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Synthesis and Anticancer Activity Evaluation of Azepinobisindoles; The Isomeric Iheyamine A-derivatives

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ABSTRACT

Azepinobisindole derivatives, the isomeric Iheyamine skeleton, were prepared and their anticancer activity evaluation were investigated against two human cancer cell lines, *Hepatocellular carcinoma* (HepG2) and human cervical cancer line (HeLa) as well as the normal cell line (Vero cell line) using MTT assay. The anticancer activity results indicated that 2-methoxy-5-methyl-5H-azepino[2,3-b:4,5-b]diindole was the most active derivative against tested cell lines. Additionally, molecular docking study in silico the possible inhibitory effect of cyclin-dependent kinase 2 (CDK2) by the azepinoindole revealed that all synthesized compounds fit well in the binding cavity of CDK2.

Keywords: Azepinobisindole, Isomeric Iheyamine, Anticancer activity, CDK2, Molecular docking.

INTRODUCTION

Bisindole alkaloids constitute an important class of secondary metabolites with characteristic cytotoxicity and pharmacological activities.[1-5] Azepinobisindole alkaloids are a class of bisindole alkaloids containing a structural moiety of azepine (seven-membered ring) core, which demonstrated diverse biological activities especially anticancer effect. In the past years, number types of indole-fused azepines have been found with potential success in drug discovery. For example, Hyrtimomine A was reported to show anticancer activity against human epidermoid carcinoma KB cells and murine leukemia L1210 cells and showed antimicrobial activity against *Candida albicans* and *Cryptococcus neoformans* by Tanaka *et al.,*[6] Another example was Trigonoliimines C, which was reported with a modest anti-HIV-1 activity[7] and the anticancer activity against human cervical cancer HeLa cells and human histiocytic lymphoma U-937 cells was also reported by Han *et al.,*[8] As illustrated in Fig. 1, Iheyamine A (1), the azepinobisindole isolated from a colonial marine ascidian *Polycitorella* sp. by Sasaki *et al.,* was

reported to exhibit moderately cytotoxicity against murine leukemia P388 cells, human lung carcinoma A549 cells and human colon adenocarcinoma HT29 cells with IC_{50} in the level of 1 µg/mL.[9] The synthesis of Iheyamine A was firstly accomplished by Lindsay *et al.,* using cross-Mannich reaction.[10] Recently, Abe and Yamada accomplished the synthesis of Iheyamine A via dehydrative Mannich-type reaction.[11] Moreover, Bremner *et al.,* synthesized the 5H-azepino[2,3-b:4,5-b]-diindole system, which was the isomer of Iheyamine A.[12] However, these structure motifs have not been studied for biological activities and their core structure resemble to Hytimomine A, which could be giving promising result for anticancer activity. Therefore, a series of 5H-azepino[2,3-b:4,5-b]-diindole was synthesised and evaluated for cytotoxic activity. Molecular docking study against cyclic-dependent kinase 2 (CDK2) were also investigated the inhibitory ability of the designed compounds as well as the understanding of the antitumor results.

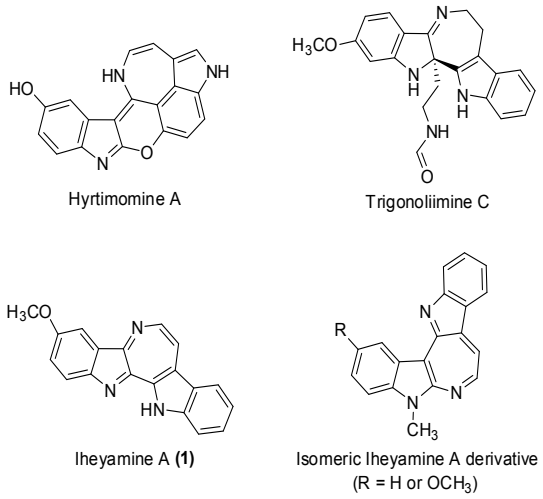


Fig. 1. Azepinobisindole alkaloids Hytimomine A, Trigonoliimine C, Iheyamine A (1) and isomeric Iheyamine A derivatives

MATERIALS AND METHODS

General Methods

Melting points were determined with a Stuart Scientific SMP 2 melting point apparatus and were uncorrected. FT-IR spectra were recorded using a Perkin Elmer Spectrum 100. NMR spectra were recorded on a Bruker Advance 300 (300 MHz for proton NMR and 75 MHz for carbon NMR) using tetramethylsilane (TMS) as an internal reference. Mass spectra were determined on a POLARIS Q or HEWLETT PACKARD 5973.

Synthesis method

Isatin was purchased from FlukaTM and 5-substituted isatins (2b–d) were prepared according to Sandmeyer isatin synthesis method.[13]

General procedure for synthesis of 3a–g

Isatin or 5-substituted isatin (7 mmol) and K_2CO_3 (10 mmol) were added to a dry CH_3CN solution (25 mL) and stirred for 30 min at RT. Methyl iodide or benzyl chloride (7.5 mmol) was added to the solution and then refluxed at 80°C for 3 hour. The mixture was cooled to RT and the solvent was removed. The residue was dissolved in CH_2Cl_2 (30 mL) and then washed with H_2O (3x30 mL). The organic extracts were dried over anh Na_2SO_4 and concentrated to give the crude brown solid. The purification of the crude solid using flash-column chromatography (silica gel, hexane/EtOAc (2:1) gave the pure compounds 3a–g.

N-methylisatin (3a)

Orange solid; 62%; m.p. 128-129°C (lit.[14] 129-130°C); IR (cm^{-1}): 1721, 1603, 1466, 1365, 1325; ^1H NMR ($CDCl_3$): δ 3.26 (s, 3H, CH_3), 6.90 (d, J = 8.2 Hz, 1H, ArH), 7.14 (t, J = 7.4 Hz, 1H, ArH), 7.58-7.64 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 26.2, 109.9, 117.5, 123.9, 125.3, 138.4, 151.5, 158.3, 183.4.

