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Synthesis of novel C4-benzazole naphthalimide derivatives with potent anti-tumor properties against murine melanoma

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Abstract

Novel C4-benzazole naphthalimide derivatives were synthesized and tested *in vitro* and *in vivo* as anti-cancer drugs. Among these synthetic molecules, compounds **9** and **10** exhibited cytotoxicity against murine B16F10 melanoma cells. In addition, the above-mentioned compounds significantly suppressed lung tumor metastasis with no visible sign of toxicity.

Keywords: benzazole, B16F10 cells; melanoma; MTS assay; naphthalimide; topoisomerase II.

1. Introduction

Malignant melanoma, an aggressive type of skin cancer with high metastatic potential, is a cause of growing public concern worldwide. Malignant melanoma can be cured by a number of treatments, such as surgery, radiation, and chemotherapy.[1-3] However, once it metastasizes to other parts of the body, effective therapies

are lacking.[4] At present, alkylating agents such as dacarbazine, cisplatin, and temozolomide are the most common chemotherapy drugs for the treatment of stage IV melanoma.[5] However, the long-term response rate with systematic chemotherapeutic agents is poor, while incidence rate and number of deaths from melanoma have been increasing continuously. Therefore, there remains an unmet need for novel medicines for effective treatment of melanoma.

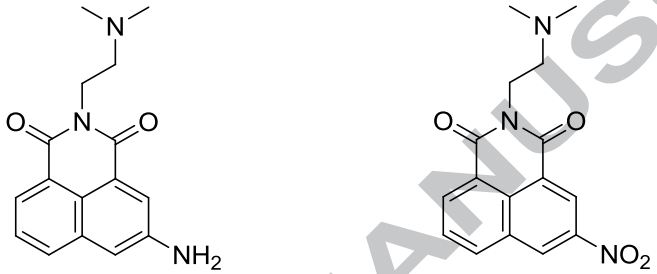


Figure 1. Naphthalimide-based antitumor agents.

Naphthalimide is a main scaffold in various luminophores owing to its flat and stable conjugated aromatic system.[6] Therefore, its derivatives are widely applied in dyes,[7] liquid crystals,[8] chemosensors,[9] fluorescent probes,[10] *etc*. In particular, the flat structure of naphthalimide derivatives allows then intercalate into DNA.[11] Thus these derivatives, such as amonafide[12] and mitonafide,[12b,13] (Figure 1) have shown promise against various cancer cell lines *in vitro* and have been tested in clinical trials. However, amonafide and mitonafide have failed in trials due to toxicity.[14] Recently, some sulphonamide and sulphonyl-hydrazone naphthalimide derivatives have shown potential cytotoxic properties against murine B16F10 melanoma cells with IC_{50} around 50 μM and 300 μM.[15]

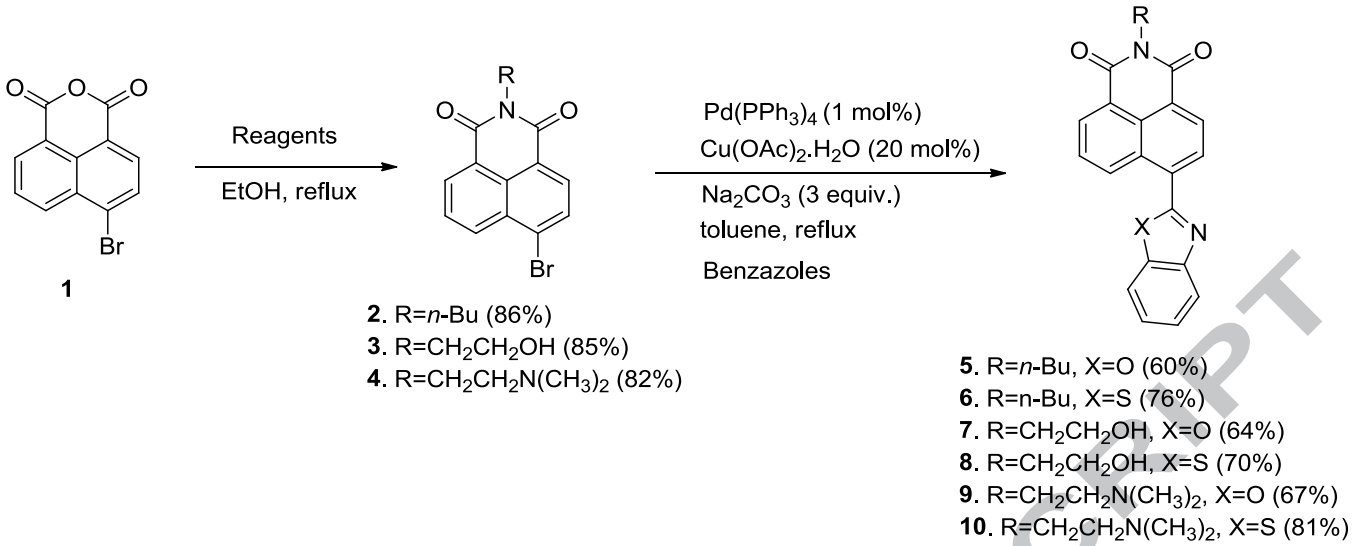
Some naphthalimide analogs have been demonstrated to inhibit melanoma tumor growth in a subcutaneous

