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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 6846–6851
Bioorganic & Medicinal Chemistry

Highly efficient antitumor agents of heterocycles containing sulfur atom: Linear and angular thiazonaphthalimides against human lung cancer cell in vitro

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Received 12 June 2007; revised 2 July 2007; accepted 6 July 2007

Abstract—A novel series of aminothiazonaphthalimides, $\mathbf{A}_{1-2}$ and $\mathbf{B}_{1-2}$, has been regioselectively synthesized. The linear compounds $\mathbf{B}_{1-2}$ were evaluated to be far more active than their angular isomers $\mathbf{A}_{1-2}$ in antitumor evaluation. The linear compounds **C–F**, derived from compound $\mathbf{B}_1$, all showed highly efficient antitumor activities against A549 and P388 cell lines. Also, cytotoxicities of these four analogues against two tumor cells were highly dependent on the length of the side chains. The compound $\mathbf{A}_1$ or $\mathbf{B}_1$, with two methylene units in the side chain, was more cytotoxic than its corresponding homologue $\mathbf{A}_2$ or $\mathbf{B}_2$, with one more methylene unit.

1. Introduction

The design of highly efficient antitumor agents is of significant importance in organic and medicinal chemistry. DNA-intercalating agents are those antitumor agents that intercalate DNA via chromophores.[1] They are characterized by a planar chromophore, generally a tri- or tetracyclic ring system and one or two flexible basic side chains. Naphthalimides are the structural examples showing high antitumor activities upon a variety of murine and human tumor cells.[1–3]

Novel naphthalimides with an extra aromatic ring fused to the naphthalimide skeletons after complex multi-step synthesis have been reported.[3] Most of these active naphthalimides showed cytotoxic activities of the order of $10^{-6}-10^{-7}$ M. The presence of an extra aromatic ring was proved to improve the affinity of the intercalator for the DNA molecule, consequently to a greater cytotoxic potency. Compound azonafide, for example, was averagely 40-fold more cytotoxic against a panel of human tumor cell lines than its unfused parent compound amonafide (Fig. 1).[3a] Interestingly, azonafide also showed much stronger cytotoxicity than its angular

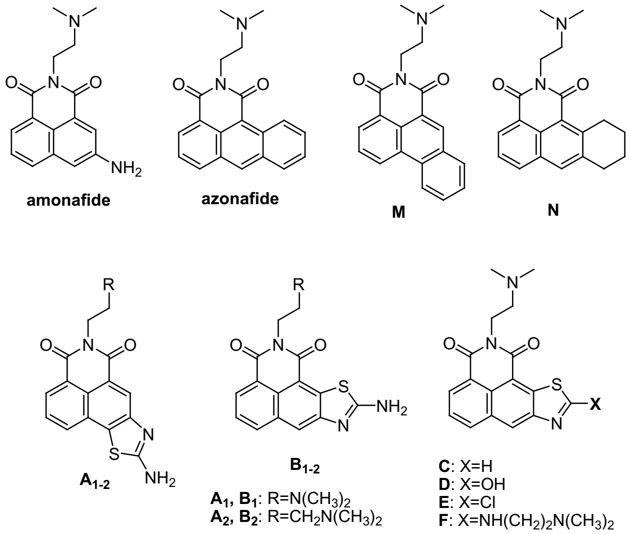

Figure 1. Structures of the reported and novel synthesized naphthalimides.

Keywords: Photocleaver; Sulfur; Heterocycle; Antitumor; Naphthalimide; Intercalation; Thiazole; DNA.

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doi:10.1016/j.bmc.2007.07.008

phenanthracene analogue **M**,[3b] indicating that the linear chromophore is preferred. Unfortunately, only one kind of such linear agent, **N**,[3b] bearing a tetrahydroanthracene nucleus instead of anthracene was further reported, which exhibited averagely lower cytotoxicity than azonafide. Here raises the question: whether the linear anthracene nucleus of azonafide is essential for higher antitumor acitivity? If this is not the case, two advantages turn up: (1) the complexed multi-step synthesis of either azonafide or tetrahydroanthracene can be avoided; (2) much more flexibility to modify the parent structure to develop novel series of antitumor agents.

