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ANTIPROLIFERATIVE ACTIVITY OF HYBRID COMPOUNDS BETWEEN 6-METHOXY-3-(4-METHOXYPHENYL)-1*H*-INDOLE AND 4-PHENYLPIPERIDINE AGAINST HCT-116 AND HL-60 CELLS

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This paper is dedicated to Professor Dr. Lutz F. Tietze, University of Göttingen, on the celebration of his 75th birthday.

Abstract – Indole derivatives have been potential anticancer drugs. Methyl 6-methoxy-3-(4-methoxyphenyl)-1*H*-indole-2-carboxylate, in particular, was reported as a potent antiproliferative agent against MCF-7, NCI-H460, and A375-C5 tumor cells. In this study, the 3-arylindole-2-carboxylate exhibited weak activity against HCT-116 colon tumor and HL-60 promyelocytic leukemia cells. To develop the potent antiproliferative indole derivatives against HCT-116 and HL-60 cells, we synthesized 6-methoxy-3-(4-methoxyphenyl)-1*H*-indoles with various 2-substituents and assessed their activity. The 4-phenylpiperidine derivatives attenuated the tumor cells viability. Furthermore, their calculated structure resembled that of the antiproliferative loperamide derivatives.

Chemotherapy is a major cancer treatment method. However, its application is quite limited for several cancer types and stages because of the narrow therapeutic dose settings[1] and the acquired drug-resistance[2] to current chemotherapeutic agents. Several potent and safer anticancer drugs are developed by natural products and synthetic compounds.[3]

Aromatic heterocyclic derivatives, especially indole alkaloids, were reviewed to exhibit great anticancer potential (Figure 1, A).[4] Dragmacidin D (**1**) inhibited the growth of the P388 and A549 tumor cells.[5] Indole-2-carboxylic acid benzylidene-hydrazides **2** were developed through cell-based high-throughput screening assay for the induction of apoptosis.[6] Most recently, methyl 6-methoxy-3-(4-methoxyphenyl)-1*H*-indole-2-carboxylate (**3**) was reported as a significantly potent antiproliferative agent against MCF-7, NCI-H460, and A375-C5 tumor cells.[7] The indicated inhibitory concentration of methyl 3-arylindole-2-carboxylate **3** was extremely lower than the concentrations of other potent antitumor compounds,[8-12] including the tubulin polymerization inhibitors with an indole nucleus.[12] Therefore, the compound is considered as an antitumor agent.[7]

The *N*-substituted 4-arylpiperidin-4-ol unit in loperamide (**4**) was reported to play an important role in antiproliferative activity against tumor cells, and the piperidine derivative **5b** was developed as a more potent antitumor agent than loperamide (Figure 1, B).[13] In this study, 2,3,6-trisubstituted indole derivatives hybridized between the 6-methoxy-3-(4-methoxyphenyl)-1*H*-indole unit and the 4-arylpiperidine unit were synthesized, and their antiproliferative effects were assessed against HCT-116 colon tumor and HL-60 leukemia cells.