xenograft model with fewer side effects.[16] In particular, it has been reported that the naphthalimide derivatives produced by modifying the *N*,*N*-dimethyl portion of mitonafide,[16,17] have been utilized in treatment of melanoma. We are aware that core structures with incorporating benzoxazole, benzothialole or benzoimidazole moieties are pervasively found in potential drugs,[18] and that these benzazole-based molecules are used as clinical drugs with therapeutic potency.[19] We are interested in combining of benzazoles at the C-4 position of naphthalimide derivatives in order to provide a wider spectrum of applications. In order to quickly assemble the two portions, we coupled benzazoles and 4-bromonaphthalimide derivatives via palladium-copper catalysis.[20] Our main goal is pursuing novel potential chemotherapeutic agents for treatment of melanoma.

2. Results and discussion

2.1 Chemistry-Synthesis of C4-benzazole naphthalimide derivatives compounds 5-10

The syntheses of compounds **5-10** are depicted in Scheme 1. We utilized commercially available compound **1** and modified its anhydride portion by reaction with *n*-butylamine, 2-hydroxyethylamine, and *N*,*N*-dimethylethylenediamine under reflux in EtOH to afford **2**, **3**, and **4**, respectively, in high yields. It is worthwhile to point out that the antitumor agents amonafide and mitonafide were also possess an *N*,*N*-dimethylethylenediamine portion. Compounds **2, 3** and **4** were directly coupled with either benzoxazoles or benzothialoles by palladium-copper catalysis[20] to provide compounds **5–10**.



Scheme 1. Syntheses of C4-benzazole naphthalimide derivative compounds **5–10**

2.2 Biological evaluation

2.2.1 In vitro cytotoxic effect of compounds 5-10

To evaluate the effect of synthetic compounds on cell viability, B16F10 cells were treated with various concentrations of compounds **5–10** for 2 days. The cytotoxicity of compounds **5–10** against murine B16F10 melanoma cells was examined by MTS assay. As shown in Figure 2, the cytotoxicity of these compounds was highly dependent on the structure of the side chain. Compounds **5-8** at concentrations ranging from 0.01 to 40 μM did not exhibit cytotoxic activity. In contrast, compounds **9** and **10** dramatically decreased cell viability in a dose-dependent manner (IC_{50} of compound **9**: 7.8 ± 0.05 μM; IC_{50} of compound **10**: 4.5 ± 0.06 μM) showing almost the same degree of cytotoxicity as amonafide (IC_{50} of amonafide: 6.7 ± 1.11 μM). Therefore, compounds **9** and **10** were selected for further studies.

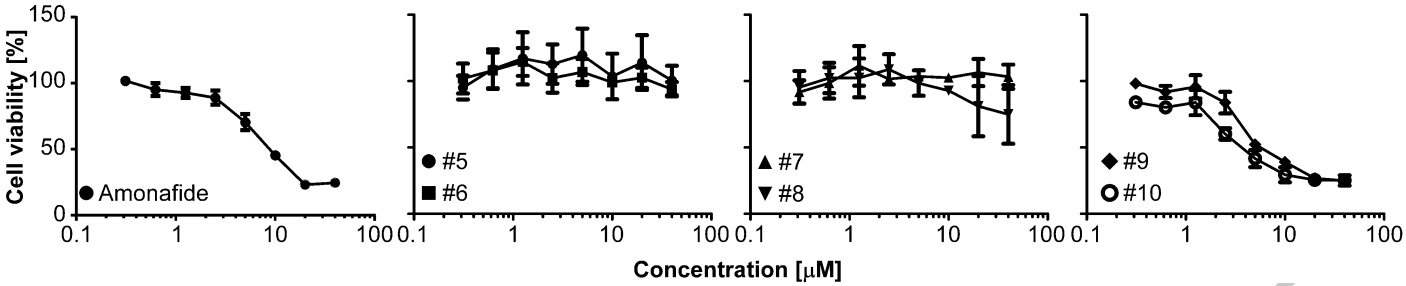


Figure 2. The *in vitro* cytotoxicity of a range of doses of compounds **5**-**10** to B16F10 melanoma cells. Cell viability was examined after 2 days incubation by MTS assay. Optical density of DMSO control groups was taken as 100% of cell viability. Values are presented as the mean ± SEM from three independent experiments. Data are the results of three independent experiments with each assay performed in duplicate within each experiment.

