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Original article

Unprecedented synthesis, *in vitro* and *in vivo* anti-cancer evaluation of novel triazolonaphthalimide derivatives

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ABSTRACT

An efficient synthesis method for fusing triazole ring onto the naphthalimide core was described. The anti-cancer activities of the generated triazolonaphthalimide derivatives were evaluated with five cancer cell lines. The compounds generally displayed higher potency than amonafide. **4d**,**4e** carrying two amino side chains showed the strongest cytotoxicities. N-oxide **5**, a prodrug of **4a**, was designed and synthesized. The agent was expected to be activated under the hypoxic condition in tumor tissue. Compared with **4a**, **5** manifested much lower cytotoxicity both in cancer cell lines and human normal cells in the *in vitro* assays. However, N-oxide **5** performed potent anti-cancer activity *in vivo* using S-180 sarcoma bearing mice. All the results suggested that **5** was a promising anti-cancer agent.

1. Introduction

Since 1973, naphthalimides have received most attention and been investigated broadly as DNA intercalators, generally showed high anti-cancer activities against a broad spectrum of cell lines, and also displayed manifold mechanisms of action besides acting as topoisomerase II poisons. Amonafide, elinafide and bisnafide (Fig. 1) were three comparatively successful naphthalimide derivatives which acceded to different phases in clinical trial as potential anti-cancer agents. Superior to most anti-tumor agents, naphthalimides haven't been affected by multi-drug resistance according to all clinical trials. However, heretofore no naphthalimide derivative has ever reached the antineoplastic market because of dose-limiting toxicity [1,2]. It is thus meaningful and valuable for more investigation with naphthalimide.

One approach to enhance the cytotoxicity of naphthalimides was to fuse one or more aromatic rings to naphthalene core. This modification was first explored by Sami et al in 1993 [3] who reported a series of compounds synthesized from anthracene nucleus, which showed significantly improved potency than amonafide, such as the representative compounds azonafide and ethonafide [4]. Since then, many groups were devoted into the modification of employing different aromatic heterocycles to the naphthalimide core; such investigation generally obtained improved anti-cancer activities and/or lower side effect [5–11]. Some representative modifications were showed in Fig. 2.

Triazole is an important class of heterocycles and occupies a momentous position in the field of organic and pharmaceutical chemistry, owning to its broad spectrum of biological activities [12–14]. Derivatives of benzotriazole were broadly employed in antifungal [15], antidepressant [16], antilipolysis [17], antibacterial [18], anti-cancer agents [19,20], etc. The introduction of triazole moiety to naphthalene core can lead to a larger intercalating plane and higher capacity of hydrogen bonding because of the nitrogen atoms in the triazole ring, which increased the affinity for DNA to obtain higher cytotoxic activity. The synthesis of benzotriazole is rather difficult, and mainly through azide-alkyne cycloaddition [21–24] or metal catalyzed triazene intramolecular cyclization [25,26]. Herein we designed and synthesized a series of triazolonaphthalimides (**4a**–**e**, **4′a**–**e**, Scheme 1) via a novel method, which was treating amonafide and its analog using nitrosonium tetrafluoroborate ($NOBF_4$) and amines under mild conditions.

Furthermore, we focused on enhancing the selectivity between cancer cells and normal cells, which existed as a fundamental cause for low therapeutic efficacy in the chemotherapeutic field. One tactic was to develop tumor-activated prodrug, which can be trigged in tumor tissue but stayed non-toxic in normal cells [27]. A unique property of solid tumor cells was its hypoxia because of

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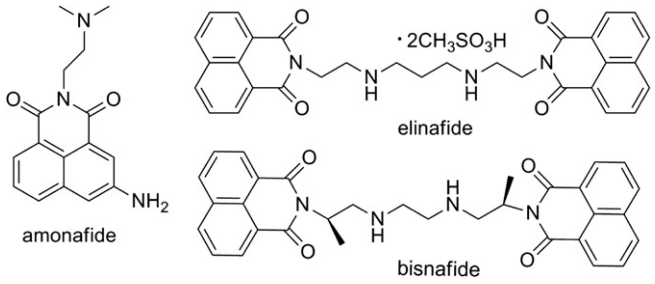


Fig. 1. Representative naphthalimide derivatives in clinical trial.

poorly organized tumor vasculature [28], this feature was often employed in developing prodrugs which depending on hypoxia to be activated. Oxidation of tertiary amine was a common strategy to afford anti-cancer prodrug, because N-oxide can be bioreduced to the corresponding amine under the hypoxic condition in tumor cells and displayed cytotoxicity, while stable in normal cells [11,29,30]. Qian et al reported a novel series of N-oxide of naphthalimides as prodrug in 2007 [11], and another article about novel aliphatic N-oxide of naphthalimides as fluorescent makers recently [29]. Thus, to fulfill this requirement, we chose **4a** to prepare the corresponding N-oxide derivative **5** to evaluate its activity in cancer cells, normal cells *in vitro* and S-180 sarcoma bearing ICR mice *in vivo*.

