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Synthesis and Cytotoxicity of Novel 2,2'-Bis- and 2,2',2"-Tris-indolylmethanes-Based Bengacarboline Analogs

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Tungstosilicic acid hydrate was employed as an efficient catalyst for the synthesis of bis-indolylmethanes **4** using the Friedel-Crafts reaction of *N*-sulfonyl tryptamine **5** with various aromatic aldehydes, except 3-formylindole. In the excluding case, tris-indolylmethane **7** was formed via a sequential addition-elimination-addition process. The bioactivity test revealed that the phenolic hydroxyl group plays an important role in cytotoxicity; it demonstrated that *ortho*- and *para*-hydroxy bis-indolylmethane (BIM) analogs (**4b** and **4d**) displayed cytotoxic potency toward HepG2 (human hepatocellular liver carcinoma cell line) and MOLT-3 (human lymphoblastic leukemia cell line) cancer cell lines. Significantly, both analogs showed slightly higher inhibitory efficacy than the control drug, etoposide, in HepG2 cells, and the analog **4d** exhibited the most potent activity against MOLT-3 cell lines, with an IC_{50} value of 1.62 μg/mL.

Key words: Bis-indolylmethane, Tris-indolylmethane, Bengacarboline, Heteropoly acid, Cytotoxicity

INTRODUCTION

An indole moiety is present in a variety of biologically active natural products and synthetic compounds. Its pharmacological properties include cytotoxic, antiviral, antimicrobial, antiparasitic, anti-inflammatory, anti-serotonin, Ca-releasing, and calmodulin-antagonistic activities (Gul and Hamann, 2005; Kochanowska-Karamyan and Hamann, 2010; Ishikura et al., 2010; Ruiz-Sanchis et al., 2011). Additionally, general bis- and tris-indolylmethanes (BIMs and TIMs) structures have also been found, and they possess numerous activities, especially anticancer potency (Shiri et al., 2010). Among the indole family, bengacarboline **1** (Fig. 1), isolated from the Fijian ascidian *Didemnum* sp., displayed *in vitro* cytotoxicity, with a mean IC_{50} of 0.9 μg/mL, on a wide range of tumor cell lines. It acted as an inhibitor of topoisomerase II at 32 μM (Foderato et al., 1997). The synthesis of bengacarboline **1** and its derivatives (**2** and **3**) were reported (Fig. 2) (Pouilhès et al., 2003, 2008); in those studies, the analogs (**2** and **3**) were shown to be more potent cytotoxic agents than the parent compound (Pouilhès et al., 2008). The cytotoxic effect of the analogs was demonstrated by the ability to induce an accumulation of cells in the S phase of DNA synthesis (Pouilhès et al., 2008). Based on the basic skeleton, the promising lead molecules have inspired us to examine a related structure required for medicinal application. Accordingly, BIM **4** (Fig. 2) is a compound of choice due to the valuable

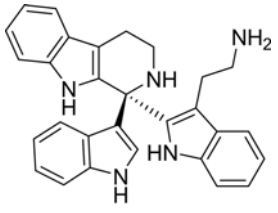

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Fig. 1. Structure of bengacarboline.

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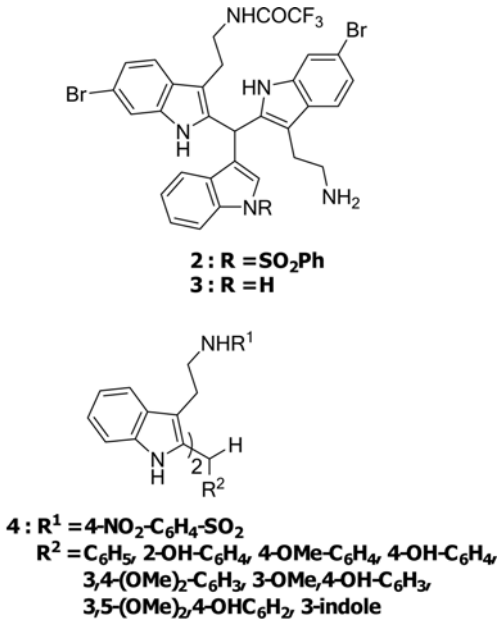