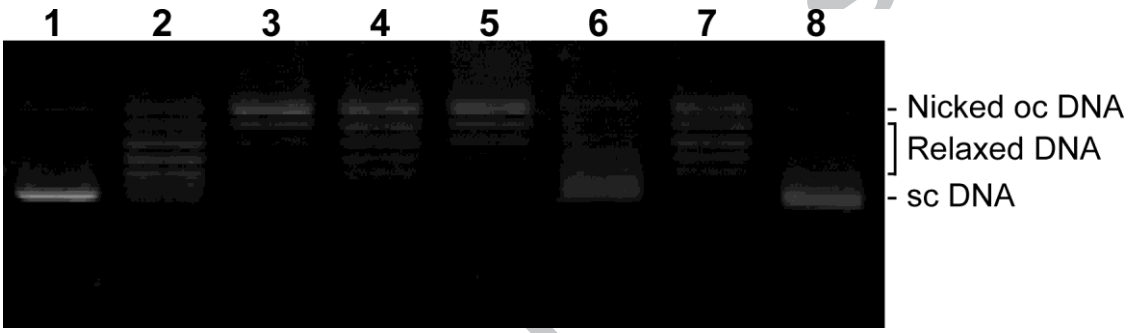


Figure 3. Inhibitory effects of compounds **9** and **10** on topoisomerase II mediated DNA relaxation. Supercoiled pHOT-1 plasmid DNA was incubated with topoisomerase II enzyme in the presence of various concentrations of indicated compounds. Intercalation was evaluated by conversion of nicked oc DNA and relaxed DNA into supercoiled DNA. Lane 1: supercoiled pHOT-1 DNA, lane 2: no drug, lane 3: 1% DMSO, lane 4: 100 μM of VP16, lane 5: 10μM of compound **9**, lane 6: 100 μM of compound **9**, lane 7: 10 μM of compound **10**, lane 8: 100 μM of compound **10**. oc, open circular; sc, supercoiled.

Because some amonafide derivatives exert their anti-cancer activity by poisoning topoisomerase II,[21] we investigated the mechanism of B16F10 melanoma cells death induced by compounds **9** and **10**. We used *in vitro* DNA relaxation assay to investigate topoisomerase II inhibitory activity. As shown in Figure 3, intercalation by compounds **9** and **10** into DNA was detectable at 10 μM. In addition, complete intercalation into samples could be observed by treating with compound **10** (100 μM), but not with compound **9**. This indicated that compound **10** at high concentration might have a better inhibitory effect on topoisomerase II activity *in vitro*.

2.2.2 Effect on mouse melanoma lung metastatic models

To examine the therapeutic effect of compounds **9** and **10** against pre-established B16F10 lung metastases, mice were intravenously (iv) injected with 2 x 10^5 B16F10 tumor cells and treated with 2 mg/kg of compound **9** or **10**, respectively, or 5% DMSO vehicle control. The number of lung nodules in each individual mouse in each group is shown in Figure 4. Mice treated with compound **9** showed a reduction in the number of lung metastases (mean 41; range 2-90) compared with mice receiving the DMSO vehicle control (mean 60; range 37-94). In contrast, 2 mg/kg of compound **10** had a marked effect in reducing metastasis to the lung, with a mean of 30 lung nodules (range 3-59).

To investigate the toxicity of compounds **9** and **10**, mice were intraperitoneally (ip) injected daily with 2 mg/kg of compound **9** or **10** or 5% DMSO vehicle control. As shown in Figure 5, no significant decrease in body weight was observed in mice treated with high doses of compound **9** or **10**. We conclude that compounds **9** and **10** are very safe.

