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Synthesis and *in vitro* cytotoxic activity of novel indazole analogues of curcumin against MCF-7, HeLa, WiDr, and vero cell lines

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ABSTRACT

We prepared six novel curcumin indazole analogs and confirmed their structures by Fourier transform infrared, nuclear magnetic resonance, and mass spectra. Subsequently, their cytotoxicity was tested using the Michigan Cancer Foundation (MCF-7) proliferation assay against the Michigan Cancer Foundation (MCF-7), HeLa, WiDr, and vero cell lines. This study found that the compounds we prepared were more active against WiDr than HeLa and MCF-7. The activity of 3b, 3c, 3d, and 5a against WiDr (colorectal carcinoma) cells was higher than curcumin and tamoxifen. Their selectivity index (SI) indicated that several synthesized compounds showed more selectivity (SI value > 2) than positive controls tamoxifen and doxorubicin (SI value < 2.00). Three compounds (3a, 3b, and 3c) showed high SI against WiDr cells (3.74, 5.27, and 4.39, respectively). Compound 3b produced the highest cytotoxic activity, especially against WiDr cells (IC_{50} = 27.20 µM) with excellent selectivity (SI = 5.27). Therefore, the compound should be further developed as an anticancer agent for colorectal carcinoma.

INTRODUCTION

The annual report in 2020 indicated a high prevalence of breast cancer, cervical cancer, and colorectal cancer worldwide (American Cancer Society, 2020). The development of monocarbonyl analogs of curcumin to be tetrahydro-indazole structures showed good antioxidant and antitumor against Michigan Cancer Foundation (MCF-7), WI38, Hep G2, and vero cells (Bayomi *et al*., 2015; Bayomi *et al*., 2013). Indazole compounds are scarcely found in nature, generally prepared by organic synthesis. The formation of the indazole ring significantly improved the biological activity of the molecules (Gaikwad *et al*., 2015; Plescia *et al*., 2010; Shrivastava *et al*., 2016; Thangadurai *et al*., 2012; Thirupalu *et al*., 2014; Zhang *et al*., 2018). Recently, *in silico* studies using pharmacophore modeling and docking methods indicated that several asymmetric hexahydro-2H-indazoles were potentially active as ERα inhibitors (Hariyanti *et al*., 2021). Therefore, we prepared six novel curcumin indazole analogs and evaluated them for their cytotoxicity against breast (MCF-7), cervical (HeLa), and colorectal (WiDr) cancer cells. To evaluate their selectivity, the compounds' cytotoxicity was also tested against normal vero cells.

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MATERIALS AND METHODS

General procedures

All chemicals (E. Merck, Germany, or Sigma-Aldrich, USA) were obtained commercially. Purity tests and reactions monitoring were carried out using the thin layer chromatography procedure. Melting points (m.p.) were assessed by the melting point instrument (Bibby Sterilin, UK) and were not corrected. Infrared (IR) spectra were scanned in a Kalium Bromide mixture on the Shimadzu FTIR-8400S Spectrometer (Japan). Nuclear magnetic resonance (NMR) spectra were run in a $CDCl_3$ solution on a JEOL JNM 500 Spectrometer (Peabody, USA). Mass spectra were found using electrospray ionization (+) mode UNIFI-Waters Liquid Chromatography-Mass Spectrometry (MS)/MS (USA). 3–(4–Methoxyphenyl/3,4-dimethoxyphenyl)–3,3a,4,5,6,7–

hexahydro–2H–indazole (**1a-b**), cyclovalone (**4a**), and 2,6-bis-[(E)-4-hydroxybenzylidene]cyclohexanone (**4b**) used as starting materials were prepared according to the reported method (Hayun *et al*., 2017; Minu *et al*., 2009; Rahmawati *et al*., 2020).

Preparation of curcumin tetrahydro-indazole analogs (3a-d)

The preparation of **3a** with a yield of 61.0% has been reported previously (Hariyanti *et al*., 2020). Preparation of compounds **3b-d** was carried out according to the procedures of compound **3a** by reacting **1b** that replaced **1a** with *p*-methoxy-benzaldehyde, vanillin, or 3,4-dimethoxy-benzaldehyde (**2a-c**).