Fig. 2. Structure of bengacarboline analogs.

bioactivity of the BIM family and its comparable structure with the bengacarboline analogs (**2** and **3**). The general strategy for BIM synthesis is an acid-promoted Friedel-Crafts reaction of indole with aldehyde (Shiri et al., 2010). A wide variety advances in reaction conditions using various catalysts have been published (Shiri et al., 2010). In this regard, heteropolyacids (HPAs) have gained considerable attention in organic synthesis as versatile catalysts with environmentally benign properties (Timofeeva, 2003; Ueda and Kotsuki, 2008). They are normally stronger than conventional Lewis acid (e.g., $AlCl_3$) and mineral acids (e.g., H_2SO_4 and HCl). Moreover, several advantages of HPA include its commercial availability, ease of handing, and extremely low toxicity (Timofeeva, 2003; Ueda and Kotsuki, 2008). Application of the catalysts for the synthesis of BIMs has been documented (Murugan et al., 2005; Sivaprasad et al., 2006; Azizi et al., 2007; Heravi et al., 2008). In this article, a novel series of BIMs **4**-related bengacarboline were synthesized using tungstosilicic acid hydrate ($H_4O_{40}SiW_{12}$·aq) as a catalyst, and their cytotoxicities were evaluated.

MATERIALS AND METHODS

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined using a Griffin melting point apparatus and were uncorrected. Column chromatography was carried out using silica gel 60 (70-230 mesh ASTM). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254} aluminum sheets. ^{1}H- and ^{13}C- nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE 300 NMR spectrometer (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C). Mass spectra were recorded on a Bruker Daltonics (microTOF).

Synthesis of 4-nitro-*N*-(2-(1*H*-indol-3-yl)ethyl)-benzenesulfonamide (5)

A solution of tryptamine (1.6022 g, 10 mmol) in dichloromethane (50 mL) was added dropwise to a stirred mixture of 4-nitrobenzenesulfonyl chloride (2.2162 g, 10 mmol) and sodium carbonate (1.4839 g, 14 mmol) in dichloromethane (20 mL). The reaction mixture was stirred at room temperature overnight, and distilled water (20 mL) was added. The organic phase was separated, and the aqueous phase was extracted with dichloromethane (2 × 30 mL). The organic extracts were combined and washed with water (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The crude product was further purified by column chromatography on silica gel using 1:9 acetone-hexane to obtain the pure product.

Brown solid; yield 85%; mp 134-135ºC; IR (UATR) cm^{-1}: 3411, 3307, 1607, 1526, 1347, 1159. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.79 (t, J = 7.1 Hz, 2H, ArCH_2), 3.11 (q, J = 6.8 Hz, 2H, CH_2NH), 6.92 (t, J = 7.4 Hz, 1H, C5-ArH), 7.00 (t, J = 7.7 Hz, 1H, C6-ArH), 7.09 (s, 1H, C2-ArH), 7.25 (d, J = 8.0 Hz, 1H, C7-ArH), 7.36 (d, J = 7.7 Hz, 1H, C4-ArH), 7.91 (d, J = 8.7 Hz, 2H, C2'-ArH and C6'-ArH), 8.12 (t, J = 5.4 Hz, 1H, NHSO$_2$), 8.25 (d, J = 8.7 Hz, 2H, C3'-ArH and C5'-ArH), 10.79 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 25.8, 43.8, 111.1, 111.8, 118.4, 118.7, 121.3, 123.6, 124.7, 127.3, 128.2, 136.6, 146.6, 149.6. TOF-MS m/z: 346.0869 (Calcd for $C_{16}H_{16}N_3O_4S$: 346.0856).

General procedure for the synthesis of Bis- and Tris-indolylmethanes (4a-g, 7)

A mixture of *N*-sulfonyltryptamine **5** (0.5 mmol), aldehyde **6** (0.3 mmol), and $H_4O_{40}SiW_{12}$Þaq (20 mol%) in acetonitrile (5 mL) was stirred at room temperature for 2-6 h. After complete conversion, as indicated by TLC, the reaction mixture was concentrated in a vacuum and purified by silica gel column chromatography using 1:9 acetone-hexane to obtain the pure product.