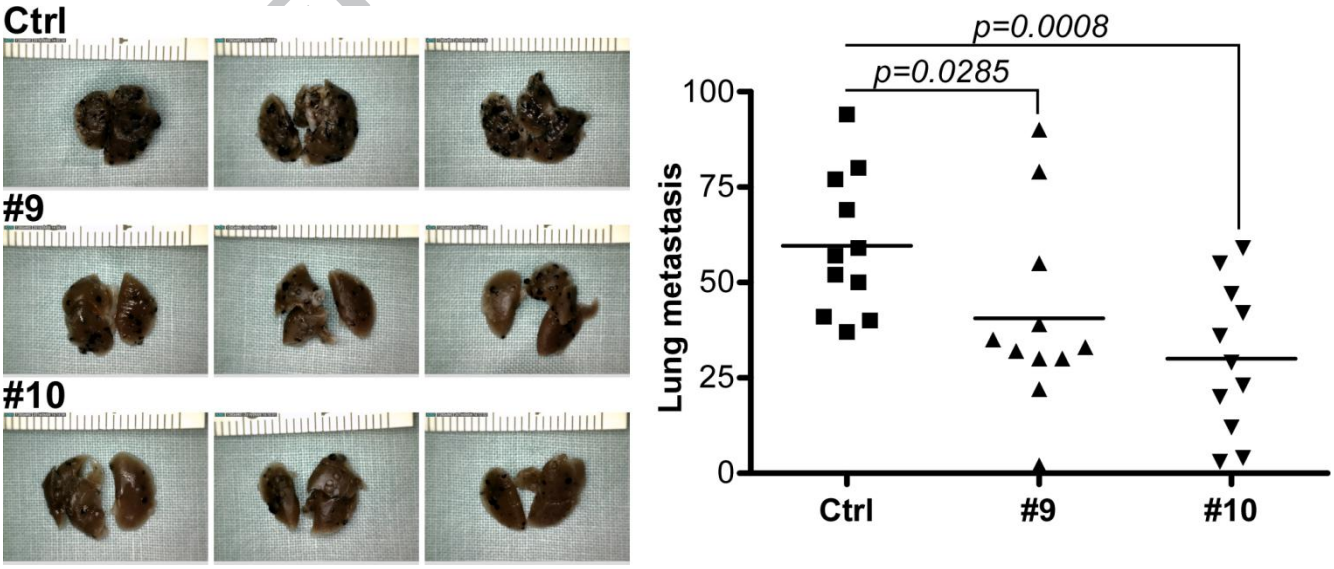


Figure 4. Lung metastases after i.v. injection of B16F10 tumor cells. 2x10^5 B16F10 cells were injected into each mouse via tail vein on day 0. Compounds **9** and **10** 2 mg/kg vs 5% DMSO vehicle control was given ip

daily on days 1–14 (n=11/group). The amount of tumor seeding was counted as total numbers of black nodules presented in the lungs under microscopy. Both compounds **9** and **10** significantly reduced tumor seeding and growth on day 14. Ctrl 5% DMSO vehicle control; #9 compound **9**; #10 compound **10**.

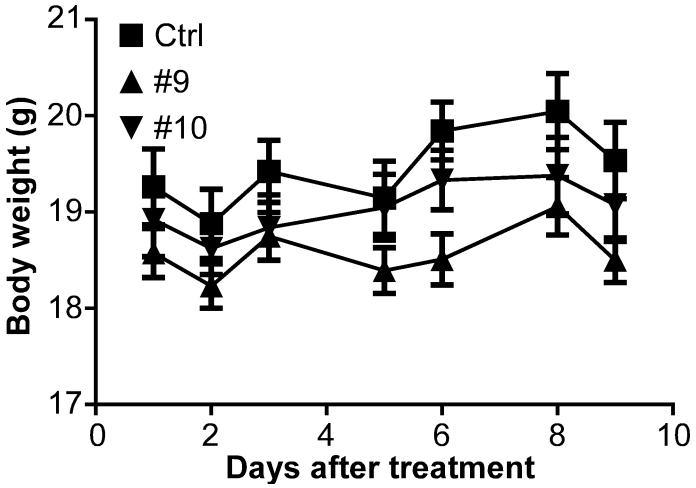


Figure 5. Average body weights of C57BL/6 mice during 9-day toxicity study. Mice (n=5/group) were ip injected daily with 2 mg/kg of compound **9** or **10** or 5% DMSO vehicle control, and their body weights were measured on the indicated days. There was no difference in the weights of 5% DMSO vehicle control, or treated compounds **9** and compound **10** mice. Ctrl, 5% DMSO vehicle control; #9 compound **9**; #10 compound **10**.

3. Conclusion

Compounds **9** and **10** possess the same naphthalimide moiety as the anti-cancer agent amonafide and exhibited comparable biological activity against B16F10 melanoma cells *in vitro*. Their differences in structure are at the C-4 benzazoles of **9** and **10** and the nitro as well as the amine groups in amonafide and mitonafide at C-3, respectively. We believe these functional groups to contribute a certain degree of their activity at both sides. Our work is based on a syngeneic lung metastatic model which models cancers that are more difficult to treat. Compound **9** and **10** significantly reduced B16F10 lung metastasis in C57BL/6 mice without obvious weight loss. Recently, some clinically approved chemotherapeutic drugs not only directly killed tumors but also elicited an immune response against tumors.[25] Our unpublished results demonstrate that compounds **9** and **10** also enhance innate cell function *in vitro*. Whether these cells play

roles in the anti-cancer effects mediated by compounds **9** and **10** is a topic that is currently under investigation.

