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ORIGINAL PAPER

Synthesis and biological evaluation of 1,3,4-oxadiazole-linked bisindole derivatives as anticancer agents

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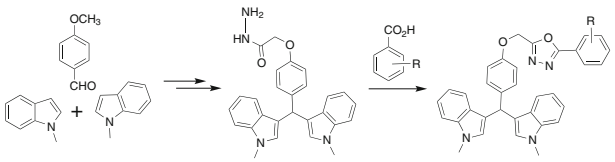
Abstract A series of ten 1,3,4-oxadiazole-linked bisindole derivatives have been synthesized. All compounds were evaluated for anticancer activity against four human cancer cell lines (MCF-7, KB, Colo-205, and A-549). Most of these new compounds exhibited significant anticancer activity as compared to etoposide. Compounds' GI_{50} values range from <0.1 to 3.9 μM, while the positive control etoposide has a GI_{50} in the range of 0.13–3.08 μM in the cell lines employed. Among them, four compounds showed a higher activity than etoposide.

Graphical abstract



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Keywords Nortopsentins A-C · Hamacanthin B · Zibotentan (ZD4054) · Bisindoles · 1,3,4-Oxadiazoles · Cytotoxicity

Introduction

Cancer is the second leading cause of mortality in developed countries. Currently, chemotherapy is the most important treatment for cancer and the ultimate goal is to destroy the cancer cells without any harmful effect on the normal cells. Within the past decade, advances in our understanding of the cell cycle have presented new targets that may allow for the development of more selective chemotherapeutic agents—agents that target only cancer cells. Cancer chemotherapy has achieved significant success through the discovery of various new drugs. Despite of this progress, the discovery of most potent anticancer agents is a challenging issue in cancer chemotherapy for the future generations.

The indole fragment is featured widely in a variety of therapeutic agents with different pharmacological activities, such as anticancer [1], antioxidant [2], antirheumatoidal [3], and anti-HIV [4, 5]. Recently, bisindole alkaloids consisting of two indole moieties bound to a spacer through their third position were found to exhibit a wide spectrum of potent biological activities including antifungal, antitumor, antiviral, antimicrobial, anti-inflammatory, and cytotoxic activities [6–11]. The bisindole alkaloids can bear either an acyclic chain or six-membered carbocyclic or five-membered heterocyclic ring or six-membered heterocyclic ring between two indole rings. The 2,4-bis(3′-indolyl)imidazole (Nortopsentins A-C, **1**) [12] exhibit in vitro cytotoxicity against P388 cells, and 3,5-bis(3′-indolyl)pyrazinone (Hamacanthin B, **2**) [13, 14] (Fig. 1), exhibits cytotoxic

activities against a wide range of human tumor cell lines. The bisindole marine drugs, such as 3,5-bis(3′-indolyl)-pyrazoles [15], 2,5-bis(3′-indolyl)furans [10], 3,5-bis(3′-indolyl)isoxazoles [10], 2,5-bis(3′-indolyl)pyrroles [16], 2,5-bis(3′-indolyl)pyrazine [17], and 2,4-bis(3′-indolyl)thiazole [18, 19] demonstrate strong inhibitory effects against a variety of tumor cell lines, including leukemia, non-small cell lung cancer, ovarian cancer, colon cancer, renal cancer, and breast cancer. Among the indole derivatives, bis(indolyl)methanes act as drug targets in cancer chemotherapy that include growth inhibition, apoptosis, and antiangiogenic activities due to overlapping and unique responses in multiple cancer cell lines and tumors [20].

On the other hand, 1,3,4-oxadiazoles have recently drawn much attention due to its diverse pharmacological activities. Among heterocyclic compounds, 1,3,4-oxadiazole has become an important construction motif for the development of new drugs. Its derivatives are reported to show a broad range of biological activities, such as anticancer [21], antibacterial [22], antifungal [23], and antiviral [24]. One of the most important drug Zibotentan (ZD4054) (**3**) (Fig. 1) is a 1,3,4-oxadiazole moiety containing anticancer agent [25].