2. Chemistry

To obtain the target compounds (**4a**–**e**, **4′a**–**e**), we first synthesized the precursor **3** and **3′** following three steps according to the reported methods [31,32] (Scheme 1). First, commercially available 1,8-naphthalic acid anhydride was treated with 65% HNO_3 in concentrate H_2SO_4 to give compound **1**. The reduction of **1** can be achieved by adding in portion to a stirring $SnCl_2$–HCl (conc.) solution and refluxing at 80 °C for several hours. Then the mixture was filtrated and purified by washing with 1M HCl and water subsequently to obtain amine **2**. Amonafide **3** and its analog **3′** were prepared after treatment of **2** with N,N-dimethylethylenediamine or N,N-dimethyl-1,3-propanediamine in refluxing ethanol, respectively.

The formation of compound **4** from **3** was in fact a happy accident. At the beginning, we were trying to synthesize triazene using

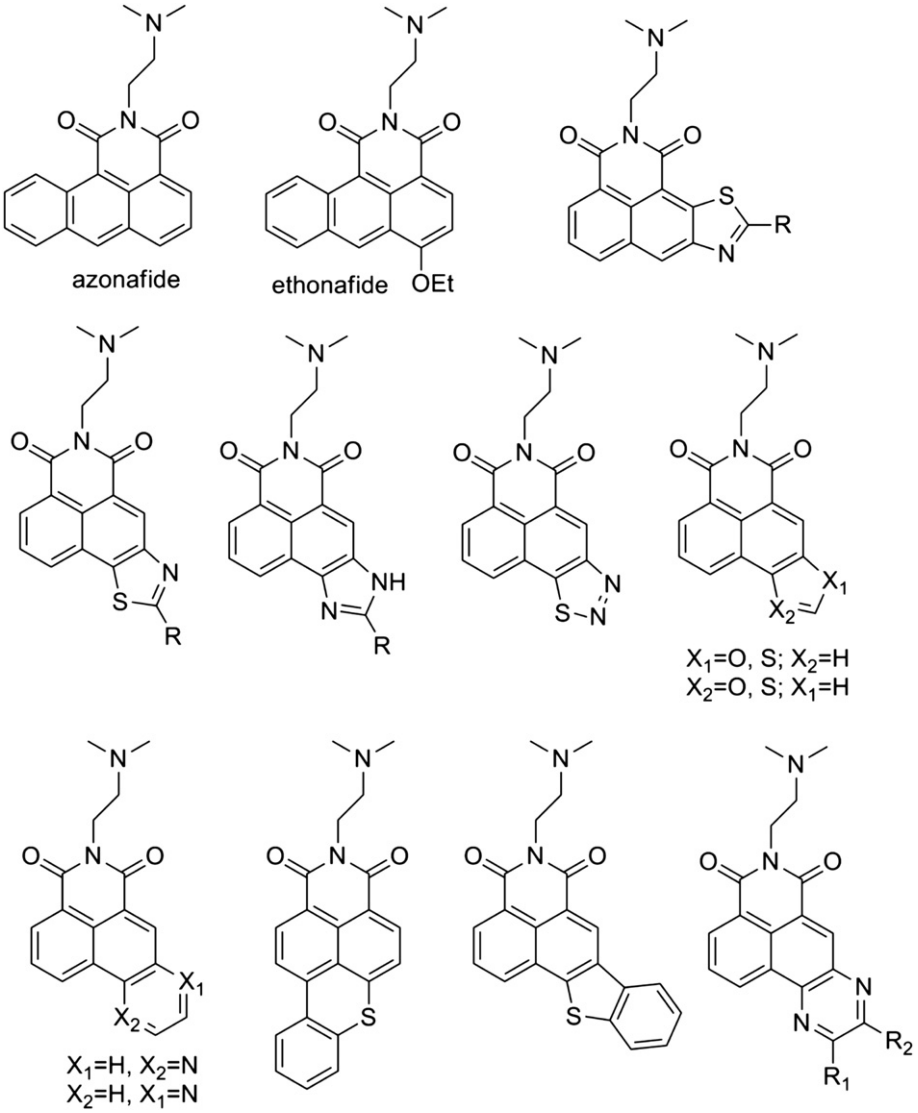
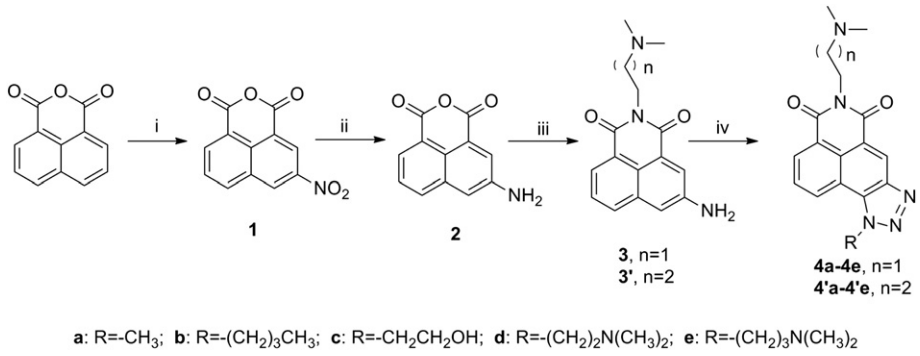


Fig. 2. Aromatic heterocycle fused naphthalimide derivatives.



Scheme 1. Reagents and conditions: (i) HNO_3 (65%), conc. H_2SO_4, 0°C-rt; (ii) $SnCl_2$, conc. HCl (37%), 80°C; (iii) corresponding amine, EtOH, reflux; (iv) a: $NOBF_4$, CH_3CN, N_2, -5°C; b: corresponding amine, Et_2O/H_2O, -5°C- 0°C.