4-([(7E)-3–(3,4–dimethoxyphenyl)–4,5,6,7–tetrahydro–3aH–indazol-7-ylidene]methyl)-2-methoxyphenol (3b)

Pale yellow powder, yield 35.2%, and m.p. 240°C–242°C. Fourier Transform Infrared (FT-IR), υ/cm^{-1}: 3,217 (OH), 3,059 (C-H Ar stretch), 2,956 (C-H Al stretch), 1,582 (C=N), 1,519 (C=C ring Ar stretch), 1,460 C-H Al bend), 1,253 (C-O-Ar asymmetrical stretch), and 1,162 (C-O-Ar symmetrical stretch). ^{1}H-NMR, δ/ppm: 7.24 (d, 1H, *J* 3 Hz), 6.94 (d,02H, *J* 8 Hz), 7.17 (dd,01H, *J* 8 Hz), 6.89 (dd,02H, *J* 8 Hz), 3.85 (t,01H), 3.91 (d, 6H), 3.92 (s, 3H), 2.81 (m,04H), 1.97 (m,02H). ^{13}C-NMR, δ/ppm: 149.35 (1C), 149.21 (1C), 148.64 (1C), 148.36 (1C), 145.53 (1C), 144.13 (1C); 129.9 and 114.8 (C=$C_{ethylenic}$), 1,122.4 (2C_{Ar}); 123.9, 119.9, 112.8,0,111.1,0110.9, and 110.2 (1C_{Ar}, respectively); 56.0, 55.1, and 55.06 (1C_{O-Me}, respectively);227.2, 31.2, 24.2, and 22.2 (1C_{Al}, respectively). HR-MS:-m/z 393.18003 $[M+H]^+$; calculated for $C_{23}H_{24}N_2O_4$ = 392.17361 [M]; Error = - 2.3 ppm.

(7E)–3–(3,4–dimethoxyphenyl)–7–(4–methoxybenzylidene)–4,5,6,7–tetrahydro–3aH–indazole (3c)

Pale yellow powder, yield 74.3%, and m.p. 244°C–246°C. FT-IR, υ/cm^{-1}: 3,020 (C-H Ar stretch), 2,920 (C-H Al stretch), 1,561 (C=N), 1,519 (C=C ring Ar stretch), 1,460 (C-H Al bend), 1,261 (C-O-Ar asymmetrical stretch), and 1,136 (C-O-Ar symmetrical stretch). ^{1}H-NMR, δ/ppm: 7.23 (d, 1H, *J* 1.5 Hz), 6.89 (d, 2H, *J* 9 Hz), 7.17 (dd, 1H, *J* 8 Hz), 6.97 (s, 1H), 6.94 (dd, 1H, *J* 8 Hz), 6.87 (d, 1H, *J* 8.5 Hz),03.92 (s, 3H),03.91 (d,06H),02.81 (m, 4H), 1.88 (m,02H). ^{13}C-NMR, δ/ppm: 149.22 (1C), 148.65 (1C), 148.29 (1C), 145.73 (1C), 144.02 (1C); 129.6 and 122.4 (C=$C_{ethylenic}$); 126.2, 123.9, 119.9, 114.8, 112.8,0111.2,0111.1, 110.95, and 110.92 (1C_{Ar}, respectively); 56.1, 56.05, and 55.0 (1C_{O-Me}, respectively); 27.2, 31.2,024.7, and 22.3 (1C_{Al}, respectively). HR-MS:-m/z 409.21152 $[M+CH_3OH+H]^+$; calculated for $C_{23}H_{24}N_2O_3$= 376.17896 [M]; Error = 0.3 ppm.

(7E)–3–(3,4–dimethoxyphenyl)–7–(3,4–dimethoxybenzylidene)–4,5,6,7–tetrahydro–3aH–indazole (3d)

