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Synthesis and Preliminary Biological Study of Bisindolylmethanes Accessed by an Acid-Catalyzed Hydroarylation of Vinylindoles

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Abstract

An acid-catalyzed hydroarylation reaction of vinyl indoles is reported, which tolerates a wide range of heterocycles as the exogenous nucleophile such as indoles, pyrroles, and indolizines. The method rapidly accesses the biologically relevant bisindolylmethane scaffold in good to excellent yields. Evaluation of the biological activity of several synthesized analogues reveals cytotoxic activity against and selectivity for the MCF-7 breast cancer cell line.

Keywords

Acid catalysis; Bisindolyl methanes; Hydroarylation; Heterocycles; Breast Cancer

1. Introduction

Bisindolylmethane scaffolds are present in many natural products isolated from both terrestrial and marine natural sources.¹ These natural products exhibit a broad range of biological activity including anticancer activity against several common cancer cell-lines.² Naturally occurring bisindolylmethanes such as vibrindole A (**1**) are useful in the treatment of fibromyalgia, chronic fatigue, and irritable bowel syndrome.³ Additionally, diindolylmethane (**2**) inhibits the proliferation of both estrogen dependent and independent breast cancer cell lines.⁴ Synthetic bisindolylmethanes are not only useful in biological applications,^{2,5} but also have found use in materials chemistry.⁶ Due to their extensive utility, a number of methods have been reported for the synthesis of symmetrical bisindolylmethanes, whereas the efficient synthesis of unsymmetrical bisindolylmethanes remains a challenge.

Recently, various groups have reported the synthesis of unsymmetrical bisindolylmethanes using a leaving group strategy (Scheme 1a).⁷ Although this strategy is useful, it has some limitations in terms of substrates that can be employed. For example, substrates with an aryl

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group at the benzylic position of the indole are preferred presumably due to the additional stabilization they impart to the cationic alkylideneindoleninium intermediate **A**.^{1,8,9} Moreover, intermediate **A** is a proposed key intermediate in various synthetic transformations to access functionalized indole core structures including unsymmetrical bisindolylmethanes.^{8,9} Hence, an efficient and simple method to access the alkylideneindoleninium intermediate would be attractive. Considering this, we became interested in developing new methods to access bisindolylmethanes, which would provide an alternative to existing methods. Herein, we report the use of readily accessible vinyl indole derivatives as precursors to alkylideneindoleninium intermediates of type **A**. It is proposed that the protonation of a vinyl indole with a Brønsted acid will afford intermediate **A**, which will subsequently react with an exogenous nucleophilic indole derivative to yield unsymmetrical bisindolylmethanes (Scheme 1b).¹⁰

2. Results and Discussion

The parent substrate 3-vinyl-1*H*-indole (**3**) was selected for initial optimization. In contrast to previous reports, substrate **3** does not have any additional stabilization of the putative cationic intermediate **A**, which makes it a more challenging substrate to functionalize. When **3** was treated with 20 mol% of *p*-TsOH in either dichloromethane (DCM), dichloroethane (DCE) or toluene, complete conversion of **3** was observed within a few hours with <10% yield of the desired product and poor mass balance (Table 1, entries **1–3**). This observation alludes to the tendency of substrate **3** to polymerize under acidic conditions. Further evaluation of the reaction conditions revealed that the addition of the Lewis basic solvent dimethylacetamide (DMA) as a co-solvent improved the yield of the desired product (Table 1, compare entries **4** and **5**). The reaction in DMA as the sole solvent gave the desired product in 89% GC yield (Table 1, entry **6**). Further optimization of acid and nucleophile loadings gave the optimal conditions, in which 81% isolated yield of the desired product was obtained (Table 1, compare entries **7** and **8**).

The scope of the exogenous nucleophile was evaluated using 3-vinyl-1*H*-indole (**3**) as the standard substrate. Unprotected 1*H*-indole as a nucleophile gave the natural product vibrindole **A** (**1**) in 80% isolated yield, without precautions to exclude air or moisture from the system (Table 2, entry **2a**). It should be noted that >90% of the remaining nucleophile was recovered.

N-methyl indole also gave the corresponding 3,3'-bisindolylmethane in good yield (entry **2b**). Furthermore, various substituted indoles are well-tolerated under the reaction conditions including sterically demanding 2-substituted indoles (entries **2c–2g**). The 3,2'-bisindolylmethanes are obtained using this method as demonstrated by successful use of 3-methyl indole to give product **2h** although a higher reaction temperature was required. A pyrrole and an indolizine can be used as alternative nucleophiles to give the corresponding bisheteroarylmethanes in good yields (entries **2i** and **2j**). Finally, a disubstituted vinylindole is also a component reaction partner giving the desired product in excellent yield (entries **2k**).

