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

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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

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-iota catalysis as another boondocks that incorporates the benefits of both homogeneous and "heterogeneous catalysis" has gathered boundless consideration since its fundamental money in 2011. 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 techniques. In the past years, these compounds have been well-established with their various functioning biological activities of antimicrobial, antimalarial, anti-inflammatory, anticancer and antihyperlipidemic, antihyperglycemic, cholesterol esterase inhibitor, 15-hydroxyprostaglandin dehydrogenase(15-PGDH) inhibitor, and bacterial arylamine N-Acetyltransferases (NATs) inhibitor. 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 activities comprehensive of antibacterial, calming, anticancer, antitubercular. 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 Diazocompounds 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

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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.

![Chemical Structures](image)

Fig.-1: Structures of the indazole nucleus

![Chemical Structures](image)

Pazopanib (I)

![Chemical Structures](image)

Niraparib (III)

![Chemical Structures](image)

Bendec (IV)

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 $^1$H NMR, $^{13}$C-NMR spectra and CHN analyzer shown in Table-1 and 2.

![Chemical structures](image)

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$_3$CN (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$_3$OH were added directly and sonicated for 15 min. $E$-hydrazone esters 2a-h were treated with 10%CuI, Cs$_2$CO$_3$ 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](image)

**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 μg/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 μg/ml, 12.08 μg/ml, and 29.06 μg/ml individually. The cytotoxic exercises of combined mixtures were contrasted and the action showed by the reference drug Doxorubicin.

2193
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<th>Entry</th>
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<tr>
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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), 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-d3) 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-d3) δ [ppm] 164.7, 160.3 (d, J1F = 244 Hz), 132.1 (d, J3F = 8 Hz), 131.6 (d, J3F = 4 Hz), 130.3, 125.5 (d, J4F = 3 Hz), 118.9 (d, J2F = 18 Hz), 116.6 (d, J2F = 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-d3) 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-d3) 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-d3) 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-d3) 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-d3) 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-d3) δ [ppm] 197.7, 164.3, 137.3, 135.6, 134.3, 129.4, 128.8, 60.5, 26.1, 13.6.
The present study described the two-step synthesis and anti-cancer activity of derived substituted indazoles. 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 fomazan 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.

REFERENCES

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