Pale yellow powder, yield 56.1%, and m.p. 236°C–238°C. FT-IR, υ/cm^{-1}: 3,020 (C-H Ar stretch), 2,947 (C-H Al stretch), 1,591 (C=N), 1,514 (C=C ring Ar stretch), 1,450 (C-H Al bend), 1,255 (C-O-Ar asymmetrical stretch), and 1,142 (C-O-Ar symmetrical stretch). ^{1}H-NMR, δ/ppm: 6.88-(s, 2H), 7.19 (dd,01H, *J* 8 Hz), 6.91 (s, 2H), 6.87 (s,01H), 6.94 (dd, 2H, *J* 8 Hz), 3.93 (s, 3H),03.92 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 2.81 (m, 4H), 1.88 (m, 2H). ^{13}C-NMR, δ/ppm: 149.27 (1C), 148.94 (1C), 148.71 (1C), 148.16 (1C),0129.85 (1C),0128.0 (1C); 122.12 and 121.3 (C=$C_{ethylenic}$); 119.42, 109.92, and 111.04 (2C_{Ar}, respectively); 112.66 and 111.28 (1C_{Ar}, respectively); 56.08 and 56.04 (2C_{O-Me}, respectively); 31.15, 27.24,024.81, and 22.38 (1C_{Al}, respectively). HR-MS :-m/z 407.19619 $[M+H]^+$; calculated for $C_{24}H_{26}N_2O_3$ = 406.18926 [M]; Error = - 0.7 ppm.

Preparation of curcumin hexahydro-indazole analogs (5a–b)

Preparation of **5a-b** was carried out by mixing 10 mmol of the synthesized cyclovalone (**4a**) for **5a** and its *p*-hydroxy analog (**4b**) for **5b**, with 100 mmol hydrazine monohydrate in 10 ml of glacial acetic acid, refluxed at 120°C for 3 hours until completed reaction, added on to crushed ice, filtered off till the suspension was obtained, and washed using cold water to afford the solid product. Recrystallization was done from a suitable solvent to provide the pure compound **5a–b**.

1-[(7E)-3-–(4-hydroxy,3–methoxyphenyl)–7–(4-hydroxy,3–methoxybenzylidene)-3,3a,4,5,6,7-hexahydro–2H–indazol-2-yl]-ethan-1-one (5a)

Pale yellow powder, yield 23.7%, and m.p. 105°C–107°C. FT-IR, υ/cm^{-1}: 3,200–3,525 (OH), 3,059 (C-H Ar stretch), 2,956 (C-H Al stretch), 1,635 (C=O), 1,599 (C=N), 1,518 (C=C ring Ar stretch), 1,450 (C-H Al bend), 1,273 (C-O-Ar asymmetrical stretch), and 1,150 (C-O-Ar symmetrical stretch). ^{1}H-NMR, δ/ppm: 7.14 (s, 1H), 6.92 (d, 2H, *J* 2-Hz), 6.75 (d,01H, *J* 2 Hz), 6.86 (s,01H), 6.78 (dd,01H, *J* 8Hz), 6.88 (d, 1H, *J* 8Hz), 3.90 (d, 6H), 2.19 (s, 3H), 4.83 (d, 1H), 3.06 (d, 1H), 2.39 (s, 2H), 2.32 (m, 1H), 2.43 (m, 1H), 2.96 (m, 1H), 1.94 (m, 2H). ^{13}C-NMR, δ/ppm: 170.64 (1$C_{C=O}$), 159.08 (1$C_{C=N}$);0146.82,0146.38, 145.61, 145.06 (1C_{Ar-O}, respectively); 0128.98 and 128.19 (C=$C_{ethylenic}$); 134.11, 128.64, 123.47, 118.63, 114.86, 114.46, 112.38, and 108.48 (1C_{Ar}, respectively); 67.99 (1C_{C-N}); 57.43 and 56.08 (1C_{O-Me}, respectively); 38.96, 30.15, 29.06,024.49, and 22,49 (1C_{Al}, respectively). HR-MS:-m/z 423.19132 $[M+H]^+$; calculated for $C_{24}H_{26}N_2O_5$ = 422.18417 [M]; Error = - 0.2 ppm.

1-[(7E)-3-(4-hydroxyphenyl)–7–(4-hydroxybenzylidene)-3,3a,4,5,6,7–hexahydro–2H–indazol-2-yl]-ethan-1-one (5b)