Encouraged by these results, substituted vinyl indole **4** was prepared in high yield by condensation of indole with the corresponding cyclic ketone.¹¹ As expected, substrate **4** underwent smooth hydrofunctionalization with *N*-Me-indole as the exogenous nucleophile, to give the desired product in excellent yield (Table 3, entry **3a**). The bisindolylmethane obtained (**3a**) not only has one quaternary carbon but a similar bisindolylmethane, reported by Wang and coworkers **5**, also exhibits *in-vivo* activity against lung cancer cell lines.¹² Additionally, both electron-rich and electron-deficient indoles are well-tolerated (entries **3b–3c**) as well as the use of a pyrrole and an indolizine as nucleophiles (entry **3d–3e**).

In light of the previously established biological activity of diindolylmethane **2** against a breast cancer cell line¹³ and our recent discovery of cytotoxic indole containing compounds,¹⁴ the biological activity of the newly synthesized bisindolylmethanes was probed in MCF-7 breast cancer cell line. Upon evaluating various derivatives in this whole cell assay, several compounds including compounds **2c** and **3a** were found to reduce cell count in comparison to the DMSO control (Figure 2a). Excitingly, compound **3a** displays excellent differential activity between MCF-7 and MCF-10A (normal breast) cells. Even at the highest concentration evaluated (100 μ M), **3a** does not generally affect the growth of MCF-10A cells whereas it leads to an EC₅₀ of 4.3 μ M in MCF-7 cells (Figure 2b). Of additional interest, an EC₅₀ of ~50 n M is observed for compound **2j** although no selectivity for either cell line is observed (Figure 2b).

3. Conclusions

In conclusion, we have successfully developed a simple and efficient reaction to access biologically relevant bisindolylmethanes from vinyl indoles. This method offers a distinct alternative to access alkylideneindoleninium intermediates in situ, which can in turn be subjected to the reaction with various nucleophiles to provide functionalized indoles. Broad scope and ease of the reaction highlight this method. Future work is focused on the further evaluation of analogues of compound **3a** and the identification of its biological target.

4. Experimental Section

4.1. General Method

Toluene, dichloromethane, and THF were dried before use by passing through a column of activated alumina. All other reagents (including indoles) were purchased from commercial sources and used without further purification. Yields were calculated for material judged homogeneous by thin-layer chromatography and NMR. Thin-layer chromatography was performed with EMD silica gel 60 F254 plates eluting with the solvents indicated, visualized by a 254 nm UV lamp, and stained either with potassium permanganate, *p*-anisaldehyde, phosphomolybdic acid or vanillin. Flash column chromatography was performed with EcoChrom MP Silitech 32-63D 60Å silica gel, slurry packed with solvents indicated in glass columns. Nuclear magnetic resonance spectra were acquired at 400 MHz for ¹H, and 100 MHz for ¹³C. Chemical shifts for proton nuclear magnetic resonance (¹H NMR) spectra are reported in parts per million downfield relative to the line of CHCl₃ singlet at 7.26 ppm. Chemical shifts for carbon nuclear magnetic resonance (¹³C NMR) spectra are reported in parts per million downfield relative to the center-line of the CDCl₃ triplet at 77.2 ppm. The abbreviations s, d, t, q, td, dd and m stand for the resonance multiplicities singlet, doublet, triplet, quartet, triplet of doublet, doublet of doublet and multiplet, respectively. IR spectra were recorded using a Nicolet FT-IR instrument. Reported melting points are uncorrected. Glassware for all reactions was oven-dried at 110 °C and cooled while purging with nitrogen prior to use.

4.2. General Procedure for Substrate Scope (Table 2)

To a 2.5 dram vial equipped with a stir bar was added 5.13 mg of *p*-TsOH (0.030 mmol, 0.100 equiv.), 351.3 mg of *N*-H-indole (3.00 mmol, 10.0 equiv.) and 42.9 mg of **1** (0.300 mmol, 1.00 equiv.). To this 1.5 mL of DMA was added *via* syringe. The vial was capped and was stirred for given time at room temperature. The crude mixture was purified with flash silica-gel column chromatography. (*Note: In case of liquid nucleophiles, p-TsOH was added last*)

4.2.1. Characterization data for 3,3'-(ethane-1,1-diyl)bis(1H-indole) (Entry 2a)—Followed same procedure as general procedure. Yield = 80% (average of two run).