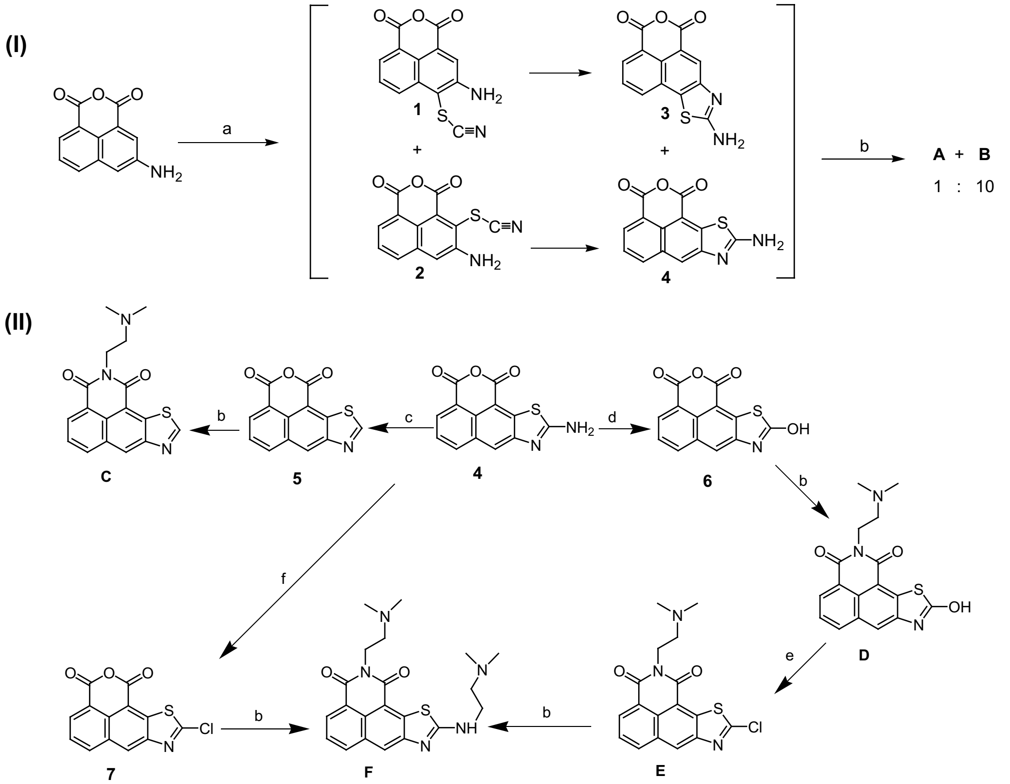
In order to answer the above question and develop novel highly cytotoxic agents, we herein report the simple but efficient synthesis and antitumor evaluation of a novel series of aminothiazonaphthalimides $\mathbf{A}_{1\text{–}2}$ and $\mathbf{B}_{1\text{–}2}$ (Fig. 1), with an aminothiazole ring angularly or linearly fused to the naphthalimide skeletons. The angular and linear isomers were obtained at the same time which enabled us to compare their cytotoxicities to study how this difference in geometry would influence their antitumor potencies.

To broaden the scope of such kind of compounds, the easy derivation of the most active compound $\mathbf{B}_1$ (see below) to compounds **C–F** by replacing the amino group with other substituent groups was further reported. The antitumor activities of these compounds were also evaluated and compared with their parent compounds.

2. Results and discussion

2.1. Synthesis and spectra data

It has been known that aromatic hydrocarbons having strong electron-donating groups such as –OH or $–NH_2$ groups reacted with thiocyanogen (formed in situ from KSCN and Br_2) to yield aryl thiocyanates resulting in *ortho* and/or *para* thiocyanato products.[4] The thiocyanate group could react with the neighborhood amine group to form an aminothiazole ring which has been proven valuable in medicinal chemistry.[5] Upon thiocyanation of 3-amino-1,8-naphthalic anhydride in acetic acid at room temperature, the thiocyanate group was introduced to one of two *ortho* positions of the amino group, resulting in isomers of **1** and **2**. The reactivity of the thiocyanate group with the amine group leads to selective formation of an aminothiazole ring. Thus, isomers of **3** and **4**, precursors of **A** and **B**, were obtained in a single step with high yield which were then condensed with corresponding amine in ethanol to form a pair of isomers, $\mathbf{A}_1$, $\mathbf{B}_1$ or $\mathbf{A}_2$, $\mathbf{B}_2$ with the ratio about 1:10 (Scheme 1(I)). To broaden the scope of such kind of linear compounds, the most cytotoxic compound $\mathbf{B}_1$ (see below) was modified to compounds **C–F** by replacing the amino group with other substituent groups as shown in Scheme 1(II). Aminothiazonaphthalic anhydride **4** (containing slight amount of **3**) was used as starting material. Diazonium salts prepared from **4** were key intermediates. All these synthetic steps were easy and



