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Original article

Natural tanshinone-like heterocyclic-fused *ortho*-quinones from regioselective Diels–Alder reaction: Synthesis and cytotoxicity evaluation

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ABSTRACT

A series of new natural tanshinone-like oxoheterocyclic-fused *ortho*-quinone derivatives were synthesized from readily available benzofuranol and *N*-substituted dienes *via* IBX oxidation–cycloaddition–aromatization procedure. The regiospecific Diels–Alder cycloaddition reactions of *N*-dienes were achieved efficiently with a variety of dienophiles. It is found that the amide moiety in the molecular could be preserved or eliminated by control of the aromatization conditions. Selected oxoheterocyclic-fused *ortho*-quinones as well as several thioheterocyclic-fused *ortho*-quinones we obtained before were evaluated for their cytotoxicities on different cancer cell lines and the Structure–Activity Relationship (SAR) was discussed.

1. Introduction

It's well known that many natural and synthetic heterocyclic quinones possess a variety of bioactivities. For example, naturally occurring tanshinones from *Salvia miltiorrhiza* Bunge exhibits interesting physiologic properties including effects on cardiac function [1–4], antioxidant activity [5–7], cytotoxicity [8–12], apoptosis induction [13,14], etc., and have attracted particular attention from medicinal chemists and clinicians. We have engaged in a program directed at searching for new bioactive *ortho*-quinoid compounds for several years [15–17]. Recently, we described regioselective cycloaddition reaction of *o*-quinones with electron-rich *N*-dienes for synthesis of novel thioheterocyclic-fused *ortho*-quinones [18]. As an extension of our previous works, the synthesis of oxoheterocyclic-fused *ortho*-quinones **I** as novel analogues of natural tanshinones (Fig. 1) was planned.

Other than the reported procedures [19–23], we present herein our results concerning an alternative strategy based on IBX oxidation–regiospecific cycloaddition–aromatization (DDQ and silica gel) sequence which we developed previously [18]. Cycloaddition reaction was adopted to construct the functionalized polycyclic systems, the *N*-substituted dienes were believed to be able to provide a rapid and powerful strategy for many complex natural products and synthetic alkaloids with high regioselectivity [24], therefore, *N*-substituted diene derivatives **II**–**IV** (Fig. 1) were used in this paper for the efficient construction of functional oxoheterocyclic-fused *ortho*-quinone derivatives with general structure **I** (Fig. 1). To the best of our knowledge, there are few reports on the cycloaddition of *ortho*-benzofuranoquinones with *N*-substituted dienes.

2. Chemistry

These key steps of our strategy involved intramolecular cyclization, IBX oxidation, cycloaddition and subsequent aromatization, starting from readily available benzofuran-5-ol **5a**–**e** and **13** (Schemes 1 and 2).

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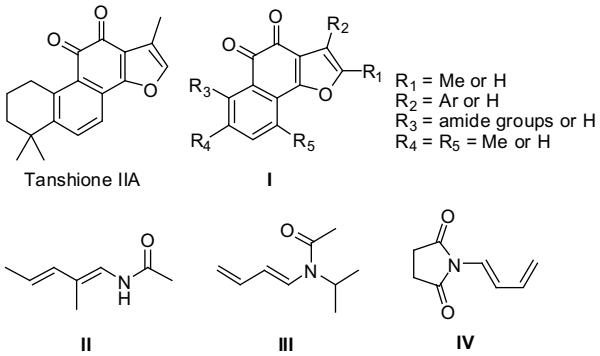


Fig. 1.

2.1. Synthesis of substituted benzofuran-5-ol (*5a–e, 13*)

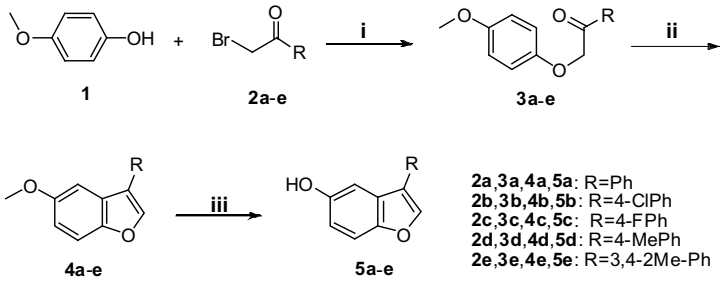
Compounds **5a**–**e** were obtained in high overall yield (**5a** 81%, **5b** 70%, **5c** 82%, **5d** 69%, **5e** 78%) through three steps (condensation, cyclization [25] and deprotection) by starting from the inexpensive and readily available compounds **1** and **2a**–**e** as outlined in Scheme 1. Compound **13** was synthesized in high yield in four steps (condensation in 98% yield, rearrangement [26] in 96% yield, cyclization [27] in 88% yield and deprotection in 86% yield) using the readily available compounds **1** and **9** as the starting materials as shown in Scheme 2.

2.2. Synthesis of substituted benzofuran-4,5-dione (*6a–e, 14*)

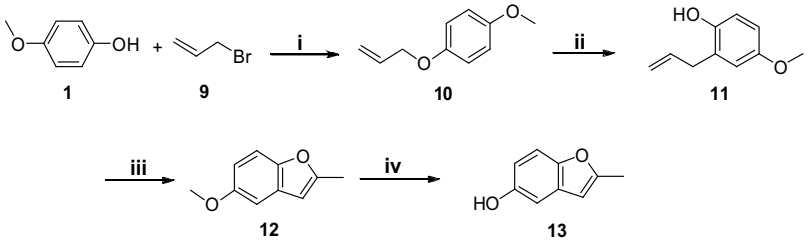
As part of our ongoing program dedicated to the construction of new bioactive molecules, we reported our strategy in 2005 for the synthesis of thioheterocyclic-fused *o*-quinones based on IBX oxidation and Diels–Alder reactions [18]. Herein our first effort was focused on extending this strategy to the synthesis of oxaheterocyclic-fused *o*-quinones by *o*-Iodoxybenzoic acid (IBX) oxidation–Diels–Alder reaction–aromatization sequence. As anticipated, IBX also worked very well in these reactions of regiospecifically and almost quantitatively transforming benzofuan-5-ol **5a**–**e** and **13** into *o*-benzofuranoquinones **6a**–**e** and **14**, as shown in Scheme 3. On the other hand, the isolation of the *o*-benzofuranoquinone intermediates was not pursued for their volatility and high reactivity. However, we found that these *o*-benzofuranoquinone intermediates could remain stable in dry benzene solution for several weeks.