Bis-indolylmethane 4a

Prepared according to the general procedure to give the title compound as a light brown solid in 70% yield; mp 179-180ºC; IR (UATR) cm^{-1}: 3393, 3323, 1606, 1527, 1348, 1161. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.80-3.10 (m, 8H, 4×CH_2), 6.18 (s, 1H, CHPh), 6.80-7.31 (m,

13H, Ar*H*, 2×N*H*SO_2), 7.45 (d, *J* = 7.1 Hz, 2H, Ar*H*), 7.80 (d, *J* = 8.7 Hz, 4H, 2×C2'-Ar*H*, 2×C6'-Ar*H*), 8.16 (d, *J* = 8.7 Hz, 4H, 2×C3'-Ar*H*, 2×C5'-Ar*H*), 9.68 (s, 2H, 2×N*H*). ^{13}C-NMR (75 MHz, acetone-d_6) δ 25.0, 40.4, 43.5, 43.6, 108.6, 111.2, 111.3, 118.0, 119.0, 121.2, 124.1, 127.0, 128.0, 128.5, 128.7, 135.2, 135.3, 135.9, 136.0, 141.0, 146.4, 149.7. TOF-MS *m/z*: 779.1944 (Calcd for $C_{39}H_{35}N_6O_8S_2$: 779.1952).

Bis-indolylmethane 4b

Prepared according to the general procedure to give the title compound as an orange solid in 63% yield; mp 210-211°C; IR (UATR) cm^{-1}: 3400, 3300, 1607, 1527, 1348, 1159. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.80-3.10 (m, 8H, 4×CH_2), 6.35 (s, 1H, C*H*), 6.68 (t, *J* = 5.5 Hz, 2H, 2×N*H*SO_2), 6.78 (t, *J* = 7.3 Hz, 1H, Ar*H*), 6.91-7.19 (m, 9H, Ar*H*), 7.39 (d, *J* = 7.2 Hz, 2H, Ar*H*), 7.74 (d, *J* = 8.8 Hz, 4H, 2×C2'-Ar*H*, 2×C6'-Ar*H*), 8.10 (d, *J* = 8.8 Hz, 4H, 2×C3'-Ar*H*, 2×C5'-Ar*H*), 8.97 (s, 1H, O*H*), 9.69 (s, 2H, 2×N*H*). ^{13}C-NMR (75 MHz, acetone-d_6) δ 24.8, 34.9, 43.3, 107.9, 111.2, 115.6, 117.9, 118.9, 120.1, 120.9, 123.9, 127.5, 127.8, 128.4, 128.5, 129.7, 135.5, 135.8, 146.3, 149.6, 154.2. TOF-MS *m/z*: 817.1711 (Calcd for $C_{39}H_{34}N_6NaO_9S_2$: 817.1721).

Bis-indolylmethane 4c

Prepared according to the general procedure to give the title compound as a pale orange solid in 38% yield; mp 169-170°C; IR (UATR) cm^{-1}: 3387, 3317, 1607, 1527, 1348, 1160. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.80-3.05 (m, 8H, 4×CH_2), 3.73 (s, 3H, OCH_3), 6.08 (s, 1H, C*H*), 6.72-7.19 (m, 12H, Ar*H*, 2×N*H*SO_2), 7.41 (d, *J* = 7.7 Hz, 2H, Ar*H*), 7.76 (d, *J* = 8.8 Hz, 4H, 2×C2'-Ar*H*, 2×C6'-Ar*H*), 8.13 (d, *J* = 8.8 Hz, 4H, 2×C3'-Ar*H*, 2×C5'-Ar*H*), 9.61 (s, 2H, 2×NH). ^{13}C-NMR (75 MHz, acetone-d_6) δ 25.9, 40.5, 44.4, 55.5, 109.1, 112.1, 114.8, 118.8, 119.8, 121.9, 124.9, 128.8, 129.4, 130.4, 133.6, 136.6, 136.8, 147.1, 150.5, 159.6. TOF-MS *m/z*: 831.1855 (Calcd for $C_{40}H_{36}N_6NaO_9S_2$: 831.1877).

