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Novel efficient anticancer agents and DNA-intercalators of 1,2,3-triazol-1,8-naphthalimides: design, synthesis, and biological activity

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ABSTRACT

Two novel series of 3-1,2,3-triazol-1,8-naphthalimides **5a**–**e**, **7a**–**e** were synthesized easily by employing 'click reaction'. Their bioactivities were evaluated. Compounds **5a**–**e** were found to be more toxic against MCF-7 cells while **7a**–**e** were more potent against 7721 cells, in particular **7a**, the IC_{50} value of which against cell lines of MCF-7 and 7721 was 0.348 μM and 0.258 μM, respectively. Due to the phenyl group linked to 1,2,3-triazole, compound **5a** not only showed higher DNA affinity but also more efficient DNA damaging ability than compound **7a**.

1. Introduction

Triazole derivatives had attracted much attention in chemistry, biology, and medicine fields because of their stable metabolism, high selectivity, and less adverse reaction. They were widely used as antifungal agents, anticonvulsant agents, and especially popular in anticancer field.[1–3] As shown in Fig. 1, Compound (a) as aromatase inhibitor could reduce the growth-stimulatory effect of estrogens in estrogen-dependent breast cancer.[4] Compound (b) effectively inhibited epithelia proliferation.[5] Contragestazol (c) had outstanding effect on suppressing the Oophoroma cells.[6] All these compounds were under clinical trial for cancer therapy.

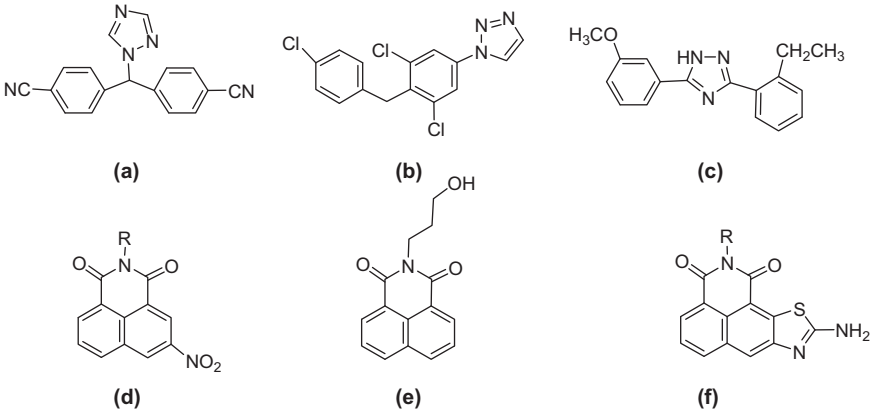

Fig. 1. Some excellent compounds of triazole and naphthalimide derivatives.

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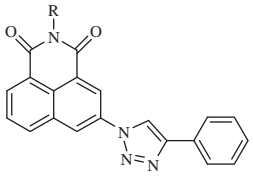
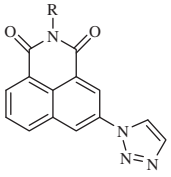
In oncotherapy area, DNA is often referred to be one of the most promising biological targets of developing anti-tumor agents to specifically constrain tumor cell growth.[7] Design and synthesis of novel and effective DNA-targeted compounds, especially DNA-intercalators have important theoretical significance and application value. Studying the interaction between DNA-intercalating molecules and DNA, in particular, effects of structural characters of molecular on the interaction, could offer novel insights into the design of new DNA targeting anti-tumor drugs.[8] Naphthalimide was a significant DNA-targeted matrix for its planar chromophore and modifiable structure. Successful examples including Amonafide (**d**), NI (**e**), and 2-aminothiazonaphthalimides (**f**).[9–11]

Fusing heterocycles to naphthalene nucleus had been widely reported, but few attentions had been paid to the linkage of functional rings with a single covalent bond,[12] much less combination of 1,2,3-triazole and naphthalimides. 1,2,3-Triazole compounds do not exist in natural substances, but they have been artificially synthesized with many ways due to their attractive features, such as large dipole moment,[13] stable to metabolic degradation, capable of hydrogen bonding,[14] and they also could react with protein in different forms. What is more, it is often referred as the classical bioisosteres of amide for its similar space structure and electrical effect to amide.[15] It is known that 3-amino-naphthalimide (Amonafide), which had been used in clinic II, was effective to various mammary cancers.[16] Unfortunately its adverse side effects blocked its further research. Herein, our group continued to expand the diversity of naphthalimide derivatives, so novel series of 3-1,2,3-triazol-1,8-naphthalimides **5a**–**e** and **7a**–**e** (Fig. 2) were designed and synthesized. 1,2,3-Triazole instead of amino was linked to 3-site of naphthalimide by 'click reaction', which was a mildness approach with high yields under environmental-friendly condition.[17] In doing so, we hoped that new properties could be endowed to the whole molecule and the horizon for designing naphthalimides could be broadened.