2.3. Synthesis of ortho-naphthofuranoquinone derivatives *8a–e, 9a–e, 16, 17, 20* and *21*

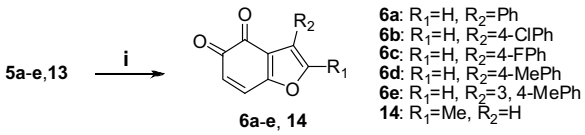
Our first plan is to finish the construction of the naphthofuranoquinone skeleton using cycloaddition between *ortho*-benzofuranoquinones and *N*-diene **II** as key step. However, because of the low stability of the intermediate *ortho*-benzofuran-4,5-diones, these compounds were not isolated from the reaction mixture, and a one-pot reaction was first designed. After the IBX oxidation process was completed at room temperature, the *N*-diene **II** was directly added to the reaction mixture, and the mixture was heated to 45 °C for a given time, after which DDQ was added in for the aromatization at the same temperature. However, the reaction gave unpredictable and complicated products. We then adopted a multistep strategy. After the completion of IBX oxidation process, the reaction mixture was first diluted with water, and the organic content was then extracted into benzene. After the benzene solution containing benzofuran-4,5-diones was dried with anhydrous sodium sulfate, *N*-diene **II** was added for cycloaddition. The reaction was carried out at 45 °C for 16 h and monitored by TLC. The products of the reaction were subsequently aromatized into the final *ortho*-naphthofuranoquinones at 45 °C by DDQ or silica gel. After evaporation of the solvent and purification by chromatography on silica gel ($CHCl_3$/CH_3OH), two types of compounds **8a**–**e**, **16** and **9a**–**e**, **17** were obtained in high yields. The cycloaddition reactions of *N*-dienes of types **III** and **IV** with *ortho*-benzofurano*quinones* **6a** and **6b** were also carried out (Scheme 4). Interestingly, the reaction rates of these dienes were much slower than



Scheme 1. *Conditions*: (i) K_2CO_3, KI, Acetone, r.t., 16 h; (ii) PPA or Amberlyst 15, Toluene, r.t., 2–5 h; (iii) Ac_2O–HBr (48%), 140 °C, 4 h.



Scheme 2. *Conditions*: (i) K_2CO_3, KI, Acetone, r.t., 10 h; (ii) N_2, r.t., 2 h; (iii) $PdCl_2$ (2 mol%), $Cu(OAc)_2$, LiCl, DMF–H_2O, r.t., 5 h; (iv) Ac_2O–HBr (48%), 140 °C, 1.5 h.



Scheme 3. *Conditions*: (i) IBX, DMF, r.t., 16 h.

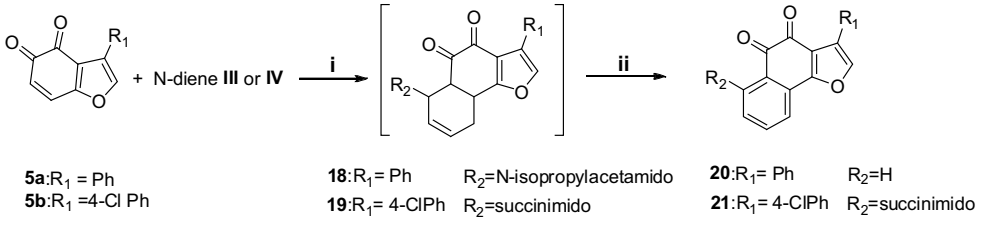
that of *N*-diene **II** even at elevated temperatures. TLC analysis of the reaction mixture showed that the reaction could not be completed even after refluxing for 24 h. 1.5 equiv of DDQ was added under reflux for aromatizing the intermediates **18** and **19**, and the aromatization procedure was completed within 2 h for **18** and 6 h for **19** respectively, giving compound **21** in 50% yield, and **20** in 30% yield. For the reaction of diene **III**, the amide group was eliminated from **18** during the reaction process, giving **20** as the only product. The possible reason for this might be the effect of steric hindrance of larger isopropyl which caused the easy removal of amide group. For diene **IV**, two carbonyls connected to N atom significantly decreased the electron donating capability, and the reactivity of diene **IV** was also remarkably decreased. Furthermore, *ortho*-benzofuranoquinones tended to decompose when it was heated for a long time, this should be the possible cause for the low yield of compound **20**.

EA, HRMS, ^{1}H NMR, ^{13}C NMR were used for final products' characterization. For the regioisomer identification, the HMBC of **17**, **9d** and **e** was recorded, in each case, the HMBC spectra show apparent correlation of an aromatic H (δ_H: 7.75, 7.62 and 7.77 referring to **17**, **9d** and **e** respectively) with a carbonyl C (δ_C: 181.48, 180.06 and 181.43 referring to **17**, **9d** and **e** respectively), indicating structure isomers 2,7,9-trimethylnaphtho[1,2-*b*]furan-4,5-dione (**17**), 7,9-dimethyl-3-*p*-tolylnaphtho[1,2-*b*]furan-4,5-dione (**9d**) and 7,9-dimethyl-3-(3,4-dimethyl)naphtho[1,2-*b*]furan-4,5-dione (**9e**) formed (Scheme 5, H-6 and C-5 show correlation in HMBC). Furthermore, we obtained the single crystal of **9a** and **b**, the X-ray data show clearly the structures as 7,9-dimethyl-3-phenylnaphtho[1,2-*b*]furan-4,5-dione (**9a**) and 7,9-dimethyl-3-(4-chlorophenyl)naphtho[1,2-*b*]furan-4,5-dione (**9b**) (Fig. 2). The structure of **16**, **8a** and **b**, **8d** and **e** could therefore be confirmed. Regioisomers **8c**, **9c** and **21** were judged by deduction.