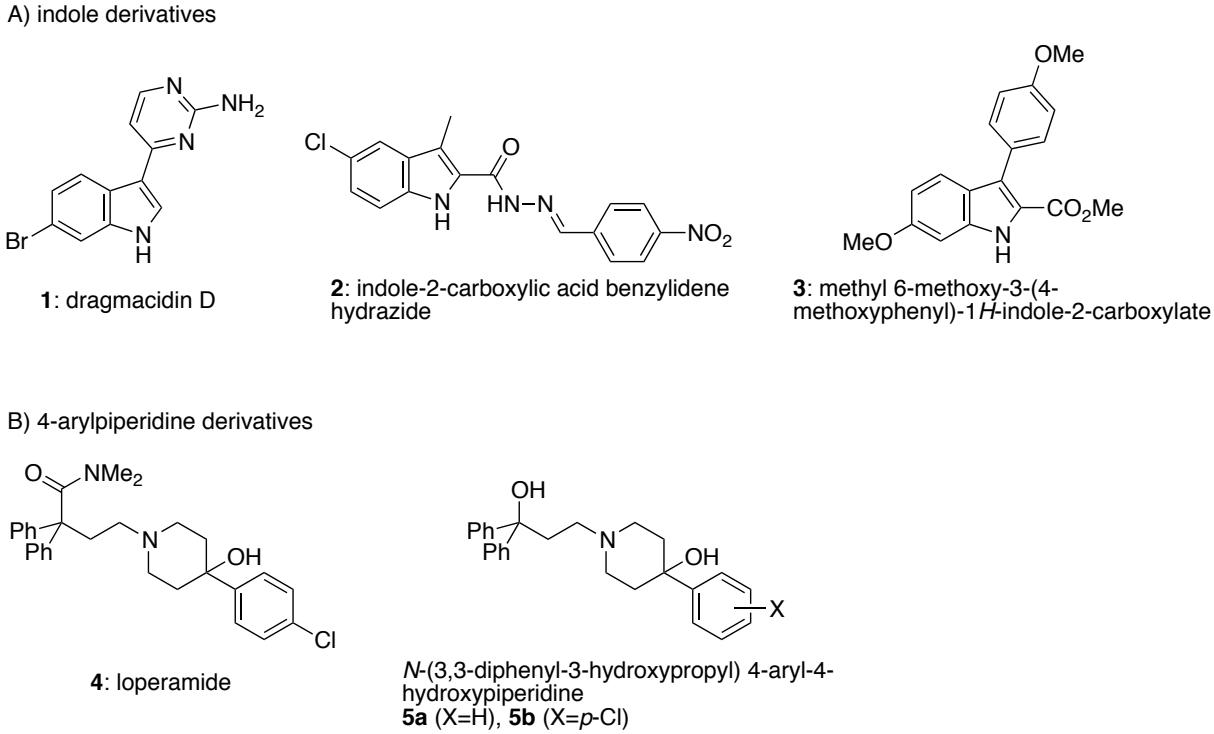
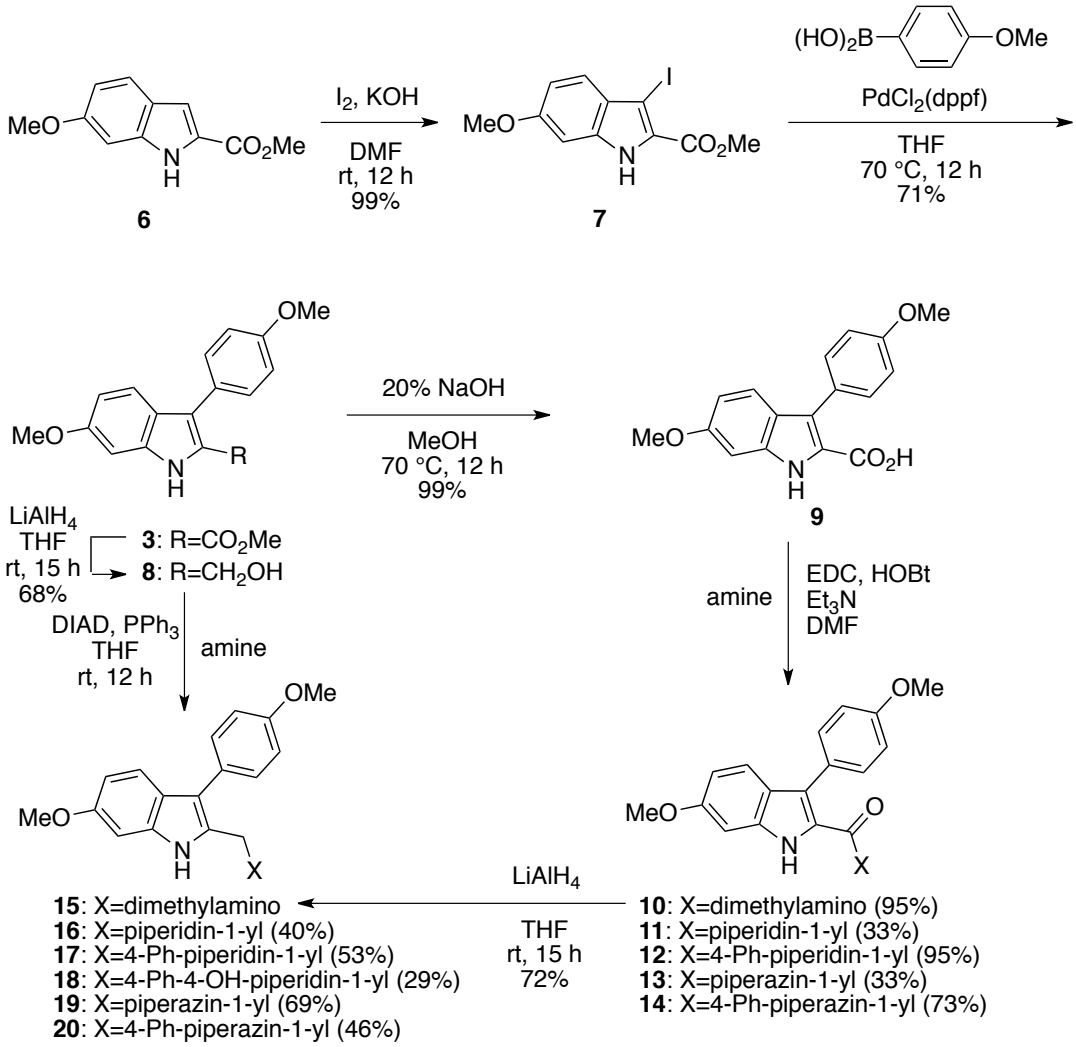


Figure 1. Structures of the antiproliferative indole derivatives (A) and the 4-arylpiperidine derivatives (B)

To synthesize indole-2-carboxamide and 2-aminomethylindole derivatives, 6-methoxyindole **6** was prepared based on Moody azide pyrolysis.[14,15] As shown in Scheme 1, the treatment of **6** with I_2 in the

presence of KOH afforded 3-iodoindole **7** (99%), which was subjected to a Suzuki-Miyaura coupling reaction[16] with 4-methoxyphenylboronic acid in the presence of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($PdCl_2$(dppf)) to give the methyl 3-arylindole-2-carboxylate **3** in 71% yield. Subsequent hydrolysis of methyl ester of **3** with aqueous 20% NaOH in MeOH afforded indole-2-carboxylic acid **9** in excellent yield. The carboxylic acid **9** was treated with amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *N*-hydroxybenzotriazole (HOBt), and triethylamine (Et_3N) to give the indole-2-carboxamides **10**-**14**. Next, we attempted to directly synthesize the 2-aminomethylindole from the indole-2-carboxamide through reduction. Treatment of *N*,*N*-dimethylindole-2-carboxamide **10** with lithium aluminum hydride ($LiAlH_4$) gave the dimethylaminoindole **15** in 72% yield, but other indole-2-carboxamides **11**-**14** could not be converted into the desired 2-aminomethylindoles **16**, **17**, **19**, and **20**. On the other hand, Masada and coworkers reported the synthesis of (piperidin-1-ylmethyl)indoles from (indol-2-yl)methanol and piperidines using Mitsunobu reaction.[17] Therefore, we investigated the synthesis of the desired 2-