5-methoxy-N-methylisatin (3b)

Red solid; 82%; m.p. 171-173°C (lit.[15] 175-176°C); IR (cm^{-1}): 1722, 1625, 1485, 1356, 1287, 1226; ^1H NMR ($CDCl_3$): δ 3.23 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.81 (d, J = 9.3 Hz, 1H, ArH), 7.14-7.18 (m, 2H, ArH)); ^{13}C NMR ($CDCl_3$): δ 26.2, 56.0, 109.6, 110.9, 117.8, 124.6, 145.4, 156.6, 158.3, 183.8.

5-bromo-N-methylisatin (3c)

Orange solid; 70%; m.p. 167-169 °C (lit.[15] 163-164°C); IR (cm^{-1}): 1722, 1603, 1439, 1349, 1321, 711; ^1H NMR ($CDCl_3$): δ 3.24 (s, 3H, CH_3), 6.80 (dd, J = 1.5, 7.4 Hz, 1H, ArH), 7.70 (s, 1H, ArH), 7.71 (dd, J = 1.8, 7.5 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 26.4, 111.6, 116.7, 118.6, 128.1, 140.6, 150.2, 157.5, 182.2.

5-nitro-N-methylisatin (3d)

Yellow solid; 76%; m.p. 195-197°C (lit.[16] 197-199°C); IR (cm^{-1}): 1742, 1607, 1515, 1467, 1326,

1291; ^{1}H NMR (DMSO-d_6): δ 7.37 (d, J = 8.8 Hz, 1H, ArH), 8.24 (d, J = 2.4 Hz, 1H, ArH), 8.55 (dd, J = 2.4, 8.7 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6): δ 27.0, 111.4, 118.3, 119.4, 133.5, 143.4, 156.1, 159.4, 181.7.

N-benzylisatin (3e)

Orange solid; 79%; m.p. 127-130°C (lit.[17] 131°C); IR (cm^{-1}): 1728, 1608, 1468, 1347, 1307; ^{1}H NMR ($CDCl_3$): δ 4.94 (s, 2H, CH_2), 6.78 (d, J = 8.0 Hz, 1H, ArH), 7.09 (td, J = 0.8, 7.6 Hz, 1H, ArH), 7.30-7.37 (m, 5H, ArH), 7.48 (td, J = 1.4, 7.8 Hz, 1H, ArH), 7.62 (dd, J = 0.8, 7.5 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 44.0, 111.0, 117.6, 123.9, 125.3, 127.4 (2C), 128.1 129.0 (2C), 134.5, 138.4, 150.7, 158.3, 183.3.

5-methoxy-N-benzylisatin (3f)

Red Solid; 52%; m.p. 123-124°C (lit.[18] 127-128°C); IR (cm^{-1}): 1721, 1618, 1490, 1335, 1269, 1237; ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 3H, OCH_3), 4.91 (s, 2H, CH_2), 6.67 (d, J = 8.6 Hz, 1H, ArH), 7.03 (dd, J = 2.7, 8.6 Hz, 1H, ArH), 7.15 (d, J = 2.7 Hz, 1H, ArH), 7.29-7.38 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$): δ 44.0, 55.9, 109.6, 112.0, 118.1, 124.6, 127.4 (2C), 128.1, 129.0 (2C), 134.6, 144.6, 156.5, 158.4, 183.6.

5-bromo-N-benzylisatin (3g)

Orange solid; 92%; m.p. 101-103°C (lit.[19] 106-108°C); IR (cm^{-1}): 1729, 1600, 1468, 1351, 1324, 696; ^{1}H NMR ($CDCl_3$): δ 4.93 (s, 2H, CH_2), 6.67 (d, J = 8.4 Hz, 1H, ArH), 7.29-7.39 (m, 5H, ArH), 7.58 (dd, J = 2.1, 8.4 Hz, 1H, ArH), 7.72 (d, J = 2.1 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 44.2, 112.7, 116.8, 118.8, 127.4 (2C), 128.2, 128.4, 129.2 (2C), 134.0, 140.5, 149.4, 157.5, 182.1.

General procedure for synthesis of 5a-g

N-substituted isatin (3a-g) (0.9 mmol) and tryptamine (4) (1 mmol) in EtOH (5mL) in the presence of (0.2 mL) glacial acetic acid and 4 Å molecular sieve were stirred at RT for 24 hours. The precipitate was removed by filtration and the filtrate was concentrated and purified by column chromatography (silica gel, hexane/EtOAc (2:1) to give compounds 5a-g.

Spiro[(1-methyl-3H-indole-2(1H)-one)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (5a)

Cream solid; 73%; m.p. 232-233°C (lit.[12] 239-241°C); IR (cm^{-1}): 3139, 1714, 1609, 1469, 1347, 1296; ^{1}H NMR ($CDCl_3$): δ 2.95 (t, J = 5.7 Hz, 2H, CH_2), 3.24 (s, 3H, CH_3), 3.31 (dt, J = 5.4, 13.3 Hz, 1H, CH_2), 3.81 (dt, J = 6.1, 13.3, 1H, CH_2), 6.91 (d, J = 7.8, 1H, ArH), 7.03 (td, J = 0.9, 7.5, 1H, ArH), 7.08-7.12 (m, 3H, ArH), 7.17 (dd, J = 0.8, 7.8 Hz, 1H, ArH), 7.36 (td, J = 1.3, 7.7 Hz, 1H, ArH), 7.46 (br s, 1H, NH), 7.51-7.58 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 21.1, 25.6, 39.0, 60.5, 107.7, 110.0, 111.5, 117.5, 118.6, 121.4, 122.3, 123.7, 126.2, 128.9, 129.1, 130.5, 135.2, 142.6, 175.6.