Pale yellow powder, yield 42.2%, and m.p. 146°C–148°C. FT-IR, υ/cm^{-1}: 3,200–3,500 (OH), 3,012 (C-H Ar stretch), 2,943 (C-H Al stretch), 1,647 (C=O), 1,579 (C=N), 1,512 (C=C ring Ar stretch), 1,450 (C-H Al bend), and 1,263 (C_{Ar}-O stretch). ^{1}H-NMR, δ/ppm: 6.74 (m,01H, *J* 2 Hz), 7.19 (dd, 2H, *J* 8 Hz), 6.91 (s,01H), 6.84 (dd,01H, *J* 6 Hz), 7.05 (m,01H, *J* 8 Hz), 6.78 (dd, 2H, *J* 8 Hz), 2.13 (s, 3H), 4.07 (m,01H), 3.06 (d,01H), 2.72 (m,02H), 2.0 (s,01H), 1.8 (m,01H), 1.21 (m, 2H). ^{13}C-NMR, δ/ppm: 170.6 (1$C_{C=O}$), 159.97 (1$C_{C=N}$); 156.63 and 155.55 (1C_{Ar-O}, respectively); 0114.16 and0115.20 (C=$C_{ethylenic}$); 131.36, 130.71, 128.88, 128.12, 126.99, 121.35, 115.70, 115. 63, 115. 28 and 115.20 (1C_{Ar}, respectively); 67.43 (1C_{C-N}); 30.05, 29.76, 29.13, 27.20 and 24.67 (1C_{Al}, respectively). HR-MS:-m/z 363.17051 $[M+H]^+$; calculated for $C_{23}H_{22}N_2O_3$ = 362.16304 [M]; Error = +0.8 ppm.

***In vitro* cytotoxicity**

The prepared compounds were tested for cytotoxicity against MCF-7, HeLa, WiDr, and vero cell lines (American Type

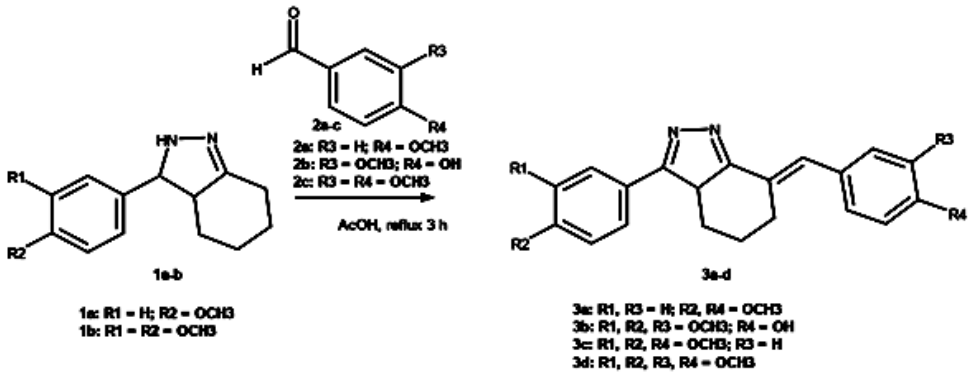

Figure 1. Preparation reaction of compounds **3a-d**.

Culture Collection ([ATCC) HTB-22, ATCC CCL-2, ATCC CCL-218, and ATCC CCL-81] using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay procedure (ATCC, 2020). Fetal bovine serum (5%), penicillin (100 U/ml), and streptomycin (100 µg/ml) were added to the 96-well plates containing cultivated cells in suitable growth media (Dulbecco's Modified Eagle's medium for WiDr and vero and Roswell Park Memorial Institute 1640 medium for MCF-7 and HeLa) and incubated for 24 hours until the culture reached 60% confluence. After replacing the growth media with new media, a series of concentrations (3.125–200 µg/ml) of test compounds were added. The cells were reincubated for 48 hours (the growth media were replaced every day with new media). After that, a 10 µl MTT solution was added and incubated for 4 hours; the media were discarded and the formazan formed in 100 µl ethanol was dissolved. Finally, the dissolved formazan was measured using an Enzyme-Linked Immunosorbent Assay reader at λ 595 nm (Bahuguna *et al*., 2017). The IC_{50} values were determined from the curve of concentrations versus inhibitions (%). GraphPad Prism 8 v. 8.02 (www.graphpad.com) was used for analysis.