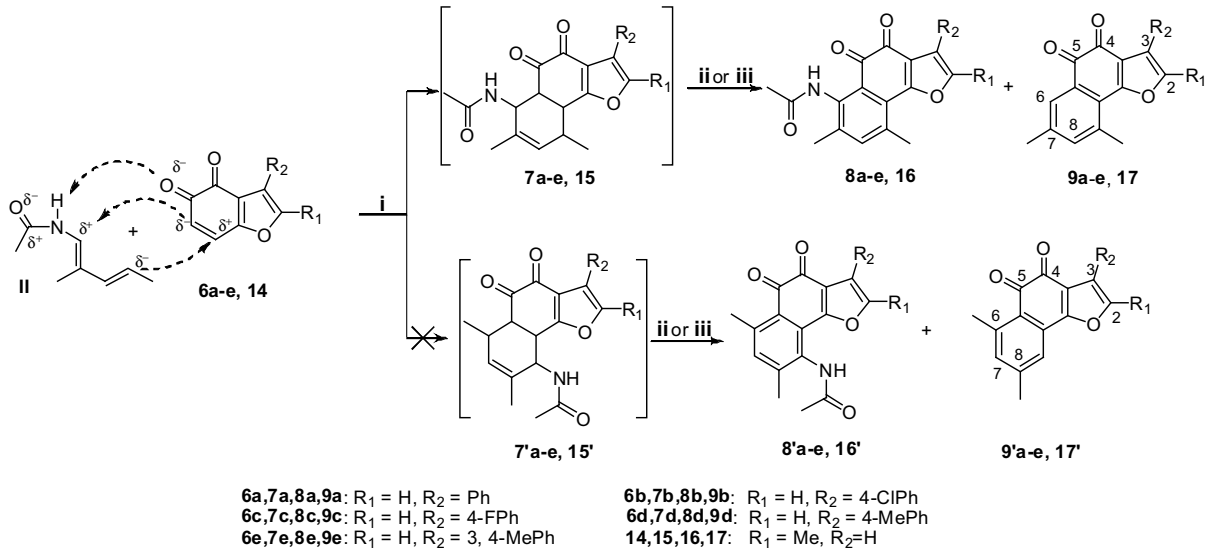
3. Results and discussion

3.1. Regioselectivity and reactivity of the cycloaddition reactions

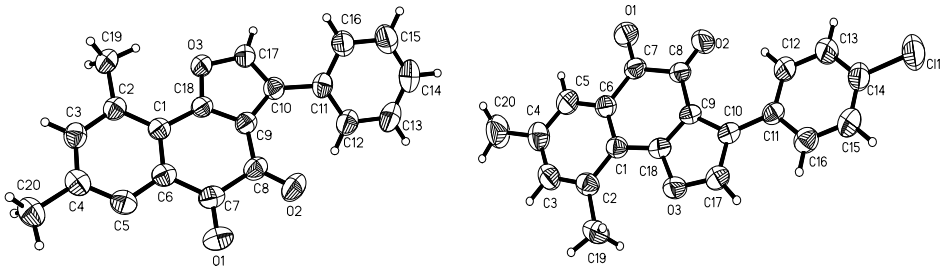
N-Dienes **II**–**IV** were unsymmetrical dienes, and the cycloaddition reaction could provide a mixture of regioisomers. However, structural analysis of the aromatized products showed that the aromatization reaction gave only compounds **8a**–**e**, **16**, **21** and **9a**–**e**, **17** which should be originated from the single regioisomers **7a**–**e**, **15**, **18**, and **19** (Schemes 4 and 5 and Table 1) [28]; and their structural isomers **8′a**–**e**, **16′** and **9′a**–**e**, **17′** were not detected. These results indicated that the cycloaddition reactions were essentially regiospecific. These observations on regioselectivity and different reactivities of the different *N*-dienes could be explained by the electronic effects of the diene induced by the carbonyl (from **II**–**IV**) and/or hydrogen bonding (**II**) (Scheme 5). The intermolecular hydrogen bond-between the amide group (NH) of the type **II** *N*-diene and the carbonyl oxygen of *ortho*-quinones, made the transition structure in



Scheme 4. *Conditions*: (i) Benzene, r.t., 24 h; (ii) DDQ, benzene, r.t., 24 h.



Scheme 5. *Conditions*: (i) benzene, 45 °C, 16 h; (ii) DDQ, benzene, 45 °C, 16 h; (iii) silica gel, 45 °C, 16 h.



9a **9b**

Fig. 2. X-ray crystal structure of **9a** (CCDC No. 254246) and **9b** (CCDC No. 260179).

a more ordered state and been easier to form. This was proved to be true from the NMR signal assignments of the aromatized products and the single-crystal structure analysis.

3.2. Facile control of the aromatization procedure

Noticeably, our experimental results (Table 1, Schemes 4 and 5) showed that different method of aromatization had great effect on the ratio of products. These results made it possible to fully control the structures of the final products. During the aromatization process, there were two competitive reactions at the same time: one would be the thermal elimination of amide groups, and the other would be the dehydrogenation of the cycloaddition intermediate by oxidant DDQ or oxygen species from the air. When DDQ is used as dehydrogenation reagent in solvent, the reaction happens in homogeneous phase. Thus, dehydrogenation procedure with DDQ finished smoothly and quickly, leaving the amide groups intact. However, when the cycloaddition intermediates were loaded on silica gel, the reaction proceeded heterogeneously. In this case it was not easy for the cycloaddition products to react with oxygen from the air. As a result, the dehydrogenation proceeded slowly, and the thermal elimination reaction was the predominant one. In addition, the hydrogen bonding interaction between amide groups and the Si–OH groups on the surface of silica gel could accelerate the elimination of amide groups. Therefore, when aromatization was carried out on silica gel, the products with amide groups eliminated were the major products.

