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Novel DNA intercalators without basic side chains as efficient antitumor agents: Design, synthesis and evaluation of benzo-[*c*,*d*]-indol-malononitrile derivatives

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ABSTRACT

Several 2-(substituted benzo[*c*,*d*]indol-2(1*H*)-ylidene)malononitriles have been designed and synthesized. Their DNA binding, antitumor and DNA damaging properties were evaluated. All the compounds exhibited efficient antitumor activities with preference to be against the tumor cell line 7721 rather than the tumor cell line MCF-7. Compound 1f could intercalate into DNA entirely presumably by the good conjugation of carbonyl group with benzo[*c*,*d*]indol moiety. What's more, **1f** exhibited potent toxicity against MCF-7 cells with IC_{50} at 0.003 μM and against 7721 cells at 0.115 μM, respectively.

1. Introduction

As cancer cells are highly proliferative tissues, the DNA becomes one of the most promising biological targets of developing antitumor agents to specifically constrain tumor cell growth.[1] In recent years, much attention has been paid to design and synthesize new and efficient DNA-targeted antitumor agents.[2,3] DNA-intercalating molecules are those which intercalate DNA base pairs via electron-deficient chromophores with flexible side chains. These specially designed groups confer DNA sequence selectivity and allow aromatic heterocycles to position at proper sites or interact with topoisomerases so as to interfere with DNA replication and transcription.[4–6] Several reported bifunctional DNA binders, which carry two such functional groups, have proven this mechanism by the increase of DNA binding affinity and biological activity.[7,8] DNA intercalators have been investigated for more than 40 years,[9] successful examples including (a) amonafide,[10] (b) DACA,[11] (c)8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carbonitrile[12] and (d) ametantrone[13] (Fig. 1).

Early in 1920s, benzo[*c*,*d*]indol-2(1*H*)-one derivatives were originally developed as dyes, and then used as electronic typing materials. Until recently, their prominent bioactivities have been discovered. Benzo[*c*,*d*]indol-2(1*H*)-one has three active sites, 1–3 (Fig. 1h). Many researches focus on optimizing its structure to broaden its application. Appelt[14] reported that naphthostyril (Fig. 1e) derivatives showed good binding affinity for thymidylate synthase (TS) as well as amazing inhibitory activity against them. By substituting the hydrogen atom of lactam with different cylic amines, López-Rodríguez[15] designed a series of 5-HT_7R antagonists (Fig. 1f), which exhibited satisfactory affinities and pharmacological properties. Our group[16] synthesized a novel DNA intercalator tailing with straight amine side chain (Fig. 1g), which manifested good DNA binding and anticancer activities.

In terms of the biological activities of benzo[*c*,*d*]indolone derivatives, regular design concept is that the lactam group on the tricyclic rings can form hydrogen bond with its targets (protein or the backbone of nucleic acids) and its planarity would facilitate its embedding to DNA base pairs. Planarity is one of the most important factors of intercalators.[17] However, most researches focused on restructuring of sites of 1 and 3 (Fig. 1h), but paid little attention to the alteration of the carbonyl group (site 2). Although the benzo[*c*,*d*]indolone derivatives were widely used as protein inhibitors, their interaction with DNA was rarely reported. Therefore, our group focused on the modification of the carbonyl group (site 2) to design and synthesis of novel DNA-targeted benzo[*c*,*d*]indolone derivatives. Unlike reported DNA intercalators with amine side chains, compounds we report here only carry alkyl groups in an attempt to expand the diversity of intercalators' structures and substituting groups.