5a R=$CH_2CH_2N(CH_3)_2$
5b R=$CH_2CH_2N(CH_2CH_3)_2$
5c R=$CH_2CH_2CH_2CH_3$
5d R=CH_2CH_2OH
5e R=$CH_2CH_2N(CH_2CH_2)_2NH$

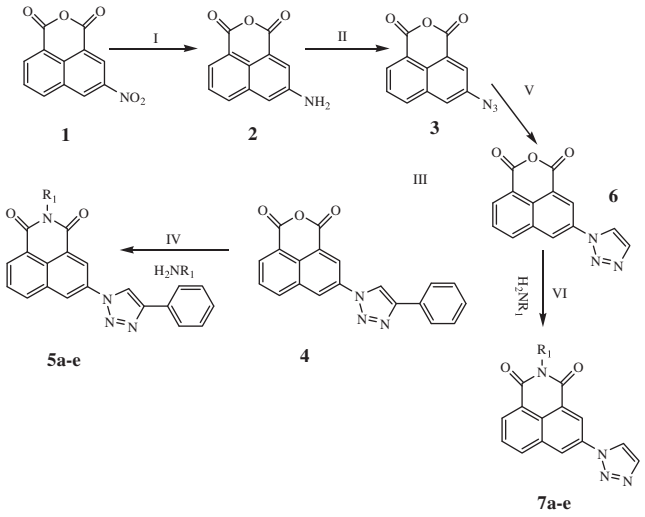
7a R=$CH_2CH_2N(CH_3)_2$
7b R=$CH_2CH_2N(CH_2CH_3)_2$
7c R=$CH_2CH_2CH_2CH_3$
7d R=CH_2CH_2OH
7e R=$CH_2CH_2N(CH_2CH_2)_2NH$

Fig. 2. The structures of 1,2,3-triazol-naphthalimide derivatives.

2. Results and discussion

2.1. Synthesis and spectra

Preparation processes of all these compounds **5a**–**e** and **7a**–**e** were shown in Scheme 1. 3-Nitro-1,8-naphthalic anhydride **1** was reduced with hydrogen to afford compound **2**.[18] Afterward, compound **2** was reacted with sodium nitrite in glacial acetic acid and concentrated hydrochloric acid at 0–5 °C for 1 h, subsequently sodium azide was dropped into the mixture and compound **3** was obtained. Cyclization of 3-azido-naphthalic (**3**) with alkyne under copper(II) and sodium-ascorbate catalysis yielded compound **4** and **6** with high yield. Without further purification, the obtained anhydride compounds were condensed with the corresponding amine in ethanol to form the corresponding targeted naphthalimides **5a**–**e** and **7a**–**e**. Structures of all the compounds were confirmed by ^{1}H NMR, HRMS, and IR spectra. The above experiments provided an approach of the linkage of 1,2,3-triazole to 3-site of naphthalimide by 'click reaction' with high yields and simple purification steps, which could further expand the variety of naphthalimide derivatives.



a R=$CH_2CH_2N(CH_3)_2$ **b** R=$CH_2CH_2N(CH_2CH_3)_2$ **c** R=$CH_2CH_2CH_2CH_3$
d R=CH_2CH_2OH **e** R=$CH_2CH_2N(CH_2CH_2)_2NH$

Scheme 1. Synthesis of 1,2,3-triazole-naphthalimides **5a**–**e** and **7a**–**e**. Reagents and conditions: (I) 10% Pd/C, H_2, DMF, 60 °C, 86.7% yield; (II) $NaNO_2$, HCl, CH_3COOH, NaN_3, H_2O, 0–5 °C, 86.9% yield; (III) phenylacetylene, $CuSO_4{\cdot}5H_2O$, sodium-ascorbate, H_2O/BuOH, rt, 92.3% yield; (IV) RNH_2, CH_3CH_2OH, refluxed; (V) acetylene, $CuSO_4{\cdot}5H_2O$, sodium-ascorbate, H_2O/*t*-BuOH, rt, 92.3% yield; (VI) RNH_2, CH_3CH_2OH, refluxed.

The UV–vis and Fluorescent data of **5a**–**e** and **7a**–**e** were measured and shown in Table 1. The different substitutes of **5a**–**e** and **7a**–**e** were found to have only slight effect on the UV–vis spectra, with the similar value of intensities around 4.15. Maximal absorption of **5a**–**e** was slightly higher than that of **7a**–**e** because of the impact of phenyl. It also can be seen that the maximal emission of these compounds was around 400 nm and corresponding fluorescence quantum yields of **5a**–**e** were low relatively. Such low yields might be caused by the rapid non-radicalization transitions, such as IST from a singlet to the triplet, vibrational relaxation, and internal or external conversion due to the vitality of single bond connecting triazole and naphthalimide.

Table 1
UV–vis and fluorescent data of **5a**–**e** and **7a**–**e**

Compound	UV λ_{max}[a] (lg ε)	FL λ_{max}[a] (Φ[b])	Stokes' shift/nm	Excitation wavelength λ/nm
5a	342 (4.15)	401 (<0.001)	59	342
5b	342 (4.16)	399 (<0.001)	57	342
5c	343 (4.18)	400 (<0.001)	57	343
5d	343(4.17)	401 (<0.001)	58	343
5e	342 (4.11)	400 (<0.001)	58	342
7a	339 (4.27)	402 (0.006)	63	339
7b	338 (4.15)	402 (0.005)	64	338
7c	339 (4.18)	403 (0.008)	64	339
7d	338 (4.17)	403 (0.004)	65	338
7e	339 (4.11)	404 (0.003)	65	339

[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard (Φ=0.55).