In continuation of these efforts, in the present study, we report the synthesis of a series of 1,3,4-oxadiazole-linked bisindole derivatives **12a–12j**. All these derivatives were evaluated for their anticancer activity against a panel of four human cancer cell lines and five promising compounds were shown to be more potent than positive control of etoposide.

Results and discussion

Chemistry

The synthesis of the new 1,3,4-oxadiazole-linked bisindole derivatives **12a–12j** is shown in Scheme 1. The intermediate two equivalents of *N*-methylindole (**4**) react with 4-methoxybenzaldehyde (**5**) in presence of the Lewis acid $FeCl_3$ in acetonitrile at room temperature for 2 h to afford **6**. The latter undergoes demethylation with BBr_3 at −78 °C for 1 h to afford **7**. The compound **7** reacts with ethyl bromoacetate in acetone in presence of K_2CO_3 and refluxing for 6 h to afford the ether **8**, which is reacted with hydrazine hydrate in ethanol by refluxing for 6 h to afford the acidhydrazide **9** in good yield. The acidhydrazide was coupled with different substituted aromatic acids **11a–11j** in $POCl_3$ at 170 °C for 6 h to afford the 1,3,4-oxadiazole-linked bisindoles **12a–12j** in good yields.

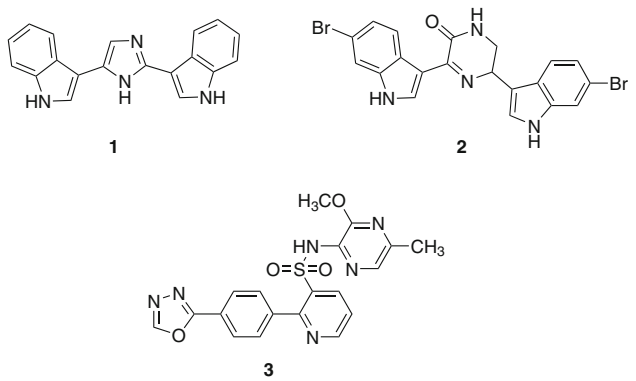


Fig. 1 Structures of Nortopsentin (**1**), Hemacanthin (**2**), and Zibotentan (ZD4054) (**3**)

Biological evaluation–in vitro cytotoxicity

The compounds **12a–12j** were evaluated for their anticancer activity in selected human cancer cell lines, that is breast (MCF-7), oral (KB), colon (Colo-205), and lung (A-549) origin by employing the sulforhodamine B (SRB) assay method [26]. The results are summarized in Table 1 and compared to the standard drug etoposide. Most of these new compounds exhibited significant anticancer activity compared to etoposide. GI_{50} values of compounds **12a–12j** range from <0.1 to 3.9 μM, while the positive control etoposide shows a GI_{50} range of 0.13–3.08 μM in the cell lines employed. Among them, the compounds **12b**, **12c**, **12d**, **12g**, and **12h** showed a higher activity than etoposide.