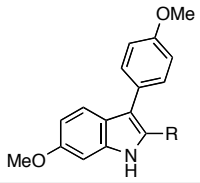


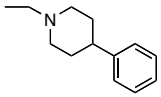
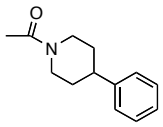
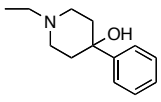
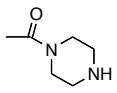
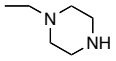
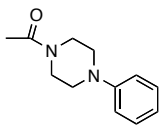
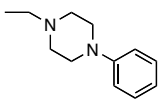
Scheme 1. Synthesis of the indole-2-carboxamide derivatives and the 2-aminomethylindole derivatives

aminomethylindoles by this reaction. The reduction of **3** with $LiAlH_4$, followed by treatment of the resulting alcohol **8** (68%) with amines in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (PPh_3) gave the 2-aminomethylindoles **16**-**20** in moderate yield.

Methyl ester **3** was reported to have a strong antiproliferative activity against MCF-7 breast cancer carcinoma cells, NCI-H460 non-small cell lung cancer cells, and A375-C5 melanoma cells with concentrations of 50% cell growth inhibition (GI_{50}) values of 0.37, 0.33, and 0.25 μM, respectively.[7] Although Abreu *et al*. described that ester **3** exhibited promising activity as an antitumoral agent,[7] our data of 50% inhibitory concentration (IC_{50}) by ester **3** exceeded 50 μM against both HCT-116 and HL-60 tumor cells (Table 1). The HCT-116 and HL-60 cells' viability values by treatment with 50 μM of ester **3** exhibited a small decrease, 71.1% and 61.0%, respectively (data not shown). These findings suggested that indole-3-carboxylate **3** exhibited weak antiproliferative activity against HCT-116 and HL-60 cells.

Table 1. Thr antiproliferative effects of indole-2-carboxamide and 2-aminomethylindole derivatives on HCT-116 and HL-60 cells



Compd	R	IC_{50} values (μM)		Compd	R	IC_{50} values (μM)	
		HCT-116 cells	HL-60 cells			HCT-116 cells	HL-60 cells
3		> 50	> 50	**15**		40.45 ± 0.66	22.66 ± 1.46
9		> 50	> 50	**16**		12.05 ± 2.13	18.05 ± 1.43
10		> 50	> 50				
11		> 50	> 50	**17**		4.89 ± 0.07	10.95 ± 0.39
12		> 50	> 50	**18**		3.93 ± 0.09	6.65 ± 0.27
13		> 50	> 50	**19**		17.05 ± 2.16	22.52 ± 1.29
14		> 50	> 50	**20**		> 50	> 50

The 4-aryl-4-hydroxypiperizine derivative **5a** was used as a reference compound (IC_{50}: HCT-116 = 46.73 ± 1.27 μM; HL-60 cells = 46.81 ± 0.36 μM). The data are expressed as mean ± sem (n = 3 or higher for at least one out of three similar experiments).

The antiproliferative activity of certain indole derivatives that inhibited tubulin polymerization were reported to depend on the types of tumor cells.[18] The IC_{50} values for the antiproliferative activities treatment with all the tested 2-carbonyl indole derivatives **3** and **9**-**14** were greater than 50 μM. However, the IC_{50} values against HCT-116 and HL-60 cells treated with the 2-methyl derivative **15** consisting of *N*,*N*-dimethylaminomethyl as a 2-substituent in indole were 40.45 and 22.66 μM, respectively. These data suggested that the aminomethyl group as a 2-substituent in indole could provide improved antiproliferative activity as compared to that of the corresponding carbonyl group. Previously, it was reported that *N*-alkylated-4-arylpiperidine moiety played an important role in the antiproliferative activity of loperamide (**4**).[13] The 4-arylpiperidine derivatives **17**, **18** exhibited significant antiproliferative activities against both HCT-116 and HL-60 cells, whereas the simple piperidine derivative **16** exhibited less potential. The tendency for the antiproliferative activity of 2-piperidinemehylindoles **16**-**18** resembled that of the loperamide derivatives.[13] Furthermore, the 4-phenylpiperidin-4-ol derivative **18** had significantly greater antiproliferative activity than that of the corresponding loperamide derivative **5a**, with IC_{50} values against HCT-116 and HL-60 cells of 46.73 and 46.81 μM, respectively. The 2-piperazinemethylindole derivatives **19**, **20** had decreased or completely lost their activity.

The three-dimensional structure of antiproliferative 2-piperidinemethylindole **18** was examined by