RESULTS AND DISCUSSION

Chemistry

Compounds **3b-d** were prepared by condensation **1b**-and-benzaldehyde derivatives-**2a-c** in a yield of 35.2%, 56.1%, and 74.3% (Fig 1), while compounds **5a-b** were prepared by condensation symmetrical bis-benzylidene-cyclohexanone **4a-b** and hydrazine monohydrate in a yield of 23.7% and 42.2% (Fig 2). The reactions were carried out in glacial acetic acid and reflux temperature to afford novel products 4,5,6,7-tetrahydro-3aH-indazole (**3b-d**) and 2-acetylated-3,3a,4,5,6,7-hexahydro-2H-indazole (**5a-b**). The high temperature applied in the reaction most likely lowered the stability of the starting materials (Weerawatanakorn *et al.,* 2015), causing low yields. The application of mild conditions resulted in a higher yield; however, the products were different from those above (Bayomi *et al*., 2015; Nuriev *et al*., 2016; Raut *et al*., 2020).

The prepared compound structures were elucidated based on spectral analysis. No sharp peak at 3,290–3,300 cm^{-1} appeared in the FT-IR spectra, indicating the disappearance of the amine group. The bands at 3,012–3,059 and 2,920–2,956 cm^{-1} confirmed the presence of C-H aromatic and aliphatic bonds. The C=N azole, C=C aromatic ring, and C-O-C ether bonds were observed at 1,732–1,734, 1,447–1,665, and 1,140–1,265 cm^{-1}, respectively. Broad peaks at 3,217 and 3,200–3,500 cm^{-1} indicated a hydroxyl group's presence in the **3b** and **5a-b** compounds, while a strong peak at 1,635–1,647 cm^{-1} confirmed the C=O of acetamide in the **5a** and **5b** compounds. In the ^{1}H-NMR spectra, 7–9 protons for the two aromatic rings and one ethenyl chain appeared at 6–7 ppm, and a typical proton OCH_3 group appeared at around 3.8 ppm. Structural analysis was also supported by ^{13}C-NMR and the mass spectral data, which confirmed the suitability to the targeted compounds (MarvinSketch 20.8.0, 2020; Silverstein *et al*., 2005).

In vitro cytotoxicity

The prepared compounds (**3a-d** and **5a-b**) were evaluated for their cytotoxicity against four cell lines (MCF-7, HeLa, WiDr, and vero) using an MTT assay procedure. Curcumin, tamoxifen, and doxorubicin were used as a comparable compound

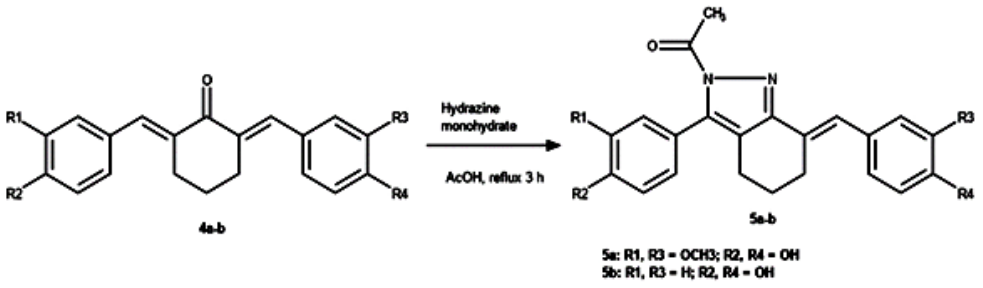

Figure 2. Preparation reaction of compounds **5a-b**.

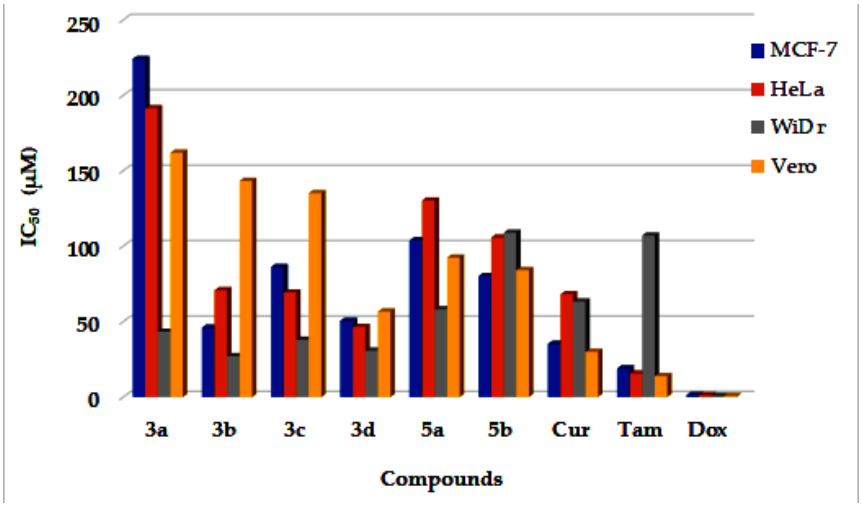

Figure 3. Cytotoxic activity (IC_{50}, µM) values for the prepared compounds, curcumin, tamoxifen, and doxorubicin on MCF-7, HeLa, WiDr, and vero cells, respectively.