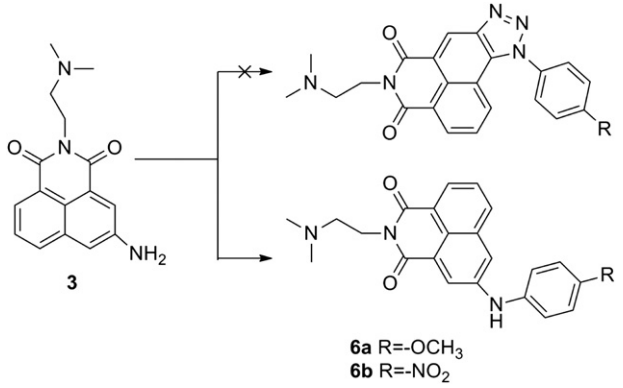
$NOBF_4$ to produce nitrene intermediate, which would be trapped by amine to give triazene product [33–36], but instead we only got the ring-fused structure **4a**. The mechanism of this reaction was still not clear, we can only speculate that triazene was first formed as intermediate, then through electrophilic intramolecular cyclization, triazole was produced. Because of steric hindrance, the angularly fused isomer was predominant over the linear one, which was verified by the NMR data. Considering the various bioactivities of triazole, we decided to synthesize a series of triazolonaphthalimide derivatives taking advantage of this unique transformation by employing various amines (**4a**–**e**, **4′a**–**e**). The reactions underwent smoothly with aliphatic amines, but were frustrated when anilines were used. N-aryl substituted product **6a**,**b**, instead of the triazole derivatives, were obtained (Scheme 2). The mechanism and scope of this reaction are still under investigation.

N-oxide **5** can be readily synthesized from **4a** by oxidation with m-CPBA in CH_2Cl_2. It should be noted that 1 eq K_2CO_3 and MeOH should be added to quench the reaction after the reaction was finished, otherwise, the yield would be very low (Scheme 3).

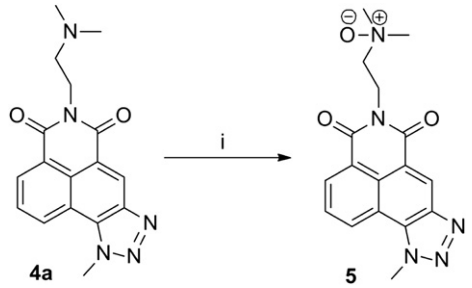
3. Results and discussion

3.1. Cytotoxic activity

We determined the cytotoxic potency of compounds **4**, **4′**, **5** and **6** against five cancer cell lines, which were HCT-116 (colorectal carcinoma), PC-3 (prostate carcinoma), U87 MG (brain tumor), Hep G2 (liver cancer), SK-OV-3 (ovarian cancer),with the *in vitro* cell growth assay. The results were listed in Table 1. All the compounds



Scheme 2. Synthesis condition of N-aryl substituted amonafide: a. $NOBF_4$, CH_3CN, N_2, -5°C; b. corresponding amine, Et_2O/H_2O, -5°C- 0°C.



Scheme 3. Reagents and conditions: CH_2Cl_2, m-CPBA, 0°C, 0.5 h; then rt, 0.5 h.

Table 1
IC_{50} values of triazolonaphthalimide compounds over five cancer cell lines.[a]

Compound	IC_{50} (μM)				
	HCT-116	PC-3	U87 MG	Hep G2	SK-OV-3
4a	0.65	1.58	0.45	0.68	1.17
4b	1.82	4.77	1.46	1.01	2.55
4c	1.42	4.29	1.22	1.07	2.53
4d	0.19	0.73	0.13	0.17	0.35
4e	0.15	0.71	0.14	0.18	0.40
4′a	1.35	3.21	1.14	0.97	2.00
4′b	6.34	15.49	6.32	5.77	8.93
4′c	7.73	24.98	4.31	5.21	9.64
4′d	0.39	3.28	0.37	0.78	0.64
4′e	0.35	1.20	0.30	0.29	0.46
6a	1.24	1.62	2.52	0.90	2.34
6b	0.62	0.41	0.62	0.41	1.32
5	>10.0	>10.0	>10.0	>10.0	>10.0
amonafide	4.55	8.05	2.80	1.41	8.83

[a] MTT assays were used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiment.

except **4′b**,**c** and **5** exhibited much stronger anti-cancer activities than the parental amonafide by several to dozens folds. The results demonstrated that fusing one more ring onto the naphthalene core increased the DNA binding potency and enhanced anti-cancer ability of the amonafide derivatives.

Table 2
IC_{50} values of compounds **4a** and **5** against human normal cells L-02 and HK-2 proliferation.[a]

Compound	IC_{50} (μM)	
	L-02	HK-2
4a	1.77	0.99
5	141.9	107.7

[a] MTT assays were used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiment.