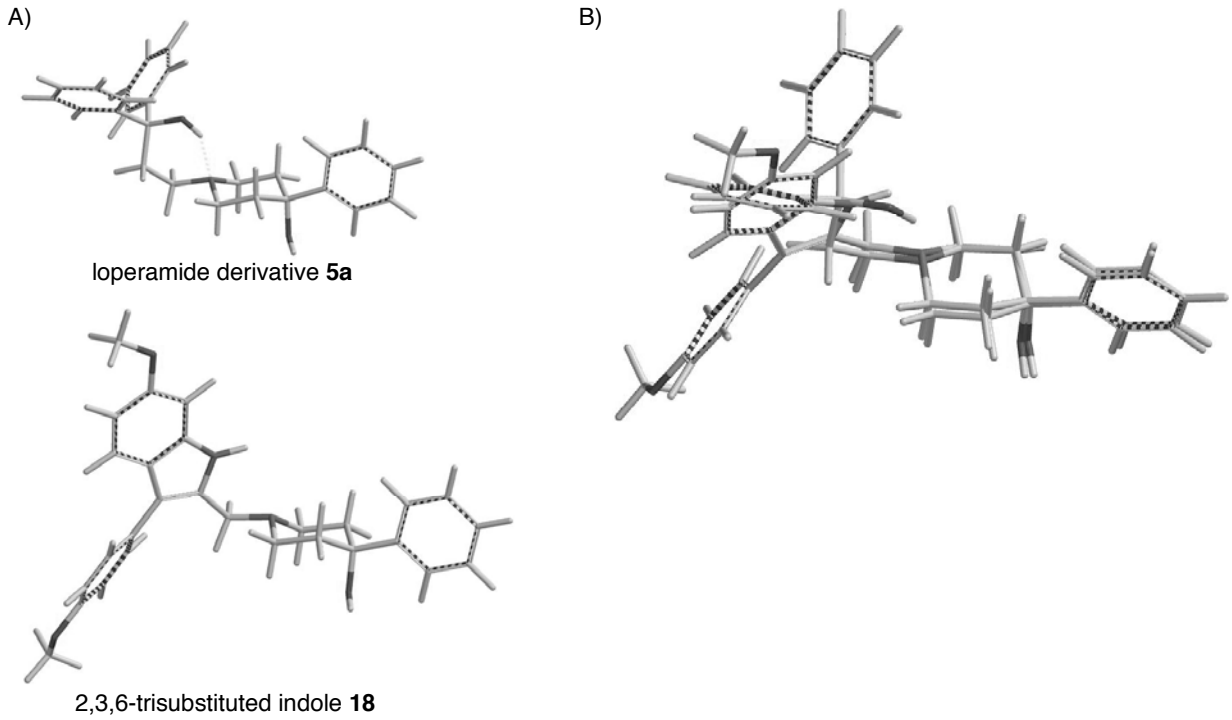


Figure 2. Molecular simulation of loperamide derivative **5a** and 2,3,6-trisubstituted indole derivative **18**. A) The calculated three-dimentional structures were drawn using the tube model, based on DFT calculations at B3LYP/6-31G(d) in Spartan 14. B) The overlying structure of indole derivative **18** on the structure of the loperamide derivative **5a**.

performing density functional theory (DFT) analysis based on the B3LYP/6-31G(d) level in Spartan 14[19,20] and was compared with the structure of loperamide derivative **5a** (Figure 2.). In the structure of the loperamide derivative **5a**, the hydroxyl group in the propanol moiety could form a hydrogen bond with the nitrogen atom in the piperidine (Figure 2, A). Aligning each compound by the 4-phenylpiperidin-4-ol moieties, the indole's phenyl ring in compound **18** was spatially closed with the phenyl ring in the loperamide derivative **5a** (Figure 2.B). The similarity analysis between the chemical functional descriptors (CFDs) of the indole's phenyl and the 4-phenylpiperidin-4-ol moieties in compound **18** and the corresponding moieties in **5a** provided at score of 0.70.

In the present study, 6-methoxy-3-(4-methoxyphenyl)-1*H*-indoles with various 2-substituents were synthesized, their antiproliferative activity was assessed, and their structure was calculated by DFT analysis. Although the reported 3-arylindole-2-carboxylate **3** exhibited weak activity against the HCT-116 and HL-60 cells, the derivatives with 4-phenylpiperidine moiety exhibited potent antiproliferative activity against both the cells. Furthermore, it was suggested that the antiproliferative activity of the synthesized 4-phenylpiperidin-4-ol derivative **18** could reflect its structural similarity with the antiproliferative loperamide derivatives.

EXPERIMENTAL

General method

All non-aqueous reactions were carried out under an atmosphere of nitrogen in dried glassware unless otherwise noted. Solvents were dried and distilled according to standard protocols. Analytical thin-layer chromatography was performed with Silica gel $60PF_{254}$ (Merck). Silica gel column chromatography was performed with Silica gel 60 (70-230 mesh, Canto Co. Lit.). All melting points were determined on Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a JEOL AL-300 at 300 MHz. Chemical shifts are reported relative to Me_4Si (δ 0.00). NMR spectra were measured with $CDCl_3$ unless otherwise noted. Multiplicity is indicated by one or more of the following: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); br (broad). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a JEOL AL-300 at 75 MHz. Chemical shifts are reported relative to $CDCl_3$ (δ 77.0) and DMSO-d_6 (δ 39.7). Infrared spectra were recorded with ATR method using a Shimadzu FTIR-8000 spectrophotometer and Technologies DuraScop. Low and High-resolution mass spectra were recorded on JEOL JMS-700 spectrometers by direct inlet system.

Methyl 3-iodo-6-methoxyindole-2-carboxylate (7). A solution of I_2 (3.6 g, 14.2 mmol) in DMF (10 mL) was added to a solution of the methyl indole-2-carboxylate **6** (2 g, 9.80 mmol) and powdered KOH (549 mg, 9.80 mmol) in DMF (10 mL) under cooling with ice-water. After stirring at rt for 12 h, the

mixture was poured into a solution of NH_3 (50 mL) and $NaHSO_3$ (500 mg, 3.50 mmol) in water (500 mL). The precipitates were filtrated, which was recrystallized from EtOAc to give the 3-iodoindole **7** (4.6 g, 99%). mp 163-164 °C (EtOAc); IR (ATR) ν: 3328, 1674 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.10 (1H, br s), 7.43 (1H, d, *J*=8.9 Hz), 6.88 (1H, dd, *J*=8.9, 2.2 Hz), 6.9 (1H, d, *J*= 2.2 Hz), 3.97 (3H, s), 3.87 (3H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ:161.3, 159.9, 137.1, 126.0, 126.0, 124.3, 113.3, 93.5, 66.6, 55.6, 51.9; MS m/z: 331 (M^+); HR-MS (EI) Calcd for $C_{11}H_{10}INO_3$: 330.9705. Found: 330.9716.