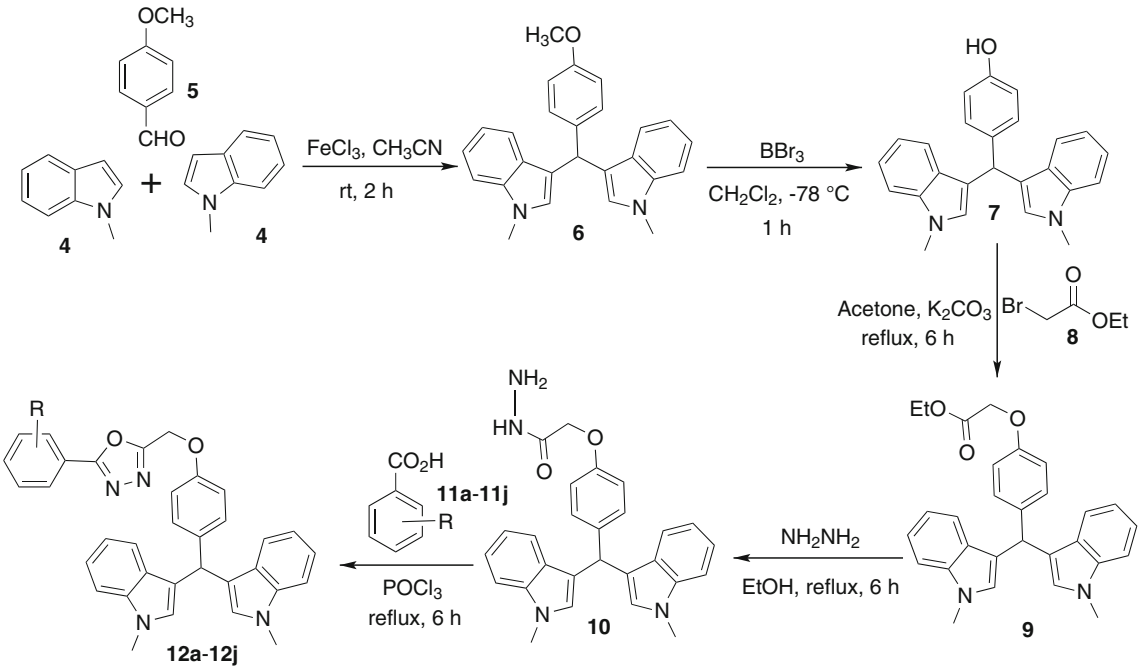
Conclusion

In conclusion, new 1,3,4-oxadiazole-linked bisindole derivatives **12a–12j** were synthesized. All the ten derivatives were evaluated for anticancer activity against four human cancer cell lines (MCF-7, KB, Colo-205, and A-549). Most of these new compounds exhibited significant anticancer activity as compared to the standard drug etoposide. Among them, the compounds **12b**, **12c**, **12d**, **12g**, and **12h** showed a higher activity than etoposide.

Experimental

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. Proton and ^{13}C NMR spectra were recorded on a Gemini Varian-VXR-unity (400 MHz) instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. Mass

Scheme 1



a, R = H; **b**, R = 3,4,5-trimethoxy; **c**, R = 4-methoxy; **d**, R = 3-methoxy; **e**, R = 4-nitro; **f**, R = 3-nitro; **g**, R = 4-hydroxy; **h**, R = 4-fluoro; **i**, R = 4-chloro; **j**, R = 4-bromo

Table 1 Cytotoxic activity (GI_{50}/μM) of compounds **12a**–**12j**

Compound	Breast MCF-7	Oral KB	Colon Colo-205	Lung A-549
12a	1.9	2.7	3.8	0.1
12b	<0.1	<0.1	1.3	<0.1
12c	0.12	–	0.13	<0.1
12d	0.14	<0.1	<0.1	<0.1
12e	–	3.9	2.9	2.1
12f	4.5	2.3	–	–
12g	<0.1	0.11	–	<0.1
12h	<0.1	0.18	0.15	<0.1
12i	3.6	–	–	2.9
12j	–	2.4	2.3	–
Etoposide	2.11	0.31	0.13	3.08

ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electrothermal melting point apparatus.

3-[(4-Methoxyphenyl)(1-methyl-1H-3-indolyl)methyl]-1-methyl-1H-indole (**6**, $C_{26}H_{24}N_2O$)

A mixture of 10 g *N*-methylindole (**4**, 76.2 mmol, 1.0 eq), 4.6 cm^3 4-methoxybenzaldehyde (**5**, 38.11 mmol, 0.5 eq), and $FeCl_3$ (5 mol %) in 40 cm^3 CH_3CN was stirred at room temperature for 2 h. After completion of the reaction as monitored by TLC the solvent was removed under vacuum, quenched with a saturated solution of $NaHCO_3$, and the products were extracted into ethyl acetate (3 × 30 cm^3). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuum. Purification by column chromatography using ethyl acetate/*n*-hexane (15:85) afforded 15.6 g pure

compound **6** (56 % yield). Yellow solid; m.p.: 148–150 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.68$ (s, 6H), 3.75 (s, 3H), 5.47 (s, 1H), 6.68 (s, 2H), 6.79 (d, 2H, $J = 8.21$ Hz), 6.98 (d, 2H, $J = 8.21$ Hz), 7.29 (d, 2H, $J = 8.03$ Hz), 7.35 (t, 2H), 7.56 (t, 2H), 7.89 (d, 2H, $J = 8.10$ Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 153.8$, 138.7, 135.9, 133.3, 130.1, 124.7, 120.8, 119.1, 109.3, 97.3, 56.4, 48.3, 32.3 ppm; MS (ESI): $m/z = 381$ ($[M + H]^+$).