2.2. Cytotoxicity

The anti-tumor activity of the compounds **5a**–**e** and **7a**–**e** was evaluated in vitro against MCF-7 (human mammary cancer cell), Hela (human cervical carcinoma cell), and 7721 (human liver

cancer cell) by MTT tetrazolium assay. The IC_{50} values were listed in Table 2 and activities of Amonafide were as a control. It can be seen from Table 2 that compounds **5a**–**e** were to be more toxic against MCF-7 than Hela and 7721 cells, and **5a** bearing *N*,*N*-dimethylethylenediamine was the most effective compound. Its inhibition abilities against three cell lines were 5.6-fold, 2.4-fold, and 8.5-fold higher than that of Amonafide under the same experimental conditions, respectively. Unlike **5a**–**e**, compounds **7a**–**e** were more toxic against 7721 than MCF-7 and Hela cells, especially **7d** had 2.9-fold and 5.4-fold more selectively for 7721 than cell lines of MCF-7 and Hela. To further analyze the data in Table 2, the strong resemblance between **5a**–**e** and **7a**–**e** was found that their potent were closely related with the side chains, which further demonstrated that the presence of a basic terminal group in the side chain was essential for cytotoxic activity.[19] Compounds **5c**,**d** and **7c**,**d** whose side chains had no basic terminal were less active than Amonafide. The better cytotoxic activity of **5a**–**c**, **5e**, and **7a**–**c**, **7e** hinted that 1,2,3-triazole could enhance the cytotoxicity and improve anti-tumor ability of naphthalimides compared to the activities with the famous Amonafide.

Table 2
Anti-tumor activities of compounds against MCF-7, Hela, and 7721 cells

Compound	Cytotoxicity (IC_{50}, μM)		
	MCF-7	Hela	7721
5a	0.301	0.725	0.502
5b	0.862	0.775	>100
5c	>100	>100	>100
5d	33.71	71.4	>100
5e	1.0	0.194	2.55
7a	0.348	0.357	0.258
7b	0.692	0.78	0.354
7c	>100	>100	>100
7d	45.6	85	15.6
7e	24.6	1.55	1.28
Amonafide	1.68	1.73	4.27

2.3. DNA intercalating property

DNA intercalating property of compound **5a** and **7a**, which was typical for all compounds was evaluated. As **5a** had very weak fluorescence, its UV–vis spectra was used to investigate the interaction between the compound and DNA.[20] If the compound can intercalate DNA, the UV–vis curve of their complex will be induced bathochromic shift and hypochromicity.[21,22] UV–vis curve of **5a** was shown in Fig. 3. As DNA concentration was increased, **5a** showed significant hypochromicities and slight bathochromic shifts indicating that compound can insert into the base pairs of DNA. Its binding constant was calculated with the following equation:

$$[\mathrm{DNA}]/(\varepsilon_a-\varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_b-\varepsilon_f) + 1/(K_b(\varepsilon_b-\varepsilon_f))$$

ε_a——average molar extinction coefficient of the solution ($A/C_{\mathbf{5a}}$)
K_b——the binding constant
ε_b——molar extinction coefficient of totally binding **5a**
ε_f——molar extinction coefficient of free **5a**

K_b could be calculated out with the equation. In this way, the binding constant of **5a** with DNA was $5.10\times10^5\ M^{-1}$, which was relatively higher during DNA-intercalation, indicating that **5a** had a good ability of DNA affinity.[23]

The Scatchard binding constant between CT-DNA and compound **7a** was determined by Fluorescence spectroscopy technique (in 30 mM Tris–HCl buffer, pH 7.5).[24] As shown in Fig. 4, the emission intensity of the **7a** was strong quenched and had a slightly bathochromic shift upon the addition of CT-DNA, suggesting that the transition of energy or electron was occurred between compounds and the base pairs of DNA. Its Scatchard binding constant was determined to be $3.51\times10^5\ M^{-1}$ (Fig. 5), which was a little lower

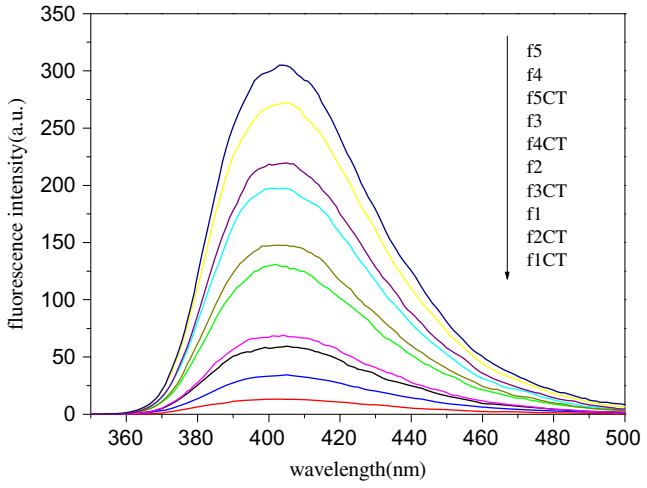


Fig. 4. Fluorescence spectra before and after interaction of compound **7a** and CT-DNA. Curves F and F-CT corresponded to compound **7a** before and after being mixed with DNA. Numbers 1–5 indicated the concentration of **7a**, 2, 5, 10, 20, 40 μM, respectively. DNA applied was 50 μM.