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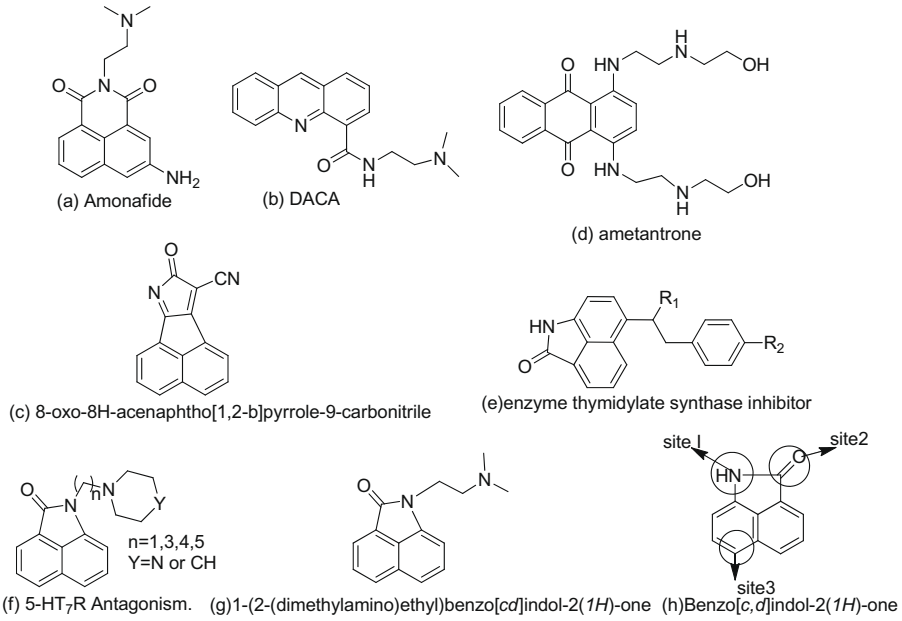

Figure 1. Structures of some reported compounds.

Cyano group is known to be a good photophore for one-electron DNA oxidation due to its forceful electron withdraw ability.[18] Therefore, we introduced dicyano moiety to the site of oxygen atom, not only enlarging the conjugated plane but also reducing electron density so as to facilitate binding of the electron-rich DNA. Although amine side chains have been widely used as essential toxic groups in DNA intercalators,[19] we demonstrated here electron-deficient chromophore with non-basic side chains (**1a–g**) also behaved as efficient antitumor agents with intercalation to DNA. Several unsaturated hydrocarbons were linked to nitrogen atom (**1a–c**) to increase electrostatic interaction between compounds and acidic DNA molecules through the similar mechanism as amine side chains follows. Meanwhile, compounds **1d–e** were synthesized to behave as potential DNA alkylating agents and result in inhibition of tumor growth by cross-linking of basic groups.[20] Furthermore, the intercalator conjugated with acylamide had been reported to have better antitumor activity,[21–23] so compound **1f–g** were designed as new anticancer agents by changing lactam to acylamide (Fig. 2).

2. Results and discussion

2.1. Synthesis and spectra data

Preparation of the compounds (**1a–g**) was shown in Figure 2. Benzo[*c,d*]indol-2(1*H*)-one was reacted with propanedinitrile in toluene at 100 °C in the presence of $POCl_3$ for 4 h to give intermediate **2**, which was a Knoevenagel Condensation. Then **2** underwent three different routes to give all the compounds. (Route 1): **2** was reacted with the corresponding bromo-reactants in acetonitrile with K_2CO_3 as catalyst to afford compounds **1a–c**; (route 2): dihaloalkane was used as reactant as well as solvent to combine with **2** to result in compounds **1d–e**; (route 3): acyl chloride was added to the mixture of **2** and acetic acid at 0–5 °C, then heating to reflux for 5 h to give **1f–g**. Structures of all these final products were confirmed by ^{1}H NMR, HRMS and IR.

The different substitutes were found to have slight effect on the UV–vis spectra, with maximal absorptions around 440 nm with different intensities (Table 1). One exception is that **1b** exhibited

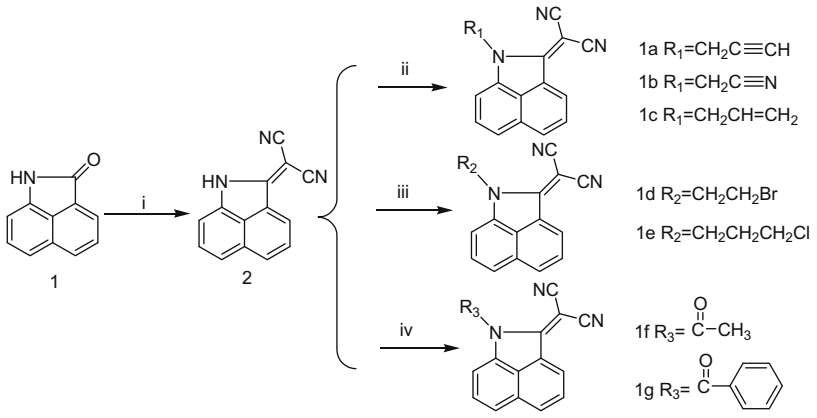

Figure 2. Structures and synthesis of target compounds. Regents and conditions: (i) $CNCH_2CN$, toluene, $POCl_3$, 100 °C, 83% yield; (ii) correspond bromo-reactants, acetonitrile, K_2CO_3, 80 °C; (iii) dihaloalkane, K_2CO_3, 100 °C; (iv) acyl chloride, acetic acid, reflux.