Methyl 6-methoxy-3-(4-methoxyphenyl)indole-2-carboxylate (3). An aqueous 2N Na_2CO_3 solution was added to a mixture of 3-iodoindole **7** (55 mg, 0.16 mmol), 4-methoxyphenylboronic acid (36.5 mg, 0.24 mmol) and $PdCl_2$(dppf) (16 mg, 0.02 mmol) in DMF (15 mL) at rt under N_2 atmosphere. After stirring at 70 °C for 12 h, the reaction mixture was quenched with water, and then the mixture was extracted with EtOAc (40 mL). The organic layer was washed with brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 20 g) using EtOAc-hexane (1:4, v/v) as an eluent to give 3-(4-methoxyphenyl)indole **3** (37 mg, 71%). mp 149–150 °C (EtOAc); IR (ATR) ν: 3336, 1670 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.97 (1H, br s), 747-7.51 (3H, m), 6.98 (2H, d, *J*=8.8 Hz), 6.79-6.82 (2H, m), 3.88 (3H, s), 3.88 (3H, s), 3.81 (3H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ:162.3, 159.3, 158.9, 136.8, 131.6, 125.7, 124.8, 122.7, 122.4, 121.0, 113.4, 112.3, 93.4, 55.5, 55.2, 51.6. MS *m/z*: 311 (M^+); HR-MS (EI) Calcd for $C_{18}H_{17}NO_4$: 311.1158. Found: 311.1149.

[6-Methoxy-3-(4-methoxyphenyl)indol-2-yl]methanol (8). A solution of 3-(4-methoxyphenyl)indole **3** (86 mg, 0.25 mmol) in THF (10 mL) was added dropwise to a suspension of $LiAlH_4$ (19 mg, 0. 50 mmol) in THF (10 mL) under cooling with ice-water. After stirring at rt for 15 h, the reaction mixture was quenched with water, and then the mixture was filtered through Celite pad. The filtrate was extracted with EtOAc (30 mL). The organic layer was washed with brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 30 g) using EtOAc-hexane (3:7, v/v) as an eluent to give the alcohol **8** (58 mg, 68%). mp 175-176 °C (EtOAc-MeOH); IR (ATR) ν: 3733, 3398 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.30 (1H, br s), 7.53 (1H, d, *J*=8.7 Hz), 7.38 (2H, d, *J*=8.8 Hz), 7.00 (2H, d, *J*=8.8 Hz), 6.89 (1H, d, *J*=2.2 Hz), 6.80 (1H, dd, *J*=8.7, 2.2 Hz), 4.87 (2H, d, *J*=5.7 Hz), 3.87 (6H, s), 1.74 (1H, br s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 157.4, 155.6, 136.3, 133.9, 129.8, 127.5, 120.9, 119.2, 114.1, 112.7, 109.1, 94.5, 55.2, 55.1, 55.1; MS *m/z*: 283 (M^+); HR-MS (EI) Calcd for $C_{17}H_{17}NO_3$: 283.1208. Found: 283.1222.

6-Methoxy-3-(4-methoxyphenyl)indole-2-carboxylic acid (9). A solution of 20% NaOH (8 mL) was added to a suspention of 3-(4-methoxyphenyl)indole **3** (100 mg, 0.32 mmol) in MeOH (20 mL) and heated at 70 °C for 12 h. After cooling to an ambient temperature, the mixture was acidified with a 10% HCl solution. The resulting precipitates were filtrated and washed with water. The crude compound was

recrystallized with EtOAc-MeOH to afford the indole-2-carboxylic acid **9** as a white crystal (100 mg, 99%). mp 225-227 °C (EtOAc-MeOH); IR (ATR) ν: 3733, 3398, 1650 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.5 (1H, s), 7.41 (2H, d, *J*=8.3 Hz), 7.32 (1H, d, *J*=8.8 Hz), 6.97 (2H, d, *J*=8.3 Hz), 6.90 (1H, s), 6.71 (1H, d, *J*=8.8 Hz), 3.79 (3H, s), 3.78 (3H, s); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 163.5, 158.8, 158.7, 137.7, 132.2, 126.7, 123.0, 122.7, 122.3, 122.2, 113.9, 112.4, 94.6, 55.9, 55.8. MS *m/z*: 297 (M^+); HR-MS (EI) Calcd for $C_{17}H_{15}NO_4$: 297.1001. Found: 297.0974.

***N*,*N*-Dimethyl-6-methoxy-3-(4-methoxyphenyl)indole-2-carboxamide (10).** A solution of EDC (40 mg, 0.21 mmol) in DMF (3 mL) and Et_3N (94 μL, 0.68 mmol) was added dropwise to a mixture of indole-2-carboxylic acid **9** (50 mg, 0.17 mmol)**,** dimethylamine (27 mg, 0.34 mmol) and HOBt (28 mg, 0.21 mmol) in DMF (3 mL) at -20 °C, and then the mixture was stirred at the same temperature for 30 min. After being gradually raised up to rt, the reaction mixture was stirred for 8 h. The reaction mixture was quenched with water, and then the mixture was extracted with EtOAc (10 mL). The organic layer was washed with brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 20 g) using EtOAc-hexane (1:4 v/v) as an eluent to give the indole-2-carboxamide **10** (52 mg, 95%). mp 187–188 °C (EtOAc); IR (ATR) ν: 3278, 1592 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.71 (1H, br s), 7.60 (1H, d, *J*=8.7 Hz), 7.41 (2H, d, *J*=8.9 Hz), 7.00 (2H, d, *J*=8.9 Hz), 6.87 (1H, d, *J*=2.1 Hz), 6.82 (1H, dd, *J*=8.7, 2.2 Hz), 3.87 (6H, s), 2.95 (3H, br s), 2.57 (3H, br s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 165.3, 158.6, 157.9, 136.6, 130.1, 127.3, 127.2, 125.3, 121.1, 120.8, 118.3, 114.3, 111.3, 94.0, 55.6, 55.3; MS *m/z*: 324 (M^+); HR-MS (EI) Calcd for $C_{19}H_{20}N_2O_3$: 324.1474. Found: 324.1485.