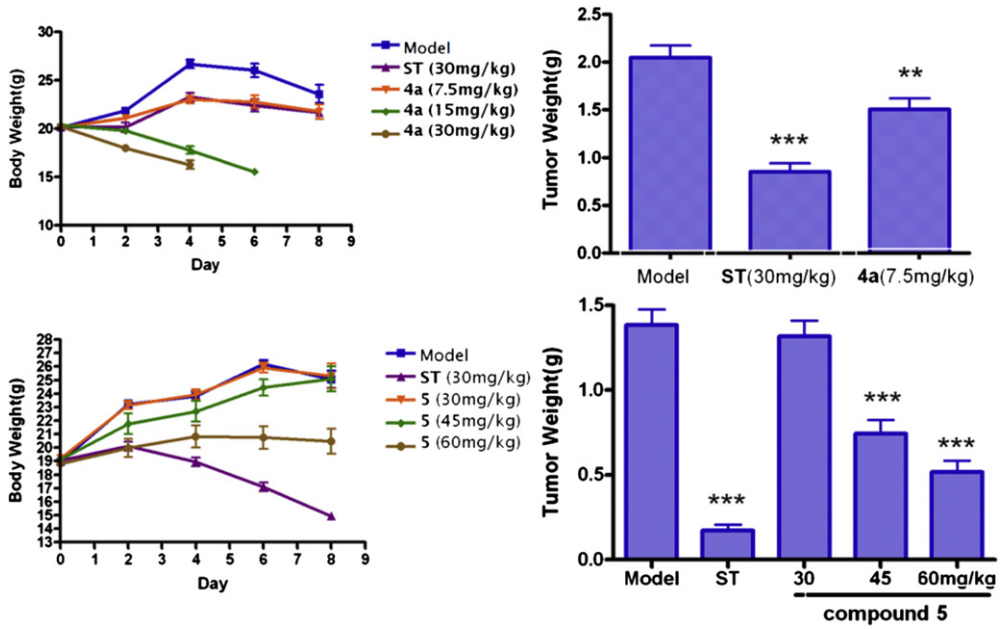


Fig. 3. Inhibitory effects of **4a** and **5** against sarcoma S-180 bearing ICR mice. Each compound was injected intraperitoneally every day over seven consecutive days: model (0.9% physiological saline), **ST** (amonafide, 30 mg/kg), **4a** (7.5 mg/kg, 15 mg/kg, 30 mg/kg), **5** (30 mg/kg, 45 mg/kg, 60 mg/kg). The weights of mice were recorded every two days (left line graphs). Sarcoma S-180 were excised and weighed 24h after the last time of injection (right column graphs). Each value was the mean±S.E.of ten data. TTest: **, $p<0.01$;***, $p<0.001$ vs Model.

Comparing to compounds **4′a**–**e**, **4a**–**e** generally showed better anti-cancer activities, respectively. The result indicated that the length of the side chain is important for the cytotoxicity of the compounds. It also proved the best length of the side chain is two carbons between the two nitrogen atoms [1]. Compared to **4a** which has only a methyl group attached to the triazole moiety, **4b** and **4c** have longer alkyl chains (n-butyl and hydroxyethyl) and showed weaker cytotoxicities. **4d**,**e**, which possessed a terminal amino group (*N*,*N*-dimethylethanamine and *N*,*N*-dimethylpropan-1-amine) to the triazole moiety, exhibited better potency than **4a**–**c**. These results indicated that two basic amino side chains can significantly improve the cytotoxicity of the naphthalimides. Compounds **6a** and **6b** which are N-phenyl amonafide also showed delightful activities [37,38]; especially **6b** which has a comparable activity with **4a**. Further investigation is undertaking by synthesizing more analogs and evaluating their cytotoxicities.

3.2. Prodrug designing and testing

Compound **4a** displayed stronger cytotoxicity than amonafide, which encouraged us to prepare its prodrug analog N-oxide **5** and measure the potency of **5**. N-oxide **5** showed poor cytotoxicity against all five cell lines as we predicted (Table 1), revealed that it was unable to be bioreduced to amine form in the cancer cells. We subsequently conducted a proliferative inhibition assay with human normal liver cells (L-02) and renal proximal tubular epithelial cells (HK-2). As shown in Table 2, compound **4a** with a tertiary amine side chain manifested obvious toxic effect on L-02 and HK-2 with IC_{50}s of 1.77 and 0.99 μM, respectively. The N-oxide **5** showed a remarkable decrease in cytotoxicity by about 100 folds. It indicated that N-oxide **5** would be a promising prodrug if only N-oxide can be bioreduced to compound **4a** in the hypoxic condition of tumor tissues.

3.3. In vivo anti-cancer activity evaluation

Since N-oxide **5** showed low cytotoxic activity in the *in vitro* assay, the next step was to employ an *in vivo* assay to investigate if compound **5** has the *in vivo* anti-cancer activity, since it could be possibly transformed to the active form. A study using sarcoma S-180 bearing mice was performed; mice body weights and S-180 tumor weights were used as evaluating indicators which were illustrated in Fig. 3. To examine the efficiency of prodrug **5**, both **4a** and **5** were evaluated in three dose levels (7.5, 15 and 30 mg/kg for **4a**, 30, 45 and 60 mg/kg for **5**, intraperitoneal). Compared with amonafide (ST, 30 mg/kg), **4a** showed similar tumor growth suppression activity when administered at low dose (7.5 mg/kg, 26.25% suppression). However, when the dose was increased to 15 and 30 mg/kg, mice were died before termination of the tests. Obviously, **4a** was more toxic to the animals than the parental amonafide. **5** displayed dose-dependent tumor suppression activity when administered at 30, 45 or 60 mg/kg once a day, with 4.83%, 46.18% or 62.74% tumor growth suppression compared with the control group, respectively.