The ¹H-NMR spectrum of product was matched with literature

4.2.2. Characterization data for 3-(1-(1H-indol-3-yl)ethyl)-1-methyl-1H-indole (Entry 2b)—Followed same procedure as general procedure. Yield = 81% (average of two run), R_f = 0.30 w/ 20% EtOAc/Hex, pale green solid, M.P. = 51–53 °C. ¹H-NMR (400 MHz, CDCl₃) δ = 7.37 (s, 1 H), 7.66 (d, J = 7.9 Hz, 2 H), 7.35 (dt, J = 7.2 Hz, J = 0.9 Hz, 2 H), 7.30–7.22 (m, 2 H), 7.13 (ddd, J = 7.9 Hz, J = 7.0 Hz, J = 0.8 Hz, 2 H), 6.91 (d, J = 2.3 Hz, 1 H), 6.82 (s, 1 H), 4.75 (q, J = 7.1 Hz, 1 H), 3.72 (s, 3 H), 1.88 (d, J = 7.1 Hz, 3 H). ¹³C-NMR {¹H} (100 MHz, CDCl₃) δ = 137.5, 136.8, 127.5, 127.1, 126.2, 121.9, 121.5, 121.4, 120.3, 120.0, 119.9, 119.2, 118.6, 111.2, 109.3, 32.7, 28.3, 22.2. IR 3411, 2967, 1455, 1335, 760 cm⁻¹. HRMS C₁₉H₁₉N₂ (M+H)⁺ calcd. 275.1543, obsvd. 275.1554.

4.2.3. Characterization data for 3-(1-(1H-indol-3-yl)ethyl)-1,2-dimethyl-1H-indole (Entry 2c)—Followed same procedure as entry 2b except 432.0 mg of indole (3.00 mmol, 10.0 equiv.) was added. Yield = 85% (average of two run), R_f = 0.35 w/ 20% EtOAc/Hex, colorless solid, M.P. = 52–54 °C. ¹H-NMR (400 MHz, CDCl₃) δ = 7.81 (s, 1 H), 7.64 (d, J = 7.9 Hz, 1 H), 7.45 (d, J = 7.9 Hz, 1 H), 7.31 (dd, J = 12.4 Hz, J = 8.1 Hz, 2 H), 7.19–7.15 (m, 2 H), 7.06–7.00 (m, 3 H), 4.72 (qd, J = 7.2 Hz, J = 1.1 Hz, 1 H), 3.67 (s, 3 H), 2.40 (s, 3 H), 1.89 (d, 3 H). ¹³C-NMR {¹H} (100 MHz, CDCl₃) δ = 136.9, 136.8, 131.2, 127.4, 127.1, 121.9, 121.8, 121.1, 120.3, 119.8, 119.5, 119.1, 118.5, 115.2, 111.1, 108.7, 29.6, 29.3, 21.2, 10.7. IR 3419, 1457, 1233, 765 cm⁻¹. HRMS C₂₀H₂₁N₂ (M+H)⁺ calcd. 289.1699, obsvd. 289.1701.

4.2.4. Characterization data for 3-(1-(1H-indol-3-yl)ethyl)-1-methyl-2-phenyl-1H-indole (Entry 2d)—Followed same procedure as general procedure except 616.8 mg of 1-methyl-2-phenyl-1H-indole (3.00 mmol, 10.0 equiv.) and additional 1 mL of DMA were used. Yield = 55% (average of two run), R_f = 0.32 w/ 20% EtOAc/Hex, colorless solid, M.P. = 178–180 °C. ¹H-NMR (400 MHz, CDCl₃) δ = 7.80 (s, 1 H), 7.67 (d, J = 7.9 Hz, 1 H), 7.49–7.43 (m, 3 H), 7.39 (dd, J = 7.7 Hz, J = 1.6 Hz, 2 H), 7.34 (t, J = 8.6 Hz, 1 H), 7.28 (t, J = 7.6 Hz, 1 H), 7.21 (td, J = 7.6 Hz, J = 1.1 Hz, 1 H), 7.13–7.09 (m, 1 H), 7.05–6.94 (m, 3 H), 4.55 (dq, J = 7.2 Hz, J = 0.8 Hz, 1 H), 3.60 (s, 3 H), 1.86 (d, J = 7.2 Hz, 3 H). ¹³C-NMR {¹H} (100 MHz, CDCl₃) δ = 137.7, 137.2, 136.7, 132.4, 130.9, 130.8, 128.4, 128.1, 126.8, 121.8, 121.7, 121.4, 121.3, 120.9, 119.9, 118.9, 118.8, 117.4, 110.9, 109.5, 30.9, 28.6, 21.7. IR 3414, 2969, 1466, 740 cm⁻¹. HRMS C₂₅H₂₃N₂ (M+H)⁺ calcd. 351.1856, obsvd. 351.1843.