[6-Methoxy-3-(4-methoxyphenyl)indol-2-yl](piperidin-1-yl)methanone (11). The same procedure as above was carried out using indole-2-carboxylic acid **9** (100 mg, 0.34 mmol) to give the amide **11** (41 mg, 33%). IR (ATR) ν: 3262, 1624 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.88 (1H, br s), 7.40 (1H, d, *J*=8.7 Hz), 7.29 (2H, d, *J*=8.7 Hz), 6.92 (2H, d, *J*=8.7 Hz), 6.80 (1H, d, *J*=2.2 Hz), 6.82 (1H, dd, *J*=8.7, 2.2 Hz), 3.78 (3H, s), 3.77 (3H, s), 2.32-2.34 (4H, m), 1.48-1.51 (4H, m), 1.36-1.38 (2H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 157.9, 156.3, 135.9, 130.9, 130.6, 127.4, 122.3, 119.6, 115.2, 113.8, 109.3, 94.4, 55.7, 55.2, 54.6, 54.5, 26.0, 24.2; MS *m/z*: 364 (M^+); HR-MS (EI) Calcd for $C_{22}H_{24}N_2O_3$: 364.1787. Found: 364.1773.

[6-Methoxy-3-(4-methoxyphenyl)indol-2-yl](4-phenylpiperidin-1-yl)methanone (12). The same procedure as above was carried out using indole-2-carboxylic acid **9** (50 mg, 0.17 mmol) to give the amide **12** (70 mg, 95%). mp 239–240 °C (EtOAc); IR (ATR) ν: 3737, 1736 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.82 (1H, br s), 7.59 (1H, d, *J*=8.7 Hz), 7.46 (2H, d, *J*=8.9 Hz), 7.18-7.36 (3H, m), 6.99-7.05 (4H, m), 6.89 (1H, d, *J*=2.1 Hz), 6.83 (1H, dd, *J*=8.7, 2.1 Hz), 3.88 (3H, s), 3.87 (3H, s), 4.45-4.93 (1H,

m), 2.43-2.70 (3H, m), 0.60-1.95 (5H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 164.2, 159.0, 157.9, 145.3, 136.7, 130.8, 128.4, 126.8, 126.7, 126.4, 125.4, 121.1, 120.9, 117.9, 114.4, 111.4, 94.1, 55.6, 55.3, 42.5, 32.4; MS *m/z*: 440 (M^+); HR-MS (EI) Calcd for $C_{28}H_{28}N_2O_3$: 440.2030. Found: 440.2012.

[6-Methoxy-3-(4-methoxyphenyl)indol-2-yl](piperazin-1-yl)methanone (13). The same procedure as above was carried out using indole-2-carboxylic acid **9** (50 mg, 0.17 mmol) to give the amide **13** (18 mg, 33%). mp 211–212 °C (EtOAc); IR (ATR) ν: 3737, 3313, 1736 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.54 (1H, br s), 7.56 (1H, d, *J*=8.8 Hz), 7.39 (2H, d, *J*=8.7 Hz), 6.99 (2H, d, *J*=8.7 Hz), 6.87 (1H, d, *J*=1.8 Hz), 6.83 (1H, dd, *J*=8.8, 1.8 Hz), 3.88 (3H, s), 3.87 (3H, s), 3.39 (4H, br s), 2.52 (4H, br s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 164.4, 158.9, 157.9, 136.5, 130.5, 126.8, 124.9, 121.1, 120.8, 118.0, 114.4, 111.4, 94.1, 55.6, 55.3, 45.5; MS *m/z*: 364 (M^+); HR-MS (EI) Calcd for $C_{21}H_{22}N_3O_3$: 364.1661. Found: 364.1648.

[6-Methoxy-3-(4-methoxyphenyl)indol-2-yl](4-phenylpiperazin-1-yl)methanone (14). The same procedure as above was carried out using indole-2-carboxylic acid **9** (60 mg, 0.20 mmol) to give the amide **14** (65 mg, 73%). mp 205-207 °C ($CHCl_3$); IR (ATR) ν: 3297 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.20 (1H, br s), 7.58 (1H, d, *J*=8.8 Hz), 7.43 (2H, d, *J*=8.8 Hz), 7.19-7.25 (2H, m), 7.00 (2H, d, *J*=8.8 Hz), 6.89 (1H, d, *J*=2.0 Hz), 6.80-6.86 (1H, m), 6.82 (1H, dd, *J*=8.8, 2.0 Hz), 6.77 (2H, d, *J*=8.8 Hz), 3.85 (3H, s), 3.82 (3H, s), 3.54 (4H, br s), 2.78 (4H, br s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 164.2, 159.0, 158.0, 150.9, 136.8, 130.6, 129.1, 126.7, 124.7, 121.1, 120.8, 120.5, 118.3, 116.7, 114.5, 111.5, 94.0, 55.6, 55.3, 49.1; MS *m/z*: 441 (M^+); HR-MS (EI) Calcd for $C_{27}H_{27}N_3O_3$: 441.2052. Found: 441.2038.

