Synthesis, Cytotoxicities and DNA-Binding Affinities of Benzofuran-3-ols and Their Fused Analogs

Zhong-Zhen Zhou,[a,#] Min Zou,[a,#] Jia Zhou,[a] Chun-Qiong Zhou,[a] Yan-Hong Deng,[a] Ming-Hui Chen,[a] Chun-Ping Gu,[a] Zhi-Hong Jiang,[a,b] Wen-Hua Chen,*[,a] and Shu-Wen Liu*[,a]

[a] *School of Pharmaceutical Sciences, Southern Medical University; Guangzhou 510515, China: and* [b] *School of Chinese Medicine, Hong Kong Baptist University; Kowloon Tong, Hong Kong, China.*
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A series of benzofuropyrazoles 2a—i were synthesized in 10—92% from the reaction of 2-aroylbenzofuran-3-ols 1a—i with hydrazine hydrate, and screened for their antitumor activities toward four human solid tumor cell lines, including gastric carcinoma cells MKN45, hepatocellular carcinoma cells HepG2, breast cancer cells MCF-7, and lung cancer cells A549. The results indicated that both compounds 1a—i and 2a—i displayed moderate antitumor activities. Among them, compound 2e exhibited potent inhibitory activity toward all the four tumor cell lines. In addition, compounds 1e and 2e showed strong DNA-binding affinities, and induced an increase in the viscosity of calf-thymus DNA, suggesting that they might act as an intercalator.

Key words benzofuran; benzofuropyrazole; cytotoxicity; DNA binding

Some serious modern human diseases, such as human immunodeficiency virus (HIV), cancers and diabetes, are increasingly threatening the public health. Among them, cancers are definitely the scourge on human being.[1)] Therefore, finding novel, potent and selective antitumor agents has become one of the major goals in modern medicinal chemistry. From the viewpoint of the efficiency in new drug discovery, chemical modification of natural products that display multiple biological activities may be one viable approach. A typical example of this is the rational structural modification of the antibiotics netropsin and distamycin leading to the development of lexitropsins.[2)]

In this field, we have keenly become interested in benzofurans that are ubiquitous in nature and attractive heterocycles for new drug discovery.[3,4)] It is reported that benzofurans exhibit a wide range of pharmacological activities, including antitumor and antiviral activities (*e.g.*, naturally occurring cyclopenta[*b*]benzofurans),[5)] potent, reversible and non-selective aromatase inhibitory effect (*e.g.*, 1-[(benzofuran-2-yl)phenylmethyl]imidazoles),[6)] and selective cytotoxicity against tumorigenic cell lines.[7)] Thus, it is anticipated that 2-aroylbenzofuran-3-ols that we recently reported,[8)] may display promising antitumor activities. On the other hand, many antitumor agents feature a pyrazolyl group as one of the main constituents. Such fused pyrazole-containing antitumor agents, for example, indenopyrazole derivatives,[9)] benzofuro[3,2-*c*]pyrazolyl-3-amine[10,11)] and 3-phenylbenzofuropyrazoles,[12)] show high antitumor activities. These observations, together with the combination principles of drug design, make us reasoning that incorporating of benzofurans with pyrazoles may lead to potent antitumor agents.

The primary objectives of the work reported in this paper, therefore, were two-folds. Firstly, we described the synthesis of fused pyrazole-containing 2-aroylbenzofurans, *i.e.* benzofuropyrazoles **2a**—**i**, from 2-aroylbenzofuran-3-ols **1a**—**i**[8)] (Chart 1), and assayed the antitumor activities of both compounds **1a**—**i** and **2a**—**i** toward four human tumor cells, including gastric carcinoma cells MKN45, hepatocellular carcinoma cells HepG2, breast cancer cells MCF-7, and lung cancer cells A549. In addition, to gain further insight into their biological activities, we investigated the DNA-binding affinities of compounds **1e** and **2e** by means of spectrophotometric titrations and viscosity measurements.