4.2.5. Characterization data for 3-(1-(1H-indol-3-yl)ethyl)-5-bromo-1H-indole (Entry 2e)—Followed same procedure as general procedure except 588.1 mg of 5-bromo-1H-indole (3.00 mmol, 10.0 equiv.) was used. Yield = 46% (average of two run), R_f = 0.30 w/ 20% EtOAc/Hex, colorless solid, M.P. = 47–50 °C. ¹H-NMR (400 MHz, CDCl₃) δ = 7.88 (s, 2 H), 7.71–7.70 (m, 1 H), 7.54 (dd, J = 7.9 Hz, J = 0.7 Hz, 1 H), 7.36 (dt, J = 8.1 Hz, J = 0.8 Hz, 1 H), 7.26–7.16 (m, 3 H), 7.06 (ddd, J = 7.9 Hz, J = 7.0 Hz, J = 0.9 Hz, 1 H), 6.91 (td, J = 2.2 Hz, J = 0.8 Hz, 2 H), 4.61 (q, J = 7.1 Hz, 1 H), 1.79 (d, J = 7.1 Hz, 3 H). ¹³C-NMR {¹H} (100 MHz, CDCl₃) δ = 136.8, 135.3, 128.8, 126.9, 124.7, 122.6, 122.3, 122.0, 121.5, 119.8, 119.2, 112.6, 112.5, 111.3, 28.2, 21.7. IR 3405, 1453, 1092, 739 cm⁻¹. HRMS C₁₈H₁₅BrN₂ (M)⁺ calcd. 338.0429, obsvd. 338.0419.

4.2.6. Characterization data for 3-(1-(1H-indol-3-yl)ethyl)-5-methoxy-1H-indole (Entry 2f)—Followed same procedure as general procedure except 588.1 mg of 5-

bromo-1*H*-indole (3.00 mmol, 10.0 equiv.) was used. Yield = 76% (average of two run), R_f = 0.25 w/ 20% EtOAc/Hex, colorless solid, M.P. = 57–59 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ = 7.86 (s, 1 H), 7.75 (s, 1 H), 7.63 (d, J = 7.9 Hz, 1 H), 7.32 (d, J = 8.1 Hz, 1 H), 7.23–7.18 (m, 2 H), 7.11–7.06 (m, 2 H), 6.89–6.85 (m, 3 H), 4.66 (q, J = 7.1 Hz, 1 H), 3.80 (s, 3 H), 1.83 (d, J = 7.1 Hz, 3 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 153.7, 136.8, 132.0, 127.4, 127.0, 122.2, 121.9, 121.6, 121.5, 121.4, 119.9, 119.1, 111.9, 102.0, 56.0, 28.3, 21.8. IR 3403, 1452, 1206, 1091, 739 cm^{-1} . HRMS $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}$ ($\text{M}+\text{H}$) $^+$ calcd. 291.1492, obsvd. 291.1489.

4.2.7. Characterization data for 3-(1-(1*H*-indol-3-yl)ethyl)-7-ethyl-1*H*-indole

(Entry 2g)—Followed same procedure as general procedure except 435.6 mg of 7-ethyl-1*H*-indole (3.00 mmol, 10.0 equiv.) was used. Yield = 62% (average of two run), R_f = 0.35 w/ 20% EtOAc/Hex, pale green solid, M.P. = 50–52 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ = 7.78–7.76 (m, 2 H), 7.61 (d, J = 7.9 Hz, 1 H), 7.49–7.47 (m, 1 H), 7.34 (d, J = 8.1 Hz, 1 H), 7.19 (td, J = 7.6 Hz, J = 0.9 Hz, 1 H), 7.10–7.04 (m, 3 H), 6.90–6.86 (m, 2 H), 4.69 (q, J = 7.1 Hz, 1 H), 2.85 (q, J = 7.6 Hz, 2 H), 1.83 (d, J = 7.1 Hz, 3 H), 1.38 (t, J = 7.6 Hz, 3 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 136.8, 135.6, 127.1, 126.8, 126.5, 122.3, 121.9, 121.8, 121.4, 121.0, 120.4, 119.9, 119.4, 119.1, 117.6, 111.2, 28.4, 24.1, 21.9, 13.9. IR 3403, 1455, 1293, 1091, 739 cm^{-1} . HRMS $\text{C}_{20}\text{H}_{21}\text{N}_2$ ($\text{M}+\text{H}$) $^+$ calcd. 289.1699, obsvd. 289.1681.

4.2.8. Characterization data for 2-(1-(1*H*-indol-3-yl)ethyl)-3-methyl-1*H*-indole

(Entry 2h)—Followed same procedure as general procedure except 393.6 mg of 3-methyl-1*H*-indole (3.00 mmol, 10.0 equiv.) was used. Yield = 54% (average of two run), R_f = 0.41 w/ 20% EtOAc/Hex, colorless solid, M.P. = 64–66 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ = 7.99 (m, 1 H), 7.59–7.56 (m, 2 H), 7.35 (t, J = 8.2 Hz, 2 H), 7.21–7.08 (m, 3 H), 6.99 (t, J = 7.5 Hz, 1 H), 4.72 (q, J = 7.1 Hz, 1 H), 2.45 (s, 3 H), 1.76 (d, J = 7.1 Hz, 3 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 138.7, 136.7, 135.0, 129.8, 126.9, 122.5, 121.4, 120.9, 119.8, 119.7, 119.5, 119.0, 118.2, 111.2, 110.6, 105.9, 28.6, 20.6, 8.7. IR 3412, 1455, 1335, 740 cm^{-1} . HRMS $\text{C}_{19}\text{H}_{19}\text{N}_2$ ($\text{M}+\text{H}$) $^+$ calcd. 275.1543, obsvd. 275.1554.