Table 1
UV–vis spectra of the compounds

Compound	λ_{max} (nm)	log ξ	ξ (10^4 L/mol cm)
1a	446	4.21	1.62
1b	377	3.94	0.87
1c	445	4.26	1.82
1d	452	4.24	1.74
1e	446	4.03	1.07
1f	440	4.30	1.99
1g	447	4.30	1.99

In absolute DMSO.

remarkable blue shift to 377 nm due to the strong electron-withdrawing ability of cyano group, increasing the energy of electronic transition.

2.2. Studies on mechanism of interaction with DNA

The affinities of the target compounds for CT DNA were determined by spectroscopic technique and viscosity measurement.

2.2.1. UV–vis spectra

It is widely accepted that if the compound can intercalate DNA, the UV–vis curve of their complex will be induced bathochromic shift and hypochromicity.[24,25] The UV–vis spectra were measured in two ways: (1) Fixed the concentration of compounds (C_{com} = 25 μM), increasing the concentration of CT DNA; (2) kept DNA at 50 μM, changing compounds' concentration.

In the first way, as the DNA concentration was increased, all the compounds showed significant hypochromicities and slight bathocromic shifts (1–3 nm). The most attractive spectrum was **1f**, 2-(1-acetylbenzo[*c*,*d*]indol-2(1*H*)-ylidene)malononitrile (Fig. 3). When DNA was added, **1f** was forced to lose all of its own absorption peaks. The reason could be that the acetyl group was conjugated with the whole aromatic heterocycle, leading **1f** to be a big planar rigid system which could insert into the double helix entirely, so that its spectrum could not be observed. However, the molecular volume of **1g** was bigger than that of **1f**, so it did not have the same behavior. In the second way, with titration of DNA at 50 μM, bathochromic shift and hypochromicity could be found in all the compounds. One typical curve was **1a** (Fig. 4).

When binding with DNA, distinguishable changes happened to **1a** with different relative concentration. When R = 0.1, the maximal isosbestic point was at 452 nm; when R <0.1, DNA decreased its absorption, this was hypochromicity; when R >0.1, hyperchromicity occurred. It could be concluded that different binding mode existed during the increase of **1a**'s concentration. At low compound/DNA ratio, a complete intercalation mode dominated the whole process, so the intensity was reduced; at high compound/DNA ratio, part of the compounds might just stack along DNA surface which induced hyperchromicity. We also used **1a** to calculate binding constant, reaching 1.65 × 10^5 mol^{-1} L, suggesting good binding ability.

The UV–vis spectra indicated that the binding mode for compounds with DNA was of intercalation.

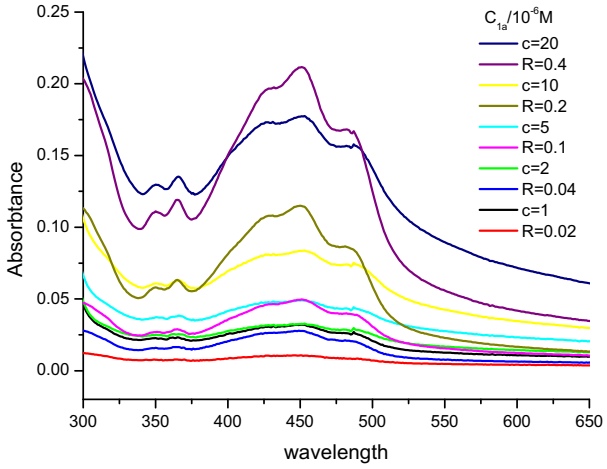

Figure 4. Absorbance changes after interaction of **1a** and calf thymus DNA (C_{DNA} = 50 μM, $R = C_{com}/C_{DNA}$, R value varies at 0.02, 0.04, 0.1, 0.2, 0.4).