4. Experimental

All chemicals were purchased from commercial providers and used without purification. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR were recorded on a Bruker 600 MHz spectrometer. Chemical shifts were reported in parts per million (ppm) and referred to the residual of deuterium solvent: ^{1}H NMR ($CDCl_3$, 7.26 ppm; CD_3OD, 3.3ppm; d_6-DMSO, 2.5 ppm); ^{13}C NMR ($CDCl_3$, 77.0 ppm; CD_3OD, 49.15 ppm; d_6-DMSO, 39.51 ppm). Melting points were determined on a Fargo MP-2D apparatus and not corrected. HRMS were recorded on Finnigan MAT-95S. Purification conducted by flash column chromatography on either SiO_2 (Chromatorex GS 60-40 (230-400 mesh) Fuji Silysia Chemical LTD) or NH-SiO_2 (Chromatorex, MB 100-40) except as otherwise stated. All solid products were filtrated by suction manner and washed with appropriate solvents.

4.1. Synthetic methods

6-Bromo-2-butyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**2**). Compound **1** (0.415 g, 1.5 mmol) and *n*-butylamine (0.163 mL, 1.65 mmol) in EtOH (8 mL) was heated at 70 ^{o}C under nitrogen atmosphere for 3 h. This mixture was cooled to room temperature and filtered. The solid was collected and washed with 95% EtOH to afford a pale-yellow, needle-like solid (0.422 g, 1.27 mmol) in 86% yield. Mp 196–198 ^{o}C. (*lit.*[22] 109–110 ^{o}C from chlorobenzene) ^{1}H NMR (600 MHz, CD_3OD): δ 8.65 (d, J=7.2 Hz, 1H), 8.56 (d, J=8.4 Hz, 1H), 8.41 (d, J=7.8 Hz, 1H), 8.03 (d, J=7.8 Hz, 1H), 7.84 (t, J=8.4 Hz, 1H), 4.17 (t, J=7.8 Hz, 2H), 1.71 (quint, J=7.2 Hz, 2H), 1.45 (sixtet, J=7.2 Hz, 2H), 0.98 (t, J=7.2 Hz, 3H). ^{13}C NMR (150 MHz,

CD_3OD): δ 163.6 (x2), 133.2, 132.0, 131.2, 131.1, 130.6, 130.2, 129.0, 128.1, 123.2, 122.3, 40.4, 30.2, 20.4, 13.8. HRMS (EI) calculated for $C_{16}H_{14}BrNO_2$ ($[M]^+$): 331.0208. Found: 331.0204.

6-Bromo-2-(2-hydroxyethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**3**). Compound **1** (1.00 g, 3.61 mmol) and 2-aminoethanol (0.244 g, 4.322 mmol) in EtOH (20 mL) was refluxed under nitrogen atmosphere for 6 h. At the end of reaction time, the mixture was filtrated and the solid was washed with 95% EtOH to afford a pale-yellow solid (0.979 g, 3.07 mmol) in 85% yield. Mp 209–211 ^oC. (*lit.*[23] 203–204 ^oC) ^1H NMR (600 MHz, $CDCl_3$): δ 8.68 (dd, *J*=7.2, 1.0 Hz, 1H), 8.59 (dd, *J*=8.5, 1.0 Hz, 1H), 8.43 (t, *J*=7.8 Hz, 1H), 8.06 (d, *J*=7.8 Hz, 1H), 7.86 (dd, *J*=8.5, 8.5 Hz, 1H), 4.45 (t, *J*=10.8 Hz, 2H), 3.99 (t, *J*=10.8 Hz, 2H), 2.08 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 164.5, 164.4, 133.6, 132.4, 131.5, 131.2, 130.7, 129.1, 128.2, 122.8, 121.2, 61.7, 42.9. HRMS (EI) calculated for $C_{14}H_{10}BrNO_3$ ($[M]^+$): 318.9844, Found: 318.9840.

6-Bromo-2-(2-(dimethylamino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione (**4**). Compound **1** (1.00 g, 3.61 mmol) and *N*,*N*-dimethylethylenediamine (0.382 g, 4.332 mmol) was refluxed under nitrogen atmosphere for 4 h. The mixture was filtrated and the solid was washed with 95% EtOH to provide a pale-yellow solid (1.025 g, 2.952 mmol) in 82% yield. Mp 152–154 ^oC. (*lit.*[24] 142.5–143.5 °C) ^1H NMR (600 MHz, CD_3OD): δ 8.61 (d, *J*=7.2 Hz, 1H), 8.60 (d, *J* = 8.4 Hz, 1H), 8.37 (d, *J*=7.8 Hz, 1H), 8.13 (d, *J*=7.8 Hz, 1H), 7.91 (t, *J*=8.1 Hz, 1H), 4.30 (t, *J*=7.2 Hz, 2H), 2.69 (t, *J*=7.2 Hz, 2H), 2.35 (s, 6H). ^{13}C NMR (150 MHz, CD_3OD): δ 165.3, 165.2, 134.4, 133.1, 132.6, 132.4, 132.0, 131.2, 130.4, 129.6, 124.6, 123.8, 57.9, 45.9 (x2), 38.9. HRMS (EI) calculated for $C_{16}H_{15}BrN_2O_2$ ($[M]^+$): 346.0317, Found: 346.0309.