Spiro[(5-methoxy-1-methyl-3H-indole-2(1H)-one)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (5b)

Beige solid; 87%; m.p. 243-245°C (lit.[12] 255-256°C); IR (cm^{-1}): 3292, 1708, 1600, 1496, 1341, 1296, 1231; ^{1}H NMR ($CDCl_3$): δ 2.98 (t, J = 5.7 Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.34 (dt, J = 5.4, 13.2 Hz, 1H, CH_2), 3.72 (s, 3H, OCH_3), 3.88 (dt, J = 6.3, 13.2 Hz, 1H, CH_2), 6.81-6.91 (m, 3H, ArH), 7.08-7.19 (m, 3H, ArH), 7.29 (br s, 1H, NH), 7.57 (d, J = 5.9 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 22.0, 26.6, 40.0, 55.9, 62.0, 109.4, 111.2, 111.6, 111.9, 114.7, 118.4, 119.4, 122.3, 127.0, 129.9, 132.7, 136.4, 136.9, 156.6, 176.7.

Spiro[(5-nitro-1-methyl-3H-indole-2(1H)-one)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (5d)

Cream solid; 89%; m.p. 279-281°C; IR (cm^{-1}): 3259, 1702, 1606, 1509, 1489, 1324, 1293; ^{1}H NMR ($CDCl_3$): δ 3.01 (t, J = 5.6, 2H, CH_2), 3.35 (s, 3H, CH_3), 3.40-3.43 (m, 1H, CH_2), 3.79 (dt, J = 5.7, 13.5, 1H, CH_2), 7.05 (d, J = 8.7, 1H, ArH), 7.09-7.19 (m, 3H, ArH), 7.59 (d, J = 6.4, 1H, ArH), 8.08 (d, J = 2.3, 1H, ArH), 8.35 (dd, J = 2.3, 8.7, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 21.9, 27.1, 61.1, 109.7, 111.6 (2C), 118.6, 119.1, 120.0, 122.1, 126.9, 127.0, 130.5, 133.5, 136.5, 143.1, 150.8, 177.6; HREI-MS (m/z) calculated for $C_{19}H_{17}N_4O_3$ $(M+H)^+$ 349.1295, found 349.1308.

Spiro[(1-benzyl-3H-indole-2(1H)-one)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (5e)

Cream Solid; Yield 85%; m.p. 142-146°C; IR (cm^{-1}): 3295, 1714, 1606, 1461, 1347, 1293; 1H NMR ($CDCl_3$): δ 2.98 (t, J = 5.7 Hz, 2H, CH_2), 3.37 (dt, J = 5.3, 13.2 Hz, 1H, CH_2), 3.92 (dt, J = 6.2, 13.2 Hz, 1H, CH_2), 4.86 (d, J = 15.5 Hz, 1H, CH_2), 5.00 (d, J = 15.5 Hz, 1H, CH_2), 6.85 (d, J = 7.8 Hz, 1H, ArH), 7.00 (td, J = 0.9, 7.5 Hz, 1H, ArH), 7.07-7.16 (m, 3H, ArH), 7.19 (d, J = 7.4 Hz, 1H, ArH), 7.25 (td, J = 1.3, 7.8 Hz, 1H, ArH), 7.29-7.40 (m, 5H, ArH), 7.54-7.61 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 22.1,

40.1, 44.0, 61.5, 109.6, 111.0, 112.6, 118.6, 119.7, 122.5, 123.4, 124.8, 127.3, 127.6 (2C), 127.9, 129.0 (2C), 129.7, 130.2, 131.5, 135.8, 136.3, 142.6, 176.9; HREI-MS (m/z) calculated for $C_{25}H_{22}N_3O$ $(M+H)^+$ 380.1757, found 380.1761.

Spiro[(5-bromo-1-methyl-3H-indole-2(1H)-one)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (5c)

Beige solid; Yield 89%; m.p. 240-242°C; IR (cm^{-1}): 3267, 1708, 1607, 1459, 1348, 1229, 688; ^1H NMR ($CDCl_3$): δ 2.98 (t, J = 5.7 Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.32 (dt, J = 5.5, 13.2 Hz, 1H, CH_2), 3.83 (dt, J = 5.9, 13.2 Hz, 1H, CH_2), 6.82 (d, J = 8.3 Hz, 1H, ArH), 7.09-7.20 (m, 3H, ArH), 7.30 (br s, 1H, NH), 7.34 (d, J = 1.9 Hz, 1H, ArH), 7.50 (dd, J = 2.0, 8.3 Hz, 1H, ArH), 7.56 (d, J = 6.5 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 19.8, 24.4, 37.8, 59.3, 108.0, 108.8, 110.6, 113.7, 116.4, 117.5, 120.5, 124.8, 125.7, 126.9, 130.4, 131.2, 134.0, 140.3, 173.9; HREI-MS (m/z) calculated for $C_{19}H_{17}BrN_3O$ $(M+H)^+$ 382.0550, found 382.0555.

Spiro[(5-methoxy-1-benzyl-3H-indole-2(1H)-one)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (5f)

Beige solid; Yield 84%; m.p. 180-183°C; IR (cm^{-1}): 3290, 1601, 1710, 1494, 1336, 1229, 1202; ^1H NMR ($CDCl_3$): δ 2.99 (t, J = 5.7 Hz, 2H, CH_2), 3.36 (dt, J = 5.3, 13.1 Hz, 1H, CH_2), 3.66 (s, 3H, OCH_3), 3.95 (dt, J = 6.3, 13.1 Hz, 1H, CH_2), 4.83 (d, J = 15.4 Hz, 1H CH_2), 5.0 (d, J = 15.4 Hz, 1H, CH_2), 6.75-6.80 (m, 3H, ArH), 7.09-7.18 (m, 3H, ArH), 7.30 (br s, 1H, NH), 7.31-7.36 (m, 5H, ArH), 7.58 (d, J = 6.9 Hz, 1H ArH); ^{13}C NMR ($CDCl_3$:) δ 22.0, 40.1, 44.0, 55.7, 61.9, 110.2, 111.1, 111.3, 112.5, 114.8, 118.6, 119.6, 122.5, 127.2 (2C), 127.5, 127.9, 128.9 (2C), 130.2, 132.6, 135.7, 135.9, 136.3, 156.5, 176.7; HREI-MS (m/z) calculated for $C_{26}H_{24}N_3O_2$ $(M+H)^+$ 410.1863, found 410.1873.