4-[Bis(1-methyl-1H-3-indolyl)methyl]phenol (**7**, $C_{25}H_{22}N_2O$)

BBr_3 solution (12.4 cm^3, 131.5 mmol, 5.0 eq) was added drop wise over a period of 30 min to a vigorously stirred solution of 10 g 3-[(4-methoxyphenyl)(1-methyl-1*H*-3-indolyl)methyl]-1-methyl-1*H*-indole (**6**, 26.3 mmol, 1.0 eq) in anhydrous dichloromethane under nitrogen at −78 °C (dry ice/acetone bath). After the mixture was stirred for an additional 30 min, excess reagent was decomposed by careful addition of 10 cm^3 methanol followed by 15 cm^3 5 % HCl. The resulting mixture was allowed to warm to 0 °C and the organic layer was removed. The aqueous layer was extracted with 500 cm^3 dichloromethane, the organic layers combined, washed with 100 cm^3 brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to afford the crude compound **7**, which on column chromatography with ethyl acetate/*n*-hexane (2:8) afforded 8.4 g pure compound **7** (86 % yield). Yellow solid; m.p.: 156–158 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.71$ (s, 6H), 5.73 (s, 1H), 6.53 (s, 2H), 6.78 (d, 2H, $J = 8.34$ Hz), 6.99 (d, 2H, $J = 8.34$ Hz), 7.30 (d, 2H, $J = 8.01$ Hz), 7.38 (t, 2H), 7.67 (t, 2H), 7.87 (d, 2H, $J = 10.2$ Hz), 10.20 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 151.8$, 138.5, 135.7, 133.7, 129.8, 124.6, 120.6, 119.2, 118.9, 114.8, 107.9, 97.1, 47.9, 32.2 ppm; MS (ESI): $m/z = 367$ ($[M + H]^+$).

Ethyl 2-[4-[bis(1-methyl-1H-3-indolyl)methyl]phenoxy]-acetate (**9**, $C_{29}H_{28}N_2O_3$)

4-[Bis(1-methyl-1*H*-indol-3-yl)methyl]phenol (**7**, 8 g, 21.8 mmol, 1.0 eq) was dissolved in 10 cm^3 of dried acetone, followed by addition of 24 cm^3 ethyl bromoacetate (**8**, 21.8 mmol, 1.0 eq) and 9 g K_2CO_3 (65.4 mmol, 3.0 eq). The reaction mixture was heated under reflux for 4 h. After completion of the reaction, K_2CO_3 was removed by filtration and the solvent was evaporated under vacuum to afford crude product. The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (1:9) to afford 9.2 g pure compound **9** (94 %). Yellow solid; m.p.: 162–164 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.69$ (t, 3H), 3.62 (s, 6H), 3.99 (q, 2H), 4.98 (s, 2H), 5.70 (s, 1H), 6.43 (s, 2H), 6.67 (d, 2H, $J = 8.00$ Hz), 6.89 (d, 2H, $J = 8.00$ Hz), 7.12 (d, 2H, $J = 8.45$ Hz), 7.29 (t, 2H), 7.45 (t, 2H), 7.86 (d, 2H, $J = 8.11$ Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 171.1$, 153.8, 139.1, 137.3, 134.1, 130.1, 124.8, 122.2, 120.1, 119.8, 114.9, 108.1, 97.3, 66.6, 62.5, 48.0, 31.9, 14.2 ppm; MS (ESI): $m/z = 453$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]-phenoxy]ethanohydrazide (**10**, $C_{27}H_{26}N_4O_2$)