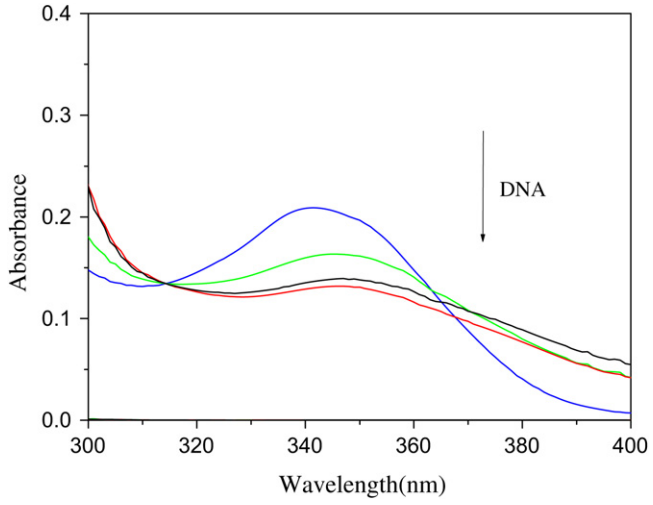


Fig. 3. Interaction of **5a** and calf thymus DNA. Absorption changes of **5a** during addition of calf thymus DNA (0, 50, 100, 200 μM) in 30 mM Tris–HCl (pH 7.5) solution.

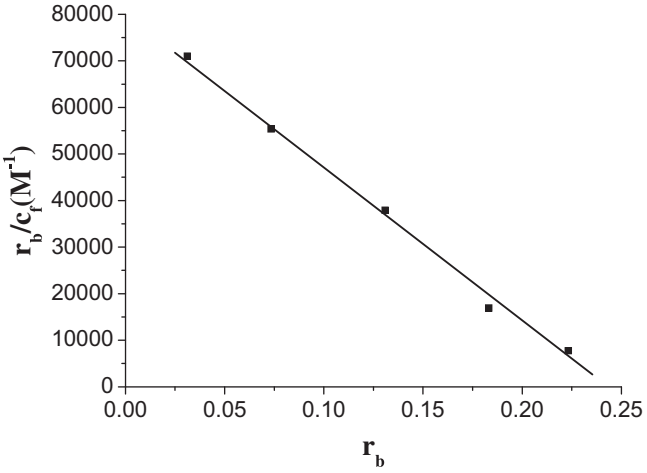


Fig. 5. Scatchard plots of spectrophotometric titration of CT-DNA to **7a** in Tris–HCl buffer.

than *N*-(*N*′,*N*′-dimethylamino-ethyl)-3-(4-phenyl-[1,2,3]-triazol-1-yl)-naphthalimide (**5a**). The results suggested that the **5a**–DNA complex was more stable with the aid of large π-conjugated systems formed by the phenyl linked to the 1,2,3-triazole of **5a**.

2.4. DNA photo-damaging property

Besides the anti-tumor activities and binding abilities of these novel naphthalimides with DNA, their photodamage abilities to DNA under light irradiation were also evaluated. The gel mobility assay is sensitive to change of DNA length or conformation, since the electrophoretic mobility of nucleic acid is proportional to the length of the nucleic acid molecules. Therefore, DNA damaging activities of all compounds were evaluated in 20 mM Tris–HCl (pH 7.5) in the presence of supercoiled plasmid DNA pBR322 under the irradiation with 365 nm-UV light for 2 h. All the compounds could apparently cleave the closed supercoiled DNA into relaxed and open circular form (form II) according to Fig. 6. Compared lane 3 and lane 7, it is easily found that **5a** had a higher DNA damaging ability than **7a** indicating that the phenyl in **5a** could assist it with a more efficient approach for action between compound and DNA.

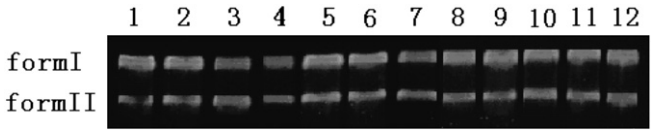


Fig. 6. Agarose gel (1%) electrophoresis assay of plasmid pBR322 DNA cleaved by compounds **5a**–**e** and **7a**–**e**. Lane 1: DNA alone (no hv); lane 2: DNA alone; lanes 3–12: DNA and compounds **5a**–**e**, **7a**–**e** at concentration of 100 mM, respectively. Irradiation for 2 h in 30 mM Tris–HCl (pH 7.5).

2.5. Viscosity measurement

To further assure the binding mode of drug and DNA, compounds **5a** and **7a** were studied on CT-DNA viscosity. The viscosity of mixture was increased obviously on the addition of compounds according to Fig. 7, which powerfully proved that compounds could intercalate into DNA and then caused the distance between adjacent base pairs to be largened and the helix to be unwound and stiffened, and so the viscosity increased.[25] It could be seen that **5a** aroused a greater viscosity's change than **7a** did, in other words, compound **5a** had a higher DNA damaging capacity. The result was parallel to their DNA binding constants.