4.2 General procedure of palladium-copper-catalyzed direct coupling of compounds 2-4 with benzazoles.

6-(Benzo[*d*]oxazol-2-yl)-2-butyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**5**). To a mixture of compound **2** (0.050 g, 0.150 mmol), benzoxazole (0.0536 g, 0.450 mmol), $Pd(PPh_3)_4$ (0.0017 g, 0.0015 mmol), $Cu(OAc)_2 \cdot H_2O$ (0.0060 g, 0.030 mmol) and Na_2CO_3 (0.0477 g, 0.4503 mmol) was added toluene (2 mL). The solution was refluxed for 6 h under nitrogen atmosphere. The solid was removed by filtration through celite. The filtrant was diluted with EtOAc and 20% HCl. The organic layer was separated, dried over $MgSO_4$ and purified by flash column chromatography (EtOAc/Hexane=1/8–1/4; R_f=0.55, EtOAc/Hexane=1/6) to afford a yellow-green solid (0.0342 g, 0.0923 mmol). Yield: 60%. Mp 208–211 ^oC. ^1H NMR (600 MHz, $CDCl_3$) : δ 9.97 (d, *J*=8.6 Hz, 1H), 8.72–8.70 (m, 2H), 8.67 (d, *J*=7.8 Hz, 1H), 7.95 (t, *J*=7.6 Hz, 1H), 7.92 (d, *J*=7.8 Hz, 1H), 7.69 (d, *J*=7.8 Hz, 1H), 7.48 (t, *J*=7.4 Hz, 1H), 7.46 (t, *J*=7.8 Hz, 1H), 4.21 (t, *J*=7.8 Hz, 2H), 1.76 (quint, *J*=7.3 Hz, 2H) , 1.47 (sextet, *J*=7.3 Hz, 2H), 1.00 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 164.0, 163.6, 160.9, 150.3, 142.1, 133.1, 131.6, 130.0, 129.1, 128.8, 128.7, 128.5, 126.4, 125.1, 125.0, 123.0, 120.8, 110.8, 40.4, 30.2, 20.4, 13.8. HRMS (EI) calculated for $C_{23}H_{18}N_2O_3$ ($[M]^+$) : 370.1317. Found: 370.1320.

6-(Benzo[*d*]thiazol-2-yl)-2-butyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**6**). Same procedure as **5**. To a mixture of compound **2** (0.100 g, 0.3021 mmol), benzothiazole (0.034 g, 0.252 mmol), $Pd(PPh_3)_4$ (0.0030 g, 0.0025 mmol), $Cu(OAc)_2 \cdot H_2O$ (0.010 g, 0.0503 mmol) and Na_2CO_3 (0.0533 g, 0.503 mmol) was added

toluene (4 mL). Purification by flash column chromatography (EtOAc/Hexane=1/8–1/4; R_f=0.4, EtOAc/Hexane=1/4) afforded a yellow-green solid (0.0342 g, 0.0923 mmol). Yield: 76%. Mp 154–156 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.43 (d, *J*=8.6 Hz, 1H), 8.68 (t, *J*=7.6 Hz, 2H), 8.24 (d, *J*=8.2 Hz, 1H), 8.19 (d, *J*=7.6 Hz, 1H), 8.01 (d, *J*=8.0 Hz, 1H), 7.88 (t, *J*=7.9 Hz, 1H), 7.61 (t, *J*=7.6 Hz, 1H), 7.52 (t, *J*=7.6 Hz, 1H), 4.21 (t, *J*=7.7 Hz, 2H), 1.76 (quint, *J*=7.6 Hz, 2H), 1.47 (sextet, *J* =7.6 Hz, 2H), 1.00 (t, *J*=7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) : δ 165.5, 164.0, 163.6, 154.2, 136.1, 135.4, 132.9, 131.7, 130.2, 129.6, 129.1, 128.9, 128.3, 126.8, 126.2, 124.2, 124.0, 122.9, 121.6, 40.4, 30.2, 20.4, 13.8. HRMS (ESI) calculated for $C_{23}H_{19}N_2O_2S$ ($[M+H]^+$): 387.1167. Found: 387.1155.