2.2.2. Fluorescence spectra

Ethidium bromide (EB) has no fluorescence itself, but when incubated with DNA, it will intercalate exclusively into DNA and its fluorescence intensity will be enhanced greatly. If the compound can replace EB from DNA, its fluorescence will be quenched, indicating that the drug has stronger intercalation ability than EB.[26] **1a** was employed to evaluate the potential affinity capabilities (Fig. 5). It can be seen from the curve **1a**D in Figure 5, **1a** appeared little change in fluorescence while interacting with DNA, eliminating the effect of **1a** on the change of fluorescence. Then, **1a** was added to the complex of EB and DNA, distinctive decrease happened to the intensity of EB, which gave another proof for the deducement of intercalation model.

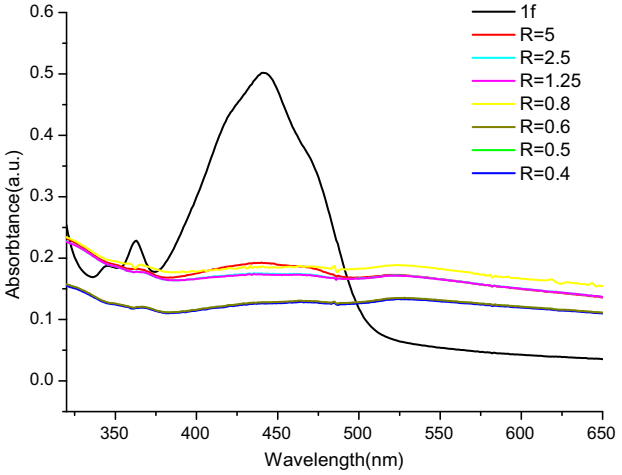

Figure 3. Absorbance changes after interaction of **1f** and calf thymus DNA (C_{com} = 25 μM, $R = C_{com}/C_{DNA}$, R value varies at 5, 2.5, 1.25, 0.8, 0.6, 0.5, 0.4).

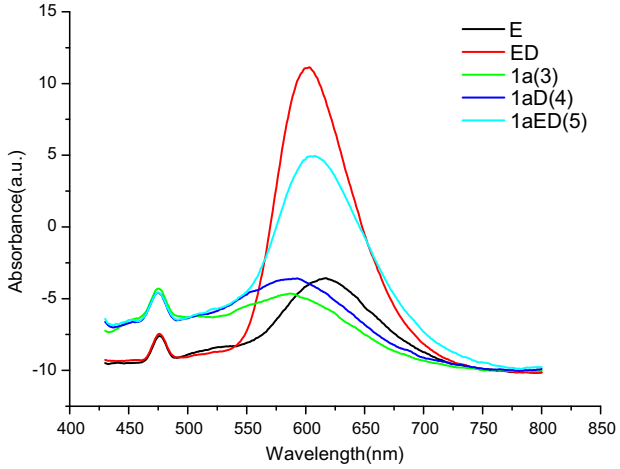

Figure 5. Fluorescence spectrum of E, ED, **1a**, **1a**D and **1a**ED [E: EB only; ED: EB+DNA; **1a**D: **1a**+DNA; **1a**ED: **1a**+EB+DNA (E = 5 μM, D = 10μM, **1a** = 5 μM)].

2.2.3. Viscosity measurement

In the absence of crystal figures, viscosity measurement is the most effective method to explore the binding mode of drug and DNA which is more persuasive than spectra.[27] Generally, when the molecule intercalates into DNA, the distance between adjacent base pair will be largened, causing lengthening, unwinding and stiffening of the helix and usually accompanied by an increases in solution viscosity. When drug interacts with DNA in other ways (electrostatic attraction or groove binding), the DNA length will remain and the viscosity of the complex solution has minor change. Take **1a** as an example to study its effect on CT DNA viscosity. In Figure 6, the increase in viscosity of DNA solution was observed versus the increase in concentration of **1a**, implying intercalation binding by further evidence.