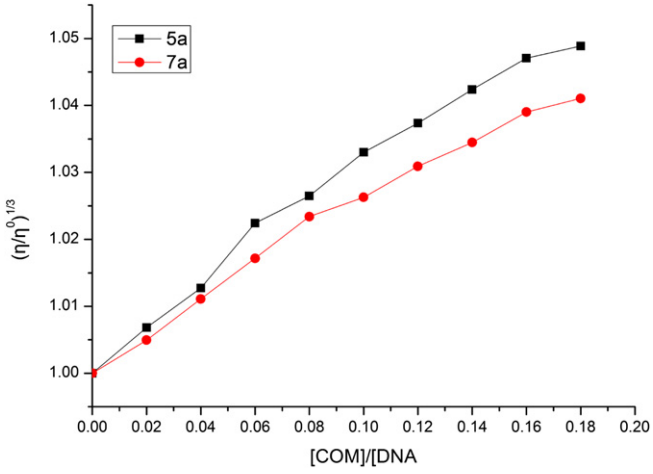


Fig. 7. Effect of increasing amounts of compounds **5a** and **7a** on the relative viscosities of CT-DNA at 25 °C, respectively. [DNA]=100 μM in Tris–HCl (30 mM, pH 7.5). η is the viscosity of DNA in the presence of the compounds and η^0 is in the absence of the compounds.

3. Conclusions

The present work focused on the design, synthesis, and quantitative evaluation of two kinds of 1,2,3-triazol-1,8-naphthalimide derivatives as anti-tumor and DNA-photocleaving agents. An easy approach of linkage of 1,2,3-triazole or 4-phenyl-1,2,3-triazole rings to the 3-site of naphthalimide instead of amino by using 'click reaction' was offered. This reconstruction improved the cytotoxicity of the compounds. Based on the testing of anti-tumor activity, **5a**–**e** were found to be more cytotoxic against MCF-7 than cell lines of Hela and 7721 while **7a**–**e** had a more selectivity on 7721 cells. In particular, compound **5a**, bearing *N*,*N*-dimethylethylenediamine with the values of IC_{50} against three cell lines was 5.6-fold, 2.4-fold, and 8.5-fold lower than that of Amonafide, and other compounds bearing aminoalkyl side chains were all more potent than Amonafide. Under the impact of phenyl, compound **5a** not only showed the higher DNA binding affinity (the binding constant was $5.10\times10^5\ M^{-1}$) but also more efficient DNA damaging ability than compound **7a**.

4. Experimental section

4.1. Materials and methods

All the solvents were analytical grade. The CT-DNA and the closed supercoiled pBR322 DNA was purchased from Takara Biotech Co., Ltd (Dalian).

Melting points were determined by an X-6 micro-melting point apparatus and were uncorrected. Infrared (IR) spectra were measured on a Nicolet 20DXB FR-I infrared spectrometer, with samples analyzed as KBr disks. High-resolution mass spectra (HRMS) were obtained on a HPLC Q-TOF MS (Micro) spectrometer. The purity of compounds was checked by ascending TLC on Merck's silica gel plates (0.25 mm) with fluorescent baking. NMR measurements (data reported in ppm) were performed on a Varian INOVA spectrometer operating at 400 MHz or an AVANCE spectrometer operating at 500 MHz, respectively. Chemical shifts δ are reported in parts per million downfield from tetramethylsilane, *J* values are in hertz, and the splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. UV spectra were recorded on HP 8453 UV–visible spectrophotometer.

4.2. Synthesis

*4.2.1. 3-Amino-naphthalic anhydride (**2**).* 3-Nitro-naphthalic anhydride (7.5 g) and Pd/C (1.2 g) were suspended in DMF (100 mL) under 1.5 MPa hydrogen gas and were stirred at 60 °C for 3 h, then cooled and the catalyzer Pd/C was removed by filtration. The filter cake was washed by DMF until filtrate was achromatic. The obtained filtrate was poured into the icewater and the yellow precipitation (5.7 g) was gained. Yield (86.7%). Mp: >300 °C.

*4.2.2. 3-Azido-naphthalic anhydride (**3**).* 3-Amino-naphthalic anhydride (4.1 g) was suspended in the mixture of concentrated HCl (30 mL) and AcOH (30 mL). A solution of sodium nitrite (3.5 g) in water (15 mL) was added dropwise within 20 min. After stirring reaction for 1 h under the temperature not beyond 5 °C, the solution of NaN_3 (3.1 g) in water (10 mL) was added dropwise within 0.5 h under the ice-water bath condition. Withdrew the ice-water bath, the reaction mixture was continued stirred for 2 h at room temperature. Then the yellow precipitation was obtained when the mixture was poured into icewater (300 mL). The desired compound (4.0 g) was obtained after filtration, washing, and drying. Yield: 86.9%, ^{1}H NMR ($CDCl_3$-d_6, 400 MHz): δ (ppm): 7.84 (t, *J*=8.0 Hz, 1H), 7.88 (d, *J*=1.6 Hz, 1H), 8.3 (d, *J*=8.0 Hz, 1H), 8.27 (d, *J*=1.6 Hz, 1H),

8.55 (d, J=8.0 Hz, 1H). IR (KBr, cm^{-1}): 3050, 2155, 1770, 1730, 1601, 1521. HRMS (m/z): $C_{12}H_5N_3O_3$, calcd: 239.0331, found: 239.0324.