Table 1. IC_{50} and SI values of the prepared compounds, curcumin, tamoxifen, and doxorubicin against MCF-7, HeLa, WiDr, and vero cell lines.

Compounds	IC_{500}(µM)[a]				SIb		
	HeLa	MCF-7	WiDr	Vero	HeLa	MCF-7	WiDr
3a	>100	>100	43.28	>100	<2	<2	3.74
3b	70.80	45.97	27.20	>100	2.02	3.12	5.27
3c	69.30	86.24	37.96	>100	<2	<2	4.39
3d	46.36	50.56	30.77	56.68	<2	<2	<2
5a	>100	>100	58.19	92.47	<2	<2	<2
5b	>100	80.26	>100	84.12	<2	<2	<2
Curcumin	68.09	35.30	63.41	30.01	<2	<2	<2
Tamoxifen	15.62	19.01	>100	13.91	<2	<2	<2
Doxorubicin	1.27	1.40	0.66	0.75	<2	<2	<2

[a]: Mean of triplicate experiments; [b] : SI value = ratio between IC_{50} of vero (normal) cell and IC_{50} of cancer cell.

and positive control. Tamoxifen is a drug commonly used in breast cancer treatment, while doxorubicin is most useful for treating broad cancers such as leukemia, neuroblastoma, and ovary, lung, and breast cancer (Thorn *et al*., 2011).

The results indicated that the prepared compounds had low to medium cytotoxic activity (Table 1 and Fig. 3). The IC_{50} values against MCF-7 cells were between 45.97 and 86.24 µM. Compound **3b** had the highest cytotoxicity against MCF-7, but its activity was lower than curcumin. In contrast, tamoxifen and doxorubicin exhibited high cytotoxicity against MCF-7. The IC_{50} values against HeLa cells were between 46.36 and 100 µM. Compound **3d** had the highest cytotoxicity against HeLa cells. The cytotoxicity of **3d** was higher than that of curcumin but lower than tamoxifen and doxorubicin. The IC_{50} values against WiDr cells were between 27.20 and 58.19 µM. These results indicated that the synthesized compounds were more cytotoxic against WiDr than against HeLa and MCF-7 cells. -Compound **3b** had the highest cytotoxicity against WiDr cells. This activity was better than curcumin and tamoxifen but lower than doxorubicin.

The cytotoxicity of compounds **3b**, **3c**, and **3d** containing three and four methoxy groups was higher than compounds **3a**, **5a**, and **5b** containing less than three methoxy groups. The results were in line with the finding previously reported in asymmetrical analogs of curcumin and 4-amino chalcone derivatives that the methoxy group's number and position influence cytotoxic activity (Prasetyaningrum *et al*., 2018; Novilla *et al*., 2019).

The IC_{50} values against vero cells were between 56.68 and >100 µM. These data indicated that most synthesized compounds had low toxicity against normal cells (Burger *et al*., 2004; Schmitz *et al*., 1993). Compound **3b** showed high selectivity against MCF-7, HeLa, and WiDr cells [selectivity index, (SI) = 3.12, 2.02, and 5.27]. In contrast, compounds **3a** and **3c** only showed selectivity against WiDr cells (SI = 3.74 and 4.39). The selectivity of these compounds was higher than curcumin and the positive controls (tamoxifen and doxorubicin). The compound with an SI less than two indicates general toxicity. The higher the SI, the more selective the compound (Burger *et al*., 2004; Badisa *et al*.,

2009; Kurnia *et al*., 2019). The IC_{50} value of curcumin against MCF-7 cells was 35.03 µM, whereas in a previous study it was 8.62 µM (Li *et al*., 2015). This difference may be due to the different methods used for the testing or different laboratory conditions. The resistance factor also affects the sensitivity of the drug to cells.