Results and Discussion

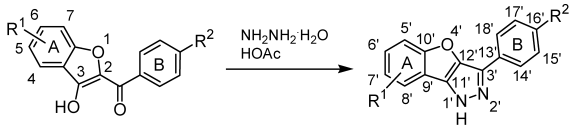
Synthesis of Benzofuropyrazoles 2a—i Compounds **2a**—**i** were prepared according to the reported protocols (Chart 1).[13,14)] Thus, one-step reaction of compounds **1a**—**i** with hydrazine hydrate in refluxing acetic acid afforded compounds **2a**—**i** in 10—92%. These compounds were fully characterized by MS (low resolution (LR) and high resolution (HR)) and NMR (^{1}H and ^{13}C) (see Experimental). They afforded mass spectra with the *m*/*z* values corresponding to $[M+H]^+$. Their NMR spectra were in full agreement with the given structures. The purity of each compound was judged from its clean NMR and one spot on TLC developed by different eluting solvents.

Biological Activities of Compounds 2a—i The *in vitro* antitumor activities of compounds **1a**—**i** and **2a**—**i** against the four cancer cell lines MKN45, HepG2, MCF-7 and A549, were screened by a cell proliferation assay using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).[15)] Their inhibition percentages at the concentration of 100 μM are shown in Fig. 1.

As can be seen from Fig. 1, most of compounds **2a**—**i** exhibited moderate antiproliferative activities, in particular toward HepG2, MCF-7 and A549 cells. In contrast, most of compounds **1a**—**i** were active in inhibiting MKN45, but weak toward the other three cells. Among them, compound **2e** showed strong inhibition toward all the four tumor cells. Further analysis on the structure–activity correlations indicates that compounds **2e**—**h** bearing methoxy groups at ring A were more active than or comparable to their corresponding compounds **2a**—**d** without methoxy groups, suggesting that methoxy group plays a crucial role in modulating the inhibitory activity. However, the presence of a methoxy group at ring B led to a decrease in the activity, that is, compounds **2b** and **f** that were methoxylated at ring B were much less ac-

* To whom correspondence should be addressed. e-mail: whchen@smu.edu.cn; liusw@smu.edu.cn

# These authors contributed equally to this work.



1a-i **2a-i**

Compound	R^1	R^2	Compound	R^1	R^2	Yield (%)
1a	H	H	**2a**	H	H	92
1b	H	MeO	**2b**	H	MeO	80
1c	H	Me	**2c**	H	Me	84
1d	H	Cl	**2d**	H	Cl	68
1e	5-MeO	H	**2e**	7′-MeO	H	88
1f	5-MeO	MeO	**2f**	7′-MeO	MeO	82
1g	5-MeO	Me	**2g**	7′-MeO	Me	89
1h	5-MeO	Cl	**2h**	7′-MeO	Cl	87
1i	7-NO_2	Me	**2i**	5′-NO_2	Me	10

Chart 1. Synthetic Route for Compounds **2a**—**i**

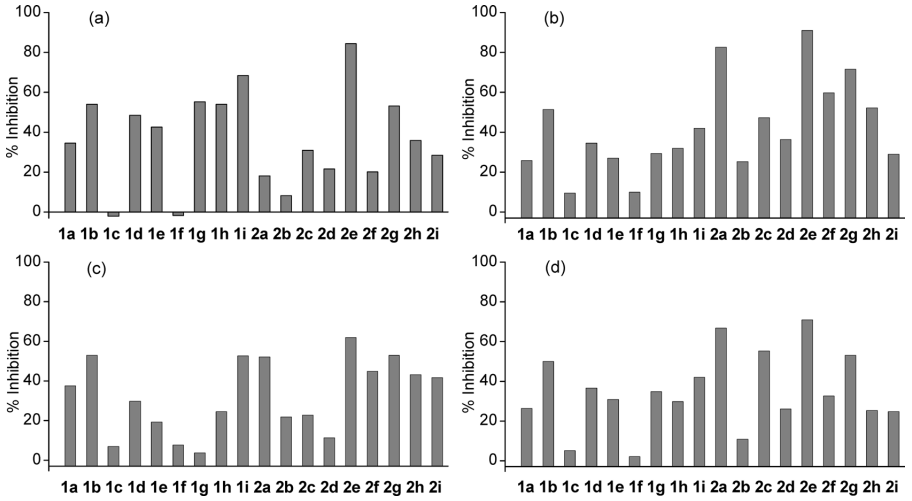


Fig. 1. Antiproliferative Activities of Compounds **1a**—**i** and **2a**—**i** (100 μM) toward MKN45 (a), HepG2 (b), MCF-7 (c), and A549 (d) Cells after 48 h Incubation

tive than their corresponding compounds **2a** and **e** that were not methoxylated at ring B, respectively. No clear structure–activity relationship was found in the other substituents.