Bis-indolylmethane 4d

Prepared according to the general procedure to give the title compound as a red solid in 48% yield; mp 121-122°C; IR (UATR) cm^{-1}: 3401, 3313, 1608, 1528, 1348, 1160. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.85-3.10 (m, 8H, 4×CH_2), 6.08 (s, 1H, C*H*), 6.75 (d, *J* = 8.4 Hz, 2H, C3"-Ar*H*, C5"-Ar*H*), 6.84 (br t, 2H, 2×N*H*SO_2), 6.94-7.07 (m, 6H, Ar*H*), 7.20 (d, *J* = 7.3 Hz, 2H, Ar*H*), 7.44 (d, *J* = 7.7 Hz, 2H, Ar*H*), 7.78 (d, *J* = 8.7 Hz, 4H, 2C2'-Ar*H*, 2C6'-Ar*H*), 8.16 (d, *J* = 8.7 Hz, 4H, 2C3'-Ar*H*, 2C5'-Ar*H*), 8.51 (s, 1H, O*H*), 9.62 (s, 2H, 2×N*H*). ^{13}C-NMR (75 MHz, acetone-d_6) δ 25.1, 39.7, 43.4, 43.5, 108.2, 110.0, 111.3, 115.5, 118.0, 119.0, 121.1, 124.1, 128.0, 128.5, 129.6, 131.6, 135.8, 135.9, 146.3, 149.7, 156.3, 156.4. TOF-MS *m/z*: 817.1713 (Calcd for $C_{39}H_{34}N_6NaO_9S_2$: 817.1721).

Bis-indolylmethane 4e

Prepared according to the general procedure to give the title compound as a light brown solid in 52% yield; mp 139-140°C; IR (UATR) cm^{-1}: 3387, 3317, 1607, 1527, 1348, 1160. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.90-3.10 (m, 8H, 4×CH_2), 3.68, 3.80 (2s, 6H, 2×OCH_3), 6.14 (s, 1H, C*H*), 6.72 (dd, *J* = 8.2 and 1.9 Hz, 1H, Ar*H*), 6.84-7.06 (m, 8H, Ar*H*, 2×N*H*SO_2), 7.21 (d, *J* = 7.1 Hz, 2H, Ar*H*), 7.45 (d, *J* = 7.1 Hz, 2H, Ar*H*), 7.79 (d, *J* = 8.9 Hz, 4H, 2×C2'-Ar*H*, 2×C6'-Ar*H*), 8.18 (d, *J* = 8.7 Hz, 4H, 2×C3'-Ar*H*, 2×C5'-Ar*H*), 9.71 (s, 2H, 2×N*H*). ^{13}C-NMR (75 MHz, acetone-d_6) δ 25.1, 40.3, 43.5, 48.9, 55.1, 108.2, 111.3, 111.8, 112.5, 117.9, 119.0, 120.6, 121.0, 124.1, 127.9, 128.5, 133.4, 135.8, 135.9, 146.3, 148.5, 149.5, 149.7. TOF-MS *m/z*: 861.1949 (Calcd for $C_{41}H_{38}N_6NaO_{10}S_2$: 861.1983).

Bis-indolylmethane 4f

Prepared according to the general procedure to give the title compound as a red solid in 57% yield; mp 186-187°C; IR (UATR) cm^{-1}: 3386, 3316, 1607, 1527, 1348, 1159. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.90-3.10 (m, 8H, 4×CH_2), 6.13 (s, 1H, C*H*), 6.64 (d, *J* = 8.2 Hz, 1H, Ar*H*), 6.75 (d, *J* = 8.2 Hz, 1H, Ar*H*), 6.80-7.07 (m, 7H, Ar*H*, 2×N*H*SO_2), 7.21 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.45 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.80 (d, *J* = 8.6 Hz, 4H, 2×C2'-Ar*H*, 2×C6'-Ar*H*), 8.18 (d, *J* = 8.6 Hz, 4H, 2×C3'-Ar*H*, 2×C5'-Ar*H*), 9.66 (s, 2H, 2×N*H*). ^{13}C-NMR (75 MHz, acetone-d_6) δ 25.1, 40.3, 43.5, 55.4, 108.2, 111.3, 112.3, 115.2, 117.9, 119.0, 121.0, 124.1, 127.9, 128.6, 132.2, 135.9, 145.7, 146.4, 147.7, 149.7. TOF-MS *m/z*: 825.2023 (Calcd for $C_{40}H_{37}N_6O_{10}S_2$: 825.2007).