2-(*N*,*N*-Dimethylaminomethyl)-6-methoxy-3-(4-methoxyphenyl)indole (15). A solution of alcohol **8** (16 mg, 0.32 mmol) in THF (5 mL) was added dropwise to a suspension of $LiAlH_4$ (13 mg, 0.32 mmol) in THF (5 mL) under cooling with ice-water. After stirring at rt for 15 h, the reaction mixture was quenched with water, and then the mixture was filtered through Celite pad. The filtrate was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography using EtOAc-hexane (3:7, v/v) as an eluent to give the indole **15** (37 mg, 72%). IR (ATR) ν: 3737 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.90 (1H, br s), 7.47 (1H, d, *J*=8.7 Hz), 7.36 (2H, d, *J*=8.7 Hz), 6.99 (2H, d, *J*=8.7 Hz), 6.87 (1H, d, *J*=2.2 Hz), 6.77 (1H, dd, *J*=8.7, 2.2 Hz), 3.87 (3H, s), 3.86 (3H, s), 3.68 (2H, s), 2.26 (6H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 157.9, 156.4, 136.1, 130.9, 130.6, 127.3, 122.3, 119.7, 115.3, 113.9, 109.3, 94.4, 55.7, 55.3, 55.0, 45.3. MS *m/z*: 310 (M^+). HR-MS (EI) Calcd for $C_{19}H_{22}N_2O_2$: 310.1681. Found: 310.1667.

6-Methoxy-3-(4-methoxyphenyl)-2-(piperidin-1-ylmethyl)indole (16). A solution of DIAD (1.9 M in toluene 0.16 mL, 0.30 mmol) was added to a mixture of alcohol **8** (70 mg, 0.25 mmol), PPh_3 (79 mg, 0.30 mmol) and piperazine (26 mg, 0.30 mmol) in THF (2 mL) under stirring at rt. After stirring at the same temperature for 12 h, the reaction mixture was quenched with water, and then the mixture was extracted with EtOAc. The EtOAc layer was washed with water and brine and dried over Na_2SO_4. The solvent was

removed under reduced pressure, and then the resulting residue was purified by column chromatography (silica gel, 30 g) using EtOAc-hexane (3:7, v/v) as an eluent to give 2-aminomethylindole **16** (28 mg, 40%). IR (ATR) ν: 3737 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.81 (1H, br s), 7.48 (1H, d, *J*=8.6 Hz), 7.37 (2H, d, *J*=8.8 Hz), 6.99 (2H, d, *J*=8.8 Hz), 6.85 (1H, d, *J*=2.1 Hz), 6.76 (1H, dd, *J*=8.6, 2.1 Hz), 3.84 (3H, s), 3.83 (3H, s), 3.65 (2H, s), 2.36-2.38 (4H, m), 1.52-1.59 (4H, m), 1.41-1.44 (2H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 157.9, 156.3, 135.9, 130.9, 130.6, 127.4, 122.3, 119.6, 115.2, 113.8, 109.3, 94.4, 55.7, 55.2, 54.6, 54.5, 26.0, 24.2. MS *m/z*: 350 (M^+). HR-MS (EI) Calcd for $C_{22}H_{26}N_2O_2$: 350.1994. Found: 350.1984.

6-Methoxy-3-(4-methoxyphenyl)-2-(4-phenylpiperidin-1-ylmethyl)indole (17). The same procedure as above was carried out using alcohol **8** (30 mg, 0.11 mmol) to give 2-aminomethylindole **17** (16 mg, 53%). IR (ATR) ν: 3521 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.75 (1H, br s), 7.48 (1H, d, *J*=8.6 Hz), 7.38 (2H, d, *J*=8.5 Hz), 7.20-7.33 (5H, m), 7.01 (2H, d, *J*=8.5 Hz), 6.90 (1H, d, *J*=2.1 Hz), 6.77 (1H, dd, *J*=8.6, 2.1 Hz), 3.87 (3H, s), 3.86 (3H, s), 3.75 (2H, s), 2.98-3.04 (2H, m), 2.43-2.57 (1H, m), 2.10-2.15 (2H, m), 1.73-1.85 (4H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.0, 156.4, 146.1, 136.0, 130.7, 128.4, 127.4, 126.8, 126.2, 122.4, 119.8, 115.5, 114.0, 109.4, 94.5, 60.4, 55.8, 55.3, 54.4, 54.2, 42.5, 33.4. MS *m/z*: 426 (M^+). HR-MS (EI) Calcd for $C_{28}H_{30}N_2O_2$: 426.2307. Found: 426.2316.

6-Methoxy-3-(4-methoxyphenyl)-2-(4-hydroxy-4-phenylpiperidin-1-ylmethyl)indole (18). The same procedure as above was carried out using the alcohol **8** (50 mg, 0.18 mmol) to give the 2-aminomethylindole **18** (23 mg, 29%). IR (ATR) ν: 3737, 3613 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.82 (1H, br s), 7.47-7.51 (3H, m), 7.34-7.39 (5H, m), 7.00 (2H, d, *J*=8.7 Hz), 6.91 (1H, d, *J*=2.2 Hz), 6.77 (1H, dd, *J*=8.6, 2.2 Hz), 3.87 (3H, s), 3.86 (3H, s), 3.82 (2H, s), 2.79-2.84 (2H, m), 2.50-2.59 (2H, m), 2.11-2.22 (2H, m), 1.73-1.79 (2H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 157.6, 156.1, 147.5, 135.8, 135.7, 130.3, 128.0, 126.8, 124.1, 121.8, 119.5, 119.4, 115.6, 113.6, 109.1, 94.1, 70.6, 55.4, 54.9, 53.3, 49.0, 37.7. MS *m/z*: 442 (M^+). HR-MS (EI) Calcd for $C_{28}H_{30}N_2O_3$: 442.2256. Found: 442.2244.