To better understand their pharmacological activities, we measured the cytotoxicity IC_{50} values of the compounds that showed strong inhibition, together with those of their corresponding derivatives/precursors for comparison, that is, compounds (**1**—**2**)**a**, (**1**—**2**)**b**, (**1**—**2**)**e**, and (**1**—**2**)**i** (Table 1). Here, the IC_{50} value represents the concentration of one compound resulting in a 50% inhibition in cell growth after 48 h incubation, and was the mean value of three repeated experiments. Doxorubicin was used as a positive control.

It can be seen that compound **2e** was the most active in inhibiting MKN45 and HepG2 with the IC_{50} values being 39±3 μM and 34±2 μM, respectively. Compound **2a** (IC_{50}=32±7 μM) was similar with compound **2e** in inhibiting HepG2. Under our assay conditions, no compound was active toward MCF-7 Cells. Compounds **1i** and **2e** were similar in inhibiting A549. The fact that compound **2e** was slightly more potent than doxorubicin in inhibiting MCF-7 and exhibited high inhibitory activity toward all the four tumor cell lines, suggests that it may be exploitable as a lead compound for further structural optimization in search for potent antitumor agents.

Table 1. Antiproliferative Activities of Compounds (**1**—**2**)**a**, (**1**—**2**)**b**, (**1**—**2**)**e**, and (**1**—**2**)**i**

Compound	Cytotoxicity IC_{50} (Mean±S.D., μM)[a]			
	MKN45	HepG2	MCF-7	A549
1a	>100	>100	>100	>100
1b	>100	134±14	>100	>100
1e	70±10	>100	>100	>100
1i	>100	>100	>100	42±2
2a	>100	32±7	>100	>100
2b	>100	>100	>100	>100
2e	39±3	34±2	112±7	90±11
2i	>100	>100	>100	>100
Doxorubicin	1.1±0.3	3.0±0.4	232±28	3.5±0.1

a) The IC_{50} values of all the other compounds that were not listed, were estimated to be greater than 100 μM.

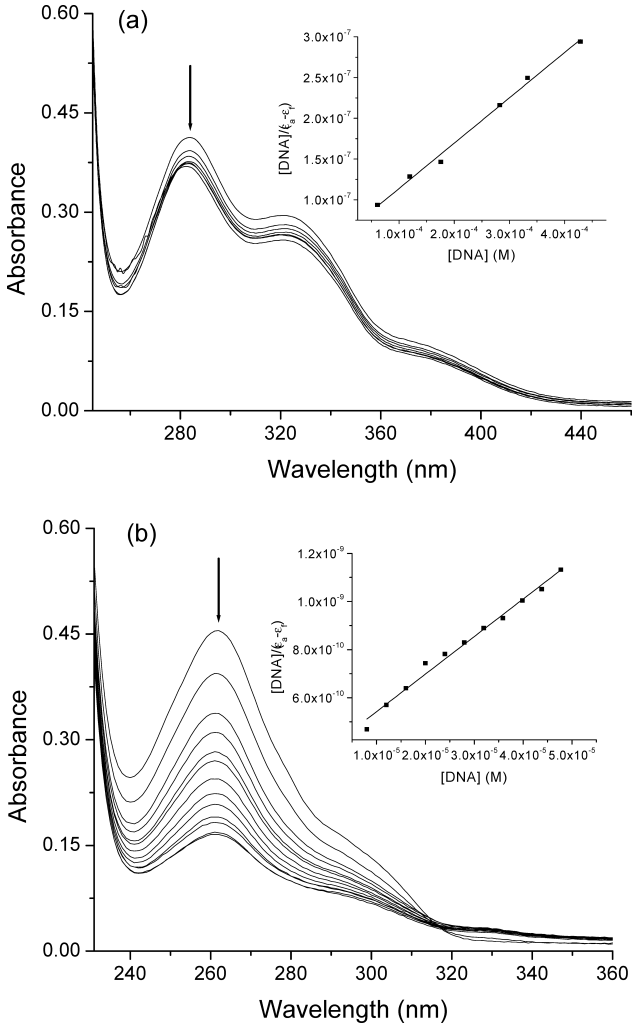


Fig. 2. Spectrophotometric Titrations of (a) **1e** (3.0×10^{-5} M) and (b) **2e** (6.8×10^{-6} M) with CT DNA of Increasing Concentrations (0—4.3×10^{-4} M for **1e**; 0—4.4×10^{-5} M for **2e**) in 5 mm Tris–HCl Buffer (5 mM NaCl, pH 7.0) at Room Temperature

The arrows indicate the absorbance changes upon increasing DNA concentration. The insets are the plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$ *vs.* [DNA].