Bis-indolylmethane 4g

Prepared according to the general procedure to give the title compound as a red solid in 54% yield; mp 172-173°C; IR (UATR) cm^{-1}: 3385, 3298, 1607, 1528, 1348, 1160. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.85-3.05 (m, 8H, 4×CH_2), 3.70 (s, 6H, 2×OCH_3), 6.13 (s, 1H, C*H*Ar), 6.66 (s, 2H, C2"-Ar*H*, C6"-Ar*H*), 6.81-7.48 (m, 11H, Ar*H*, 2×N*H*SO_2, O*H*), 7.79 (d, *J* = 8.7 Hz, 4H, 2C2'-Ar*H*, 2C6'-Ar*H*), 8.18 (d, *J* = 8.7 Hz, 4H, 2C3'-Ar*H*, 2C5'-Ar*H*), 9.72 (s, 2H, 2×N*H*). ^{13}C-NMR (75 MHz, acetone-d_6) δ 25.1, 40.8, 43.4, 55.8, 106.3, 108.2, 111.3, 117.9, 118.9, 121.0, 124.1, 127.9, 128.6, 131.3, 135.1, 135.9, 146.3, 148.1, 149.7. TOF-MS *m/z*: 877.1918 (Calcd for $C_{41}H_{38}N_6NaO_{11}S_2$: 877.1932).

Tris-indolylmethane 7

Prepared according to the general procedure to give the title compound as a light brown solid in 31% yield; mp 212-213ºC; IR (UATR) cm^{-1}: 3383, 3314, 1607, 1526, 1347, 1159. ^{1}H-NMR (300 MHz, acetone-d_6) δ 2.85-3.10 (m, 12H, 6×CH_2), 6.56 (s, 1H, C*H*), 6.80 (br t, 3H, 3×N*H*SO_2), 6.99-7.09 (m, 6H, Ar*H*), 7.18 (d, *J* = 7.7 Hz, 3H, Ar*H*), 7.49 (d, *J* = 7.2 Hz, 3H, Ar*H*), 7.70 (d, *J* = 8.8 Hz, 6H, 3×C2'-Ar*H*, 3×C6'-Ar*H*), 8.08 (d, *J* = 8.8 Hz, 6H, 3×C3'-Ar*H*, 3×C5'-Ar*H*), 9.85 (s, 2H, 3×N*H*). ^{13}C-NMR (75 MHz, acetone-d_6) δ 25.2, 33.5, 43.3, 108.8, 111.5, 118.2, 119.2, 121.3, 124.1, 127.9, 128.7, 133.7, 136.0, 146.2, 149.7. TOF-MS *m/z*: 1068.2061 (Calcd for $C_{49}H_{43}N_9NaO_2S_3$: 1068.2086).

Cytotoxic assay

The cytotoxic assay was performed as previously described (Tengchaisri et al., 1998). Briefly, cell lines suspended in RPMI 1640 containing 10% fetal bovine serum (FBS) were seeded at 1×10^4 cells (100 μL) per well in 96-well plates and incubated in a humidified atmosphere, 95% air, 5% CO_2 at 37ºC. After 24 h, additional medium (100 μL) containing the test compound and vehicle was added to a final concentration of 50 μg/mL, 0.2% dimethyl sulfoxide (DMSO), and further incubated for 3 days. After that, the cells were fixed with EtOH-H_2O (95:5, v/v), stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH, after which absorbance was measured at 550 nm. The number of surviving cells was determined from the absorbance. Etoposide and doxorubicin were used as the reference drugs.