For compound **5** to achieve a comparable tumor suppression activity to amonafide, a much higher dosage (60 mg/kg) is needed. This phenomenon is possible due to the insufficient *in vivo* bioreduction of the prodrug [11]. Low bioreductive efficiency resulted low transformation of compound **5** to **4a**, which is actually the effective agent to inhibit hypoxic tumor growth. However, taking account of the complexity of *in vivo* metabolism process, the exact cause for low tumor suppression activity of prodrug **5** still need further investigation. Nevertheless, all the data suggest that N-oxide **5** is an efficient and low toxic anti-cancer prodrug and has the value for more investigation to improve the therapeutic efficacy.

4. Conclusion

In summary, a series of triazolonaphthalimide derivatives were synthesized via a novel method and displayed much potent cytotoxicities than the lead compound amonafide. The preliminary SAR revealed that 1) by fusing a triazole ring onto the naphthalene core can increase the intercalating plane and significantly improve the cytotoxic activity and 2) activity would be enhanced if the triazole ring possessed another basic amino side chain. The *in vitro* and *in vivo* invistigations suggest that N-oxide **5**, a prodrug synthesized from **4a**, could be bioreduced to its precursor amine form **4a** and

displayed dose-dependent tumor growth suppression activity. All the data demonstrated that this novel series of triazolonaphthalimides merited further investigation and derivatization, and the N-oxide **5** could be used as promising prodrug which possessed selectivity for hypoxic cancer cells, so an improved therapeutic efficacy.

5. Experimental section

5.1. Synthesis

All the solvents are of analytical grade. CH_3CN used in the reaction was distilled with P_2O_5 according to the reported method. ^1H NMR and ^{13}C NMR spectra were recorded with Bruker AM-400 MHz spectrometer. The chemical shifts were reported in ppm using TMS as internal standard. High resolution mass spectrometry data were measured on Bruker Apex IV FTMS. Melting points were measured on an X-5 micro-melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was developed on commercial silica gel HSGF254 plates. Column chromatography was conducted on silica gel 60 (E. Merck, 0.063–0.200 mm).

5.1.1. General procedure for synthesis and purification of *4a–e* and *4′a–e*

To obtain compounds **4a**–**e** and **4′a**–**e**, we first synthesized the precursor compound **3** and **3′** from commercially available 1,8-naphthalic acid anhydride following three steps according to the reference methods [31,32], illustrated above in Chemistry section.

Then compound **3** or **3′** (0.5 mmol) was suspended in 5 mL dry CH_3CN at −5 °C and cooled for 10 min under nitrogen atmosphere. Then nitrosonium tetrafluoroborate ($NOBF_4$, 1 mmol) was added and stirred at −5 °C for 1 h to obtain a clear solution. The resulting mixture was added dropwise into a solution of corresponding amine (4 mmol) in 3 mL Et_2O/H_2O (v/v, 10:1), and then raised the temperature to 0 °C. After the reaction was finished monitored by TLC, 1 mmol Na_2CO_3 was added and the solution was stirred for additional 30 min at room temperature. After removal of the solvent in vacuum, the crude product was purified by column chromatography (CH_2Cl_2/MeOH, 1–5‰ Et_3N) to obtain compound **4** or **4′**, respectively (yield 58–95%).

5.1.2. 5-[2-(Dimethylamino)ethyl]-10-methylbenzo[de] [1–3] triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione (*4a*)

Light yellow solid, yield: 95%; m.p. 214–216 °C. ^1H NMR (400 MHz, DMSO) δ_H(ppm): 8.66 (1H, d, J = 8.2 Hz), 8.55 (1H, s), 8.35 (1H, d, J = 7.3 Hz), 7.86 (1H, t, J = 7.8 Hz), 4.60 (3 H, s), 4.07 (2 H, t, J = 6.5 Hz), 2.58 (2 H, t, J = 6.4 Hz), 2.30 (6 H, s). ^{13}C NMR (100 MHz, DMSO): 163.3, 162.9, 143.0, 131.7, 130.2, 129.0, 128.3, 126.6, 124.1, 122.8, 119.5, 118.3, 56.6, 45.6 (2C), 38.2, 38.1. HR-ESI-MS: Calcd for $C_{17}H_{17}N_5O_2$ $[M + H]^+$: 324.1455; Found: 324.1453.

5.1.3. 5-[3-(Dimethylamino)propyl]-10-methylbenzo[de] [1–3] triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione (*4′a*)

Yellow solid, yield: 68%; m.p. 207–208 °C. ^1H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.11 (1H, s), 8.68 (2 H, dd, J = 7.8, 0.8 Hz), 7.94 (1H, t, J = 7.7 Hz), 4.74 (3 H, s), 4.23 (2 H, t, J = 7.6 Hz), 2.46 (2 H, t, J = 6.8 Hz), 2.27 (6 H, s), 2.00–1.81 (2 H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 163.7, 163.3, 143.9, 131.9, 130.6, 127.9, 127.6, 127.2, 125.6, 123.9, 120.2, 118.6, 57.2, 45.2 (2C), 39.1, 38.0, 25.9. HR-ESI-MS: Calcd for $C_{18}H_{19}N_5O_2$ $[M + H]^+$: 338.1613; Found: 338.1612.