2.3. Damage of plasmid DNA

DNA damaging activities of **1a–g** were evaluated in 20 mM Tris–HCl (pH 7.5) in the presence of supercoiled plasmid DNA pBR322 under dark for 12 h as well as light irradiation for 3 h. Exposed under 365 nm light, all the compounds could cleave the closed supercoiled DNA into relaxed, open circular form (Form II) and compounds **1a–c** could cleave closed supercoiled plasmid DNA into linear form (Form III) (Fig. 7). These results seemed to be in accordance with our prediction that under photo-irradiation, the electron-deficient compounds owing to the dicyano group could facilitate the electron transferring from DNA to themselves, causing oxidation of DNA.[18] The compounds and DNA formed D–A complex in this process, where compounds acted as electron acceptor (A) while DNA as electron donor (D).[28] Thus, it might be concluded that the cleaving process was charge-transfer mechanism.

More interestingly, the reaction mixtures containing closed supercoiled plasmid DNA and compounds at 4°C for 12 h under dark, more linear DNA (Form III) were observed (Fig. 8). This phenomenon hinted that photo-irradiation might not the essential condition for cleavage. It might be caused by hydrolysis mechanism, which was similar to our previous report using naphthalimide derivatives as DNA cleavers.[29]

2.4. Cytotoxicity

The antitumor activity of the compounds, **1a–g**, were evaluated in vitro against MCF-7 cells (human mammary cancer cell) and 7721 cells (human liver cancer cell) by MTT tetrazolium assay.

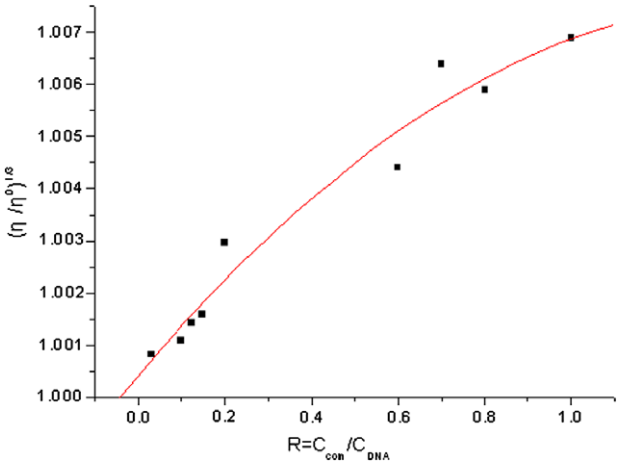

Figure 6. Effect of increasing amounts of compounds **1a** on the relative viscosity of CT DNA at 25 °C. [DNA] = 100 μM in Tris–HCl (30 mM, pH 7.5). η is the viscosity of DNA in the presence of the compounds and η^0 is the absence of the compounds.

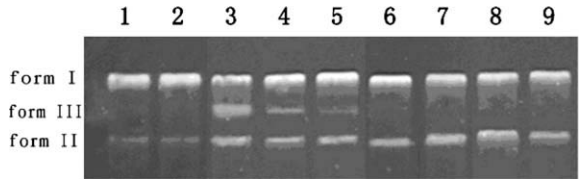

Figure 7. The photocleavage of plasmid DNA pBR322 (25 ng/μL) by compounds, **1a–g**, in 20 mM Tris–HCl, pH 7.5 under light irradiation for 3 h. Lane 1: DNA alone; lane 2: DNA under light irradiation for 3 h; lane 3–9, DNA plus 50 μM compounds under light irradiation for 3 h.

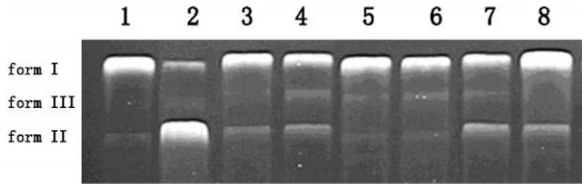

Figure 8. The cleavage of plasmid DNA pBR322 (25 ng/μL) by compounds, **1a–g**, in the buffer of Tris–HCl (20 mM, pH 7.5) at 4 °C for 12 h under no photo-irradiation. Lane 1: DNA alone no *hv*, lane 2–8: DNA plus 50 μM compounds at 4 °C for 12 h.

The IC_{50} values were listed in Table 2 and revealed that all the compounds possessed desirable antitumor activities with higher efficiency against 7721 cells than MCF-7 cells, except that **1f** was the strongest inhibitor against MCF-7 cells than 7721 cells with IC_{50} of 0.003 μM and 0.115 μM, respectively. The IC_{50} of most of the compounds could be as low as 10^{-7} M against 7721 cells, 10-times lower than most reported compounds with IC_{50} of around 10^{-6} M.