RESULTS AND DISCUSSION

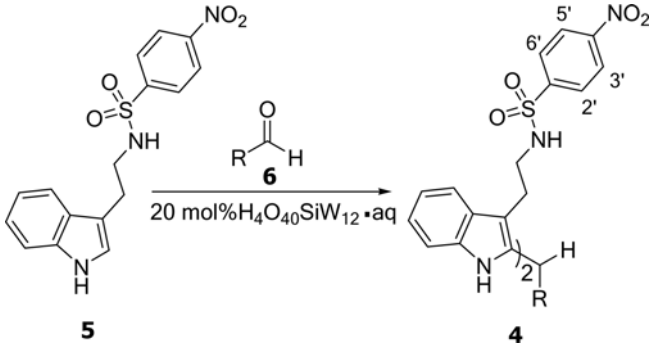
Chemistry

N-sulfonyltryptamine **5** was prepared by treatment of tryptamine with *p*-nitrobenzenesulfonyl chloride in the presence of Na_2CO_3. The alkylation of *N*-sulfonyltryptamine **5** with benzaldehyde was performed using 20 mol% catalyst at room temperature. Double nucleophilic attack of the indole carbon on the electrophilic center of benzaldehyde gave the anticipated BIM **4a** in 70% yield within 2 h (Table I; entry 1). The ^{1}H-NMR spectra of BIM **4a** was characterized by the presence of a methine proton as a singlet at 6.18 ppm and a typically symmetrical signal of two *N*-sulfonyltryptamine moieties. The structure of BIM **4a** was further confirmed by ^{13}C-NMR, 2D-NMR, infrared (IR), and high-resolution mass spectrometry (HRMS). The success of forming **4a** prompted us to extend the reaction of indole **5** with a range of other aldehydes **6**, as shown in Table I. The reaction was performed under similar conditions and furnished BIMs **4**, except **4h**, in moderate yield. To the best of our knowledge, these BIMs have not been reported. Surprisingly, treatment of indole **5** with 3-formylindole provided tris(2-indolyl) methane **7** instead of the expected BIM **4h**. The reaction required elevated temperature (60ºC) to consume **5** within 9 h. However, the reaction was not completed at room temperature, even when it was prolonged to 48 h. The ^{1}H-NMR spectra of TIM **7** illustrated a methine proton as a singlet at 6.56 ppm and a triple set of symmetrical *N*-sulfonyltryptamine moieties. However, the formation of the TIM system using 3-formylindole has also been observed (Chakrabarty and Sarkar, 2002; Hazra et al., 2008). Mechanistically, as shown in Scheme 2, the formation of indoleninium intermediate III may be considered to take place through rearrangement and elimination of water. Subsequently, nucleophilic addition of indole **5** on intermediate III led to BIM **4** (Biswas and Jackson, 1969; Chakrabarty and Sarkar, 2002; Chakrabarty et al., 2002). In the case of using 3-formylindole (R=indole), the formation of TIM **7** was proposed to proceed via a loss of a molecule of indole, followed by re-addition of the *N*-sulfonyltryptamine **5** (Chakrabarty and Sarkar, 2002).

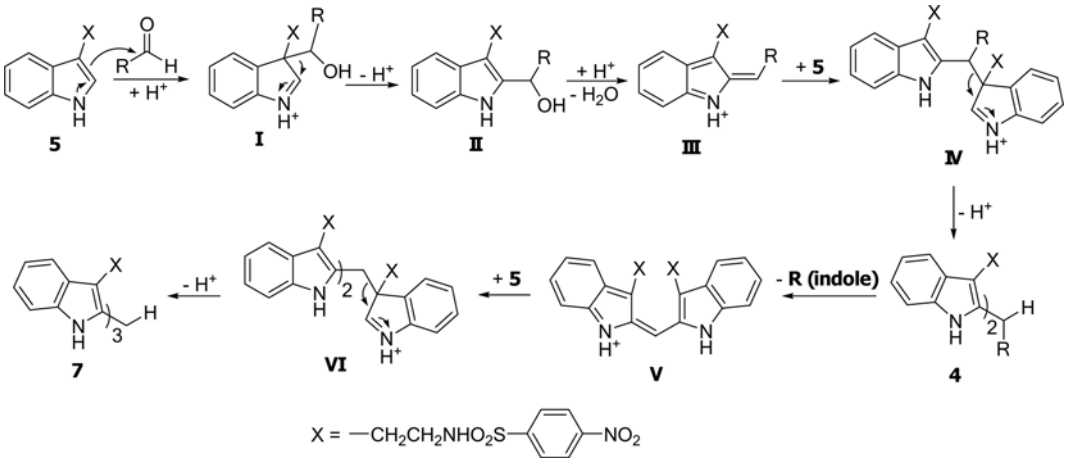
Table I. Reaction[a] of indole **5** with aldehydes **6** using tungstosilicic acid hydrate