4.2.9. Characterization data for 3-(1-(1*H*-pyrrol-2-yl)ethyl)-1*H*-indole (Entry 2i)

—Followed same procedure as general procedure except 201.2 mg of 1*H*-pyrrole (3.00 mmol, 10.0 equiv.) was added. Yield = 55% (average of two run), R_f = 0.43 w/ 20% EtOAc/Hex, colorless liquid, $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ = 7.94–7.78 (m, 2 H), 7.48 (d, J = 7.9 Hz, 1 H), 7.37 (d, J = 8.2 Hz, 1 H), 7.22 (td, J = 7.6 Hz, J = 0.9 Hz, 1 H), 7.11–7.07 (m, 1 H), 6.97 (d, J = 2.4 Hz, 1 H), 6.59 (q, J = 2.1 Hz, 1 H), 6.21 (q, J = 2.9 Hz, 1 H), 6.17–6.16 (m, 1 H), 4.45 (q, J = 7.1 Hz, 1 H), 1.74 (d, J = 7.1 Hz, 3 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 136.7, 129.2, 126.7, 122.3, 121.4, 120.2, 119.7, 119.6, 116.3, 111.3, 108.1, 104.3, 30.0, 21.3. IR 3401, 1291, 1091, 743 cm^{-1} . HRMS $\text{C}_{14}\text{H}_{14}\text{N}_2$ ($\text{M}+\text{H}$) $^+$ calcd. 211.123, obsvd. 211.1224.

4.2.10. Characterization data for methyl 3-(1-(1*H*-indol-3-yl)ethyl)indolizine-1-carboxylate (Entry 2j)

—Followed same procedure as general procedure except 525.5 mg of methyl indolizine-1-carboxylate (3.00 mmol, 10.0 equiv.) was used. Yield = 68% (average of two run), R_f = 0.28 w/ 20% EtOAc/Hex, colorless solid, M.P. = 77–79 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ = 8.22 (d, J = 9.0 Hz, 1 H), 8.11 (s, 1 H), 7.68 (d, J = 7.1 Hz, 1 H), 7.61 (d, J = 7.9 Hz, 1 H), 7.35 (d, J = 8.1 Hz, 1 H), 7.31 (s, 1 H), 7.23–7.19 (m, 1 H), 7.12 (t, J = 7.5 Hz, 1 H), 6.98 (dd, J = 9.0 Hz, J = 6.6 Hz, 1 H), 6.59 (d, J = 2.4 Hz, 1 H), 6.52 (td, J = 6.8 Hz, J = 0.7 Hz, 1 H), 4.57 (q, J = 7.0 Hz, 1 H), 3.93 (s, 3 H), 1.85 (d, J = 7.0 Hz, 3 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 165.7, 136.8, 136.3, 129.1, 126.4, 124.0, 122.3, 121.8, 121.5, 119.8, 119.6, 118.8, 117.9, 113.6, 112.1, 111.6, 102.4, 51.0, 28.6, 20.8.

IR 3314, 2966, 1662, 1214, 735 cm^{-1} . HRMS $\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}$)⁺ calcd. 319.1441, obsvd. 319.1453.

4.2.11. Characterization data for 3-(1-(1H-indol-3-yl)propyl)-1-methyl-1H-indole (Entry 2k)—Followed same procedure as general procedure except reaction was ran at 70 °C. Yield = 84% (average of two run), R_f = 0.40 w/ 20% EtOAC/Hex, colorless solid, M.P. = 88–90 °C. ^1H -NMR (400 MHz, CDCl_3) δ = 7.82 (s, 1 H), 7.64 (dt, J = 7.9 Hz, J = 0.9 Hz, 2 H), 7.33–7.29 (m, 2 H), 7.26–7.32 (m, 2 H), 7.07 (ddd, J = 8.0 Hz, J = 7.0 Hz, J = 1.1 Hz, 2 H), 6.99 (d, J = 1.6 Hz, 1 H), 6.86 (s, 1 H), 4.41 (t, J = 7.4 Hz, 1 H), 3.72 (s, 3 H), 2.27 (quintet, J = 7.4 Hz, 2 H) 1.05 (t, J = 7.4 Hz, 3 H). ^{13}C -NMR { ^1H } (100 MHz, CDCl_3) δ = 137.4, 136.7, 128.2., 127.8, 127.4, 126.5, 121.8, 121.6, 121.4, 120.6, 120.0, 119.1, 119.0, 118.6, 111.2, 109.3, 36.0, 32.7, 29.1, 13.3. IR 3406, 2963, 1584, 1451, 1335, 1095, 738 cm^{-1} . HRMS $\text{C}_{20}\text{H}_{21}\text{N}_2$ ($\text{M}+\text{H}$)⁺ calcd. 289.1699, obsvd. 289.1705.