6-(Benzo[*d*]oxazol-2-yl)-2-(2-hydroxyethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7). Compound **3** (0.100 g, 0.3124 mmol), benzoxazole (0.116 g, 0.937 mmol), $Pd(PPh_3)_4$ (0.0036 g, 0.0031 mmol), $Cu(OAc)_2 \cdot H_2O$ (0.0125 g, 0.0625 mmol) and Na_2CO_3 (0.0662 g, 0.635 mmol) in toluene (5 mL). Purification by flash column chromatography (EtOAc/CH_2Cl_2=1/4–1/1; R_f=0.3, EtOAc/CH_2Cl_2=1/1) afforded a yellow-green solid (0.072 g, 0.201 mmol). Yield: 64%. Mp 194–198 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.01 (d, *J*=8.4 Hz, 1H), 8.74–8.72 (m, 2H), 8.69 (d, *J*=7.7 Hz, 1H), 7.97 (t, *J*=8.0 Hz, 1H), 7.92 (d, *J*=7.6 Hz, 1H), 7.69 (d, *J*=7.8 Hz, 1H), 7.49 (t, *J*=7.1 Hz, 1H), 7.46 (t, *J*=7.3 Hz, 1H), 4.49 (t, *J*=5.4 Hz, 2H), 4.02 (t, *J*=5.4 Hz, 2H), 1.59 (s, -OH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 164.9, 164.5, 160.8, 150.3, 142.1, 133.6, 132.0, 130.4, 129.2, 129.1, 128.9, 128.5, 126.5, 126.5, 125.2, 124.6, 122.6, 120.9, 110.8, 61.7, 42.9. HRMS (EI) calculated for $C_{21}H_{14}N_2O_4$ $[M+H]^+$: 358.0954. Found: 358.0957.

6-(Benzo[*d*]thiazol-2-yl)-2-(2-hydroxyethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**8**). Compound **3** (0.100 g, 0.3142 mmol), benzothiazole (0.1267 g, 0.937 mmol), $Pd(PPh_3)_4$ (0.0036 g, 0.0031 mmol), $Cu(OAc)_2 \cdot H_2O$ (0.010 g, 0.050 mmol) and Na_2CO_3 (0.0662 g, 0.625 mmol) in toluene (4 mL). Purification by flash column chromatography ($EtOAc/CH_2Cl_2$=1/4–1/1; R_f=0.4, $EtOAc/CH_2Cl_2$=1/1) afforded a yellow-green solid (0.082 g, 0.219 mmol). Yield: 70%. Mp 231–234 ^oC. ^1H NMR (600 MHz, CD_3OD): δ 9.47 (d, *J*=8.3 Hz, 1H), 8.71 (t, *J*=7.4 Hz, 1H), 8.25 (d, *J*=8.0 Hz, 1H), 8.21 (d, *J*=7.4 Hz, 1H), 8.03 (d, *J*=8.0 Hz, 1H), 7.90 (t, *J*=7.6 Hz, 1H), 7.62 (t, *J*=7.4 Hz, 1H), 7.53 (t, *J*=7.5 Hz, 1H), 4.50 (t, *J*=4.8 Hz, 2H), 4.02 (t, *J*=4.8 Hz, 2H), 1.84 (s, -OH). ^{13}C NMR (150 MHz, CD_3OD): δ164.9, 164.5, 154.1, 133.4, 132.1, 130.6, 129.7, 129.1, 129.0, 128.4, 126.9, 126.3, 124.1, 123.9, 122.5, 121.6, 61.8, 43.0. HRMS (EI) calculated for $C_{21}H_{14}N_2O_3S$ ($[M]^+$): 374.0725. Found: 374.0717.

6-(Benzo[*d*]oxazol-2-yl)-2-(2-(dimethylamino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**9**). Compound **4** (0.1000 g, 0.2889 mmol), benzoxazole (0.103 g, 0. 864 mmol), $Pd(PPh_3)_4$ (0.0033 g, 0.0029 mmol), $Cu(OAc)_2 \cdot H_2O$ (0.0115 g, 0.0576 mmol) and Na_2CO_3 (0.061 g, 0.576 mmol) in toluene (3 mL). Purification by flash column chromatography ($NH\text{-}SiO_2$, EtOAc/Hexane=1/8–1/4; R_f=0.3, EtOAc/Hexane=1/4) afforded a yellow-green solid (0.0742 g, 0.193 mmol). Yield: 67%. Mp 238–242 ^oC. ^1H NMR (600 MHz, d_6-DMSO): δ9.86 (d, *J*=8.4 Hz, 1H), 8.70 (d, *J*=7.7 Hz, 1H), 8.65 (d, *J*=7.9 Hz, 1H), 8.62 (d, *J*=7.1 Hz, 1H), 8.09 (t, *J*=7.8 Hz, 1H), 7.99 (d, *J*=7.8 Hz, 7.91 (d, *J*=8.0 Hz, 1H), 7.56 (t, *J*=7.4 Hz, 1H), 7.51 (t, *J*=7.7 Hz, 1H), 4.18 (t, *J*=7.2 Hz, 2H), 2.57 (t, *J*=7.2 Hz, 2H), 2.23 (s, 6H). ^{13}C NMR (150 MHz, d_6-DMSO): δ163.2, 162.8, 160.4, 149.7, 141.5, 132.4, 131.2, 130.0, 129.5, 128.9, 128.3, 128.1, 127.7, 126.8,

125.3, 124.6, 122.7, 120.5, 111.2, 59.4, 45.4, 37.8. HRMS (ESI) Calculated for $C_{23}H_{20}N_3O_3$ ($[M+H]^+$) 386.1505. Found: 386.1492.