The aforementioned results may be rationalized by taking into consideration the structures of compounds **1a**—**i** and **2a**—**i**. It is known that hydrogen bonding is one factor that plays a crucial role in inhibitory activities of this class of compounds.[16,17] Thus, we believe that the moderate antitumor activities of compounds **1a**—**i** and **2a**—**i** were, at least in part due to their abilities to form hydrogen-bonding. This was supported by our preliminary study that, under our assay conditions (100 μM), bridging compound **2a** at the NH group with *n*-propyl chain to form dimeric **2a**, led to a complete loss of the anti-proliferative activity toward MKN45, MCF-7 and A549 cells, and a decrease from 83 to 47% inhibition toward HepG2 cells. In addition, the methoxy group on the ring A as a hydrogen bonding acceptor may make further contribution to the enhanced activity of compound **2e**.

DNA Binding of Compounds 1e and 2e To gain further insight into the biological activities of benzofuropyrazole **2e** and its precursor **1e**, we investigated their interaction with calf thymus (CT) DNA, by means of spectrophotometric titrations and viscosity measurement.

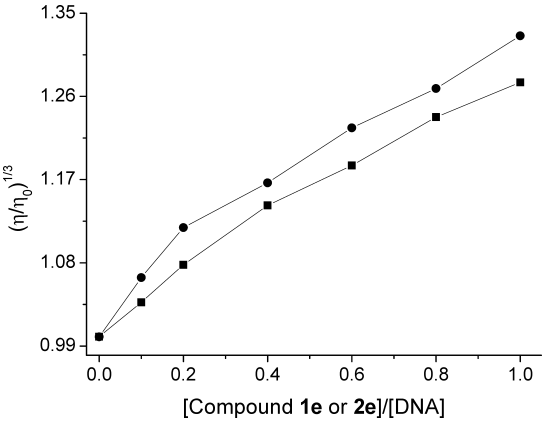


Fig. 3. Effect of Increasing Amounts of Compounds **1e** (■) and **2e** (●) on the Relative Viscosity of CT DNA (1.0×10^{-4} M) in 5 mM Tris–HCl Buffer (5 mM NaCl, pH 7.0) at 20 °C

As shown in Fig. 2, compound **1e** showed a UV–Vis absorption spectrum with a strong π–π* transition band at λ_{max}=282 nm and a moderate *n*–π* transition band at λ_{max}=324 nm, whereas compound **2e** has a strong π–π* transition band at λ_{max}=261 nm. Upon interacting with CT DNA, both compounds showed decreases in the molar absorptivity (hypochromism) and small red shifts in the wavelength (bathochromism), suggesting that they were capable of forming stable complexes with CT DNA. Their binding constants K_a's were calculated from the ratio of the slope to the intercept in the plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$ *vs.* [DNA], according to Eq. 1,[18] in which [DNA] is the concentration of CT DNA in base, ε_a, ε_f and ε_b are the extinction coefficients corresponding to A_{obsd}/[compound], for the free and fully bound compounds, respectively. The K_a values of compounds **1e** and **2e** were found to be $(9.55\pm1.18)\times10^3$ M^{-1} and $(4.04\pm0.32)\times10^4$ M^{-1}, respectively. Thus, compound **2e** showed higher DNA-binding affinity than compound **1e**, in agreement with the fact that it exhibited higher anti-proliferative activities toward the four tumor cell lines.[19]

$$[DNA]/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+1/K_a(\varepsilon_b-\varepsilon_f) \quad (1)$$

Their DNA-binding activities were further studied by viscosity measurements. It is observed that the viscosity of CT DNA increased with increasing amounts of compounds **1e** and **2e** (Fig. 3). Such behavior is in accordance with intercalation, which increases the relative viscosity because of the lengthening of the DNA double helix resulting from intercalation.[19] Thus, these two compounds may intercalate between the adjacent base pairs of CT DNA, leading to an extension in the helix and an increase in the viscosity.