The strong electron-withdrawing ability of the dicyano group played an important role in anticancer effect. The moderate activities of **1d** and **1e** might be the result of alkylation which was consistent with previous report,[30] excellent leaving groups such as chloride and bromide are required for cytotoxicity in an alkylation mechanism. The acetyl group of **1f** could form hydrogen bond with DNA bases, so it could interact with DNA forcefully and showed the most outstanding cytotoxic ability, indicating molecules that have intense interaction with DNA often put up favorable antitumor activities. The phenyl of **1g** could conjugate with carbonyl to reduce its competence of forming hydrogen bond and its volume was bigger than **1f**, therefore, its antitumor activity was much lower than **1f**. Moreover, the good potency of **1a–c** conferred new functions to unsaturated aliphatic side chains, though the exact mechanism was still under study.

The basic side chains in many anticancer agents such as naphthalimide, pyridocarbazoles and acridines, were well known strategy to enhance intercalator's antitumor activity. However, the compounds without *N*-dialkyl group basic side chains, we presented here, also exhibited good bioactivity, suggesting that this series of compounds hold entirely different structure–activity relationship (SAR) from traditional DNA intercalators.

Table 2
Antitumor activities of compounds against 7721 and MCF-7 cells

Compound	Cytotoxicity (IC_{50}, μm)	
	7721	MCF-7
1a	0.728 ± 0.02	12.5 ± 0.13
1b	0.260 ± 0.07	13.5 ± 0.15
1c	0.175 ± 0.01	4.73 ± 0.07
1d	0.210 ± 0.05	5.38 ± 0.14
1e	0.354 ± 0.08	6.93 ± 0.09
1f	0.115 ± 0.06	0.003 ± 0.0002
1g	8.80 ± 0.23	15.3 ± 0.27

In summary, comparing with existing compounds, our compounds have two distinct novelty in anticancer effects: (1) the introduction of dicyano group was the first report on modifying the carbonyl group of benzo[*c,d*]indol-2(1*H*)-one to act as DNA poisons. Its forceful electron-withdrawing ability played an important role in anticancer effect. (2) These compounds contained no basic side chains which had been widely used as functional groups in many DNA intercalators, however, they also showed attractive biological activities, which might expand the diversity of intercalators' structures and substituting groups.

3. Conclusions

The strategy we report here was the first one on restructuring the carbonyl group of Benzo[*c,d*]indol-2(1*H*)-one, which had already been used as DNA-targeted drugs. Though they did not have traditional basic side chains, they put up well chemical and biological activities. Their DNA binding properties were evaluated and manifested that the compounds could efficiently intercalate CT DNA, giving a presumption that the flat conjugated aromatic structure of compounds made the large part contribution to intercalate DNA base pairs. DNA damaging assay using plasmid DNA pBR322 showed all the compounds were efficient to photocleave supercoiled DNA, while **1a–f** could photocleave supercoiled DNA into linear forms. What's more, they could cleave DNA in darkness. The tumor cell growth inhibitory test using 7721 and MCF-7 cell lines exhibited that these compounds exhibited excellent antitumor activities and IC_{50} value of **1f** against MCF-7 cells was as low as 3 nM.

4. Experimental

4.1. Materials and methods

All the solvents were of analytic grade. The closed supercoiled pBR322 DNA was a bought from 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 with an X-6 micro-melting point apparatus and are 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 a PGENERAL TU-1901 UV–vis. spectrophotometer.

4.2. Synthesis

4.2.1. 2-(Benzo[*c,d*]indol-2(1*H*)-ylidene)malononitrile (2)

Benzo[*c,d*]indol-2(1*H*)-one (1.69 g, 0.01 mol) and malononitrile (0.6 g, 0.01 mol) were dissolved in 15 mL toluene. $POCl_3$ (1.1 mL) was added dropwise while the mixture was stirring. Then temperature of the reaction mixture was raised to 100 °C. After 4 h, the mixture was cooled and added with 10 mL methanol, filtered, and dried, separated on silica gel chromatography (CH_2Cl_2) to afford the product **2** (1.82 g, 83% yield). Mp: >300 °C. ^{1}HNMR (DMSO-d_6, 400 MHz): δ (ppm): 5.30 (s, 1H), 7.21 (d, J = 7.2 Hz, 1H), 7.58 (t, J_1 = 7.6 Hz, J_2 = 8.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.83 (t, J_1 = 8.0 Hz, J_2 = 7.2 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.55 (d, J = 8.0 Hz, 1H). IR (KBr cm^{-1}) 2216, 2237, 2197, 3427, 1642, 655. HRMS (ESI) *m/z* (M+H)$^+$ calcd for $C_{14}H_7N_3$217.0640; found: 217.0640.