Scheme 1. Synthesis of the novel thiazonaphthalimides. Reagents and conditions: (I): (a) KSCN, Br_2, AcOH, 48 h, 95% yield; (b) corresponding amine, ethanol, reflux 2 h. (II): (c) $NaNO_2$, H_2SO_4, ethanol, 0–5 °C; reflux 1 h; (d) $NaNO_2$, H_2SO_4, 0–5 °C; reflux 1 h; (e) $SOCl_2$, $CHCl_3$, reflux 20 h; (f) $NaNO_2$, concentrated HCl, $CuCl_2$, 0–5 °C; reflux 1 h.

Table 1. Absorptions and fluorescences[a, b] of these thiazonaphthalimides

Compound	UV λmax (logε)	FLλmax (Φ)
$\mathbf{A}_1$	421 (3.50)	475 (0.003)
$\mathbf{A}_2$	422 (3.73)	478 (0.004)
$\mathbf{B}_1$	421 (3.72)	476 (0.003)
$\mathbf{B}_2$	421 (3.81)	470.4 (0.004)
C	346 (3.57)	424 (0.0002)
D	413 (3.25)	469 (0.004)
E	344 (3.48)	418 (0.0001)
F	418 (3.19)	474 (0.003)

[a] In absolute DMSO.

[b] With quinine sulfate in sulfuric acid as quantum yield standard ($\phi = 0.55$.

under mild conditions. No special and expensive reagent was used. Each pure final product was successfully separated by careful column chromatography which was well identified by ^{1}H NMR, HRMS, and IR.

The UV–vis and fluorescent data for these compounds were measured and are shown in Table 1. It was found that the substituent groups on the thiazole rings had significant effects on the spectra data. The compound without substituent group (**C**) and the one with an electron-withdrawing group **(E)** had slight difference in either absorption wavelength (around 345 nm) or emission wavelength (around 420 nm). While values of both the absorption wavelength and emission wavelength of $\mathbf{A}_{1–2}$, $\mathbf{B}_{1–2}$, **D**, and **F** are all red-shifted to around 420 nm and 470 nm due to the increase of electronic pushing–pulling ICT (intramolecular charge transfer) effects caused by the introduction of the electron-donating groups.

3. Antitumor evaluation

All these aminothiazonaphthalimides were evaluated for their cytotoxicities in vitro against A549 (human lung cancer cell) and P388 (murine leukemia cell) cell lines, respectively. The results are summarized in Table 2 and compared with activities of amonafide and azonafide.

The linear compound $\mathbf{B}_1$ was found to be the most cytotoxic one which was about 600-fold more active against A549, 200-fold against P388 than its angular isomer $\mathbf{A}_1$, in good agreement with the comparison of azonafide and its angular phenantherene analogue **M**, confirming the prevalent antitumor advantage of the linear chromophore. Also $\mathbf{B}_1$ was about 10-fold more active against A549 than the lead compound azonafide, indicating the more important role of the aminothiazole moiety than phenyl moiety in cytotoxicity evaluation.

Table 2. Cytotoxicities of $\mathbf{A}_{1–2}$, $\mathbf{B}_{1–2}$, and amonafide against cell lines of A549 and P388

Compound	Cytotoxicity (IC$_{50}$, nM)	
	A549[a]	P388[b]
$\mathbf{A}_1$	500	1027
$\mathbf{A}_2$	772	1277
$\mathbf{B}_1$	0.8	5.0
$\mathbf{B}_2$	21.8	294
Amonafide	1100	200
Azonafide	10	[c] NR

[a] Cytotoxicity (CTX) against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method after 72 h.[6]

[b] CTX against murine leukemia cells (P388) was measured by micro-culture tetrazolium-formazan method after 48 h.[7]

[c] NR, not reported.