Concluding Remarks

In summary, a series of benzofuropyrazoles were synthesized and characterized with MS (LR and HR) and NMR (^{1}H and ^{13}C). Their antitumor activities were assayed against four human solid tumor cell lines MKN45, HepG2, MCF-7 and A549. The results indicated that both compounds **1a**—**i** and **2a**—**i** displayed moderate antitumor activities. Among them, compound **2e**, *i.e.* 7-methoxy-3-phenyl-1*H*-benzofuro[3,2-*c*]pyrazole, was the most active in inhibiting all the four tumor cell lines. The structure–activity correlations high-

lighted the facts that the ability to form hydrogen bonding, for example by pyrazole unit and methoxy group on the ring A, might play a crucial role in modulating inhibitory activity. In addition, compounds **1e** and **2e** were capable of forming stable complexes with CT DNA, possibly *via* an intercalative mode.

Experimental

General Electrospray ionization (ESI)-MS and HR-ESI-MS spectra were measured on Waters UPLC/Quattro Premier XE and Agilent 6460 Triple Quadrupole mass spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d_6 using Varian Mercury 400 spectrometers and TMS as an internal reference. UV–Vis absorption measurements were conducted by using a TU-1901 spectrophotometer (Purkinje Instrument Co., China) equipped with quartz cells. Viscosity experiments were carried out on an Ubbelohde viscometer. CT DNA was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and its concentration was determined spectrophotometrically using the molar extinction coefficient of 6600 mol^{-1} cm^{-1}/base at 260 nm. All the other reagents and chemicals were obtained from commercial sources and used as received unless otherwise stated.

Synthesis of Compounds 2a—i. General Procedures Hydrazine hydrate (0.65 g, 10 mmol) was added to a solution of **1a**—**i** (5.0 mmol) in acetic acid (25 ml) and the resulting mixture was refluxed for 24 h. Acetic acid was removed in vacuum and the obtained residue was purified by chromatography on a silica-gel column (petroleum ether/acetone, 10/1 by volume) to give compounds **2a**—**i**.

3-Phenyl-1*H*-benzofuro[3,2-*c*]pyrazole **2a**: (92%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.38—7.42 (m, 2H, H-6′, H-8′), 7.50 (t, J=7.8 Hz, 1H, H-7′), 7.56—7.95 (m, 6 H, Ar-H), 13.38 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 112.9 (C-11′), 119.3, 120.4, 123.3 (C-8′), 125.1, 126.0, 126.9, 127.7, 128.9, 129.2, 131.4 (C-12′), 143.9 (C-3′), 162.38 (C-10′). ESI-MS m/z: 234.78 ([M+H]$^+$). HR-ESI-MS for $C_{15}H_{11}N_2O$ ([M+H]$^+$) Calcd: 235.0871; Found: 235.0844.

3-(4-Methoxyphenyl)-1*H*-benzofuro[3,2-*c*]pyrazole **2b**: (80%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.82 (s, 3H, MeO), 7.13 (d, J=7.6 Hz, 2H, H-17′, H-15′), 7.38 (t, J=7.2 Hz, 1H, H-6′), 7.47 (t, J=7.2 Hz, 1H, H-7′), 7.71—7.91 (m, 4 H, Ar-H), 13.38 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 55.2 (MeO), 113.0, 113.7, 114.6, 119.9 (C-9′), 120.0 (C-8′), 126.3, 126.4, 131.1 (C-15′, C-17′), 143.7 (C-3′), 158.8 (C-10′), 161.9 (C-16′). ESI-MS m/z: 264.84 ([M+H]$^+$). HR-ESI-MS for $C_{16}H_{13}N_2O_2$ ([M+H]$^+$) Calcd: 265.0977; Found: 265.0940.

3-*p*-Tolyl-1*H*-benzofuro[3,2-*c*]pyrazole **2c**: (84%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.36 (s, 3H, Me), 7.36—7.38 (m, 3H, Ar-H)), 7.48 (t, J=7.6 Hz, 1H, H-7′), 7.69—7.95 (m, 4H, Ar-H), 13.26 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 20.8 (Me), 98.3 (C-11′), 123.3 (C-5′), 124.9 (C-9′), 125.1, 126.0, 126.8, 127.7 (C-13′), 128.9, 129.2, 129.7, 137.3 (C-12′), 143.8 (C-3′), 162.3 (C-10′). ESI-MS m/z: 248.70 ([M+H]$^+$). HR-ESI-MS for $C_{16}H_{13}N_2O$ ([M+H]$^+$) Calcd: 249.1028; Found: 249.1002.