A mixture of 9 g of ethyl 2-[4-[bis(1-methyl-1*H*-indol-3-yl)methyl]phenoxy]acetate (**9**, 19.9 mmol, 1.0 eq) and 39 cm^3 hydrazine hydrate (79.6 mmol, 4.0 eq) in ethanol was refluxed for 3 h. The crude product was obtained after distilling off the excess ethanol. Cooling, filtering, then washing with a little cold water yielded product **10**, which was employed in the next step without further purification. Yield 8 g (92 %); yellow solid; m.p.: 170–172 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.66$ (s, 6H), 4.98 (s, 2H), 5.56 (s, 1H), 6.43 (s, 2H), 6.68 (d, 2H, $J = 8.34$ Hz), 6.87 (d, 2H, $J = 8.34$ Hz), 7.18 (d, 2H, $J = 8.13$ Hz), 7.26 (t, 2H), 7.31 (t, 2H), 7.76 (d, 2H, $J = 9.12$ Hz), 8.01 (t, 3H), 8.34 (q, 2H) ppm; MS (ESI): $m/z = 439$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-5-phenyl-1,3,4-oxadiazole (**12a**, $C_{34}H_{28}N_4O_2$)

2-[4-[Bis(1-methyl-1*H*-indol-3-yl)methyl]phenoxy]aceto-hydrazide (**10**, 500 mg 1.4 mmol, 1.0 eq) was dissolved in 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq) and 128 mg benzoic acid (**11a**, 1.4 mmol, 1.0 eq) was added. The reaction mixture was refluxed at 140 °C for 12 h. After completion of reaction, it was neutralized with aq. $NaHCO_3$ and then extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and evaporated. The crude compound was purified by column chromatography with ethyl acetate/*n*-hexane (4:6) to afford 490 mg pure compound **12a** (82 % yield). Yellow solid; m.p.: 199–201 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.64$ (s, 6H), 5.45 (s, 2H), 5.51 (s, 1H), 6.44 (s, 2H), 6.69 (d, 2H, $J = 8.90$ Hz), 6.73 (d, 2H, $J = 8.90$ Hz), 6.89 (d, 2H, $J = 8.01$ Hz), 7.10 (t, 2H), 7.23 (t, 1H), 7.28 (t, 2H), 7.34 (t, 2H), 7.44 (d, 2H, $J = 9.01$ Hz), 7.51 (t, 2H), 7.59 (d, 2H, $J = 8.56$ Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 34.6$, 41.5, 67.1, 108.6, 118.7, 118.9, 119.7, 121.8, 124.3, 125.7, 126.2, 126.6, 126.9, 128.9, 129.7, 130.2, 139.9, 146.1, 154.8, 159.5, 161.8 ppm; MS (ESI): $m/z = 525$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**12b**, $C_{37}H_{34}N_4O_5$)

This compound **12b** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 297 mg of 3,4,5-trimethoxybenzoic acid (**11b**, 1.4 mmol, 1.0 eq), and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (1:1) to afford 610 mg pure compound **12b**