Interestingly, all these four analogues were found to be more cytotoxic against A549 than against P388. Furthermore, all these analogues were found to be more cytotoxic against A549 than the parent compound amonafide, however, $\mathbf{B}_2$, $\mathbf{A}_1$, and $\mathbf{A}_2$ were less cytotoxic against P388 than amonafide. These results showed that the introduction of an aminothiazole moiety to the naphthalimide skeleton resulted in higher cytotoxic potencies.

Also, cytotoxicities of these four analogues against two tumor cells were highly dependent on the length of the side chains. The compound $\mathbf{A}_1$ or $\mathbf{B}_1$, with two methylene units in the side chain, was more cytotoxic than its corresponding homologue $\mathbf{A}_2$ or $\mathbf{B}_2$, with one more methylene unit. The reason accounting for it probably lies that: the cytotoxicity of one compound is determined by many conflicting factors, for example, including cell membrane crossing ability and DNA binding ability. The side chains could be protonated to different extent under physiological pH due to their different basicity of corresponding nitrogen atoms. The protonation extent significantly affected the ability of the molecule to pass through lipophilic membranes to bind to DNA. Lower degree of protonation is desirable for cell penetration, but higher degree of protonation favored DNA binding. Once these factors effectively compromise, the higher antitumor potency is possible, such as $\mathbf{A}_1$ or $\mathbf{B}_1$. The similar result has been found for amonafide or azonafide homologues.[8]

Cytotoxicities of compounds **C–F**, derived from $\mathbf{B}_1$, were then evaluated and are summarized in Table 3. All these linear analogues showed highly efficient antitumor activities of the order of $10^{-8} - 10^{-9}$ M, showing much stronger cytotoxicities than most of other reported heterocyclic fused mono-naphthalimides,[3] proving the importance of the linear thiazonaphthalene chromophores. Compound **F**, with two aminoalkyl side chains, was more cytotoxic than the other three analogues **C–E**, indicating that the introduction of a second basic side chain to the chromophore would lead to a higher cytotoxicity, similarly reported in other polycyclic systems.[9]

Table 3. Cytotoxicity of compounds **C–F** against cell lines of A549 and P388

Compound	Cytotoxicity (IC$_{50}$, nM)	
	A549	P388
C	39.8	83.9
D	45	52.5
E	88	18.5
F	30.6	8.31

Substituent groups of these compounds showed significant effect on cytotoxicities against P388, but slight effect on cytotoxicities against A549. **D**, **E**, and **F** were more cytotoxic against P388 than the non-substituted compound **C**, while **C** is more cytotoxic against A549 than **D** and **E**.

However, none of these modified analogues was more cytotoxic against A549 and P388 than the parent compound $\mathbf{B}_1$, indicating a possibility that the formation of hydrogen bond between the amino group and the sugar phosphate chain assisted the stabilization of DNA-$\mathbf{B}_1$ complex to exert its most efficient antitumor activity.

4. Conclusion

In conclusion, we have described the synthesis and antitumor evaluation of a series of novel thiazonaphthalimides. Compared with other heterocyclic fused naphthalimides, these compounds exhibited the advantages of the great convenience of synthesis and derivation. Most importantly, the angular and linear isomers were evaluated at the same time that enabled us to draw the conclusion: the linear thiazonaphthalene chromophore tends to exhibit even higher antitumor activities over the angular one.

5. Experimental

5.1. Materials and methods

All the solvents were of analytic grade. The closed supercoiled pBR322 DNA was gifted by Takara Biotech Co. Ltd (Dalian). ^{1}H NMR was measured on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in DMSO/CDCl$_3$-d_6, TMS as internal standard). Mass spectra were measured on a HP 1100 LC–MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus, and uncorrected. IR spectra were recorded on a Nicolet Nexus 770 spectrometer. Fluorescence spectra were determined on a Hitachi F-4500. Absorption spectra were determined on PGENERAL TU-1901 UV–vis Spectrophotometer.