Spiro[(5-bromo-1-benzyl-3H-indole-2(1H)-one)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (5g)

Cream solid; Yield 75%; m.p. 135-138°C; IR (ATR) 3323, 1719, 1603, 1477, 1351, 1229, 1170, 691; ^1H NMR ($CDCl_3$): δ 2.99 (t, J = 5.7, 2H Hz, CH_2), 3.35 (dt, J = 5.4, 13.2 Hz, 1H, CH_2), 3.88 (dt, J = 6.1 Hz, 1H, CH_2), 4.85 (d, J = 15.5 Hz, 1H, CH_2), 4.99 (d, J = 15.5 Hz, 1H, CH_2), 6.72 (d, J = 8.3 Hz, 1H, ArH), 7.08-7.20 (m, 3H, ArH), 7.22 (br s, 1H, NH), 7.30-7.45 (m, 7H, ArH), 7.58 (d, J = 6.4 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 21.7, 39.8, 43.7, 61.2, 110.8, 110.9, 112.5, 115.8, 118.4, 119.5, 122.4, 126.8, 127.1 (2C), 127.7 (2C), 128.7 (2C), 129.0, 132.2, 133.2, 134.9, 136.0, 141.2, 176.0; HREI-MS (m/z) calculated for $C_{25}H_{21}BrN_3O$ $(M+H)^+$ 458.0863, found 458.0863.

General procedure for synthesis of 7a–g

A solution of spiroindolone (5a–g) (0.3 mmol) in dry toluene (6 mL) was stirred and cooled to -78°C under Ar atmosphere and DIBAL-H (3 mmol) was added dropwise into the solution. After 4 h, a solution of 5% aqueous NaOH (8 mL) was quenched to the mixture and extracted with EtOAc (3x15 mL). The organic extracts were combined and washed with brine, dried over anhydrous sodium sulfate and after that concentrated under reduced pressure to give oxyindoline 6a–g. (This compound was unstable and used without further purification due to the color changed on a silica gel and in $CDCl_3$.)

The oxyindoline (6a–g) was warmed in 5M HCl (10 mL) for 1 h at 90°C and then basified with 25% KOH until the pH was more than 12. The solid was filtered and purified by column chromatography (silica gel) to give isomeric lheyamine derivatives 7a–g.

5-methyl-5H-azepino[2,3-b:4,5-b']diindole (7a)

Following the general procedure, spiro[(1-methyl-3H-indole-2(1H)-ol)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (6a) was obtained as light brown viscous oil; ^1H NMR ($CDCl_3$): δ 2.86 (t, J = 5.6 Hz, 2H, CH_2), 2.93 (s, 3H, CH_3), 3.07 (dt, J =6.3, 12.8 Hz, 1H, CH_2), 3.42 (dt, J = 5.0, 12.9 Hz, 1H, CH_2), 4.92 (s, 1H, CH), 6.60 (d, J = 7.8 Hz, 1H, ArH), 6.69 (td, J = 0.8, 7.4 Hz, 1H, ArH), 6.92 (dd, J = 0.9, 7.4 Hz, 1H, ArH), 7.07-7.16 (m, 2H, ArH), 7.17-7.23 (m, 2H, ArH), 7.52 (dd, J = 1.7, 6.6 Hz, 1H, ArH), 7.72 (br s, 1H, NH).

The rearrangement of oxyindole 6a in 5M HCl gave compound 7a as green solid; 13 % (2 steps); m.p. 194-197°C (lit.[12] 193-195°C); IR (cm^{-1}): 2921, 2852, 1547, 1468, 1365; ^1H NMR ($CDCl_3$): δ 4.25 (s, 3H, CH_3), 7.45 (t, J = 7.8 Hz, 1H, ArH), 7.57-7.72 (m, 2H, ArH), 7.75 (d, J = 8.1 Hz, 1H, ArH), 7.83 (t, J = 8.1 Hz, 1H, ArH), 8.13 (d, J = 8.3 Hz, 1H, ArH), 8.35 (d, J = 7.8 Hz, 1H, ArH), 8.48 (d, J = 5.8 Hz, 1H, CH), 9.03 (d, J = 5.8 Hz, 1H, CH), 9.46 (d, J = 7.9 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 28.8, 109.4, 112.3, 119.5, 121.6 (2C), 122.3, 122.8, 126.0, 126.5, 127.6, 128.7, 132.6, 139.9, 145.5, 147.2, 147.4, 156.2, 160.2; HREI-MS (m/z) calculated for $C_{20}H_{16}N_3O$ $(M+H)^+$ 284.1182, found 284.1178.

2-Methoxy-5-methyl-5H-azepino[2,3-b:4,5-b']diindole (7b)

Following the general procedure, spiro[(5-methoxy-1-methyl-3H-indole-2(1H)-ol)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (6b) was obtained as brown viscous oil; ^{1}H NMR ($CDCl_3$): δ 2.86 (t, J = 4.9 Hz, 2H, CH_2), 2.87 (s, 3H, CH_3), 3.08 (dt, J = 6.1, 12.7 Hz, 1H, CH_2), 3.39 (dt, J = 5.2, 12.8 Hz, 1H, CH_2), 3.66 (s, 3H, OCH_3), 4.86 (s, 1H, CH), 6.53 (d, J = 8.7 Hz, 1H, ArH), 6.56 (d, J = 2.5 Hz, 1H, ArH), 6.79 (dd, J = 2.6, 8.4 Hz, 1H, ArH), 7.09-7.23 (m, 3H, ArH), 7.52 (d, J = 7.5 Hz, 1H, ArH), 7.79 (br s, 1H, NH).