CONCLUSION

A series of six curcumin indazole analogs have been prepared. All the compounds showed low to moderate cytotoxicity against MCF-7, HeLa, and WiDr cells. Compounds **3b** exhibited the greatest cytotoxicity, especially against WiDr cells with excellent selectivity. The compound should be further developed as a cytotoxic agent for colorectal adenocarcinoma.

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CONFLICT OF INTEREST

The authors confirm no conflicts of interest between the authors.

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ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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REFERENCES

American Cancer Society. Cancer facts & figures. American Cancer Society, pp 1–44, 2020. Available via https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2020.html (Accessed 1 December 2020).

Badisa RB, Darling-Reed SF, Joseph P, Cooperwood JS, Latinwo LM, Goodman CB. Selective cytotoxic activities of two novel synthetic drugs on human breast carcinoma MCF-7 cells. Anticancer Res, 2009; 29:2993–6.

Bahuguna A, Khan I, Bajpai VK, Kang SC. MTT assay to evaluate cytotoxic potential of a drug. Bangladesh J Pharmacol, 2017; 12:115–8; doi:10.3329/bjp.v12i2.30892

Bayomi SM, El-kashef HA, El-ashmawy MB, Nasr MNA, El-sherbeny MA, Abdel-aziz NI, El-Sayed MA, Suddek GM, El-Messer SM, Ghaly MA. Synthesis and biological evaluation of new curcumin analogues as antioxidant and antitumor agents: molecular modelling study. Eur J Med Chem, 2015; 101:584–94; doi:10.1016/j.ejmech.2015.07.014

Bayomi SM, El-Kashef HA, El-Ashmawy MB, Nasr NA, El-Sherbeny MA, Badria A. Synthesis and biological evaluation of new curcumin derivatives as antioxidant and antitumor agents. Med Chem Res, 2013; 22:1147–62; doi:10.1007/s00044-012-0116-9

Burger AM, Fiebig HH. Preclinical screening for new anticancer agents. In: Figg WD, McLeod HL (eds.). Handbook of anticancer pharmacokinetics and pharmacodynamics, cancer drug discovery and development, Humana Press Inc, Totowa, NJ, pp 36–7, 2004; doi:10.1007/978-1-59259-734-5_2

Gaikwad DD, Warad KD, Chapolikar AD, Devkate CG, Tayade AP, Pawar RP, Domb AJ. Synthesis of indazole motifs and their medicinal importance: an overview. Eur J Med Chem, 2015; 90:707–31; doi:10.1016/j.ejmech.2014.11.029

GraphPad Software, Inc. Available via www.graphpad.com (Accessed 17 November 2020).

Hariyanti H, Kusmardi K, Yanuar A, Hayun H. Ligand based pharmacophore modeling, virtual screening, and molecular docking studies of asymmetrical hexahydro-2H-indazole analogs of curcumin (AIACs) to discover novel estrogen receptors alpha (ERα) inhibitor. Indones J Chem, 2021; 21(1):137–47; doi:10.22146/ijc.54745

Hariyanti H, Yanuar A, Kusmardi K, Hayun H. (7E)–3–(4–Methoxyphenyl)–7–[(4–methoxyphenyl) methylidene]–4,5,6,7–tetrahydro–3aH–indazole. Molbank, 2020; 2020:M1162; doi:10.3390/M1162

Hayun H, Jatmika C, Purwati EM, Salim S, Kurniawan R, Chandra EG, Fajriawan AA, Nareswara AD. Synthesis and free radical-scavenging activities of di-mannich bases of cyclovalone derivatives. Orient J Chem, 2017; 33(6):2742–57; doi:10.13005/ojc/330607

Kurnia A, Saputri FC, Hayun H. Synthesis and anticancer potential of aminomethyl derivatives of methyl-substituted asymmetrical curcumin mono-carbonyl. J Appl Pharm Sci, 2019; 9(08):018–24; doi.10.7324/JAPS.2019.90803

Li Q, Chen J, Luo S, Xu J, Huang Q, Liu T. Synthesis and assessment of the antioxidant and antitumor properties of asymmetric curcumin analogues. Eur J Med Chem, 2015; 93:461–9; doi:10.1016/j.ejmech.2015.02.005

MarvinSketch 20.8.0. ChemAxon Ltd., 1998–2020. Available via http://www.chemaxon.com

Minu M, Thangadurai A, Wakode SR, Agrawal SS, Narasimhan B. Synthesis, antimicrobial activity and QSAR studies of new 2,3-disubstituted-3,3a,4,5,6,7-hexahydro-2H-indazoles. Bioorg Med Chem Let, 2009; 19(11):2960–4; doi:10.1016/j.bmcl.2009.04.052

MTT cell proliferation assay. Available via https://www.atcc.org/~/media/ DA5285A1F52C414E864C966FD78C9A79.ashx (Accessed 28 November 2020).