5.2. Synthesis

5.2.1. *N*-(2-(Dimethylamino)ethyl)-2-aminothiazonaphthalimide ($\mathbf{A_1}$ and $\mathbf{B_1}$). 3-Amino-1, 8-naphthalic anhydride (1 g, 4.695 mmol) and KSCN (1.78 g, 19 mmol) were dissolved in 35 mL glacial acid. A solution of Br_2 (0.76 g, 4.775 mmol) in 12 ml glacial acid was added dropwise, the mixture was stirred for 48 h, then filtered, and dried to get the yellow mixtures of 3 and 4 (1.2 g, 4.44 mmol, 95% yield). Mp: >300 °C. 0.5 g of above-obtained solid was refluxed with *N*,*N*-dimethylethyl diamine (0.2 mL) in ethanol (20 mL) for 2 h, cooled, the solvent removed, and separated on silica gel chromatography (CH_2Cl_2: MeOH = 7:1, v/v) to get pure 0.023 g $\mathbf{A}_1$ (yield: 5%) and 0.255 g $\mathbf{B}_1$ (yield: 55%). $\mathbf{A}_1$: mp > 300 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.64 (s, 6 H, NCH_3), 3.10 (s, 2 H, NCH_2), 4.32 (t, J_1 = 6.0 Hz, J_2 = 6.0 Hz, 2 H, $CONCH_2$), 7.85 (t, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 1 H, 2-H), 8.48 (t, J = 7.6 Hz, 3 H, 1-H, NH_2), 8.72 (d, J = 8 Hz, 1 H, 3-H), 8.87 (s, 1 H, 7-H). HRMS (ESI): Calcd for $C_{17}H_{17}N_4O_2S$ $[M+H]^+$: 341.1072, Found: 341.1070. IR (KBr): 2927, 2857, 1730, 1646, 1351, 779 cm^{-1}. $\mathbf{B}_1$: mp: 290–291 °C ^{1}H NMR (DMSO-d_6) δ (ppm): 2.50 (s, 6H, NCH_3), 2.91 (s, 2 H, NCH_2), 4.26 (s, 2 H, $CONCH_2$), 7.84 (t, J_1 = 8 Hz, J_2 = 8 Hz, 1 H, 2-H), 8.03 (s, 2 H, NH_2), 8.28 (d, J = 8.2 Hz, 1-H), 8.36 (d, J = 6 Hz, 1 H, 3-H), 8.40 (s, 1 H, 10-H). HRMS (ESI): Calcd for $C_{17}H_{17}N_4O_2S$ $[M+H]^+$: 341.1072, Found: 341.1073. IR (KBr): 2921, 2850, 1696, 1654, 1330, 777 cm^{-1}.

5.2.2. *N*-(2-(Dimethylamino)propyl)-2-aminothiazonaphthalimide ($\mathbf{A_2}$ and $\mathbf{B_2}$). Prepared and purified in a similar manner as that in $\mathbf{A}_1$ and $\mathbf{B}_1$, *N*,*N*-dimethylpropyl diamine was used here instead of *N*,*N*-dimethylethyl diamine. Separated on silica gel chromatography (CH_2Cl_2: MeOH = 4:1, v/v) to get pure $\mathbf{A}_2$ (yield: 6%) and $\mathbf{B}_2$ (yield: 57%). $\mathbf{A}_2$: mp: 260–261 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.62 (s, 2 H, CH_2), 2.59 (s, 6 H, NCH_3), 2.97 (s, 2 H, NCH_2), 4.11 (s, 2 H, $CONCH_2$), 7.84 (t, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 1 H, 2-H), 8.46 (d, J = 7.8 Hz, 1 H, 1-H), 8.50 (s, 2 H, NH_2), 8.73 (d, J = 8.4 Hz, 1 H, 3-H), 8.85 (s, 1 H, 7-H). HRMS (ESI): Calcd for $C_{18}H_{19}N_4O_2S$ $[M+H]^+$: 355.1229, Found: 355.1211. IR (KBr): 2920, 2850, 1720, 1650, 1330, 779 cm^{-1}. $\mathbf{B}_2$: mp: 240–241 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.82 (t, J_1 = 6.4 Hz, J_2 = 7.2 Hz, 2 H, CH_2), 2.28 (s, 6 H, NCH_3), 3.16 (s, 2 H, NCH_2), 4.06 (t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, 2 H, $CONCH_2$, 7.79 (t, J_1 = 7.8 Hz, J_2 = 8 Hz, 1 H, 2-H), 8.01 (s, 2 H, NH_2), 8.20 (d, J = 8.4 Hz, 1-H), 8.31 (d, J = 7.2 Hz, 1 H, 3-H), 8.34 (s, 1 H, 10-H). HRMS (ESI): Calcd for $C_{18}H_{19}N_4O_2S$ $[M+H^+]$: 355.1229, Found: 355.1219. IR (KBr): 2960, 2823, 1695, 1651, 1335, 779 cm^{-1}.