3.3. Cytotoxicity evaluation and SAR

To investigate the bioactivities of obtained analogues, the oxo-heterocyclic-fused *ortho*-quinones (**8a**–**e**, **9a**–**e**, **17**, **21**) obtained in the current work, as well as 5 thioheterocyclic-fused *ortho*-quinones (**s1**–**s5**, Fig. 3), which were prepared in our previous work [18], were chosen for the primary cytotoxicity evaluation on human lung adenocarcinoma cells GLC-82 and human nasopharyngeal carcinoma cell Line CNE2 using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. 10-Hydroxycamptothecin (HCPT), a clinically used antitumor agent, was also evaluated for positive control; unfortunately, most of compounds without the 6-amido substitution (**9b** and **c**, **9e**, **17**) and **8e** were found to be insoluble and the data cannot be obtained; the results are summarized in Table 2.

The obtained data (**8a**–**d**, **9a**, **9d**, **21**, **s1**–**s5**) reveal a general SAR for the cytotoxicity of tested compounds. Comparing the cytotoxicities of **8a**–**d** with that of **s1**–**s4** respectively, it seems that the replacement of O with S in the heterocyclic moiety can elevate the activity slightly. On the other hand, though **s2** has slight higher IC_{50} than other thioheterocyclic-fused *ortho*-quinones **s1**, **s3** and **s4**, no evidence shows that the different substitution on the 3-phenyl has apparent effects on their cytotoxicity to both cancer cell lines. Interestingly, **9a**, **9d** and **s5**, which without the 6-acetamido substitution, have much lower cytotoxicity to both cancer cell lines than others especially, a 6-*N*-succinimidyl moiety bearing compound **21** has much stronger toxicity than others, and has closer IC_{50} (2.0 and 1.6 μmol/L refer to CNE2 and GLC-82

Table 1
Synthesis from substituted benzofuran-5-ol to *ortho*-naphthofuranoquinones.[d]

Entry	Benzofuranoquinones	*N*-Diene	Naphthofuranoquinones	Total yields (%)	Ratio[a]
1	**5a**	**II**	**8a** + **9a**	85[b]	92/8
				82[c]	23/77
2	**5b**	**II**	**8b** + **9b**	91[b]	88/12
				78[c]	35/65
3	**5c**	**II**	**8c** + **9c**	90[b]	100/0
				90[c]	27/73
4	**5d**	**II**	**8d** + **9d**	85[b]	94/6
				92[c]	26/74
5	**5e**	**II**	**8e** + **9e**	88[b]	92/8
				94[c]	42/58
6	**13**	**II**	**16** + **17**	89[b]	86/14
				90[c]	20/80
7	**5a**	**IV**	**20**	30[b]	–
8	**5b**	**III**	**21**	50[b]	–

[a] Ratios = (products with amide group)/(products without amide group).
[b] DDQ aromatization method.
[c] Silica catalyzed aromatization method.
[d] All isolated yields were based on substituted benzofuran-5-ol.

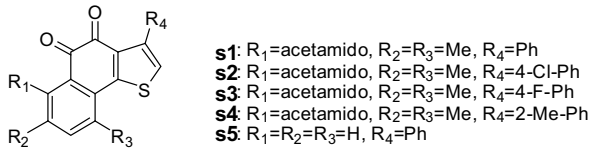


Fig. 3.

Table 2
Effect of compounds on the growth of cancer cell lines.

Compound	IC_{50}[a] (μmol/L)		Compound	IC_{50} (μmol/L)	
	CNE2	GLC-82		CNE2	GLC-82
8a	4.8	7.2	**9e**	na	na
8b	9.2	6.0	**17**	na	na
8c	nd	8.9	**21**	2.0	1.6
8d	5.6	9.5	**s1**	4.1	5.0
8e	na	na	**s2**	6.8	7.5
9a	>25	>25	**s5**	nd	20.1
9b	na	na	**s3**	4.6	3.9
9c	na	na	**s4**	4.2	nd
9d	23.3	12.0	HCPT	0.37	1.09

nd, not determined; na, data cannot be obtained owning to the poor solubility of the sample.

[a] The IC_{50} represents the compound concentration required for the reduction of the mean cell viability to 50%.

respectively) to that of clinical drug 10-hydroxycamptothecin (HCPT, $IC_{50} = 0.37$ and 1.09 μmol/L refer to CNE2 and GLC-82 respectively), indicating that the amide moiety substitution on the benzo-ring plays an important role in cancer cell growth inhibition, and further group alteration on benzo-ring would be the direction of more potent diversity generation in future work.

4. Conclusions

In this work, we successfully synthesized 14 new natural tanshinone-like *ortho*-naphthofuranoquinones *via* IBX oxidation–cycloaddition–aromatization sequences. The regiospecific cycloaddition reactions of *N*-dienes were achieved efficiently with a variety of dienophiles. By selection of different aromatization conditions, it is possible to control the amide moiety to be preserved or eliminated; the introduction of substitution on the aromatic ring provided us with a good chance to produce compounds with a variety of functional groups, and made it possible for us to establish a library of highly functionalized tanshinone analogues. Cytotoxicity evaluation of selected oxoheterocyclic-fused *ortho*-quinones as well as several thioheterocyclic-fused *ortho*-quinones indicates that most of the substrates have moderate to good cytotoxicity to cancer cell lines CNE2 and GLC-82, the SAR analysis reveals that 6-amide moiety substitution plays an important role in cancer cell growth inhibition; especially, the 6-*N*-succinimidyl moiety bearing compound **21** was found to have strongest cytotoxicity to tested cell lines, and might serve as an effective lead compound for further antitumor agent development. Further exploration focuses on more potent diversity generation mainly by the group alteration on the benzo-ring, SAR analysis and detail mechanism is under active exploration.