Nuriev VN, Vatsadze IA, Sviridenkova NV, Vatsadze SZ. Synthesis of 3,7 disubstituted hexahydro and tetrahydro-2H-indazoles from cross-conjugated dienones. Russ J Org Chem, 2016; 52:389–96; doi:10.1134/S1070428016030167

Novilla A, Mustofa M, Astuti I, Jumina J, Suwito H. Cytotoxic activity of methoxy-4-amino chalcone derivatives against leukemia cell lines. Mol Cell Biomed Sci, 2019; 3(1):34–41; doi:10.21705/mcbs.v3i1.44

Plescia S, Raffa D, Plescia F, Casula G. Synthesis and biological evaluation of new indazole derivatives. ARKIVOC, 2010; (x):163–77; doi:10.3998/ark.5550190.0011.a14

Prasetyaningrum PW, Bahtiar A, Hayun H. Synthesis and cytotoxicity evaluation of novel asymmetrical mono-carbonyl analogs of curcumin. Sci Pharm, 2018; 86(2):25; doi:10.3390/scipharm86020025

Rahmawati N, Hariyanti H, Saputri FC, Hayun H. Synthesis and preliminary in vitro anti-inflammatory evaluation of mannich bases derivatives of 4-methoxy-substituted of asymmetrical cyclovalone analogs. Indones J Pharm, 2020; 31(1):35–41; doi:10.13005/ojc/330607.

Raut S, Dhotre B, Tidke A, Pathan MA. An operationally simple and efficient synthesis of 7-benzylidene-substitutedphenyl- 3,3a,4,5,6,7-hexahydro-2H-indazole by grinding method. Curr Org Syn, 2020; 17(4):313–21; doi: 10.2174/1570179417666200406142118

Schmitz FJ, Bourden BF, Toth SI. Antitumor and cytotoxic compounds from marine organisms. In: Attaway DH, Zaborsky OR (eds.).

Marine biotechnology, pharmaceutical and bioactive natural products, Plenum Press, New York, NY, 198 p, vol. 1, 1993.

Shrivastava A, Chakraborty AK, Upmanyu N, Singh A. Recent progress in chemistry and biology of indazole and its derivatives: a brief review. Austin J Anal Pharm Chem, 2016; 3(4):1076. Available via https://austinpublishinggroup.com/analytical-pharmaceutical-chemistry/fulltext/ajapc-v3-id1076.php

Silverstein RM, Webster FX, Kiemle DJ. 2005. Spectrometric identification of organic compounds. 7th ed, John Wiley & Sons, Inc, New York, NY.

Thangadurai A, Minu M, Wakode S, Agarwal S, Narasimhan B. Indazole: a medicinally important heterocyclic moiety. Med Chem Res, 2012; 21:1509–23; doi:10.1007/s00044-011-9631-3

Thirupalu RM, Hanuman RV, Chenna KRR, Krishna V, Rami RYV. Synthesis and molecular docking studies of new substituted indazole derivatives for anti-breast cancer activity. Der Pharm Chem, 2014; 6(6):411–7; Available via https://1library.net/document/zp033loq-synthesis-molecular-docking-studies-substituted-indazole-derivatives-activity.html

Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenet Genom, 2011; 21(7):440–6; doi:10.1097/FPC.0b013e32833ffb56

Weerawatanakorn M, Wu JC, Pan MH, Ho CT. Reactivity and stability of selected flavor compounds. J Food Drug Anal, 2015; 23(2):176–90; doi:10.1016/j.jfda.2015.02.001

Zhang SG, Liang CG, Zhang WH. Recent advances in indazole-containing derivatives: synthesis and biological perspectives. Molecules, 2018; 23:2783; doi:10.3390/molecules23112783

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