5.2.3. *N*-(2-(Dimethylamino)ethyl)thiazonaphthalimide (C). The above obtained compound **4** (1 g) (containing slight amount of 3) was slowly added into mixtures of 4 mL concentrated H_2SO_4, 20 mL 95% ethanol and $NaNO_2$ (2.5 g) at 0–5 °C within 1 h, then temperature of the reaction mixture raised to reflux for 1 h, cooled and poured into 100 mL ice water, filtered, and dried to get the product of 5 (0.62 g, 65% yield). 0.5 g of above-obtained solid 5 was refluxed with *N*,*N*-dimethylethyl diamine (0.2 mL) in ethanol (20 mL) for 2 h, cooled, the solvent removed, and separated on silica gel chromatography (CH_2Cl_2: MeOH = 6:1, v/v) to afford the product C (0.32 g, 51% yield), mp: 240–241 °C ^{1}H NMR (DMSO-d_6) δ (ppm): 2.57 (s, 6H, NCH_3), 2.91 (s, 2 H, NCH_2), 4.31 (s, 2 H, $CONCH_2$), 8.00 (t, J_1 = 8 Hz, J_2 = 8 Hz, 1 H, 2-H), 8.55 (d, J = 7.2 Hz, 1-H), 8.69 (d, J = 8 Hz, 1 H, 3-H), 8.96 (s, 1 H, 10-H), 9.71 (s, 1 H, 8-H). HRMS (ESI): Calcd for $C_{17}H_{16}N_3O_2S$ $[M+H]^+$: 326.0963, Found: 326.0973. IR (KBr): 2922, 2851, 1697, 1654, 1330, 776 cm^{-1}.

5.2.4. *N*-(2-(Dimethylamino)ethyl)-2-hydroxylthiazo-naphthalimide (D). Compound **4** (4 g) (containing slight amount of 3) was slowly added in to mixtures of 4 mL concentrated H_2SO_4, 20 mL H_2O and $NaNO_2$ (0.4 g) at 0–5 °C within 1 h, then temperature of the reaction mixture raised to reflux for 1 h, cooled, filtered, and dried to get the product of 6 (3.05 g, 75% yield). 1 g of above obtain solid 6 was refluxed with *N*,*N*-dimethylethyl diamine (0.4 mL) in ethanol (40 mL) for 2 h, cooled, the solvent removed, and separated on silica gel chromatography (CH_2Cl_2: MeOH = 4:1, v/v) to afford the product D (0.63 g, 55% yield), mp: 280–281 °C, ^1H NMR (DMSO-d_6) δ (ppm): 2.56 (s, 6H, NCH_3), 2.97 (t, $J_1 = J_2 = 8$ Hz, 2 H, NCH_2), 4.26 (t, $J_1 = 8$ Hz, $J_2 = 7.2$ Hz, 2 H, $CONCH_2$), 5.765 (s, 1H, OH), 7.82 (t, $J_1 = 8$ Hz, $J_2 = 7.6$ Hz, 1 H, 2-H), 8.22 (d, $J = 8$ Hz, 1-H), 8.32 (d, $J = 7.8$ Hz, 1 H, 3-H), 8.35 (s, 1 H, 10-H). HRMS (ESI): Calcd for $C_{17}H_{16}N_3O_3S$ $[M+H]^+$: 342.0756, Found: 326.0760. IR (KBr): 2921, 2850, 1696, 1654, 1330, 777 cm^{-1}.