*4.2.3. 3-(4-Phenyl-[1,2,3]-triazol-1-yl)-1,8-naphthalic anhydride (**4**).* Combination of the 3-azido-1,8-naphthalic anhydride (2.0 g) and phenylacetylene (0.85 g) dissolved into the mixture of *t*-BuOH (25 mL) and water (25 mL). Then sodium-ascorbate (176 mg) and $CuSO_4 \cdot 5H_2O$ (20 mg) were added. The mixture was stirred for 8 h in dark at room temperature, which was traced by TCL until the reaction completed. The mixture was poured into the icewater and the appeared yellow precipitate was filtered, washed, and dried. Then the target compound (2.65 g) was obtained. Yield: 92.3%. Mp: >300 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 7.25 (t, J=7.2 Hz, 1H), 7.31 (t, J=7.2 Hz, 2H), 7.62 (m, 3H), 8.21 (m, 2H), 8.41 (s, 1H), 8.62 (s, 1H), 9.01 (s, 1H). HRMS (m/z): $C_{20}H_{11}N_3O_3$, calcd: 341.0800, found: 341.0789.

*4.2.4. N-(N′,N′-Dimethylamino-ethyl)-3-(4-phenyl-[1,2,3]-triazol-1-yl)-naphthalimide (**5a**).* A suspension of 3-(4-Phenyl-[1,2,3]-triazol-1-yl)-1,8-naphthalic anhydride **4** (0.47 g) was treated with excess *N′*,*N′*-dimethylethane-1,2-diamine (0.3 mL) in absolute EtOH (15 mL). The mixture was heated at reflux temperature until the reaction was completed (TLC). After removing organic solvent under reduced pressure, the crude mixture was purified by flash chromatography (silica gel, CH_2Cl_2/CH_3OH, 10:1, v/v) to afford the 0.46 g yellow solid **5a**. Yield: 83%. Mp: 205.3–206.1 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 2.25 (s, 6H), 2.57 (t, J=6.8 Hz, 2H), 4.17 (t, J=6.8 Hz, 2H), 7.40 (t, J=7.2 Hz, 1H), 7.51 (t, J=7.6 Hz, 2H), 7.95 (m, 3H), 8.50 (m, 2H), 8.92 (s, 1H), 8.99 (s, 1H), 9.59 (s, 1H). HRMS (m/z): $C_{24}H_{21}N_5O_2$, calcd: 411.1695, found: 411.1703. IR (KBr, cm^{-1}): 2942, 2775, 1698, 1662, 1596, 1477, 1384, 1338, 1261, 1238, 1151, 1043, 883, 781.

*4.2.5. N-(N′,N′-Diethylamino-ethyl)-3-(4-phenyl-[1,2,3]-triazol-1-yl)-naphthalimide (**5b**).* Compound **5b** was prepared and purified in a similar manner as that in **5a**, *N′*,*N′*-diethylethane-1,2-diamine was used here instead of *N′*,*N′*-dimethylethane-1,2-diamine (CH_2Cl_2/CH_3OH, 10:1, v/v). Yield: 81%. Mp: 191.1–192.3 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 0.97 (t, J=7.2 Hz, 6H), 2.50 (m, 4H), 2.68 (t, J=7.2 Hz, 2H), 4.12 (t, J=7.2 Hz, 2H), 7.42 (t, J=7.6 Hz, 1H), 7.52 (t, J=7.6 Hz, 2H), 7.98 (m, 3H), 8.50 (m, 2H), 8.93 (d, J=2.0 Hz, 1H), 9.0 (d, J=2.4 Hz, 1H), 9.62 (s, 1H). HRMS (m/z): $C_{26}H_{25}N_5O_2$, calcd: 439.2008, found: 439.2001. IR (KBr, cm^{-1}): 2971, 2803, 1702, 1664, 1627, 1517, 1477, 1380, 1361, 1290, 1257, 1203, 1172, 1050, 881, 765.

*4.2.6. N-Butyl-3-(4-phenyl-[1,2,3]-triazol-1-yl)-naphthalimide (**5c**).* Prepared and purified in a similar manner as that in **5a**, butylamine was used here instead of *N′*,*N′*-dimethylethane-1,2-diamine and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 50:1, v/v) to get pure **5c**. Yield: 85%. Mp: 195.4–196.7 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 0.94 (t, J=7.2 Hz, 3H), 1.37 (m, 2H), 1.64 (m, 2H), 4.05 (t, J=7.6 Hz, 2H), 7.41 (t, J=7.6 Hz, 1H), 7.52 (t, J=7.6 Hz, 2H), 7.97 (m, 3H), 8.51 (m, 2H), 8.93 (d, J=1.6 Hz, 1H), 9.0 (d, J=2.4 Hz, 1H), 9.62 (s, 1H). HRMS (m/z): $C_{24}H_{20}N_4O_2$, calcd: 396.1586, found: 396.1590. IR (KBr, cm^{-1}): 2950, 1708, 1669, 1630, 1510, 1472, 1378, 1361, 1293, 1245, 1201, 1172, 1055, 883, 780.