5.1.4. 5-[2-(Dimethylamino)ethyl]-10-butylbenzo[de] [1–3]triazolo [4,5-g]isoquinoline-4,6(5H,10H)-dione (*4b*)

Yellow solid, yield: 73%; m.p. 204–207 °C. ^1H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.16 (1H, s), 8.72 (1H, d, J = 7.5 Hz), 8.57 (1H, d, J = 7.8 Hz), 7.97 (1H, t, J = 7.9 Hz), 5.09 (2 H, t, J = 7.3 Hz), 4.34 (2 H, t, J = 7.0 Hz), 2.69 (2 H, t, J = 7.0 Hz), 2.37 (6 H, s), 2.16–2.06 (2 H, m), 1.52 (2 H, m), 1.04 (3 H, t, J = 7.4 Hz). ^{13}C NMR (100 MHz, DMSO): 163.3, 162.9, 142.9, 132.0, 130.1, 129.3, 127.9, 126.9, 124.2, 122.9, 119.6, 118.3, 56.2, 52.8, 45.1 (2C), 37.6, 30.7, 19.1, 13.4. HR-ESI-MS: Calcd for $C_{20}H_{23}N_5O_2$ $[M + H]^+$: 366.1927; Found: 366.1925.

5.1.5. 5-[3-(Dimethylamino)propyl]-10-butylbenzo[de] [1–3] triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione (*4′b*)

Brown solid, yield: 67%; m.p. 197–199 °C. ^1H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.17 (1H, s), 8.73 (1H, d, J = 7.4 Hz), 8.58 (1H, d, J = 8.1Hz), 7.97 (1H, t, J = 7.9 Hz), 5.09 (2 H, t, J = 7.3 Hz), 4.25 (2 H, t, J = 7.3 Hz), 2.48 (2 H, t, J = 7.2 Hz), 2.35–2.18 (6 H, m), 2.19–2.03 (2 H, m), 2.03–1.86 (2 H, m), 1.59–1.46 (2 H, m), 1.04 (3 H, t, J = 7.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 163.8, 163.4, 144.0, 131.2, 130.5, 127.9, 127.8, 127.3, 125.8, 124.0, 120.2, 118.4, 57.2, 50.7, 45.2 (2C), 39.1, 31.2, 25.9, 19.8, 13.5. HR-ESI-MS: Calcd for $C_{21}H_{25}N_5O_2$ $[M + H]^+$: 380.2079; Found: 380.2081.

5.1.6. 5-[2-(Dimethylamino)ethyl]-10-(2-hydroxyethyl)benzo[de] [1–3]triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione (*4c*)

Pale yellow solid, yield: 63%; m.p. 202–204 °C. ^1H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 8.86 (1H, s), 8.82 (1H, d, J = 8.0 Hz), 8.62 (1H, d, J = 7.0 Hz), 7.89 (1H, t, J = 7.9 Hz), 5.15 (2 H, t, J = 5.1Hz), 4.38 (1H, t, J = 5.2 Hz), 4.15 (1H, t, J = 6.9 Hz), 2.59 (2 H, t, J = 6.8 Hz), 2.31 (7 H, br). ^{13}C NMR (100 MHz, DMSO): 163.6, 163.2, 142.8, 132.0, 130.2, 129.4, 128.0, 127.0, 124.2, 122.9, 119.6, 118.3, 59.9, 56.2, 52.8, 45.1 (2C), 37.6. HR-ESI-MS: Calcd for $C_{18}H_{19}N_5O_3$ $[M + H]^+$: 354.1561; Found: 354.1561.

5.1.7. 5-[3-(Dimethylamino)propyl]-10-(2-hydroxyethyl)benzo[de] [1–3]triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione (*4′c*)

Pale yellow solid, yield: 61%; m.p. 200–201 °C. ^1H NMR (400 MHz, DMSO) δ_H(ppm): 8.98 (1H, d, J = 8.3 Hz), 8.85 (1H, s), 8.52 (1H, d, J = 7.3 Hz), 7.98 (1H, t, J = 7.9 Hz), 5.19 (2 H, t, J = 4.9 Hz), 4.09–4.00 (4 H, m), 2.89 (2 H, br), 2.54 (6 H, s), 2.07–1.89 (2 H, m). ^{13}C NMR (100 MHz, DMSO): 164.0, 163.5, 143.3, 132.4, 130.6, 129.8, 128.4, 127.5, 124.6, 123.4, 120.2, 118.7, 59.9, 55.1, 52.8, 43.0 (2C), 37.7, 23.7. HR-ESI-MS: Calcd for $C_{19}H_{21}N_5O_3$ $[M + H]^+$: 368.1716; Found: 368.1717.

5.1.8. 5,10-Bis[2-(dimethylamino)ethyl]benzo[de] [1–3]triazolo [4,5-g]isoquinoline-4,6(5H,10H)-dione (*4d*)

Yellow solid, yield: 73%; m.p. 182 °C. ^1H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.14 (1H, s), 8.70 (1H, dd, J = 7.5, 0.8 Hz), 8.63 (1H, dd, J = 8.0, 0.8 Hz), 7.94 (1H, t, J = 7.9 Hz), 5.16 (2 H, t, J = 7.2 Hz), 4.32 (2 H, t, J = 6.9 Hz), 2.99 (2 H, t, J = 7.2 Hz), 2.67 (2 H, t, J = 6.9 Hz), 2.36–2.35 (6 H, 2s). ^{13}C NMR (100 MHz, $CDCl_3$): 163.8, 163.4, 143.9, 131.5, 130.7, 127.9, 127.3, 125.9, 124.0, 120.3, 118.4, 57.8, 56.9, 49.5, 45.7, 38.5. HR-ESI-MS: Calcd for $C_{20}H_{24}N_6O_2$ $[M + H]^+$: 381.2035; Found: 381.2034.