5. Experimental

5.1. General

All reagents were commercially available. Solvents were treated using standard techniques. Reactions were monitored by TLC on glass plate coated with silica gel with fluorescent indicator (GF_{254}). Flash chromatography was performed on silica gel H. ^1H NMR, ^{13}C NMR spectra were measured on a Varian UNITY INOVA 500 MHz or 300 MHz spectrometer using TMS as an internal standard. The electrospray (ESI) MS analysis was performed on a Finnigan LCQ Deca XP ion trap mass spectrometer. FAB-MS was measured on a VG ZAB-HS analytical spectrometer. HRMS were measured on a Thermo MAT 95XP; *N*-dienes **II–IV** were obtained by using the reported method [24,29,30]; Analyses indicated by the symbols of the element functions were within 0.4% of the theoretical values for the final products **8a–e**, **9a–e**, **16**, **17**, **20** and **21**.

5.2. General procedure for 2-(4-methoxyphenoxy) -1-arylethanone (*3a–e*)

Potassium carbonate (0.05 mol) and potassium iodide (0.005 mol) were added to a mixture of 2-bromo-1-arylethanone (0.05 mol), 4-methoxyphenol (0.05 mol), and dry acetone (60 mL). The reaction mixture was covered with black cloth and was stirred at room temperature for 16 h under nitrogen atmosphere, and then was diluted with 120 mL of CH_2Cl_2. The solid salts were filtered off and the organic phase was washed with water, dried over Na_2SO_4. Evaporation gave crude compounds **3a–e** which were directly used for the next reactions without further purification.

5.3. General procedure for 5-methoxy-3-arylbenzofuran (*4a–e*)

Method A. A mixture of 2-(4-methoxyphenoxy)-1-arylethanone (**3a–e**) (about 0.01 mol), polyphosphoric acid (20 g), and toluene (60 mL) was refluxed for 5–6 h. The reaction mixture was cooled to room temperature, poured into water, and extracted with diethyl ether. The organic extracts were dried over Na_2SO_4. Evaporation gave crude compounds **4a–e** which were directly used for the next reactions without further purification.

Method B. A mixture of 2-(4-methoxyphenoxy)-1-arylethanone (**3a–e**) (0.01 mol), dry Amberlyst 15 resin (1 g), and toluene (25 mL) was refluxed for 2–6 h with azeotropic removal of water. The catalyst was removed by filtration, and the filtrate was washed with ethyl ether. Evaporation of solvent gave compounds **4a–e** which were directly used for the next reactions without further purification.

5.4. General procedure for substituted benzofuran-5-ol (*5a–e*)

Unpurified 3-aryl-5-methoxybenzofuran (about 2 mmol) was added to mixed solution (12 mL) of 48% HBr and acetic anhydride (1:1, v/v). The mixture was refluxed at 140 °C for 0.5–4 h. The reaction mixture was cooled to room temperature, neutralized with saturated sodium bicarbonate solution and extracted with diethyl ether. The organic extracts were washed with saturated sodium chloride solution and dried over Na_2SO_4. Evaporation and purification by chromatography on silica gel (Hexanes/EtOAc) gave (**5a–e**) in overall yields of 69–82%.

*3-Phenylbenzofuran-5-ol (**5a**)*. ESI-MS *m/z*: 209 ($[M-H]^-$). ^1H NMR (500 MHz, $CDCl_3$, δ ppm): 4.91 (br, s,1H), 6.86 (dd, 1H, $J = 2.5$ Hz, $J = 9.0$ Hz), 7.24 (d, 1H, $J = 2.5$ Hz), 7.34–7.37 (m, 1H), 7.39 (d, 1H, $J = 9.0$ Hz), 7.43–7.47 (m, 2H), 7.58–7.60 (m, 2H), 7.75 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 151.31,150.57, 142.10, 131.66, 128.67, 127.14, 126.99, 121.87, 113.22, 112.04, 105.53.

*3-(4-Chlorophenyl)benzofuran-5-ol (**5b**)*. ESI-MS *m/z*: 243 ($[M-H]^-$). ^1H NMR (300 MHz, d_6-acetone, δ ppm): 6.93 (dd, 1H, $J = 2.4$, $J = 9.0$), 7.28 (d, 1H, $J = 2.4$), 7.40 (d, 1H, $J = 9.0$), 7.48 (d, 2H, $J = 8.4$), 7.69 (d, 2H, $J = 8.4$), 8.05 (s, 1H). ^{13}C NMR (75 MHz, d_6-acetone, δ ppm): 154.11, 150.34, 143.17, 132.62, 131.29, 129.21, 128.79, 126.76, 120.85, 113.84, 112.16, 104.90.

*3-(4-Fluorophenyl)benzofuran-5-ol (**5c**).* ESI-MS *m/z*: 227 ([M − H]$^-$). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.56 (s, 1H), 7.28 (dd, 2H, *J* = 5.4 Hz, *J* = 8.7 Hz), 7.25 (d, H, *J* = 8.7 Hz), 7.11 (d, 1H, *J* = 2.7 Hz), 6.91 (t, 2H, *J* = 8.7 Hz), 6.84 (dd, 1H, *J* = 2.7 Hz, *J* = 8.7 Hz), 6.73 (br, s,1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 161.75 (d), 151.08, 150.54, 141.99, 128.44 (d), 127.54, 126.95, 120.96, 115.54 (d), 113.30, 112.14, 105.36.