(87 % yield). Yellow solid; m.p.: 215–217 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.67$ (s, 6H), 3.89 (s, 6H), 3.93 (s, 3H), 5.29 (s, 2H), 5.46 (s, 1H), 6.34 (s, 2H), 6.56 (s, 2H), 6. 70 (d, 2H, $J = 9.02$ Hz), 6.87 (d, 2H, $J = 9.02$ Hz), 7.17 (d, 2H, $J = 8.06$ Hz), 7.21 (t, 2H), 7.28 (t, 2H), 7.56 (d, 2H, $J = 8.34$ Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 33.6$, 41.4, 56.8, 59.9, 67.1, 105.6, 108.8, 118.5, 118.9, 119.8, 121.3, 124.5, 126.4, 126.8, 127.3, 141.4, 141.6, 144.7, 154.7, 154.9, 157.9, 162.1 ppm; MS (ESI): $m/z = 615$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-5-(4-methoxyphenyl)-1,3,4-oxadiazole
(**12c**, $C_{35}H_{30}N_4O_3$)
This compound **12c** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 213 mg of 4-methoxybenzoic acid (**11c**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (3:7) to afford 560 mg pure compound **12c** (89 % yield). Yellow solid; m.p.: 178–180 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.66$ (s, 6H), 3.89 (s, 3H), 5.31 (s, 2H), 5.43 (s, 1H), 6.29 (s, 2H), 6.56 (d, 2H, $J = 8.23$ Hz), 6.64 (d, 2H, $J = 8.23$ Hz), 6.71 (d, 2H, $J = 9.23$ Hz), 6.79 (d, 2H, $J = 8.12$ Hz), 6.84 (t, 2H), 6.90 (t, 2H), 6.99 (d, 2H, $J = 8.03$ Hz), 7.45 (d, 2H, $J = 9.23$ Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 32.8$, 41.3, 56.4, 67.1, 106.9, 116.4, 116.9, 118.3, 118.8, 119.3, 120.5, 123.8, 125.4, 125.7, 125.8, 141.2, 146.2, 155.4, 159.4, 162.6, 163.4 ppm; MS (ESI): $m/z = 555$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-5-(3-methoxyphenyl)-1,3,4-oxadiazole
(**12d**, $C_{35}H_{30}N_4O_3$)
This compound **12d** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 213 mg of 3-methoxybenzoic acid (**11d**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (2:8) to afford 511 mg pure compound **12d** (81 % yield). Yellow solid; m.p.: 189–191 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.70$ (s, 6H), 3.78 (s, 3H), 5.28 (s, 2H), 5.35 (s, 1H), 6.29 (s, 2H), 6.65 (d, 2H, $J = 8.10$ Hz), 6.70 (d, 1H, $J = 8.23$ Hz), 6.78 (s, 1H), 6.99 (d, 2H, $J = 8.10$ Hz), 7.09 (d, 1H, $J = 9.01$ Hz), 7.25 (d, 2H, $J = 8.23$ Hz), 7.27 (t, 2H), 7.30 (t, 2H), 7.35 (d, 2H, $J = 8.23$ Hz), 7.40 (t, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 32.7$, 39.9, 56.4, 66.5, 107.6, 107.9, 117.9, 118.7, 118.9, 119.4, 119.9, 123.8, 125.7, 125.8, 125.9, 126.2, 130.9, 131.2, 140.8, 146.0, 156.3, 159.3, 161.7, 162.7 ppm; MS (ESI): $m/z = 555$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-5-(4-nitrophenyl)-1,3,4-oxadiazole (**12e**, $C_{34}H_{27}N_5O_4$)
This compound **12e** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 234 mg of 4-nitrobenzoic acid (**11e**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (3:7) to afford 521 mg pure compound **12e** (80 % yield). Yellow solid; m.p.: 220–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.75$ (s, 6H), 5.30 (s, 2H), 5.62 (s, 1H), 6.29 (s, 2H), 6.72 (d, 2H, $J = 8.03$ Hz), 6.95 (d, 2H, $J = 8.03$ Hz), 7.23 (d, 2H, $J = 8.23$ Hz), 7.30 (t, 2H), 7.46 (t, 2H), 7.78 (d, 2H, $J = 9.23$ Hz), 7.89 (d, 2H, $J = 8.10$ Hz), 8.23 (d, 2H, $J = 8.23$ Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 32.6$, 41.2, 67.3, 108.6, 117.9, 118.9, 119.8, 120.3, 124.4, 124.8, 125.7, 125.3, 125.8, 126.0, 126.8, 134.8, 139.8, 144.9, 148.5, 156.7, 158.7, 163.4 ppm; MS (ESI): $m/z = 570$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-5-(3-nitrophenyl)-1,3,4-oxadiazole (**12f**, $C_{34}H_{27}N_5O_4$)
This compound **12f** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 227 mg of 3-nitrobenzoic acid (**11f**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (3:7) to afford 534 mg pure compound **12f** (82 % yield). Yellow solid; m.p.: 227–229 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.63$ (s, 6H), 5.36 (s, 2H), 5.39 (s, 1H), 6.29 (s, 2H), 6.38 (d, 2H, $J = 8.05$ Hz), 6.90 (d, 2H, $J = 8.22$ Hz), 7.23 (d, 2H, $J = 8.02$ Hz), 7.29 (t, 2H), 7.45 (t, 2H), 7.49 (d, 2H, $J = 8.44$ Hz), 7.61 (t, 1H), 8.00 (d, 1H, $J = 9.01$ Hz), 8.20 (d, 1H, $J = 10.35$ Hz), 8.49 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 33.4$, 41.2, 67.3, 106.9, 117.8, 119.6, 119.9, 120.8, 121.9, 124.4, 124.9, 125.4, 125.6, 125.9, 130.9, 131.9, 141.0, 144.8, 148.9, 155.8, 159.3, 163.9 ppm; MS (ESI): $m/z = 570$ ($[M + H]^+$).