3-(4-Chlorophenyl)-1*H*-benzofuro[3,2-*c*]pyrazole **2d**: (68%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.37—7.43 (m, 1H, H-6′), 7.46—7.51 (m, 1H, H-7′), 7.58 (d, J=8.4 Hz, 1H, H-8′), 7.64 (d, J=8.4 Hz, 1H, H-5′), 7.72—7.83 (m, 2H, H-17′, H-15′), 7.90—7.99 (m, 2H, H-14′, H-18′), 13.43 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 112.9, 113.3, 116.1 (C-9′), 119.4, 120.5, 123.4 (C-6′), 126.7 (C-13′), 129.0, 129.3, 130.2 (C-16′), 144.0 (C-12′), 145.2 (C-3′), 161.5 (C-10′). ESI-MS m/z: 268.70 ([M]$^+$). HR-ESI-MS for $C_{15}H_{10}ClN_2O$ ([M+H]$^+$) Calcd: 269.0482; Found: 269.0442.

7-Methoxy-3-phenyl-1*H*-benzofuro[3,2-*c*]pyrazole **2e**: (88%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.86 (s, 3H, MeO), 6.96—7.01 (m, 1H, H-6′), 7.33—7.40 (m, 2H, H-5′ and H-8′), 7.50—7.58 (m, 2H, H-15′, H-17′), 7.67—7.95 (m, 3H, Ar-H), 13.15 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 55.6 (MeO), 98.5 (C-11′), 112.9 (C-5′), 120.4 (C-9′), 123.3 (C-8′), 124.6 (C-12′), 126.8, 127.6, 128.9, 129.5, 137.2 (C-16′), 142.7 (C-3′), 150.4 (C-10′), 162.3 (C-7′). ESI-MS m/z: 265.09 ([M+H]$^+$), 287.07 ([M+Na]$^+$). HR-ESI-MS for $C_{16}H_{13}N_2O_2$ ([M+H]$^+$) Calcd: 265.0977; Found: 265.0939.

7-Methoxy-3-(4-methoxyphenyl)-1*H*-benzofuro[3,2-*c*]pyrazole **2f**: (82%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.82 (s, 3H, MeO), 3.86 (s, 3H, MeO), 6.95 (d, J=8.0 Hz, 1H, H-6′), 7.12 (d, J=8.6 Hz, 2H, H-17′, H-15′), 7.32—7.37 (m, 1H, H-5′), 7.66—7.88 (m, 3H, H-8′, H-14′, H-18′), 13.15 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 55.1 (MeO), 55.6 (MeO), 98.5 (C-11′), 110.8 (C-8′), 120.3, 120.5, 120.6, 121.4, 126.1 (C-13′), 139.4, 140.1, 142.3 (C-10′), 158.8 (C-7′), 163.7 (C-16′). ESI-MS m/z: 295.11 ([M+H]$^+$), 317.14 ([M+Na]$^+$). HR-ESI-MS for $C_{17}H_{15}N_2O_3$ ([M+H]$^+$) Calcd: 295.1083; Found: 295.1041.

7-Methoxy-3-*p*-tolyl-1*H*-benzofuro[3,2-*c*]pyrazole **2g**: (89%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.36 (s, 3H, Me), 3.86 (s, 3H, MeO), 6.96—7.00 (m, 1H, H-6′), 7.33—7.37 (m, 3H, Ar-H)), 7.37—7.86 (m, 3H, Ar-H), 13.08 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 20.8 (Me), 55.6 (MeO), 98.5 (C-11′), 110.7 (C-8′), 112.1 (C-5′), 119.5, 120.6, 124.5 (C-13′), 128.7, 129.4, 130.8, 136.9 (C-3′, C-16′), 143.8 (C-10′), 158.4 (C-7′). ESI-MS m/z: 279.07 ([M+H]$^+$), 301.12 ([M+Na]$^+$). HR-ESI-MS for $C_{17}H_{15}N_2O_2$ ([M+H]$^+$) Calcd: 279.1134; Found: 279.1091.