*3-p-Tolylphenylbenzofuran-5-ol (**5d**).* ESI-MS *m/z*: 223 ([M − H]$^-$). ^{1}H NMR (300 MHz, d_6-acetone, δ ppm): 2.35 (s, 3H), 6.93 (dd, 1H, *J* = 2.4 Hz, *J* = 9.0 Hz), 7.26 (d, 2H, *J* = 7.8 Hz), 7.31 (d, 1H, *J* = 2.4 Hz), 7.38 (d, 1H, *J* = 9.0 Hz), 7.55 (d, 2H, *J* = 7.8 Hz), 7.95 (s, 1H). ^{13}C NMR (75 MHz, d_6-acetone, δ ppm): 153.92, 150.37, 142.44, 137.03, 129.77, 129.49, 127.26, 127.16, 121.95, 113.62, 112.04, 105.15, 20.76.

*3-(3,4-Dimethylphenyl)benzofuran-5-ol (**5e**).* ESI-MS *m/z*: 257 ([M − H]$^-$). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.63 (s, 1H), 7.05–7.29 (m, 5H), 6.80 (dd, 1H, *J* = 2.4 Hz, *J* = 8.7 Hz), 2.22 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 151.17, 150.59, 141.82, 136.94, 135.66, 129.95, 129.13, 128.25, 127.27, 124.46, 121.88, 113.01, 111.96, 105.59, 19.78, 19.50.

*2-Methylbenzofuran-5-ol (**13**).* ESI-MS *m/z*: 147 ([[M − H]$^-$). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.15 (d, 1H, *J* = 8.7 Hz), 6.86 (d, 1H, *J* = 2.1 Hz), 6.69 (dd, 1H, *J* = 2.1 Hz, *J* = 8.7 Hz), 6.11 (s,1H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 156.24, 150.61, 149.55, 129.89, 111.31, 110.76, 105.48, 102.48, 14.02.

5.5. General procedure for substituted naphtho[1,2-b]furan-4,5-dione (**8a–e**, **9a–e**, **16**, **17**, **20**, **21**)

Substituted benzofuran-5-ol (**5a–e**, **13**) (1 mmol) and IBX (1.2 mmol) were added to dry DMF (5 mL) and the reaction mixture was stirred at room temperature for 4–5 h. The reaction mixture was diluted with 120 mL water, extracted with benzene, washed with saturated sodium chloride solution, dried over Na_2SO_4, and concentrated to 50 mL. *N*-Dienes (1 mmol) were added and the mixture was stirred at 45 °C for 16 h for *N*-diene **II** and refluxed for 36 h for *N*-dienes **II**, **IV**. The cycloaddition products were then aromatized using the following two different methods.

Method A. To one half of the above solution was added DDQ (0.75 mmol) and the mixture was refluxed for 16 h. Evaporation and purification by chromatography on silica gel ($CHCl_3$/CH_3OH) gave the target compounds **8a–e** and **16** in 75–86% yield, **21** in 20% yield, **9a–e** and **17** in 6–12% yield and **20** in 50% yield.

Method B. To the other half of solution was added silica gel (10 g, 80–120 mesh) and the solvent was removed in vacuo at 45 °C. The resulted dry silica gel was then stirred at 45 °C for 24 h. Purification by chromatography on silica gel ($CHCl_3$/CH_3OH) gave target compounds **8a–e**, **16** in 19–25% yield and **9a–e**, **17** in 55–65% yield.

*7,9-Dimethyl-3-(3,4-dimethylphenyl)-6-acetamidonaphtho [1,2-b] furan-4,5-dione (**8e**) (Red solid).* ESI-MS *m/z*: 388 ([M + H]$^+$), HRMS-EI *m/z*: calcd for $C_{24}H_{21}NO_4$, 387.1465, found 387.1459. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.25 (s, 3H), 2.26 (s, 3H), 2.29 (s, 3H), 2.31 (s, 3H), 2.67 (s, 3H), 7.18 (d, 1H, *J* = 7.5 Hz), 7.33 (s, 1H), 7.39 (dd, 1H, *J* = 7.5 Hz, *J* = 1.5 Hz), 7.45 (s, 1H), 7.61 (s, 1H), 9.91 (s, br, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 19.47, 19.58, 19.76, 21.89, 24.23, 118.43, 121.16, 125.24, 125.76, 126.37, 127.14, 129.52, 129.74, 133.14, 136.67, 137.18, 138.19, 139.98, 141.05, 142.12, 163.07, 169.02, 174.56, 184.89. Anal. ($C_{24}H_{21}NO_4$) C, H, N.

*7,9-Dimethyl-3-(3,4-dimethyl)naphtho[1,2-b]furan-4,5-dione (**9e**) (Red solid).* ESI-MS *m/z*: 331 ([M + H]$^+$). HRMS-EI *m/z*: calcd for $C_{22}H_{18}O_3$, 330.1250, found 330.1251. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.29 (s, 3H), 2.30 (s, 3H), 2.35 (s, 3H), 2.67 (s, 3H), 7.17 (d, 1H, *J* = 7.5 Hz), 7.24 (s, 1H), 7.37 (dd, 1H, *J* = 7.5 Hz, *J* = 2.0 Hz), 7.45 (s, 1H), 7.57 (s, 1H), 7.77 (s, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 19.57, 19.75, 21.06, 21.58, 118.91, 124.36, 125.81, 126.62, 127.33, 129.52, 129.65, 129.74, 135.51, 136.57, 137.02, 139.42, 140.18, 140.68, 163.33, 175.05, 181.43. Anal. ($C_{22}H_{18}O_3$) C, H.

*7,9-Dimethyl-3-p-tolylnaphtho[1,2-b]furan-4,5-dione (**9d**) (Red solid).* ESI-MS *m/z*: 317 ([M + H]$^+$). HRMS-EI *m/z*: calcd for $C_{21}H_{16}O_3$, 316.1094, found 316.1087. ^{1}H NMR (500 MHz, d_6-DMSO, δ ppm): 2.32 (s, 3H), 2.35 (s, 3H), 2.61 (s, 3H), 7.23 (d, 2H, *J* = 8.0 Hz), 7.38 (s, 1H), 7.59 (d, 2H, *J* = 8.0 Hz), 7.62 (s, 1H), 8.16 (s, 1H). ^{13}C NMR (500 MHz, d_6-DMSO, δ ppm): 20.44, 20.72, 20.93, 118.40, 123.65, 125.89, 126.48, 128.13, 128.20, 128.63, 129.70, 134.62, 137.29, 138.65, 139.53, 141.93, 162.04, 174.37, 180.06. Anal. ($C_{21}H_{16}O_3$) C, H.