4.3. General Procedure for Substrate Scope (Table 3)

To a 2.5 dram vial equipped with a stir bar was added 3.44 mg of *p*-TsOH (0.020 mmol, 0.100 equiv.), 262.4 mg of *N*-Me-indole (2.00 mmol, 10.0 equiv.) and 59.7 mg of **1** (0.200 mmol, 1.00 equiv.). To this 1.0 mL of DMA was added *via* syringe. The vial was capped and was stirred for given time at 70 °C in oil bath. The crude mixture was purified with flash silica-gel column chromatography. (*Note: In case of liquid nucleophiles, p-TsOH was added last*)

4.3.1. Characterization data for tert-butyl-4-(1H-indol-3-yl)-4-(1-methyl-1H-indol-3-yl)piperidine-1-carboxylate (Entry 3a)—Followed same procedure as the general procedure. Yield = 91% (average of two run), R_f = 0.35 w/ 66% EtOAC/Hex, colorless solid, M.P. = 133–135 °C. ^1H -NMR (400 MHz, CDCl_3) δ = 8.14 (s, 1 H), 7.52 (dd, J = 12.1 Hz, J = 9.1 Hz, 2 H), 7.31 (d, J = 8.1 Hz, 1 H), 7.26 (d, J = 8.2 Hz, 1 H), 7.16–7.08 (m, 2 H), 7.03 (d, J = 2.5 Hz, 1 H), 6.96–6.90 (m, 3 H), 3.73 (s, 3 H), 3.65–3.55 (m, 4 H), 2.62 (t, J = 5.4 Hz, 4 H), 1.52 (s, 9 H). ^{13}C -NMR { ^1H } (100 MHz, CDCl_3) δ = 155.4, 137.9, 137.3, 126.9, 126.5, 126.1, 122.3, 121.8, 121.6, 121.4, 121.2, 120.4, 118.9, 118.4, 111.4, 109.4, 79.4, 38.1, 35.9, 32.9, 28.7. IR 3303, 2947, 1667, 734 cm^{-1} . HRMS $\text{C}_{27}\text{H}_{31}\text{N}_3\text{O}_2$ ($\text{M}+\text{Na}$)⁺ calcd. 452.2308, obsvd. 452.2314.

4.3.2. Characterization data for tert-butyl-4-(1H-indol-3-yl)-4-(5-methoxy-1-methyl-1H-indol-3-yl)piperidine-1-carboxylate (Entry 3b)—Followed same procedure as the general procedure. Yield = 90% (average of two run), R_f = 0.35 w/ 66% EtOAC/Hex, colorless solid, M.P. = 186–188 °C. ^1H -NMR (400 MHz, CDCl_3) δ = 8.10 (d, J = 1.1 Hz, 1 H), 8.01 (d, J = 1.2 Hz, 1 H), 7.49 (d, J = 8.0 Hz, 1 H), 7.29 (d, J = 8.1 Hz, 1 H), 7.18 (d, J = 8.8 Hz, 1 H), 7.10–7.02 (m, 3 H), 6.90 (dd, J = 3.8 Hz, J = 1.7 Hz, 1 H), 6.75 (dd, J = 8.8 Hz, J = 2.4 Hz, 1 H), 3.66 (s, 3 H), 3.58 (bs, 4 H), 2.63–2.52 (m, 4 H), 1.49 (s, 9 H). ^{13}C -NMR { ^1H } (100 MHz, CDCl_3) δ = 155.4, 153.1, 137.3, 132.5, 129.2, 126.6, 126.1, 123.0, 122.2, 121.7, 121.6, 121.4, 121.2, 118.9, 111.8, 111.4, 11.2, 104.0, 79.5, 55.9, 37.9, 35.7, 20.7. IR 3305, 1524, 1425, 793 cm^{-1} . HRMS $\text{C}_{27}\text{H}_{31}\text{N}_3\text{O}_3$ ($\text{M}+\text{Na}$)⁺ calcd. 468.2258, obsvd. 468.2267.