4.2.2. General procedure for synthesis of 1a–c

2-(Benzo[*c,d*]indol-2(1*H*)-ylidene) (2, 0.217 g, 1 mmol) was dissolved in 10 mL acetonitrile, K_2CO_3 (0.331 g, 2.4 mmol) and relevant 1-bromo side chain (1.2 mmol) were added to the solution. Stirred and refluxed at 80 °C until the reaction was completed. The mixture was cooled, dropped into ice-water, extracted with CH_2Cl_2 and dried with $MgSO_4$. The crude product was separated on silica gel chromatography.

4.2.2.1. 2-(1-(Prop-2-ynyl)benzo[*c,d*]indol-2(1*H*)-ylidene)malononitrile (1a). Yield 70%, mp: >300 °C. ^{1}HNMR (DMSO-d_6, 400 MHz): δ (ppm): 2.50 (s, 1H), 5.23 (s, 2H), 7.24 (d, J = 7.2 Hz, 1H), 7.62 (dd, J_1 = 8.0 Hz, J_2 = 7.2 Hz, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.82 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.69 (d, J = 7.6 Hz, 1H. IR (KBr cm^{-1}) 2214, 3415, 3290, 600, 675. HRMS (ESI) *m/z* (M+H)$^+$ calcd for $C_{17}H_9N_3$ 255.0796; found: 255.0796.

4.2.2.2. 2-(1-(Cyanomethyl)benzo[*c,d*]indol-2(1*H*)-ylidene)malononitrile (1b). Yield 68%, mp: >300 °C. ^{1}HNMR(DMSO-d_6, 400 MHz): δ (ppm): 4.36 (s, 2H), 7.60 (dd, J_1 = 6.8 Hz, J_2 = 7.6 Hz, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 7.2 Hz, 1H), 7.75 (dd, J_1 = 8.4 Hz, J_2 = 7.2 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 6.8 Hz, 1H). IR (KBr cm^{-1}) 2250, 2209, 2196, 2178, 3435. HRMS (ESI) *m/z* (M+H)$^+$ calcd for $C_{16}H_8N_4$ 256.0749; found: 256.0749.

4.2.2.3. 2-(1-Allylbenzo[*c,d*]indol-2(1*H*)-ylidene)malononitrile (1c). Yield 60%, mp: 223.5 °C. ^{1}HNMR($CDCl_3$, 400 MHz): δ (ppm): 5.07 (d, J = 4.4 Hz, 2H), 5.20 (d, J = 13.2 Hz, 1H), 5.36 (d, J = 10.0 Hz, 1H), 6.07 (m, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.58 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.81 (dd, J_1 = 7.6 Hz, J_2 = 8.0 Hz, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.69 (d, J = 7.6 Hz, 1H). IR (KBr cm^{-1}) 2214, 3400, 3000, 1670, 665. HRMS (ESI) *m/z* (M+H)$^+$ calcd for $C_{17}H_{11}N_3$ 257.0953; found: 257.0953.

4.2.3. General procedure for synthesis of 1d–e

2-(Benzo[*c,d*]indol-2(1*H*)-ylidene) (**2**, 0.217g, 1mmol) was dissolved in relevant 1-bromo side chain (1.2mmol), K_2CO_3 (0.331 g, 2.4 mmol) were added to the solution as catalyst. Stirred at 100 °C for 4h. The mixture was cooled, filtrated directly to get the product. The crude product was separated on silica gel chromatography.

