spectra of 2h

¹H NMR (400 MHz, Acetonitrile-*d*3) δ [ppm] 8.10 – 8.07 (m, 2H), 7.44 – 7.41 (m, 2H), 6.72 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 2.63 (s, 3H), 1.27 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, Acetonitrile-*d*3) δ [ppm] 197.7, 164.3, 137.3, 135.6, 134.3, 129.4, 128.8, 60.5, 26.1, 13.6.

¹³C-NMR Spectra of Product (100 MHz, Chloroform-*d*) δ [ppm]

- 3a.** 160,142.4,141.3, 126.4,120.7,120.4,113.5.
3c. 160,144.6,141.3, 124.4,122.6,120.6, 113.3.
3d. 160,143.1,141.3, 127.3,121.6,115.8, 113.3,109.8.
3e. 160, 141.3,138.2, 135,129.3,127.8, 125.5,111.6.
3f. 160, 142.3,139.2, 134,128.3,125.8, 124.5,108.6.
3g. 160, 141.3,138.2, 135,129.3,127.6, 125.3,111.6.
3h. 160, 142.3,141.3, 134.7,127.4,120.3, 120.2,99.8,29.3.

Table-2: Analytical Data of Series of Compounds 2 and 3 by CHN Analyzer

Entry	Molecular Formulae	Analytical Data of CHN	
		Calcd (%)	Found (%)
2a	C ₁₀ H ₁₂ N ₂ O ₂	C, 62.49; H, 6.29; N, 14.57	C, 62.62; H, 6.38; N, 14.60
2b	C ₁₀ H ₁₁ FN ₂ O ₂	C,57.14;H, 5.27; N, 13.33	C,58.04;H,5.39;N,14.00
2c	C ₁₀ H ₁₁ BrN ₂ O ₂	C, 44.30; H, 4.09; N, 10.33	C, 44.64; H, 4.29; N, 11.00
2d	C ₁₁ H ₁₀ ClN ₃ O ₂	C, 52.50; H, 4.01;N, 16.70	C, 54.50; H, 4.81;N, 16.90
2e	C ₁₀ H ₇ F ₃ N ₂ O ₂	C, 42.57; H, 2.50; N, 9.93	C, 44.27; H, 2.89;N, 11.00
2f	C ₁₁ H ₁₁ N ₃ O ₂	C, 60.82; H, 5.10; N, 19.34	C, 61.82; H, 6.10; N, 20.14
2g	C ₁₀ H ₁₂ N ₂ O ₃	C, 57.68; H, 5.81; N, 13.45	C, 59.28; H, 6.71; N, 14.15
2h	C ₁₂ H ₁₄ N ₂ O ₃	C, 61.53; H, 6.02; N, 11.96	C, 62.33; H, 7.02; N, 12.11
3a	C ₈ H ₆ N ₂ O ₂	C, 59.26; H, 3.73; N, 17.28	C, 61.06; H, 4.73; N, 19.28
3b	C ₈ H ₅ N ₃ O ₂	C, 57.68; H, 5.81; N, 13.45	C, 59.28; H, 6.71; N, 14.15
3c	C ₈ H ₅ BrN ₂ O ₂	C, 39.86; H, 2.09; N, 11.62	C, 41.86; H, 3.19;N, 13.02
3d	C ₁₇ H ₁₄ N ₂ O	C, 57.76; H, 2.69; N, 22.45	C, 59.16; H, 3.69; N, 23.65
3e	C ₈ H ₂ F ₄ N ₂ O ₂	C, 41.04; H, 0.86; N, 11.97	C, 43.03; H, 1.16; N, 13.67
3f	C ₈ H ₃ F ₃ N ₂ O ₂	C, 59.76; H, 2.69; N, 22.45	C, 59.16; H, 3.69; N, 23.65
3g	C ₁₀ H ₇ FN ₂ O ₃	C, 41.04; H, 0.86; N, 12.97	C, 43.03; H, 1.16; N, 13.67
3h	C ₁₀ H ₈ N ₂ O ₃	C, 58.82; H, 3.95; N, 13.72	C, 59.92; H, 4.55; N, 15.12

Biological Evaluation

Cytotoxicity assay (MTT) was evaluated for all the synthesized compounds. Three different cancer cell lines viz., SKOV3 - Ovarian cancer, MCF7 - Breast cancer and HeLa Cervical cancer malignant growth was acquired from the ATCC (Bethesda, MD, USA) and kept up in DMEM enhanced with 10 % FBS, 2 mM l-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C in a 5 % CO₂ hatchery. After cultivating of cells in 96 well culture plates, permitted to join appropriately. Test mixtures of various fixations going from 1 to 50 µM were included sets of three and hatched for 24hr. The cells were then hatched with MTT (0.5 mg/mL) for 3hr and to break up the insoluble formazan precious stones 100 µl DMSO was added to each well. At long last, the absorbance of the plates was estimated utilizing a Collaboration H1 multi-mode plate per user, USA. Doxorubicin was utilized as a positive control for the examination.

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CONCLUSION

The present study described the two-step synthesis and anti-cancer activity of derived substituted indazoles. The compounds 3b, 3i and 3d are effective in anticancer activity.

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