5.1.9. 10-[2-(Dimethylamino)ethyl]-5-[3-(dimethylamino)propyl] benzo[de] [1–3]triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione (*4′d*)

Yellow solid, yield: 64%; m.p. 175–177 °C. ^1H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.14 (1H, s), 8.72 (1H, d, J = 7.8 Hz), 8.67 (1H, d, J = 7.8 Hz), 7.98 (1H, t, J = 7.9 Hz), 5.18 (2 H, t, J = 7.1Hz), 4.27 (2 H, t, J = 7.3 Hz), 3.02 (2 H, t, J = 7.1Hz), 2.85–2.71 (2 H, m), 2.52 (6 H, s), 2.38 (6 H, s), 2.16–2.01 (2 H, m). ^{13}C NMR (100 MHz, DMSO/$CDCl_3$): 163.4, 163.0, 142.9, 131.1, 130.1, 128.8, 128.2, 127.0, 124.2, 123.1, 119.8, 118.0, 57.0, 54.7, 48.3, 45.4, 45.0, 42.4, 23.2. HR-ESI-MS: Calcd for $C_{21}H_{26}N_6O_2$ $[M + H]^+$: 395.2189; Found: 395.2190.

*5.1.10. 5-[2-(Dimethylamino)ethyl]-10-[3-(dimethylamino)propyl] benzo[de] [1–3]triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione (**4e**)*

Yellowish white solid, yield: 70%; m.p. 214–216 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.21 (1H, s), 8.78 (1H, d, J = 7.9 Hz), 8.73 (1H, d, J = 7.6 Hz), 7.94 (1H, t, J = 7.9 Hz), 5.17 (2 H, t, J = 8.0 Hz), 4.35 (2 H, t, J = 7.6 Hz), 2.67 (2 H, t, J = 7.2 Hz), 2.42 (2 H, t, J = 6.5 Hz), 2.35 (6 H, s), 2.31–2.21 (8 H, br). ^{13}C NMR (100 MHz, DMSO): 164.3, 163.9, 143.6, 131.9, 130.9, 129.8, 129.0, 127.7, 124.9, 123.7, 120.6, 118.7, 55.5, 54.6, 48.3, 43.5 (2C), 43.1 (2C), 36.2, 24.5. HR-ESI-MS: Calcd for $C_{21}H_{26}N_6O_2$ $[M + H]^+$: 395.2190; Found: 395.2190.

*5.1.11. 5,10-Bis[3-(dimethylamino)propyl]benzo[de] [1–3]triazolo [4,5-g]isoquinoline-4,6(5H,10H)-dione (**4′e**)*

Yellow solid, yield: 58%; m.p. 193–195 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.10 (1H, s), 8.76 (1H, d, J = 8.0 Hz), 8.69 (1H, d, J = 7.6 Hz), 7.95 (1H, t, J = 8.0 Hz), 5.16 (2 H, t, J = 7.2 Hz), 4.30 (2 H, t, J = 8.4 Hz), 2.50 (2 H, t, J = 7.2 Hz), 2.44 (2 H, t, J = 6.4 Hz), 2.30–2.23 (14 H, br), 1.99–1.94 (2 H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 163.6, 163.2, 143.6, 131.2, 130.4, 127.8, 127.8, 127.5, 125.4, 123.6, 112.0, 118.3, 57.0, 55.8, 48.9, 45.9, 45.5 (2C), 45.1 (2C), 27.6, 25.7. HR-ESI-MS: Calcd for $C_{22}H_{28}N_6O_2$ $[M + H]^+$: 409.2344; Found: 409.2346.

*5.1.12. N,N-dimethyl-2-[10-methyl-4,6-dioxobenzo[de] [1–3] triazolo[4,5-g]isoquinolin-5(4H,6H,10H)-yl]ethanamine oxide (**5**)*

A solution of **4a** (1 g, 3 mmol) in 50 mL CH_2Cl_2 was cooled for 5 min in an ice bath, added m-CPBA (0.57 g, 3.3 mmol) and stirred for 0.5 h, then moved to room temperature for further 0.5 h. 1.1 eq K_2CO_3 and 50 mL MeOH were added to quench the reaction and stirred about 5 min, the solution became limpid. Then removed the solvent and purificated with column chromatography (aluminum oxide, neutral, 100–200 Å) can afford compound **5**. Pale yellow solid, yield: 95%; m.p. 236 °C. ^{1}H NMR (400 MHz, DMSO) δ_H(ppm): 9.29–8.89 (2 H, m), 8.64 (1H, d, J = 7.3 Hz), 8.10 (1H, t, J = 7.9 Hz), 4.77 (3 H, s), 4.58 (2 H, t, J = 6.4 Hz), 3.99 (2 H, t, J = 6.5 Hz), 3.56 (6 H, s). ^{13}C NMR (100 MHz, DMSO): 163.1, 162.7, 142.6, 131.6, 130.1, 129.1, 128.1, 126.4, 124.1, 122.3, 119.1, 118.1, 64.7, 56.0, 42.8, 37.9, 33.9. HR-ESI-MS: Calcd for $C_{22}H_{28}N_6O_2$ $[M + H]^+$: 340.1410; Found: 340.1411.