The rearrangement of oxyindole 6b in 5M HCl gave compound 7b as burgundy solid; 14% (2 steps); m.p. 148-151°C (lit.[12] 147-148°C); IR (cm^{-1}): 2922, 2852, 1539, 1497, 1369, 1261; ^{1}H NMR ($CDCl_3$): δ 4.15 (s, 3H, CH_3), 4.23 (s, 3H, OCH_3), 7.42 (dd, J = 2.6, 8.9 Hz, 1H, ArH), 7.43 (t, J = 7.5 Hz, 1H, ArH), 7.60 (d, J = 8.9 Hz, 1H, ArH), 7.81 (t, J = 7.7 Hz, 1H, ArH), 8.11 (d, J = 8.0 Hz, 1H, ArH), 8.32 (d, J = 7.7 Hz, 1H, ArH), 8.43 (d, J = 5.7 Hz, 1H, CH), 8.97 (d, J = 5.8 Hz, 1H, CH), 9.12 (d, J = 2.5 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 26.7, 54.1, 105.1, 108.1, 109.5, 116.9, 117.7, 118.2, 119.2, 119.5, 120.5, 123.4, 130.2, 132.9, 142.8, 143.9, 145.3, 153.0, 153.8, 156.4; HREI-MS (m/z) calculated for $C_{19}H_{14}N_3$ $(M+H)^+$ 314.1288, found 314.1286.

2-Bromo-5-methyl-5H-azepino[2,3-b:4,5-b']diindole (7c)

Following the general procedure, spiro[(5-bromo-1-methyl-3H-indole-2(1H)-ol)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (6c) was obtained as brown viscous oil; ^{1}H NMR ($CDCl_3$): δ 2.86 (t, J = 5.6 Hz, 2H, CH_2), 2.90 (s, 3H, CH_3), 3.06 (dt, J = 6.3, 12.8 Hz, 1H, CH_2), 3.39 (dt, J = 5.0, 12.9 Hz, 1H, CH_2), 4.91 (s, 1H, CH), 6.46 (d, J = 8.3 Hz, 1H, ArH), 7.01 (d, J = 2.0 Hz, 1H, ArH), 7.11 (td, J = 1.4, 7.1 Hz, 1H, ArH), 7.16 (td, J = 1.5, 7.3 Hz, 1H, ArH), 7.23 (d, J = 7.0 Hz, 1H, ArH), 7.31 (dd, J = 2.0, 8.3 Hz, 1H, ArH), 7.53 (d, J = 7.2 Hz, 1H, ArH), 7.70 (br s, 1H, ArH).

The rearrangement of oxyindole 6c in 5M HCl gave compound 7c as green solid; 14% (2 steps); m.p. 213-215°C; IR (cm^{-1}): 2921, 2852, 1551, 1463, 1369, 589; ^{1}H NMR ($CDCl_3$): δ 4.18 (s, 3H, CH_3), 7.45 (t, J = 7.9 Hz, 1H, ArH), 7.49 (d, J = 8.8 Hz, 1H, ArH), 7.79 (dd, J = 2.0, 8.7 Hz, 1H, ArH), 7.83 (t, J = 7.7 Hz, 1H, ArH), 8.10 (d, J = 8.1 Hz, 1H, ArH), 8.30 (d, J = 7.8 Hz, 1H, ArH), 8.37 (d, J = 5.7 Hz, 1H, CH), 8.98 (d, J = 5.7 Hz, 1H, CH), 9.70 (d, J = 2.0 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 27.0, 108.8, 113.3, 117.7, 117.8, 118.5, 119.8, 120.0, 120.9, 121.9, 123.9, 125.6, 127.4, 129.3, 130.9, 136.2, 137.8, 144.4, 145.3; HREI-MS (m/z) calculated for $C_{19}H_{13}BrN_3$ $(M+H)^+$ 362.0287, found 362.0283.

2-nitro-5-methyl-5H-azepino[2,3-b:4,5-b']diindole (7d)

Following the general procedure, spiro[(5-nitro-1-methyl-3H-indole-2(1H)-ol)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (6d) was obtained as brown viscous oil; ^{1}H NMR ($CDCl_3$): δ 2.85-2.91 (m, 2H, CH_2), 3.01-3.12 (m with s prominent, 4H, CH2, CH_3), 3.45 (dt, J = 4.6, 13.0 Hz, 1H, CH_2), 5.11 (s, 1H, CH), 6.49 (d, J = 8.9 Hz, 1H, ArH), 7.11-7.23 (m, 3H, ArH), 7.55 (d, J = 6.9 Hz, 1H, ArH), 7.60 (br s, 1H, NH), 7.78 (d, J = 2.3 Hz, 1H, ArH), 8.17 (dd, J = 2.3, 8.8 Hz, 1H, ArH).

The rearrangement of oxyindole 6d in 5M HCl gave compound 7d as green solid; 10% (2 steps); m.p. 328-330°C; IR (cm^{-1}): 2922, 2852, 1554, 1506, 1455, 1307, 1293; ^{1}H NMR ($CDCl_3$): δ 4.24 (s, 3H, CH_3), 7.49 (t, J = 7.3 Hz, 1H, ArH), 7.65 (d, J = 8.8 Hz, 1H, ArH), 7.87 (t, J = 8.2 Hz, H, ArH), 8.15 (d, J = 8.0 Hz, 1H, ArH), 8.32 (d, J = 7.4 Hz, 1H, ArH), 8.42 (d, J = 5.7 Hz, 1H, CH), 8.57 (dd, J = 2.4, 9.2 Hz, 1H, ArH), 9.04 (d, J = 5.6 Hz, 1H, CH), 10.43 (d, J = 2.1 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 29.8, 110.7, 112.0, 117.9, 118.5, 118.6, 121.1, 122.1, 123.7, 124.4, 125.4, 126.0, 126.6, 127.7, 130.7, 136.9, 141.3, 143.8, 151.3.; HREI-MS (m/z) calculated for $C_{19}H_{12}N_4O_2$ $(M+H)^+$ 329.1039, found 329.1041.