6-(Benzo[*d*]thiazol-2-yl)-2-(2-(dimethylamino)ethyl)-1*H*-benzo[de]isoquinoline-1,3(2*H*)-dione (**10**). Compound **4** (0.1595 g, 0.5 mmol), benzothiazole (0.212 g, 1.0 mmol), $Pd(OAc)_2$ (0.001 g, 0.005 mmol), PPh_3 (0.0656, 1mmol), $Cu(OAc)_2 \cdot H_2O$ (0.0199 g, 0.1 mmol) and Na_2CO_3 (0.111 g, 1.0 mmol) in toluene (8 mL). Purification by flash column chromatography (NH-SiO_2, CH_2Cl_2/Hexane=1/6–1/4–1/2; R_f=0.35, CH_2Cl_2/Hexane=1/3) afforded a yellow-green solid which was then washed with ether to produce **10** (0.151 g, 0.40 mmol). Yield: 81%. Mp 231–234 ^oC. ^1H NMR (600 MHz, CD_3OD): δ9.41 (d, J=8.5 Hz, 1H), 8.64 (t, J=6.1 Hz, 2H), 8.25 (d, J=7.5 Hz, 1H), 8.15 (d, J=8.1 Hz, 1H), 8.08 (d, J=8.0 Hz, 1H), 7.91 (t, J=8.0 Hz, 1H), 7.61 (t, J=7.5 Hz, 1H), 7.53 (t, J=7.6 Hz, 1H), 4.55 (t, J=5.4 Hz, 2H), 3.57 (t, J=5.4 Hz, 2H), 3.05 (s, 6H). ^{13}C NMR (150 MHz, CD_3OD): δ167.1, 166.0, 165.6, 155.5, 137.8, 136.9, 134.8, 133.1, 131.8, 131.2, 130.4, 130.3, 129.6, 128.3, 127.7, 125.1, 124.9, 123.8, 123.1, 57.7, 44.5, 36.9. HRMS (ESI) calculated for $C_{23}H_{19}N_3O_2S$ ($[M+H]^+$) 402.1276. Found: 402.1264.

4.3. Anti-cancer assay

4.3.1. Cell lines and mice

Murine B16F10 cell lines maintained in Dulbecco's modified Eagle's medium (DMEM), 10% heat-inactivated fetal calf serum, 2mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) were combined at 37 ^oC in a 5% CO_2 humidified atmosphere. Female C57BL/6 mice (6 to 8 weeks old)

were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All animal experiments were performed under specific pathogen-free conditions and in accordance with guidelines approved by the Animal Care and Usage Committee of Mackay Memorial Hospital (Taipei, Taiwan). The body weight of each mouse was measured at the beginning of treatment and every day during the treatment period.

4.3.2. Cell viability assay

5 x 10^3 B16F10 cells were seeded into 96-well plates and treated with the following: DMSO control or combination of various concentrations of amonafide (purchased from Abcam) with compounds **5-10** for 2 days. For the MTS assay (Promega), 40 µl of MTS reagent was added into each well. Cells were incubated at 37 ^oC for 4 h. Absorbance was detected at OD490 nm.

4.3.3. DNA intercalation assay

DNA intercalation was examined using topoisomerase II drug screening kit (TopoGen) according to the manufacturer's instructions. Intercalation reaction mixtures containing supercoiled pHOT-1 plasmid DNA and topoisomerase II enzyme in the presence of various concentrations of indicated compounds were incubated at 37 °C for 30 min. The sample contained supercoiled DNA and topoisomerase II in the presence of 100 µM etoposide VP-16 served as positive control. The reactions were terminated with 10% sodium dodecyl sulfate, followed by proteinase K treatment for 15 min at 37 °C. Samples were separated by electrophoresis on a 1% agarose gel and stained with EtBr. DNA bands were visualized by ultraviolet light.

4.3.4. Tumor treatment study

In order to generate lung metastases, 6- to 8-week-old female C57BL/6 mice were injected intravenously with $2x10^5$ B16F10 cells, a dose consistently yielding lung metastases in 100% of animals. Compounds **9** or **10** in an amount of 2mg/kg or 5% DMSO vehicle control was administered ip daily on days 1-14 to test the effect of the compounds on tumor metastasis. The amount of tumor seeding was determined based on the numbers of black nodules visible in the lungs under microscopy.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary data

Supplementary data (^{1}H and ^{13}C NMR data) for all the compounds associated with this article can be found in the online version.

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