*7,9-Dimethyl-3-p-tolyl-6-acetamidonaphtho[1,2-b]furan-4,5-dione (**8d**) (Red solid).* ESI-MS *m/z*: 372 ([M − H]$^-$). HRMS-EI *m/z*: calcd for $C_{23}H_{19}NO_4$, 373.1309, found 373.1310. ^{1}H NMR (500 MHz, d_6-DMSO, δ ppm): 2.08 (s, 3H), 2.18 (s, 3H), 2.34 (s, 3H), 2.64 (s, 3H), 7.23 (d, 2H, *J* = 7.5), 7.48 (s, 1H), 7.60 (d, 2H, *J* = 7.5), 8.22 (s, 1H), 9.65 (br, s,1H). ^{13}C NMR (500 MHz, d_6-DMSO, δ ppm): 17.90, 20.72, 21.28, 23.03, 117.85, 124.03, 124.68, 125.54, 126.31, 128.01, 128.70, 132.62, 137.40, 137.57, 138.28, 140.02, 141.29, 162.32, 168.24, 174.96, 182.35. Anal. ($C_{23}H_{19}NO_4$) C, H, N.

*7,9-Dimethyl-3-phenyl-6-acetamidonaphtho[1,2-b]furan-4,5-dione (**8a**) (Red solid).* ESI-MS *m/z*: 358 ([M − H]$^-$). HRMS-EI *m/z*: calcd for $C_{22}H_{17}NO_4$, 359.1152, found 359.1154. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.23 (s, 3H), 2.24 (s, 3H), 2.63 (s, 3H), 7.29 (s, 1H), 7.34–7.41 (m, 3H), 7.61 (s, 1H), 7.64 (d, 2H, *J* = 7.2), 9.83 (br, s,1H). ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 19.44, 21.85, 24.19, 118.30, 121.16, 125.09, 127.06, 128.33, 128.45, 128.50, 128.97, 133.18, 138.33, 140.01, 141.31, 142.10, 163.19, 168.98, 174.51, 184.68. Anal. ($C_{22}H_{17}NO_4$ with $1H_2O$) C, H, N.

*7,9-Dimethyl-3-phenylnaphtho[1,2-b]furan-4,5-dione (**9a**) (Red solid).* ESI-MS: *m/z* 303 ([M + H]$^+$). HRMS-EI *m/z*: calcd for $C_{20}H_{14}O_3$, 302.0937, found 302.0925. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.35 (s, 3H), 2.67 (s, 3H), 7.23 (s, 1H), 7.35–7.42 (m, 3H), 7.59 (s, 1H), 7.64 (d, 1H, *J* = 6.6 Hz), 7.75 (s, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 21.16, 21.68, 118.71, 124.14, 127.19, 128.30, 128.34, 129.09, 129.46, 129.63, 135.47, 139.33, 140.19, 140.81, 163.30, 174.84, 181.10. Anal. ($C_{20}H_{14}O_3$) C, H.

*2,7,9-Trimethyl-6-acetamidonaphtho[1,2-b]furan-4,5-dione (**16**) (Red solid).* ESI-MS *m/z*: 296 ([M − H]$^-$). HRMS-EI *m/z*: calcd for $C_{17}H_{15}NO_4$, 297.0996, found 297.0991. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.23 (s, 3H), 2.24 (s, 3H), 2.40 (d, *J* = 1 Hz, 3H), 2.59 (s, 3H), 6.40 (q, *J* = 1 Hz, 1H), 7.26 (s, 1H), 9.86 (br, s,1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 13.58, 19.30, 21.50, 24.11, 103.78, 121.00, 121.94, 125.25, 132.62, 137.87, 140.26, 141.88, 155.52, 161.21, 168.97, 173.98, 184.71. Anal. ($C_{17}H_{15}NO_4$) C, H, N.

*2,7,9-Trimethylnaphtho[1,2-b]furan-4,5-dione (**17**) (Red solid).* ESI-MS *m/z*: 241 ([M + H]$^+$). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.34 (s, 3H), 2.41 (d, *J* = 1 Hz, 3H), 2.62 (s, 3H), 6.44 (q, *J* = 1 Hz, 1H), 7.20 (s, 1H), 7.75 (s, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 13.60, 20.99, 21.22, 104.03, 122.60, 124.42, 129.71, 129.79, 134.92, 139.17, 139.69, 154.97, 161.53, 174.80, 181.48. Anal. ($C_{15}H_{12}O_3$) C, H.

*7,9-Dimethyl-3-(4-chlorophenyl)-6-acetamidonaphtho[1,2-b]furan-4,5-dione (**8b**) (Red solid).* ESI-MS *m/z*: 391 ([M − H]$^-$). HRMS-EI *m/z*: calcd for $C_{22}H_{16}ClNO_4$, 393.0762, found 393.0764. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.26 (s, 3H), 2.27 (s, 3H), 2.69 (s, 3H), 7.36 (s, 1H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.66 (s, 1H), 9.91 (br, s,1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 19.50, 21.86, 24.21, 118.17, 121.27, 124.99, 126.15, 127.52, 128.74, 129.70, 133.32, 134.57, 138.62, 140.21, 141.32, 142.19, 163.45, 169.02, 174.62, 184.62. Anal. ($C_{22}H_{16}ClNO_4$) C, H, N.