5-benzyl-5H-azepino[2,3-b:4,5-b']diindole (7e)

Following the general procedure, spiro[(1-benzyl-3H-indole-2(1H)-ol)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (6e) was obtained as light brown viscous oil; ^{1}H NMR ($CDCl_3$): δ 2.81-2.88 (m, 2H, CH_2), 3.00-3.09 (m, 1H, CH_2), 3.38-3.46 (m, 1H, CH_2), 4.41 (d, J = 15.2 Hz, 1H, CH_2), 4.58 (d, J = 15.1 Hz, 1H, CH_2), 5.05 (s, 1H, CH), 6.58 (d, J = 7.9 Hz, 1H, ArH), 6.67 (td, J = 0.8, 7.5 Hz, 1H, ArH), 6.93 (dd, J = 0.9, 7.4 Hz, 1H, ArH), 7.05-7.21 (m, 4H, ArH), 7.27-7.39 (m, 3H, ArH), 7.44 (d, J = 6.7 Hz, 2H, ArH), 7.51 (d, J = 7.5 Hz, 1H, ArH), 7.64 (br s, 1H, NH).

The rearrangement of oxyindole 6e in 5M HCl gave compound 7e as green solid; Yield 11% (2 steps); m. p. 143-146°C; IR (cm^{-1}): 2923, 2851, 1609, 1454, 1351; ^1H NMR ($CDCl_3$): δ 6.04 (s, 2H, CH_2), 7.17-7.35 (m, 5H, ArH), 7.46 (t, J = 7.5 Hz, 1H, ArH), 7.55-7.72 (m, 3H, ArH), 7.83 (t, J = 7.6 Hz, 1H, ArH), 8.14 (d, J = 8.1 Hz, 1H, ArH), 8.35 (d, J = 7.7 Hz, 1H, ArH), 8.66 (d, J = 5.7 Hz, 1H, CH), 9.02 (d, J = 5.7 Hz, 1H, CH), 9.65 (d, J = 7.7 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 43.3, 107.7, 110.0, 117.0, 117.5, 119.3, 119.4, 120.1, 120.5, 123.6, 124.4 (2C), 124.9, 125.1, 126.2 (2C), 126.4, 130.3, 134.7, 137.0, 143.5, 144.9, 145.0, 153.9, 157.6; HREI-MS (m/z) calculated for $C_{25}H_{18}N_3$ $(M+H)^+$ 360.1495, found 360.1485.

2-methoxy-5-benzyl-5H-azepino[2,3-b:4,5-b']diindole (7f)

Following the general procedure, spiro[(5-methoxy-1-benzyl-3H-indole-2(1H)-ol)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (6f) was obtained as brown viscous oil; ^1H NMR ($CDCl_3$): δ 2.81-2.90 (m, 2H, CH_2), 2.98-3.11 (m, 1H, CH_2), 3.35-3.45 (m, 1H, CH_2), 3.64 (s, 3H, OCH_3), 4.33 (d, J = 14.9 Hz, 1H, CH_2), 4.52 (d, J = 14.8 Hz, 1H, CH_2), 5.01 (s, 1H, CH), 6.48 (d, J = 8.5 Hz, 1H, ArH), 6.56 (d, J = 2.6 Hz, 1H, ArH), 6.71 (dd, J = 2.6, 8.5 Hz, 1H, ArH), 7.06-7.22 (m, 3H, ArH), 7.27-7.45 (m, 5H, ArH), 7.51 (d, J = 7.5 Hz, 1H, ArH), 7.71 (br s, 1H, NH).

The rearrangement of oxyindole 6f in 5M HCl gave compound 7f as burgundy solid; 13% (2 steps); m.p. 209-211°C; IR (cm^{-1}): 2923, 2852, 1544, 1497, 1314, 1268; ^1H NMR ($CDCl_3$): δ 4.11 (s, 3H, OCH_3), 5.97 (s, 2H, CH_2), 7.18-7.24 (m, 5H, ArH), 7.29 (dd, J = 2.6, 8.9 Hz, 1H, ArH), 7.38-7.49 (m, 2H, ArH), 7.80 (t, J = 7.6 Hz, 1H, ArH), 8.11 (d, J = 8.0 Hz, 1H, ArH), 8.29 (d, J = 7.7 Hz, 1H, ArH), 8.38 (d, J = 5.7 Hz, 1H, ArH), 8.91 (d, J = 5.7 Hz, 1H, ArH), 9.09 (d, J = 2.5 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 44.1, 54.4, 105.6, 109.3, 110.2, 117.4, 118.3, 118.6, 119.8, 120.1, 121.4, 124.1, 124.9 (2C), 125.7, 126.2, 126.7, 126.9 (2C), 130.8, 132.8, 135.3, 143.5, 145.0, 145.6, 154.2; HREI-MS (m/z) calculated for $C_{26}H_{20}N_3O$ $(M+H)^+$ 390.1600, found 360.1595.

2-Bromo-5-benzyl-5H-azepino[2,3-b:4,5-b']diindole (7g)

Following the general procedure, spiro[(5-bromo-1-benzyl-3H-indole-2(1H)-ol)-3,1'-(1',2',3',4'-tetrahydro-9H-pyrido[3,4-b]indol)] (6g) was obtained as red viscous oil; ^1H NMR ($CDCl_3$): δ 2.81-2.91 (m, 2H, CH_2), 3.04 (dt, J = 6.4, 13.0 Hz, 1H, CH_2), 3.40 (dt, J = 4.8, 12.9 Hz, 1H, CH_2), 4.39 (d, J = 15.1 Hz, 1H, CH_2), 4.56 (d, J = 15.1 Hz, 1H, CH_2), 5.05 (s, 1H, CH), 6.44 (d, J = 8.4 Hz, 1H, ArH), 7.02 (d, J = 2.0 Hz, 1H, ArH), 7.08-7.23 (m, 4H, ArH), 7.28-7.45 (m, 5H, ArH), 7.52 (d, J = 7.9 Hz, 1H, ArH), 7.53 (br s, 1H, NH).