5.2.5. *N*-(2-(Dimethylamino)ethyl)-2-chlorothiazonaphthalimide (E). 0.6 g of above-obtained solid D was refluxed in mixtures of $SOCl_2$ (10 mL) and $CHCl_3$ (10 mL) for 20 h, cooled, the solvent removed, and separated on silica gel chromatography (CH_2Cl_2: MeOH = 6:1, v/v) to afford the product E (0.526 g, 79% yield), mp: 278–279 °C ^1H NMR (DMSO-d_6) δ (ppm): 2.91 (s, 6H, NCH_3), 3.48 (t, $J_1 = J_2 = 8$ Hz, 2 H, NCH_2), 4.43 (t, $J_1 = 8$ Hz, $J_2 = 7.2$ Hz, 2 H, $CONCH_2$), 8.03 (t, $J_1 = 6$ Hz, $J_2 = 7.2$ Hz, 1 H, 2-H), 8.58 (d, $J = 6.4$ Hz, 1-H), 8.66 (d, $J = 8$ Hz, 1 H, 3-H), 8.85 (s, 1 H, 10-H). HRMS (ESI): Calcd for $C_{17}H_{15}ClN_3O_2S$ $[M+H]^+$: 394.0837, Found: 394.0837. IR (KBr): 2921, 2850, 1696, 1654, 1330, 776 cm^{-1}.

5.2.6. *N*-(2-(Dimethylamino)ethyl)-2-*N*′-(2-(dimethyl amino)ethyl) thiazonaphthalimide (F). 0.2 g of above-obtained solid E was refluxed with *N*,*N*-dimethylethyl diamine (0.05 mL) in ethanol (10 mL) for 2 h, cooled, the solvent removed, and separated on silica gel chromatography (CH_2Cl_2: MeOH = 4:1, v/v) to afford the product F (0.119 g, 57% yield), mp: 296–297 °C ^1H NMR (DMSO-d_6) δ (ppm): 2.55 (s, 6H, NCH_3), 2.97 (t, $J_1 = J_2 = 8$ Hz, 2 H, NCH_2), 3.25 (s, 6H, NCH_3), 3.34 (s, 4 H, CH_2), 4.26 (t, $J_1 = 8$ Hz, $J_2 = 7.2$ Hz, 2 H, $CONCH_2$), 7.83 (t, $J_1 = J_2 = 8$ Hz, 1 H, 2-H), 8.26 (d, $J = 8.4$ Hz, 1-H), 8.35 (d, $J = 7.2$ Hz, 1 H, 3-H), 8.38 (s, 1 H, 10-H). HRMS (ESI): Calcd for $C_{21}H_{27}N_5O_2S$ $[M+H]^+$: 412.1807, Found: 412.1815. IR (KBr): 2920, 2850, 1696, 1654, 1330, 778 cm^{-1}.

5.2.7. 2-Chloro thiazonaphthalic anhydride (7). Compound **4** (1 g) (containing slight amount of 3) was slowly added in mixtures of 3 mL concentrated HCl and 5 mL H_2O at 0–5 °C, then mixtures of $NaNO_2$ (0.5 g) and concentrated HCl (5 mL) were slowly added into the reaction mixture, temperature of the reaction mixture raised to reflux for 1 h, cooled, filtered, and dried to get the product of 7 (0.7 g, 65% yield).

5.2.8. *N*-(2-(Dimethylamino)ethyl)-2-*N*′-(2-(dimethyl amino) ethyl) thiazonaphthalimide (F). 0.5 g of above-obtained solid **7** was refluxed with *N*, *N*-dimethylethyl diamine (0.2 mL) in ethanol (20 mL) for 2 h, cooled, removal of solvent, and separated on silica gel chromatography (CH_2Cl_2: MeOH = 7:1, v/v) to afford the product **F** (0.361 g, 58% yield).

5.3. Spectroscopic measurements

The compounds were dissolved in absolute DMSO to give 10^{-5} M solutions which were read with Shimadzu UV for absorption spectra and with Perkin-Elmer LS 50 using quinine sulphate in sulfuric acid as quantum yield for fluorescence spectra.

5.4. Cytotoxicity in vitro evaluation

The prepared compounds were submitted to Shanghai Institute of Materia Medica for testing their cytotoxicities.

Acknowledgment

Financial supports by National Natural Science Foundation of China (20536010) (20576016) (20676021) and the National Key Project for Basic Research (2003CB114400), Fok Ying Tung Education Fundation are greatly appreciated.

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