*7,9-Dimethyl-3-(4-chlorophenyl)naphtho[1,2-b]furan-4,5-dione (**9b**) (Red solid).* ESI-MS *m/z*: 336 ([M + H]$^+$). HRMS-EI *m/z*: calcd for $C_{20}H_{13}ClO_3$, 336.0548, found 336.0545. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.36 (s, 3H), 2.66 (s, 3H), 7.24 (s, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.59 (s, 1H), 7.76 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 180.91, 174.82, 163.50, 140.85, 140.40, 139.39, 135.58,

134.25, 129.62, 129.55, 128.49, 127.58, 126.14, 123.97, 118.45, 21.70, 21.21. Anal. ($C_{20}H_{13}ClO_3$) C, H.

*7,9-Dimethyl-3-(4-flurophenyl)-6-acetamidonaphtho[1,2-b]furan-4,5-dione (**8c**) (Red solid).* ESI-MS *m*/*z*: 376 ($[M-H]^-$). HRMS-EI *m*/*z*: calcd for $C_{22}H_{16}FNO_4$, 377.1058, found 377.1055. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.26 (s, 6H), 2.67 (s, 3H), 7.09 (t, 2H, $J = 8.5$ Hz), 7.33 (s, 1H), 7.61 (s, 1H), 7.64 (dd, 2H, $J = 5.4$ Hz, $J = 8.5$ Hz), 9.88 (br, s,1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 184.69, 174.65, 169.08, 163.33, 162.95 (d), 142.19, 141.11, 140.11, 138.53, 133.31, 130.23 (d), 126.22, 125.04, 121.25, 118.23, 115.51 (d), 24.23, 21.89, 19.50. Anal. ($C_{22}H_{16}FNO_4$) C, H, N.

*7,9-Dimethyl-3-(4-flurophenyl)naphtho[1,2-b]furan-4,5-dione (**9c**) (Red solid).* ESI-MS *m*/*z*: 321 ($[M+H]^+$). HRMS-EI *m*/*z*: calcd for $C_{20}H_{13}FO_3$, 320.0843, found 320.0842. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.37 (s, 3H), 2.68 (s, 3H), 7.08 (t, 2H, $J = 8.7$ Hz), 7.25 (s, 1H), 7.58 (s, 1H), 7.63 (dd, 2H, $J = 5.4$ Hz, $J = 8.7$ Hz), 7.78 (s, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 181.26, 175.12, 163.59, 162.88 (d), 140.73, 140.47, 139.48, 135.66, 130.30 (d), 129.80, 129.66, 126.40, 125.26, 124.17, 118.70, 115.39 (d), 21.58, 21.10. Anal. ($C_{20}H_{13}FO_3$) C, H.

*3-Phenylnaphtho[1,2-b]furan-4,5-dione (**20**) (Red solid).* ESI-MS *m*/*z*: 275 ($[M+H]^+$). HRMS-EI *m*/*z*: calcd for $C_{18}H_{10}O_3$, 274.0624, found 274.0617. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.35–7.47 (m, 4H), 7.60–7.74 (m, 5H), 8.04 (d, 1H, $J = 7.5$ Hz). ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 180.20, 174.28, 161.26, 141.35, 135.33, 130.18, 130.12, 128.81, 128.35, 128.10, 127.65, 122.37. Anal. ($C_{18}H_{10}O_3$) C, H.

*6-(N-Succinimidyl)-3-(4-chlorophenyl)naphtho[1,2-b]furan-4,5-dione (**21**).* FAB-MS *m*/*z*: 406 ($[M+H]^+$). ^{1}H NMR (300 MHz, d_6-DMSO, δ ppm): 2.86–2.96 (m, 4H, $2CH_2$), 7.41 (d, 1H, $J = 8.0$ Hz), 7.51 (d, 2H, $J = 8.5$ Hz), 7.78 (d, 2H, $J = 8.5$ Hz), 7.92–7.98 (m, 2H), 8.38 (s, 1H). ^{13}C NMR (300 MHz, d_6-DMSO, δ ppm): 178.23, 176.09, 172.78, 159.62, 143.41, 135.90, 134.39, 132.70, 132.92, 129.70, 129.58, 128.20, 128.06, 125.24, 123.74, 123.25, 118.48, 28.74. Anal. ($C_{22}H_{12}ClNO_5$) C, H, N.

5.6. Cell culture

Two different human cancer lines (human lung adenocarcinoma cells GLC-82 and human nasopharyngeal carcinoma cell Line CNE2) were cultured in RPMI 1640 medium (Gibco-BRL Grand Island, NY) supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine at 37 °C in incubator with humidified atmosphere of 5.0% CO_2 and 95% air.

5.7. Cell growth inhibition assay

The growth inhibitory effects of selected compounds on different cancer cell lines were evaluated by means of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. The cells were plated at a density of 1×10^4 per well in 96-well microplates and allowed to incubate overnight. The tested compounds were added to the wells at increasing concentrations (0–100 μM). After 48 h, each well was treated with 20 μL 5 mg/mL MTT solution and the cells were further incubated at 37 °C for 4 h. At the end of incubation, the untransformed MTT was removed and 150 μL DMSO added. The microplates were well shaken to dissolve the formazan dye and the absorbance at 570 nm was measured using a microplate reader (Bio-Rad). Cytoviability of the control cells was taken as 100%. Tests were conducted in quadruplicate. For each dose, the mean cell viability was expressed as a percentage of the control. The IC_{50} represents the compound concentration required for the reduction of the mean cell viability to 50%.

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[28] Tetrahydro cycloadducts **7a**–**e**, **15**, **18**, **19** were found to be so unstable in atmosphere or on silica gel that it was difficult to be purified. Moreover, when the unstable cycloaddition products **7a**–**e**, **15**, **18**, **19** were aromatized, thermal elimination of amide group gives compounds **9a**–**e**, **17**, **20**. The regioselectivity of the cycloaddition reactions could therefore be assigned indirectly from the structure assignments of these aromatized products.
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