Entry	Aldehyde (**6**)	Product	Yield (%)
1	C_6H_5CHO	4a	70
2	2-OH-C_6H_4CHO	4b	63
3	4-OMe-C_6H_4CHO	4c	42
4	4-OH-C_6H_4CHO	4d	48
5	3,4-$(OMe)_2$-C_6H_3CHO	4e	52
6	3-OMe,4-OH-C_6H_3CHO	4f	57
7	3,5-$(OMe)_2$,4-OH-C_6H_2CHO	4g	54
8[b]	3-CHO-indole	**7** (not **4h**)	31

[a]20 mol% $H_4O_{40}SiW_{12}$·aq, CH_3CN, rt 2-6 h; [b]20 mol% $H_4O_{40}SiW_{12}$ ·aq, CH_3CN, 60ºC 9 h



Scheme 1. Synthesis of BIMs **4**.



Scheme 2. Proposed reaction mechanism of **4** and **7** formations.

Cytotoxic activity

Cytotoxicity of the synthesized BIMs **4** and TIM **7** were assayed against HuCCA-1 (human cholangiocarcinoma cancer cells), HepG2 (human hepatocellular liver carcinoma cell line), A549 (human lung carcinoma cell line) and MOLT-3 (human lymphoblastic leukemia cell line), as summarized in Table II. Unfortunately, most synthesized BIMs, **4a**, **4c**, **4e-4g**, and TIM **7** were not active toward any the tested cell lines. However, mono hydroxy analogs (**4b** and **4d**) displayed cytotoxic potency against HepG2 and MOLT-3 cells. More importantly, the hydroxy group at either the *ortho* or *para* position on the aromatic ring (R of **4**) played a significant role in the cytotoxic effect. It has been noted that the inhibitory potency of the *para* hydroxy analog (**4d**) was completely lost when the steric crowding of methoxy group appeared at the *o*-position of the hydroxy group, as presented in compounds **4f** and **4g**. It is conceivable that the compounds require H-bonding (from phenolic OH) and hydrophobic binding of the aromatic ring for their cytotoxicities. Significantly, the mono hydroxy analogs (**4b** and **4d**) displayed cytotoxicity with slightly lower IC_{50} than the control drug, etoposide, against HepG2 cells. In conclusion, we have achieved an application of tungstosilicic acid hydrate as an influential catalyst for the synthesis of BIMs **4** and TIM **7** in moderate to good yield. We found that BIMs **4** were obtained when benzaldehydes were employed. However, in the case of 3-formylindole, TIM **7** was formed via the cascade of an addition-elimination-addition process. It is noteworthy that the *ortho*-hydroxy (**4b**) and *para*-hydroxy (**4d**) groups of the phenolic moiety play an important role in their cytotoxic efficacy toward HepG2 and MOLT-3 cell lines. Significantly, the method offers several attractive advantages, including the use of a readily available and non-corrosive catalyst as well as a simple process for the synthesis of these biologically active compounds.

Table II. Cytotoxic activity of compounds **4b** and **4d** against four cancer cell lines

compound	Cytotoxic activity (IC_{50}, μg/mL)			
	HuCCA-1	HepG2	A549	MOLT-3
4a, **4c**, **4d-4g**, **7**	>50	>50	>50	>50
4b	>50	17.67	>50	3.95
4d	>50	17.50	>50	1.62
Etoposide[b]	ND[a]	18.20	ND[a]	0.019
Doxorubicin[c]	0.43	0.55	0.38	ND[a]

[a]ND, not determined; [b,c]Etoposide and doxorubicin were used as the reference drugs.

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