4.3.3. Characterization data for methyl-3-(1-(tert-butoxycarbonyl)-4-(1H-indol-3-yl)piperidin-4-yl)-1-methyl-1H-indole-5-carboxylate (Entry 3c)—Followed same procedure as the general procedure. Yield = 71% (average of two run), R_f = 0.30 w/ 66% EtOAC/Hex, colorless solid, M.P. = 266–268 °C. ^1H -NMR (400 MHz, CDCl_3) δ = 8.35 (d, J = 0.4 Hz, 1 H), 8.12 (d, J = 0.6 Hz, 1 H), 8.05 (d, J = 0.9 Hz, 1 H), 7.56 (dd, J = 8.6 Hz, J = 1.5 Hz, 1 H), 7.45 (d, J = 8.6 Hz, 1 H), 7.40 (d, J = 8.1 Hz, 1 H), 7.31 (d, J = 8.1 Hz, 1 H), 7.25 (d, J = 2.5 Hz, 1 H), 7.11–7.05 (m, 2 H), 6.90–6.86 (m, 1 H), 3.87 (s, 3 H),

3.57 (bs, 4 H), 2.64-2.51 (m, 4 H), 1.48 (s, 9 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 155.3, 137.2, 136.5, 129.7, 125.9, 125.2, 123.4, 122.7, 122.0, 121.9, 121.4, 121.0, 120.6, 119.9, 119.0, 113.7, 111.4, 79.6, 52.0, 37.9, 35.8, 28.6. IR 3303, 1667, 1509, 734 cm^{-1} . HRMS $\text{C}_{28}\text{H}_{32}\text{N}_3\text{O}_4$ ($\text{M}+\text{H}$) $^+$ calcd. 474.2401, obsvd. 474.2401.

4.3.4. Characterization data for tert-butyl-4-(1H-indol-3-yl)-4-(1H-pyrrol-2-yl)piperidine-1-carboxylate (Entry 3d)—Followed same procedure as the general procedure. Yield = 85% (average of two run), R_f = 0.32 w/ 66% EtOAc/Hex, colorless thick liquid, $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ = 8.14 (s, 1 H), 7.70 (d, J = 0.6 Hz, 1 H), 7.35 (dt, J = 7.3 Hz, J = 0.9 Hz, 1 H), 7.15 (td, J = 7.6 Hz, J = 1.1 Hz, 1 H), 7.01-6.96 (m, 2 H), 6.55 (td, J = 2.6 Hz, J = 1.5 Hz, 1 H), 6.23-6.16 (m, 2 H), 3.60 (ddd, J = 13.3 Hz, J = 7.5 Hz, J = 3.9 Hz, 2 H), 3.49-3.44 (m, 2 H), 2.47-2.41 (m, 2 H), 2.26 (ddd, J = 13.3 Hz, 8.5 Hz, J = 4.3 Hz, 2 H), 1.47 (s, 9 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 155.2, 137.7, 137.1, 125.8, 122.3, 122.1, 120.9, 120.8, 119.7, 116.6, 111.4, 108.0, 104.9, 79.5, 38.3, 36.6, 28.6. IR 3369, 2969, 2928, 1594, 1484, 1381, 789 cm^{-1} . HRMS $\text{C}_{22}\text{H}_{28}\text{N}_3\text{O}_2$ ($\text{M}+\text{H}$) $^+$ calcd. 366.2176, obsvd. 366.2182.

4.3.5. Characterization data for methyl-3-(1-(tert-butoxycarbonyl)-4-(1H-indol-3-yl)piperidin-4-yl)indolizine-1-carboxylate (Entry 3e)—Followed same procedure as the general procedure. Yield = 90% (average of two run), R_f = 0.30 w/ 66% EtOAc/Hex, colorless solid, M.P. = 197–199 $^{\circ}\text{C}$. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ = 8.62 (d, J = 1.7 Hz, 1 H), 8.17 (dt, J = 9.0 Hz, J = 1.1 Hz, 1 H), 7.84 (d, J = 7.2 Hz, 1 H), 7.54 (s, 1 H), 7.30 (d, J = 8.2 Hz, 1 H), 7.21 (d, J = 2.5 Hz, 1 H), 7.05 (ddd, J = 8.1 Hz, J = 7.1 Hz, J = 1.0 Hz, 1 H), 6.98 (d, J = 8.1 Hz, 1 H), 6.85 (ddd, J = 9.0 Hz, 6.6 Hz, J = 0.9 Hz, 1 H), 6.78 (ddd, J = 8.1 Hz, 7.1 Hz, J = 0.9 Hz, 1 H), 6.31 (td, J = 6.9 Hz, J = 1.3 Hz, 1 H), 3.95 (s, 3 H), 3.73 (bs, 2 H), 3.48 (bs, 2 H), 2.5 (m, 4 H), 1.48 (s, 9 H). $^{13}\text{C-NMR}$ $\{^1\text{H}\}$ (100 MHz, CDCl_3) δ = 165.8, 155.1, 137.1, 136.9, 129.2, 128.8, 125.4, 125.1, 122.3, 121.7, 121.6, 120.0, 119.9, 119.6, 119.3, 115.7, 111.7, 111.4, 102.5, 79.8, 51.1, 37.9, 28.6. IR 3303, 1667, 1509, 734 cm^{-1} . HRMS $\text{C}_{28}\text{H}_{32}\text{N}_3\text{O}_4$ ($\text{M}+\text{H}$) $^+$ calcd. 474.2387, obsvd. 474.2397.