*5.1.13. 2-[2-(dimethylamino)ethyl]-5-[(4-methoxylphenyl)amino]-1H-benzo[de]isoquinoline-1,3(2H)-dione (**6a**)*

Compound **6a** and **6b** was synthesized via the same procedure for compounds **4** and **4′**. After the reaction completed, the product was existed as precipitate in the resulted solution and could be obtained by filtration then purified with column chromatography.

Yellow solid, yield: 41%; m.p. 168 °C. ^{1}H NMR (400 MHz, DMSO) δ_H(ppm): 8.44 (1H, d, J = 1.9 Hz), 8.36 (1H, d, J = 8.2 Hz), 8.29 (1H, d, J = 7.2 Hz), 8.22 (1H, d, J = 1.7 Hz), 7.78 (1H, t, J = 7.8 Hz), 7.56 (2 H, d, J = 8.9 Hz), 7.04 (2 H, d, J = 9.0 Hz), 4.15 (2 H, t, J = 6.8 Hz), 3.81 (3 H, s), 2.52 (2 H, m), 2.22 (6 H, s). ^{13}C NMR (100 MHz, DMSO): 163.8, 163.6, 158.9, 142.8, 142.1, 133.8, 133.1, 128.8, 128.2, 124.3, 123.7, 122.5, 121.8, 120.6, 116.1, 115.0, 56.9, 55.8, 45.8, 38.1. HR-ESI-MS: Calcd for $C_{23}H_{23}N_3O_3$ $[M + H]^+$: 390.1812; Found: 390.1812.

*5.1.14. 2-[2-(dimethylamino)ethyl]-5-[(4-nitrophenyl)amino]-1H-benzo[de]isoquinoline-1,3(2H)-dione (**6b**)*

Yellow solid, yield: 52%; m.p. 189–191 °C. ^{1}H NMR (400 MHz, DMSO) δ_H(ppm): 10.00 (1H, d, J = 2.0 Hz), 9.44 (1H, d, J = 2.0 Hz), 8.97 (2 H, d, J = 8.0 Hz), 8.30 (1H, t, J = 8.0 Hz), 8.00 (1H, s), 7.98 (1H, s), 6.65 (2 H, d, J = 8.0 Hz), 4.47 (2 H, t, J = 5.9 Hz), 3.53 (2 H, t, J = 5.9 Hz), 2.98 (3 H, s), 2.97 (3 H, s). ^{13}C NMR (100 MHz, DMSO): 163.8, 163.7, 155.7, 134.8, 132.2, 130.4, 128.0, 127.9, 126.4, 125.7, 123.4, 123.4, 122.2, 122.2, 114.1, 112.4, 55.0, 42.9 (2C), 35.2. HR-ESI-MS: Calcd for $C_{22}H_{22}N_4O_4$ $[M + H]^+$: 405.1558; Found: 405.1557.

5.2. Materials and methods for bioactivity assays

5.2.1. Animals

Male ICR mice weighing from 16 to 18 g were purchased from Sino-British Sippr/Bk Lab.Animal Ltd.,Co. They were raised at controlled environment with a 12 h light–dark cycle, drinking and eating were provided ad libitum.

5.2.2. Preparation of test solutions

Each test solution for *in vitro* assay was prepared by diluting with DMSO. Solutions for *in vivo* assay were dissolved by 0.9% physiological saline to obtain a series of concentrations: **ST** (3 mg/mL), **4a** (0.75 mg/mL, 1.5 mg/mL, 3 mg/mL), **5** (3 mg/mL, 4.5 mg/mL, 6 mg/mL).

5.2.3. In vitro cytotoxicity evaluation

All cells used in the research were prepared at 3.5 × 104 cells/mL concentration and each 100 μL cells suspension was seeded in 96-well cell imcroplate for 24 h (37 °C, 5%CO_2). Then each solution was added and incubated for another 72 h. For the control group, equivalent concentration of DMSO (final concentration 0.5%) was added. MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide) method was employed to measure the number of surviving cells and recorded the OD value at 492nm/620 nm. The IC_{50} values were calculated using Prism Graphpad software of the triplicate experiment.

5.2.4. In vivo evaluation of anti-cancer activity

Sarcoma S-180 cells were inoculated hypodermically in male ICR mice and grew for a week. The injection volume for the first batch was 2.5×10^6 cells/animal (used for compound 5), the second batch 3×10^6 cells/animal (used for compound 4a). The rats were divided into eight groups (ten rats/group) according to their weights: model (0.9% physiological saline), **ST** (amonafide, dose 30 mg/kg, positive control), **4a** (three groups with 7.5 mg/kg, 15 mg/kg, 30 mg/kg, respectively), **5** (three groups with 30 mg/kg, 45 mg/kg, 60 mg/kg, respectively). Each test compound was injected intraperitoneally once a day over seven consecutive days. The weights of rats were recorded every two days. Sarcoma S-180 were excised and weighed 24 h after the last time of injection. Both of the changes in rats body weights and the sarcoma 180 tumor weights were summarized for further analysis.

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