3-(4-Chlorophenyl)-7-methoxy-1*H*-benzofuro[3,2-*c*]pyrazole **2h**: (87%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.86 (s, 3H, MeO), 6.96—7.02 (m, 1H, H-6′), 7.34 (d, J=13.2 Hz, 1H, H-5′), 7.60—7.93 (m, 5H, Ar-H), 13.24 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 55.6 (MeO), 98.5 (C-11′), 110.6, 111.0, 120.0, 120.1, 126.4, 129.1 (C-16′), 131.9 (C-3′), 158.7 (C-10′), 163.2 (C-7′). ESI-MS m/z: 299.05 ([M+H]$^+$). HR-ESI-MS for $C_{16}H_{12}ClN_2O_2$ ([M+H]$^+$) Calcd: 299.0587; Found: 299.0541.

5-Nitro-3-*p*-tolyl-1*H*-benzofuro[3,2-*c*]pyrazole **2i**: (10%) having ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.37 (s, 3H, Me), 7.38—7.39 (m, 2H, Ar-H), 7.58—7.59 (m, 1H, Ar-H), 7.78—7.85 (m, 2H, Ar-H), 8.21—8.37 (m, 2H, Ar-H), 13.48 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 20.8 (Me), 98.5 (C-11′), 122.2, 123.4, 124.8 (C-6′), 126.8, 129.7, 134.1 (C-5′), 137.6, 137.7, 153.7 (C-10′). ESI-MS m/z: 294.01 ([M+H]$^+$). HR-ESI-MS for $C_{16}H_{12}N_3O_3$ ([M+H]$^+$) Calcd: 294.0879; Found: 294.0849.

Biological Measurements. Cell Culture MKN45, HepG2 and MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), whereas A549 cells were cultured in 1640 with 10% FBS. Cells were passaged at 70—80% confluence, about twice a week by trypsinization.

Cell Proliferation Assay Cell proliferation was determined using an MTT assay.[15] Exponentially growing cells were plated in 96-well plates (16000 cells/well for MKN45 and MCF-7 cells, 10000 cells/well for HepG2 cells and 18000 cells/well for A549 cells) and incubated at 37 °C for 24 h for attachment. Test compounds were prepared by dissolving in dimethyl sulfoxide (DMSO) at 100 mM and diluted with the medium. Then, culture medium was changed, and cells grew in medium with the test compounds. DMSO (0.1%) was used as negative control. Cells were incubated at 37 °C for 48 h. Then the medium was replaced with MTT solution (5 mg/ml, 100 μl) followed by incubation for another 3 h. The medium was then aspirated and formazan crystals were dissolved in DMSO (100 μl) for about 10 min. The absorbance at 570 nm (Abs) of the suspension was measured by an enzyme-linked immunosorbent assay (ELISA) reader. The inhibition percentage was calculated using the following formula: % inhibition=(Abs$_{control}$−Abs$_{compound}$)/Abs$_{control}$×100%.

The IC$_{50}$ values of the test compounds and doxorubicin were measured by treating cells with drugs of varying concentrations, and analyzing by use of the prism statistical package (GraphPad Software, San Diego, CA, U.S.A.).

DNA-Binding Study. Spectrophotometric Titrations Spectrophotometric titrations were performed at room temperature by fixing the concentrations of compounds **1e** and **2e**, while gradually increasing the concentration of CT DNA.[20] Typically, to a solution of **1e** (3.0×10^{-5} M) in 5 mM Tris–HCl buffer (5 mM NaCl, pH 7.0) were added aliquots of CT DNA (3.1×10^{-3} M) solution containing **1e** (3.0×10^{-5} M) in 5 mM Tris–HCl buffer (5 mM NaCl, pH 7.0). The mixing was achieved by stirring for 2 min. Then, the corresponding absorption spectra were measured. The operations repeated until saturation reached. The spectrophotometric titrations of **2e** were conducted in a similar way.

Viscosity Measurements Viscosity experiments were carried out in 5 mM Tris–HCl buffer (5 mM NaCl, pH 7.0) at 20 °C. Thus, the solution of compound **1e** (or **2e**) (1.0×10^{-5}—1.0×10^{-4} M) was added to the solution of CT DNA (1.0×10^{-4} M). Flow time (t) of each sample was measured three times, and the average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the concentration ratios of compound **1e** (or **2e**) to CT DNA, where η is the relative viscosity of CT DNA in the presence of compound **1e** (or **2e**), and η_0 is the relative viscosity of CT DNA alone. Relative viscosity was calculated according to $\eta=t-t_0$,[19] where t_0 is the flow time of buffer solution.

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