4.4. Biological Evaluation

4.4.1. General Culture Procedure—MCF-10A and MCF-7 cells were cultured in monolayer in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DME/F-12) containing HEPES buffer and L-glutamine (HyClone) supplemented with 10% (v/v) fetal bovine serum (FBS, HyClone), 1% (v/v) penicillin/streptomycin (P/S), 1% (v/v) insulin-transferrin-selenium-X (ITS, Gibco), and 2.5 nM human epidermal growth factor, recombinant (EFG, BD Biosciences). Cells were maintained in a humidified incubator at 37 $^{\circ}\text{C}$ and 5% CO_2 .

4.4.2. Initial 3-point Screening of Compounds 2b-2k, 3a-3e—In a 96-well flat bottom plate, MCF-7 cells were seeded at 1,500 cells/well and MCF-10A cells were seeded at 9,000 cells/well using the 10% FBS medium described in the general culture procedure. The cells were then incubated in a humidified incubator at 37 $^{\circ}\text{C}$ and 5% CO_2 for 18 hours. From a 100 mM stock of each compound in molecular biology grade DMSO (Sigma) was made a 50 μM standard solution of each compound in DME/F-12 supplemented with 2% (v/v) FBS, 1% (v/v) P/S, 1% (v/v) ITS, and 2.5 nM EFG. From this 50 μM standard solution, serial dilutions of 10 μM and 1 μM were made. The media was aspirated from the cells in the 96-well plates after the initial incubation period and 100 μL of the appropriately diluted compound in media was added (day 0). The cells were incubated and on days 2, and 4, the media was aspirated and 100 μL of fresh compound-containing media was added. On day 5, an MTS assay was performed using CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega). The absorbance measurements were normalized to the

DMSO control and these values were plotted against the $\pm$ standard deviation of 2 wells per condition.

4.4.3. 12-Point Dose Response Curve of Compounds 2c, 2j, and 3a—In a 96-well flat bottom plate, MCF-7 cells were seeded at 1,500 cells/well and MCF-10A cells were seeded at 9,000 cells/well using the 10% FBS medium described in the general culture procedure. The cells were then incubated in a humidified incubator at 37 °C and 5% CO₂ for 18 hours. From a 100 mM stock of each compound in molecular biology grade DMSO (Sigma) was made a 100 μ M standard solution of each compound in DME/F-12 supplemented with 2% (v/v) FBS, 1% (v/v) P/S, 1% (v/v) ITS, and 2.5 nM EFG. From this 100 μ M standard solution, an automated liquid handler (EP Motion 5075, Eppendorf) was used to prepare the 12 necessary concentrations. The media was aspirated from the cells in the 96-well plates after the initial incubation period and 100 μ L of the appropriate compound in media was added (day 0). The cells were incubated and on days 2 and 4, the media was aspirated and 100 μ L of fresh compound-containing media was added. On day 5, an MTS assay was performed using CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega). The absorbance measurements were normalized to the DMSO control and these values were plotted against the $\pm$ standard deviation of the 3 wells per condition. The EC₅₀ values were calculated using GraphPad Prism (v5.0) software, nonlinear fit, log(inhibitor) vs. response – variable slope (four parameters).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Vibrindole A, **1**

Diindolylmethane, **2**

Figure 1.
Structure of Biologically Relevant Natural Products

b)

Figure 2.

a) Evaluation of Biological Activity of Newly Synthesized Bisindolylmethanes in MCF-7 Cells at 10 μM concentration b) Dose Response Curves for Compound **3a** (left) and **2j** (right)

a) Common strategy to synthesize unsymmetrical bisindolylmethanes

b) This Report

Scheme 1.
Different Strategies for Synthesis of BIMs

Table 1

Optimization of Reaction Conditions.

Entry	X	Y	Solvent	t (h)	% Conv. ^a	% 1 ^a
1	20	15	DCM	2	>99	<5
2	20	15	DCE	2	>99	<5
3	20	15	toluene	2	>99	9
4	20	15	9:1 toluene:DMA	16	>99	36
5	20	15	1:1 toluene:DMA	16	>99	71
6	20	15	DMA	16	>99	89
7 ^b	10	7	DMA	16	>99	73 ^c
8 ^b	10	10	DMA	16	>99	81 ^c

^a) The conversion and yield were measured by GC with an internal standard.^b) Concentration of the reaction mixture (with respect to 3): 0.2 M.^c) Isolated yield

Table 2

Scope of Hydroarylation of 3-vinyl-1H-indole.

^aYields are average of at least two runs at 0.3 mmol scale.^bReactions were performed at 70 °C.

Table 3

Scope of Hydroarylation of **4**. ^aYields are average of at least two runs at 0.2 mmol scale.^bReaction time = 30 h