The rearrangement of oxyindole 6g in 5M HCl gave compound 7g as green solid; 19% (2 steps); m.p. 213-216°C; IR (cm^{-1}): 2921, 2846, 1551, 1450, 1372, 688; ^1H NMR ($CDCl_3$): δ 5.92 (s, 2H, CH_2), 7.14-7.25 (m, 5H, ArH), 7.35 (d, J = 8.7 Hz, 1H, ArH), 7.44 (t, J = 7.7 Hz, 1H, ArH), 7.65 (dd, J = 1.5, 8.7 Hz, 1H, ArH), 7.82 (t, J = 7.6 Hz, 1H, ArH), 8.10 (d, J = 8.0 Hz, 1H, ArH), 8.26 (d, J = 7.6 Hz, 1H, ArH), 8.32 (d, J = 5.7 Hz, 1H, CH), 8.93 (d, J = 5.7 Hz, 1H, CH), 9.69 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 44.2, 109.9, 110.8, 113.9, 117.7, 118.5, 120.0, 120.5, 122.2, 123.9, 125.1 (2C), 126.0, 127.0 (3C), 127.5, 129.8, 131.3, 135.0, 135.9, 145.2, 145.5, 153.6, 157.5; HREI-MS (m/z) calculated for $C_{25}H_{17}BrN_3$ $(M+H)^+$ 438.0600, found 438.0593.

Anticancer activity

Cell culture

Herein, human cervical cancer cells (HeLa), human hepatocellular carcinoma cells (HepG2) and kidney epithelial cells from African green monkey (Vero) were cultured in 10% Fetal Bovine Serum (FBS supplemented DMEM (Dulbecco's Modified Eagle's Medium) and antibiotics. Cells were kept in a humidified 5% CO_2 atmosphere at 37°C with medium changes every 2 days until cells grown to 80-85% confluency. They were collected by trypsinization procedure with 0.25% trypsin and the viable cells were counted by tryphan blue exclusion.

Determination of anticancer activity by MTT assay

Cells were seeded on 96-well plate at a density of 1x105 cells/mL. After 24 h, the cells were administered with the test compounds at various concentrations and 20% organic solvent (acetone or DMSO) was used as a positive control. After incubation for 24 h, 100 µL of MTT (400 µg/mL) was added to each well and further incubated for 4 hours. The medium was then removed and 100 µL of DMSO, which was used to dissolve the resulting formazan crystal, was added to each well. The microplate spectrometer (biochrom EZ Read

2000) was used to measure the absorbance at 540 nm. Percentage cell viability was calculated using corrected absorbance with the following equation:

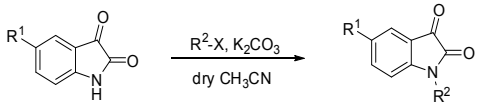
$$\% \text{ viable cells} = [(Abs_{sample} - Abs_{blank})/(Abs_{control} - Abs_{blank})] \times 100$$

Where Abs_{sample} was the absorbance of the test compound, $Abs_{control}$ was the absorbance of 1% organic solvent in medium and Abs_{blank} was the absorbance of 20% organic solvent in medium. The IC_{50} value was determined by plotting percentage cell viability versus sample concentration.

RESULTS AND DISCUSSION

Chemistry

The isatin (2a) was purchased from Sigma-Aldrich and the 5-substituted isatins (2b–d) were prepared by classical Sandmeyer isatin synthesis methods.[13] N-alkylation of isatins (2a–d) with alkyl halide in the presence of K_2CO_3 were completed the N-substituted isatin derivatives (5a–g) in 52-92% yield (scheme 1).

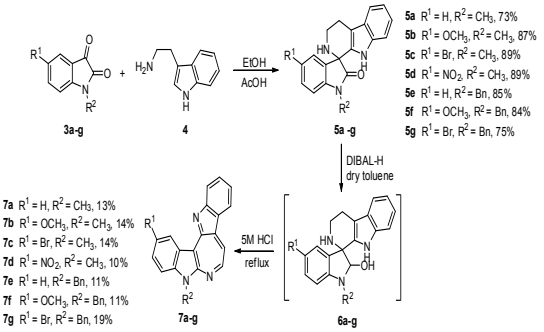


2a R^1 = H
2b R^1 = OCH_3
2c R^1 = Br
2d R^1 = NO_2

3a R^1 = H, R^2 = CH_3, 62%
3b R^1 = OCH_3, R^2 = CH_3, 82%
3c R^1 = Br, R^2 = CH_3, 70%
3d R^1 = NO_2, R^2 = CH_3, 76%
3e R^1 = H, R^2 = Bn, 79%
3f R^1 = OCH_3, R^2 = Bn, 52%
3g R^1 = Br, R^2 = Bn, 92%

Scheme 1. Synthesis of N-substituted isatin derivatives (3a–g)

The condensation reaction of N-substituted isatin derivatives (3a–g) with tryptamine (4) under acidic conditions forming the iminium ion intermediate, followed by spiro cyclization to provided racemic spiroindolone (5a–g) in good yield. Based on literature, the partial reduction of spiroindolones were achieved cleanly to the corresponding carbinolamines using sodium bis(2-methoxyethoxy) aluminium hydride.[20] However, the partial reduction using DIBAL-H in handed gave the carbinolamine (6a–g) in low yields. The carbinolamine (6a–g) were used without further purification by heating in 5 M HCl at 90°C to undergo rearrangement to the azepinodiindole (7a–g) and yields were reported from a 2-step process. The structure of compounds (7a–g) were compared to the previous synthesis isomeric Iheyamines[12] (scheme 2).



Scheme 2. Synthesis of isomeric Iheyamine 7a–g

Anticancer activity

The *in vitro* anticancer activity of the synthesized compounds (7a–g) were evaluated for cytotoxicity against HeLa, HepG2, and Vero cell lines by MTT assay.[21] All of the tested compounds exhibited good activity against both cancer cell lines and the IC_{50} values were shown in Table 1. The N-methyl derivative of azepinodiindoles (7a–c) were more potent than the N-benzyl derivatives (7e–g). The methoxy substituent on the aromatic ring of compound (7b) and bromine substituent (7c) had similar inhibitory effect against HepG2 and HeLa cell lines with the same IC_{50} value of 15.91 µg/mL. However, compound (7b) had lower activity on normal cell lines (Vero cell lines). The unsubstituted aromatic ring of azepinodiindoles (7a) and (7e) showed the selective inhibitory effect against HepG2 cell lines with no effect from the R^2 substitution. On the aromatic ring, the electron withdrawing nitro group, (7d) was the least active compound compared with other compounds containing methyl groups on R^2.