*4.2.7. N-(2-Hydroxy-ethyl)-3-(4-phenyl-[1,2,3]-triazol-1-yl)-naphthalimide (**5d**).* Prepared and purified in a similar manner as that in **5a**, 2-amino-ethanol was used here instead of *N′*,*N′*-dimethylethane-1,2-diamine and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 5:1, v/v) to get pure **5d**. Yield: 79%. Mp: 257.3–258.6 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 3.61 (d, J=5.6 Hz, 2H), 4.20 (d, J=5.2 Hz, 2H), 4.92 (s, 1H), 7.42 (t, J=6.8 Hz, 1H), 7.53 (t, J=6.8 Hz, 2H), 7.97 (m, 3 H), 8.52 (m, 2H), 8.93 (s, 1H), 9.00 (s, 1H), 9.60 (s, 1H). HRMS (m/z): $C_{22}H_{16}N_4O_3$, calcd: 384.1222, found: 384.1230. IR (KBr, cm^{-1}): 3630, 1710, 1655, 1600, 1510, 1450, 1330, 1230, 1156, 1112, 1015, 825.

*4.2.8. N-(2-Piperazin-1-yl-ethyl)-3-(4-phenyl-[1,2,3]-triazol-1-yl)-naphthalimide (**5e**).* Prepared and purified in a similar manner as that in **5a**, 2-piperazin-1-yl-ethylamine was used here instead of *N′*,*N′*-dimethylethane-1,2-diamine and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 5:1, v/v) to get pure **5e**. Yield: 76%. Mp: 250.2–251.7 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 2.50 (m, 4H, $NHCH_2$), 2.60 (t, J=6.4 Hz, 2H), 2.76 (m, 4H, NCH_2), 4.19 (t, J=6.4 Hz, 2H), 7.41 (t, J=7.2 Hz, 1H), 7.53 (t, J=7.6 Hz, 2H), 7.96 (m, 3H), 8.52 (m, 2H), 8.95 (s, 1H), 9.01 (s, 1H), 9.63 (s, 1H). HRMS (m/z): $C_{26}H_{24}N_6O_2$, calcd: 452.1961, found: 452.1986. IR (KBr, cm^{-1}): 2919, 1700, 1662, 1625, 1590, 1519, 1436, 1234, 1149, 1041, 840.

*4.2.9. N-(N′,N′-Dimethylamino-ethyl)-3-([1,2,3]-triazol-1-yl)-naphthalimide (**7a**).* Prepared and purified in a similar manner as that in **5a**, acetylene instead of phenylacetylene was used here, and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 10:1, v/v) to get pure **7a**. Yield: 83%. Mp: 226.3–227.5 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 2.24 (s, 6H), 2.51 (t, J=6.8 Hz, 2H), 4.18 (t, J=6.8 Hz, 2H), 7.96 (t, J=7.6 Hz, 1H), 8.10 (d, J=1.2 Hz, 1H), 8.53 (m, 2H), 8.93 (d, J=2.4 Hz, 1H), 9.02 (d, J=2.0 Hz, 1H), 9.16 (d, J=1.2 Hz, 1H). HRMS (m/z): $C_{18}H_{17}N_5O_2$, calcd: 335.1382, found: 335.1382. IR (KBr, cm^{-1}): 2923, 2815, 2771, 1700, 1656, 1627, 1598, 1471, 1381, 1292, 1240, 1174, 1058, 890, 790.

*4.2.10. N-(N′,N′-Diethylamino-ethyl)-3-([1,2,3]-triazol-1-yl)-naphthalimide (**7b**).* Prepared and purified in a similar manner as that in **5b**, acetylene instead of phenylacetylene was used here, and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 10:1, v/v) to get pure **7b**. Yield: 85%. Mp: 200.5–201.5 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 0.97 (t, J=6.8 Hz, 6H), 2.56 (m, 6H), 4.13 (t, J=6.8 Hz, 2H), 7.95 (t, J=7.6 Hz, 1H), 8.10 (s, 1H), 8.52 (m, 2H), 8.93 (d, J=2 Hz, 1H), 9.02 (d, J=2 Hz, 1H), 9.16 (s, 1H). HRMS (m/z): $C_{20}H_{21}N_5O_2$, calcd: 363.1695, found: 363.1701. IR (KBr, cm^{-1}): 2925, 2811, 2750, 1711, 1651, 1616, 1601, 1462, 1376, 1276, 1233, 1168, 1043, 891, 793.