4.2.3.1. 2-(1-(2-Bromoethyl)benzo[*c,d*]indol-2(1*H*)-ylidene)malononitrile(1d). Yield 77%, mp: 232.3–234.0 °C. ^{1}HNMR($CDCl_3$, 400 MHz): δ (ppm): 4.86 (t, J_1 = 6.8 Hz, J_2 = 6.4 Hz, 2H), 3.83 (t, J_1 = 6.8 Hz, J_2 = 6.4 Hz, 2H), 7.24 (d, J = 8.0 Hz, 1H), 7.61 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.86 (dd, J_1 = 8.0 Hz, J_2 = 8.4 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 8.71 (d, J = 7.6 Hz, 1H). IR (KBr cm^{-1}) 2220, 2197, 1640, 600, 2970. HRMS (ESI) *m/z* (M+H)$^+$ calcd for $C_{16}H_{10}BrN_3$ 323.0058; found: 323.0058.

4.2.3.2. 2-(1-(3-Chloropropyl)benzo[*c,d*]indol-2(1*H*)-ylidene)malononitrile(1e). Yield 71%, mp: 222.8–223.4 °C. ^{1}HNMR($CDCl_3$, 400 MHz): δ (ppm): 2.44 (m, 2H), 3.71 (t, J_1 = 6.8 Hz, J_2 = 6.8 Hz, 2H), 4.63 (t, J_1 = 6.8 Hz, J_2 = 6.8 Hz, 2H), 7.29 (d, J = 7.6 Hz, 1H), 7.81 (dd, J_1 = 8.4 Hz, J_2 = 7.2 Hz, 1H), 8.69 (d, J = 7.2 Hz, 1H), 7.61 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 1H), 7.69 (d, J = 7.6 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H). IR (KBr cm^{-1})2218, 2192, 3423, 3343, 3240. HRMS(ESI) *m/z* (M+H)$^+$ calcd for $C_{17}H_{11}ClN_3$ 293.0720; found: 293.0720.

4.2.4. General procedure for synthesis of 1f–g

2-(Benzo[*c,d*]indol-2(1*H*)-ylidene) (2, 0.217 g, 1 mmol) was dissolved in 6 mL acetic acid, acyl chloride was added dropwise, and refluxed for 3 h, then cooled. The solution was dropped into water, and adjusted the pH to 7 using NaOH to get brown solid, filtrated and dried over vacuum. The crude product was separated on silica gel chromatography.

4.2.4.1. 2-(1-Acetylbenzo[*c,d*]indol-2(1*H*)-ylidene)malononitrile (1f). Yield 71%, mp: >300 °C. ^{1}HNMR($CDCl_3$, 400 MHz): δ (ppm):

3.05 (s, 3H), 7.32 (d, J = 7.2 Hz, 1H), 7.52 (dd, J_1 = 8.0 Hz, J_2 = 7.2 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.97 (dd, J_1 = 7.2 Hz, J_2 = 8.4 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.74 (d, J = 7.2 Hz, 1H). IR (KBr cm^{-1}) 2217, 2205, 1670, 1640, 2970. HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{16}H_9N_3$ O259.0746; found: 259.0746.

4.2.4.2. 2-(1-Benzoylbenzo[*c*,*d*]indol-2(1*H*)-ylidene)malononitrile (1g). Yield 68%, mp: >300 °C. ^{1}HNMR($CDCl_3$, 400 MHz): δ (ppm): 6.39 (d, J = 7.6 Hz, 1H), 7.39 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 7.64 (dd, J_1 = 7.6 Hz, J_2 = 7.2 Hz, 2H), 7.72 (dd, J_1 = 6.0 Hz, J_2 = 7.6 Hz, 1H), 7.73 (d, J = 7.6 Hz, 2H), 7.84 (d, J = 8.4 Hz, 1H), 7.95 (dd, J_1 = 7.2 Hz, J_2 = 8.0 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.75 (d, J = 7.2 Hz, 1H). IR (KBr cm^{-1})2220, 3433, 1655, 693, 738. HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{21}H_{11}N_3O$ 321.0902; found: 321.0902.

4.3. Spectroscopic measurements

The compounds were dissolved in absolute DMSO to give 10^{-5} M solutions. Following spectra testing 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 solution was filtered through a PVDF membrane filter (pore size of 0.45 μm) to remove insoluble material, the concentration of CT DNA was 100 μM. Viscometric titrations were performed with an Ubbelodhe viscometer immersed in a thermostated bath maintained 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 the 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, v/v). 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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