Molecular Docking

Using the iGEMDOCK v2.1 software[22], molecular docking was performed in order to explore binding positions along with intermolecular interactions between compounds (7a–g) and binding site of cyclin-dependent kinase 2 (CDK2) which was key protein for cell cycle progression.[23,24] Compounds (7a–g) were docked into the active site of CDK2 co-crystallized with LZ8 (PDB ID: 2VTO). LZ8 was also redocked into the CDK2 and its binding energy and hydrogen bond was then compared to those of azepinodiindoles (7a–g). The docking results revealed that the binding position of redocked LZ8 (shown in orange in Fig. 2) was more or less the

same to that of co-crystallized LZ8 in CPX2. Table 2 showed that the binding energy of all synthesized compounds (7a–g) were -103.98 to -84.35 kcal/mol, which were higher than that of LZ8. Among these, compound 7b fit well in the binding cavity of CPK2 and had the most similar orientation and binding position to those of LZ8, as illustrated in Fig. 2. Additionally, this compound had three hydrogen bonding interaction with LEU83, LYS89 and GLN131. For 7a, 7c and 7d, it was found that they fit in the binding site of CPK2, however their orientation and binding pose were rather different to those of LZ8 (Fig. 3a). In contrast, although the azepinodiindoles (7e–g) containing Bn group for R^2 substituent had lower binding energy than 7b, they could not fit in the binding site of CDK2, especially 7g.

Table 1: Cytotoxicity of test compounds against cancer cell lines (HeLa and HepG2) and normal cell (Vero)

Compounds	R^1	R^2	IC_{50} (µg/mL)		
			HeLa	HepG2	Vero
7a	H	CH_3	71.30 ± 1.11	27.27 ± 0.38	101.01 ± 0.15
7b	OCH_3	CH_3	15.91 ± 0.36	22.62 ± 0.22	117.87 ± 0.37
7c	Br	CH_3	15.91 ± 0.30	30.46 ± 0.83	43.30 ± 0.52
7d	NO_2	CH_3	66.63 ± 0.61	95.07 ± 0.36	205.48 ± 0.26
7e	H	Bn	61.01 ± 0.99	28.57 ± 0.48	75.99 ± 0.76
7f	OCH_3	Bn	82.61 ± 0.47	63.00 ± 0.42	330.50 ± 0.72
7g	Br	Bn	149.56 ± 0.29	122.97 ± 0.14	234.38 ± 0.29
Acridine orange	-	-	4.11	6.36	5.82
Actinomycin D	-	-	NT	2.70	NT

NT: not tested

Table 2: Summary of binding energy, amino acid interactions and hydrogen bond length of azepenodiindoles (7a–g) in binding site of CDK2

Compounds	Binding energy (kcal/mol)	Amino acid residue	Hydrogen bond length (Å)
7a	-84.35	ILE10, GLY11	2.48, 2.47
7b	-89.74	LEU83, LYS89, GLN131	2.28, 2.39, 2.42
7c	-86.07	-	-
7d	-103.98	ILE10, GLU12, LYS129	2.45, 2.30, 1.63
7e	-100.44	ILE10, GLY11	2.84, 2.73
7f	-99.11	GLU12, THR14, GLN131	2.98, 2.62, 2.89
7g	-101.47	ILE10	2.69
LZ8	-114.60	PHE82, LEU83	2.61, 2.21

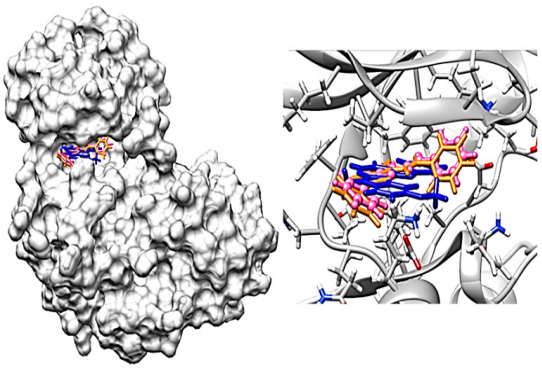


Fig. 2. Comparison of the binding of 7b (blue), redocked LZ8 (orange) and LZ8 (pink) in the cavity of CPK2 (PDB ID: 2VTO)

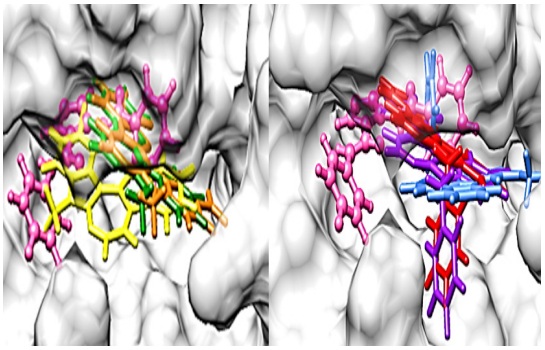


Fig. 3. The binding of 7a (green), 7c (yellow), 7d (deep orange), 7e (red), 7f (light blue), 7g (purple) and LZ8 (pink) in the cavity of CPK2 (PDB ID: 2VTO)

CONCLUSION

In summary, syntheses of isomeric Iheyamine A derivatives 7a–g can be prepared from the synthetic isatin derivatives and commercially available tryptamine. There have been achieved in 3-11% overall yield with 4-6 steps. The key reactions including Sandmeyer isatin synthesis method, N-alkylation, condensation, reduction and acid-catalyzed rearrangement. Out of the synthesized compounds 7a–g, compound 7b and 7c exhibited higher cytotoxicity against HeLa cell lines more than other compounds and exhibited cytotoxicity against HepG2 cell lines in similar to compound 7a and 7e but compound 7c is toxic to normal cell lines (Vero). Thus, the substitutions on the isomeric Iheaymine A core structure have to further investigation on their biological activities.

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