6-Methoxy-3-(4-methoxyphenyl)-2-(piperazin-1-ylmethyl)indole (19). The same procedure as above was carried out using the alcohol **8** (50 mg, 0.18 mmol) to give the 2-aminomethylindole **19** (44 mg, 69%). IR (ATR) ν: 3733, 3648 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ : 8.64 (1H, br s), 7.48 (1H, d, *J*=8.8 Hz), 7.36 (2H, d, *J*=8.8 Hz), 6.99 (2H, d, *J*=8.8 Hz), 6.89 (1H, d, *J*=2.2 Hz), 6.77 (1H, dd, *J*=8.8, 2.2 Hz), 3.87 (3H, s), 3.86 (3H, s), 3.69 (2H, s), 2.85-2.92 (4H, m), 2.37-2.48 (4H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.0, 156.3, 135.9, 130.6, 130.4, 127.3, 122.4, 119.7, 114.0, 113.9, 109.4, 94.4, 55.7, 55.3, 54.7, 54.5, 46.2; MS *m/z*: 351 (M^+); HR-MS (EI) Calcd for $C_{21}H_{25}N_3O_2$: 351.1947. Found: 351.1963.

6-Methoxy-3-(4-methoxyphenyl)-2-(4-phenylpiperazin-1-ylmethyl)indole (20). The same procedure as above was carried out using the alcohol **8** (50 mg, 0.18 mmol) to give the 2-amonomethylindole **20** (23 mg, 46%). IR (ATR) : 3421 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.59 (1H, br s), 7.42 (1H, d, *J*=8.6 Hz),

7.30 (2H, d, *J*=8.4 Hz), 7.17 (2H, t, *J*=8.4 Hz), 6.91 (2H, d, *J*=8.4 Hz), 6.74-6.83 (4H, m), 6.70 (1H, dd, *J*=8.6, 2.2 Hz), 3.77 (3H, s), 3.76 (3H, s), 3.67 (2H, s), 3.15-3.17 (4H, m), 2.49-2.58 (4H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.0, 156.4, 151.1, 136.0, 130.6, 130.0, 129.1, 127.1, 122.3, 119.8, 119.7, 116.0, 115.7, 113.9, 109.5, 94.4, 55.7, 55.3, 53.8, 53.1, 49.1. MS *m/z*: 427 (M^+). HR-MS (EI) Calcd for $C_{27}H_{29}N_3O_2$: 427.2260. Found: 427.2251.

Cell lines and cell cultures

For testing the antiproliferative cell activities, two types of cancer cell lines were used in this study: HCT-116 cells (human colon cancer) and HL-60 cells (human promyelocytic leukemia), which were purchased from the American Type Culture Collection (VA, USA). The HCT-116 and HL-60 cells were maintained in a McCOY's 5A medium with L-glutamine and 10% heat inactivated (55 °C for 30 min) fetal bovine serum (FBS) and in a RPMI-1640 medium with L-glutamine and 10% heat-inactivated FBS, respectively, at 37 °C in an atmosphere of 5% CO_2.

Cell viability assays

The HCT-116 cells' viability assay was conducted using the MTT method based on the procedure described by Mosmann.[21] Briefly, cells were placed in 96-well flat bottomed tissue culture plates with 3.0 x 10^3 cells per well in a 100 μL culture medium. This was followed by incubation at 37 °C in an atmosphere of 5% CO_2 for 24 h to allow the cells to attach onto the wells. The cells were treated with the indicated concentrations of test agents in a culture medium without FBS. Following a further 48 h incubation, 10 μL of MTT (5 mg/mL in phosphate-buffered saline) were added per well, and the plate was incubated for 4 h to allow the MTT to metabolize by cellular mitochondrial dehydrogenases. The excess MTT was aspirated and the produced formazan crystals were dissolved by adding 100 μL dimethyl sulfoxide. The absorbance of the purple formazan was read at 570 nm using a microplate reader. The results following the test agents' exposure were calculated as a percentage relative to untreated controls.

The HL-60 cells' viability assay was conducted using the WST-1 method based on the procedure described by Ishiyama.[22] The cells were seeded in 96-well flat bottomed tissue culture plates with 2.0 x 10^4 cells per well in a 100 μL of the FBS containing culture medium with the indicated concentrations of test agents. Following a further 48 h incubation, 10 μL of a mixture of WST-1/1-methoxy phenazine methosulfate solution containing 5 mM WST-1 and 0.2 mM 1-methoxy PMS in 40 mM HEPES-NaOH (pH 7.4) were added per well, and the plate was incubated for 3 h to allow the WST-1 to metabolize by cellular mitochondrial dehydrogenases. The absorbance of the yellow formazan was read at 415 nm using a microplate reader. The results following the test agents' exposure were calculated as a percentage relative to untreated controls.

Statistical calculation

The concentration-cells' viability curves were fitted to a four-parametric logistic equation using a nonlinear curve-fitting program that derived the IC_{50} values (Kaleida-graph; Synergy Software, Reading, PA). Wherever appropriate, the results were expressed as means ± sem, with n = 3 or higher in at least one out of three similar experiments.

Molecular simulation

Loperamide and the indole derivative could be accurately described by density functional theory (DFT) calculations at B3LYP/6-31G(d) in Spartan 14.[19,20] The molecular similarity analysis was conducted by comparing the chemical functional descriptors (CFDs), i.e., the common chemical features based on the hydrogen bond donor/acceptor and hydrophilic/lipophilic groups. The similarity score was defined as $[(1-R^2)/N]$-penalty, where R^2 was the root mean square distance between the molecule centers and N is the number of similarity centers. The penalties were assigned for alignments that led to unfavorable steric interactions or to incorrect orientation of the hydrogen bond donor/acceptor CFDs. The score "1" corresponded to a perfect score.[23]

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