4-[5-[4-[Bis(1-methyl-1H-3-indolyl)methyl]-phenoxymethyl]-1,3,4-oxadiazol-2-yl]phenol
(**12g**, $C_{34}H_{28}N_4O_3$)
This compound **12g** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 193 mg of 4-hydroxybenzoic acid (**11g**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (4:6) to afford 546 mg pure compound **12g** (89 % yield). Yellow solid; m.p.: 234–236 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.69$ (s, 6H), 5.27 (s, 2H), 5.31 (s, 1H), 5.38 (s, 2H), 6.72 (d, 2H, $J = 8.90$ Hz), 6.89 (d, 2H, $J = 8.04$ Hz), 6.99 (d, 2H, $J = 8.90$ Hz), 7.24 (d,

2H, $J = 8.34$ Hz), 7.29 (t, 2H), 7.34 (t, 2H), 7.76 (d, 2H, $J = 8.45$ Hz), 7.89 (d, 2H, $J = 8.90$ Hz), 10.2 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 32.8$, 41.6, 67.1, 106.8, 114.6, 117.9, 118.7, 119.3, 119.7, 121.2, 122.5, 124.4, 124.9, 125.6, 125.9, 141.0, 145.6, 156.3, 157.9, 161.3, 162.7 ppm; MS (ESI): $m/z = 541$ ($[M + H]^+$).

2-[4-[Bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-5-(4-fluorophenyl)-1,3,4-oxadiazole
(**12h**, $C_{34}H_{27}FN_4O_2$)
This compound **12h** was prepared following the method described for the preparation of the compound **12a**, employing 50 mg of **10** (1.4 mmol, 1.0 eq) with 196 mg of 4-fluorobenzoic acid (**11h**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (15:85) to afford 564 mg pure compound **12h** (91 % yield). Yellow solid; m.p.: 195–197 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.66$ (s, 6H), 5.23 (s, 2H), 5.33 (s, 1H), 5.36 (s, 2H), 6.68 (d, 2H, $J = 8.01$ Hz), 6.84 (d, 2H, $J = 8.08$ Hz), 6.94 (d, 2H, $J = 8.01$ Hz), 7.19 (d, 2H, $J = 8.34$ Hz), 7.26 (t, 2H), 7.30 (t, 2H), 7.73 (d, 2H, $J = 8.34$ Hz), 7.89 (d, 2H, $J = 8.01$ Hz), 10.34 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 32.6$, 39.9, 67.5, 106.9, 116.9, 117.9, 119.8, 119.9, 121.0, 124.6, 124.9, 125.4, 125.8, 125.9, 126.8, 141.0, 145.8, 154.7, 157.9, 162.8, 163.6 ppm; MS (ESI): $m/z = 543$ ($[M + H]^+$).