*4.2.11. N-Butyl-3-([1,2,3]-triazol-1-yl)-naphthalimide (**7c**).* Prepared and purified in a similar manner as that in **5c**, acetylene instead of phenylacetylene was used here, and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 50:1, v/v) to get pure **7c**. Yield: 86%. Mp: 191.9–193.1 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 0.94 (t, J=7.6 Hz, 3H), 1.37 (m, 2H), 1.64 (m, 2H), 4.05 (t, J=7.2 Hz, 2H), 7.94 (t, J=8.0 Hz, 1H), 8.10 (s, 1H), 8.50 (m, 2H), 8.89 (d, J=2.0 Hz, 1H), 8.99 (d, J=2.0 Hz, 1H), 9.15 (s, 1H). HRMS (m/z): $C_{18}H_{16}N_4O_2$, calcd: 320.1273, found: 320.1278. IR (KBr, cm^{-1}): 2930, 2825, 2750, 1706, 1655, 1613, 1601, 1451, 1376, 1280.

*4.2.12. N-(2-Hydroxy-ethyl)-3-([1,2,3]-triazol-1-yl)-naphthalimide (**7d**).* Prepared and purified in a similar manner as that in **5d**, acetylene instead of phenylacetylene was used here, and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 5:1, v/v) to get pure **7d**. Yield: 79%. Mp: 257.3–258.6 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 3.71 (d, J=5.6 Hz, 2H), 4.35 (d, J=5.2 Hz, 2H), 4.96 (s, 1H), 7.93 (t, J_1=8.0 Hz, 1H), 8.09 (s, 1H), 8.51 (m, 2H), 8.89 (d, J=2 Hz, 1H), 8.98 (d, J=2.4 Hz, 1H), 9.16 (s, 1H). HRMS (m/z): $C_{16}H_{12}N_4O_3$, calcd: 308.0909, found: 308.0915. IR (KBr, cm^{-1}): 3660, 1706, 1651, 1607, 1518, 1432, 1330, 1235, 1152, 1111, 1012, 821.

*4.2.13. N-(2-Piperazin-1-yl-ethyl)-3-([1,2,3]-triazol-1-yl)-naphthalimide (**7e**).* Prepared and purified in a similar manner as that in **5e**, acetylene instead of phenylacetylene was used here, and separated on silica gel chromatography (CH_2Cl_2/CH_3OH, 4:1, v/v) to get pure **7e**. Yield: 77%. Mp: 250.2–251.7 °C. ^{1}H NMR (DMSO-d_6,

400 MHz): δ (ppm): 2.53 (m, 4H, $NHCH_2$), 2.65 (t, J=7.2 Hz, 2H), 2.78 (m, 4H, NCH_2), 4.19 (t, J=7.2 Hz, 2H), 7.92 (t, J=7.6 Hz, 1H), 8.09 (s, 1H), 8.51 (m, 2H), 8.88 (d, J=2.4 Hz, 1H), 8.99 (d, J=2.0 Hz, 1H), 9.15 (s, 1H). HRMS (m/z): $C_{20}H_{20}N_6O_2$, calcd: 376.1648, found: 376.1645. IR (KBr, cm^{-1}): 2911, 1705, 1650, 1610, 1590, 1510, 1429, 1229, 1130, 1050, 833.

4.3. Spectroscopic measurements

The compounds were dissolved in absolute DMSO at the concentration of 10^{-5} M. Following spectra testings were read with Shimadzu UV for absorption spectra and with Perkin–Elmer LS 50 for fluorescence spectra.

4.4. Viscosity experiments

Calf thymus DNA was dissolved in Tris–HCl buffer (30 mM, pH 7.5) and left at 4 °C overnight. It was treated in an ultrasonic bath for 10 min, and the insoluble material was filtered through a PVDF membrane filter (pore size of 0.45 μM). The final concentration of CT-DNA was 100 μM. Viscometric titrations were performed with an Ubbelohde viscometer immersed in a thermostated bath maintained at 25 (±0.1)°C. The flow times were measured with a digital stopwatch. Each sample was measured three times, and an average flow time was calculated. Data are presented as $(\eta/\eta^0)^{1/3}$ versus [complex]/[DNA], where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flowing time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0), $\eta=(t-t_0)$.

4.5. DNA cleavage assays

The plasmid DNA cleavage experiments were performed using pBR322 DNA in Tris–HCl buffer. Reactions were performed by incubating DNA (0.05 mM bp) at 37 °C in dark in the presence/absence of the compound for the indicated time. All reactions were quenched by loading buffer. Agarose gel electrophoresis was carried out on a 1% agarose gel in 0.5×TAE (Tris–acetate–EDTA) buffer containing 0.5 μg/mL EB at 80 V for 1.5 h. The resolved bands were visualized with a UV transilluminator and quantified using Total Lab 2.01 software.

4.6. Cytotoxicity assays

MCF-7 and 7721 cell lines (1×10^5 cells/mL in 96-well culture plates) were incubated for 48 h with different concentrations of compounds dissolved in DMSO (the final volume of DMSO/medium was less than 0.001). After treatment, MTT solution (5 mg/mL in PBS) was added to each well. After 3 h incubation lysis buffer (200 g/L SDS, 50% formamide, pH 4.7) was added to each well to dissolve formazan. The absorbance was measured at 570 nm with a Microplate reader. All experiments were performed at least three times and average of the percentage absorbance was plotted against concentration. The results were expressed as percentage relative to untreated control and IC_{50} value was calculated for each compound.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2011.01.063.

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