5-(4-Chlorophenyl)-2-[4-[bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-1,3,4-oxadiazole
(**12i**, $C_{34}H_{27}ClN_4O_2$)
This compound **12i** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 219 mg of 4-chlorobenzoic acid (**11i**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (2:8) to afford 591 mg pure compound **12i** (92 % yield). Yellow solid; m.p.: 210–212 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.60$ (s, 6H), 5.21 (s, 2H), 5.29 (s, 1H), 5.33 (s, 2H), 6.69 (d, 2H, $J = 8.00$ Hz), 6.73 (d, 2H, $J = 8.04$ Hz), 6.79 (d, 2H, $J = 8.92$ Hz), 7.21 (d, 2H, $J = 8.04$ Hz), 7.30 (t, 2H), 7.34 (t, 2H), 7.71 (d, 2H, $J = 8.42$ Hz), 7.90 (d, 2H, $J = 8.90$ Hz), 10.78 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 32.8$, 41.0, 65.9, 106.8, 118.6, 118.9, 119.8, 120.7, 124.1, 124.9, 125.8, 125.9, 128.3, 128.6, 129.9, 136.8, 139.9, 145.7, 154.7, 157.8, 163.0 ppm; MS (ESI): $m/z = 560$ ($[M + H]^+$).

5-(4-Bromophenyl)-2-[4-[bis(1-methyl-1H-3-indolyl)methyl]phenoxymethyl]-1,3,4-oxadiazole
(**12j**, $C_{34}H_{27}BrN_4O_2$)
This compound **12j** was prepared following the method described for the preparation of the compound **12a**, employing 500 mg of **10** (1.4 mmol, 1.0 eq) with 281 mg of 4-bromobenzoic acid (**11j**, 1.4 mmol, 1.0 eq) and 15 cm^3 $POCl_3$ (1.6 mmol, 1.15 eq). The crude product was purified by column chromatography with ethyl acetate/*n*-hexane (2:8) to afford 602 mg pure compound **12j** (88 % yield). Yellow solid; m.p.: 224-226 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.69$ (s, 6H), 5.27 (s, 2H), 5.38 (s, 1H), 5.31 (s, 2H), 6.72 (d, 2H, $J = 8.90$ Hz), 6.89 (d, 2H, $J = 8.04$ Hz), 6.99 (d, 2H, $J = 8.90$ Hz), 7.24 (d, 2H, $J = 8.34$ Hz), 7.29 (t, 2H), 7.34 (t, 2H), 7.76 (d, 2H, $J = 8.45$ Hz), 7.89 (d, 2H, $J = 8.90$ Hz), 10.2 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 33.0$, 41.4, 67.2, 106.8, 117.8, 118.9, 119.4, 119.9, 123.9, 124.9, 125.4, 125.7, 126. 7, 129.2, 131.9, 138.9, 146.2, 154.8, 157.9, 163.3 ppm; MS (ESI): $m/z = 603$ ($[M + H]^+$).

Procedure of the SRB-assay

The compounds **12a–12j** were evaluated for their in vitro cytotoxicity in human cancer cell lines. A protocol of 48-h continuous drug exposure was used and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The cell lines were grown in DMEM medium containing 10 % fetal bovine serum and 2 mM L-glutamine and were inoculated into 96-well microtiter plates in 90 mm^3 at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity for 24 h prior to addition of experimental drugs. Aliquots of 10 mm^3 of the drug dilutions were added to the appropriate microtiter wells already containing 90 mm^3 of cells, resulting in the required final drug concentrations. For each compound four concentrations (0.1, 1, 10, and 100 μM) were evaluated and done in triplicate wells. Plates were incubated further for 48 h and the assay was terminated by the addition of 50 mm^3 of cold trichloroacetic acid (TCA) (final concentration 10 % TCA) and incubated for 60 min at 4 °C. The plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 cm^3) at 0.4 % (w/v) in 1 % acetic acid was added to each of the cells, and plates were incubated for 20 min at room temperature. The residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM Trizma base, and the absorbance was read on an ELISA plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate by plate basis for test wells relative to control wells. The above determinations were repeated three times. Percentage growth was expressed as the (ratio of average absorbance of the test well to the average absorbance of the control wells) × 100. Growth inhibition of 50 % (GI_{50}) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = GI_{50}$,

which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. In this expression, T_z is optical density at time zero, OD of control is C, and OD of test growth in the presence of drug is T_i.

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