Synthesis and Antitumor Evaluation of Thiophene Analogs of Kigelinone

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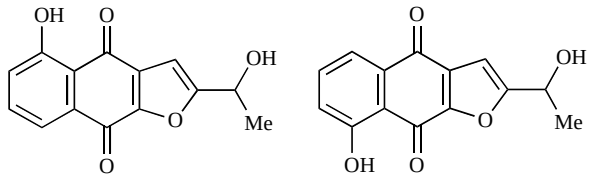
Abstract: The synthesis of kigelinone thiophene analogs and related naphtho[2,3-*b*]thiophene-4,9-quinones from 2-substituted 4,7-dimethoxybenzo[*b*]thiophenes *via* an oxidative deprotection, Diels-Alder, and oxidative aromatization reaction sequence is reported. The 2-substituted naphtho[2,3-*b*]thiophene-4,9-quinones display significant antitumor activity in the range IC_{50} 1.1-47 μM on a panel of four distinct human cancer cell lines.

Keywords: Quinones, Benzo[*b*]thiophenes, Oxidative deprotection, Diels-Alder reaction, Regioisomers, Citotoxicity.

Among the broad variety of biological active naturally occurring heterocyclic quinones [1] a series of naphthoquinones based on the naphtho[2,3-*b*]furan-4,9-quinone skeleton display potent cytotoxic activity against numerous tumor cell lines [2, 3]. Kigelinone **1** and its positional isomers **2** (Fig. (**1**)), isolated from *Tabebuia avellanedae* [3], are two representative members of this series.



1 **2**

Fig. (1). Structure of kigelinone and its positional isomer.

These naphthofuranquinones exhibit similar cytotoxic activities against leukemia cells P-388 (IC_{50}: **1** = 16 μmol/mL; **2** = 12 μmol/mL), suggesting that location of the phenolic hydroxyl substituent, at C-5 or C-8, does not have a significant influence on the biological activity in these assays [4]. Kigelinone **1**, exhibits remarkably potent inhibition against Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) [5]. Furthermore, compound **1** acts as a cancer chemopreventive agent by strongly inhibiting TPA-induced tumor promotion on mouse skin initiated with 7,12 dimethylbenz[*a*]-anthracene in two stage carcinogenesis tests [5, 6].

Following our interest in developing novel anticancer agents [7], a series of naphtho[2,3-*b*]thiophene-4,7-quinones isosterically related to the antitumor keligenone **1** and its isomer **2**, were designed and synthesized. The novel thiophene analogs were evaluated for *in vitro* anticancer activity against MRC-5 human lung fibroblast cells, and four distinct human tumor cell lines: AGS gastric adenocarcinoma, SK-MES-1 lung cancer, J82 bladder carcinoma, and HL-60 leukemia in 72-h drug exposure assays. The cytotoxicity of the compounds was measured using a conventional microculture tetrazolium reduction assay [8], using etoposide as positive control.

The synthesis of 5- and/or 8-hydroxy-2-(1-hydroxyethyl)-naphtho[2,3-*b*]thiophene-4,9-quinones was explored following the retrosynthetic sequence shown in Scheme **1**, which is based on heterocyclization and Diels-Alder reactions as the key steps.

Benzothiophene **4** was prepared from 6-nitro-2,5-dimethoxybenzaldehyde (**3**) and methyl thioglycolate according to a previously reported method [9].

Two synthetic approaches to the target benzothiophene **5** *via* the addition reaction of methylmagnesium chloride to a carbonyl group were planned using the precursors **4** or **8** (Scheme **2**). Our attempts with the first precursor were unsuccessful. Therefore aldehyde **8** was prepared from **4** [10] *via* alcohol **7** [9]. Further reaction of **8** with methylmagnesium chloride in THF gave the expected benzothiophene **9** in 90% yield.

Next, compound **9** was reacted with acetic anhydride to give benzothiophene **5** in 95% yield, which by a subsequent oxidative demethylation with CAN in acetonitrile-water produced the target benzothiophenequinone **6** in 94% yield [11]. Diels-Alder reaction of benzothiophenequinone **6** with (*E*)-1-trimethylsilyloxy-1,3-butadiene in dichloromethane at room temperature yields a nearly 50:50 mixture of regioisomers **10a**+**10b**. The ratio between these isomers was determined from the thiophene proton signals at δ 8.06 and 8.08 ppm (400 MHz).

The lack of regioselectivity of the cycloaddition between quinone **6** and the silyloxydiene can be attributed to the small difference between the LUMO eigenvectors at C-5 (0.26244 eV) and C-6 (-0.24576 eV) of dienophile **6** [12].

Acid-induced hydrolysis of cycloadduct **10a**+**10b** followed by aromatization of the alcohol intermediates with PCC gave naphthothiophenequinones **11a**+**11b** in 80% yield. Further hydrolysis of acetates **11a**+**11b** produced a 50:50

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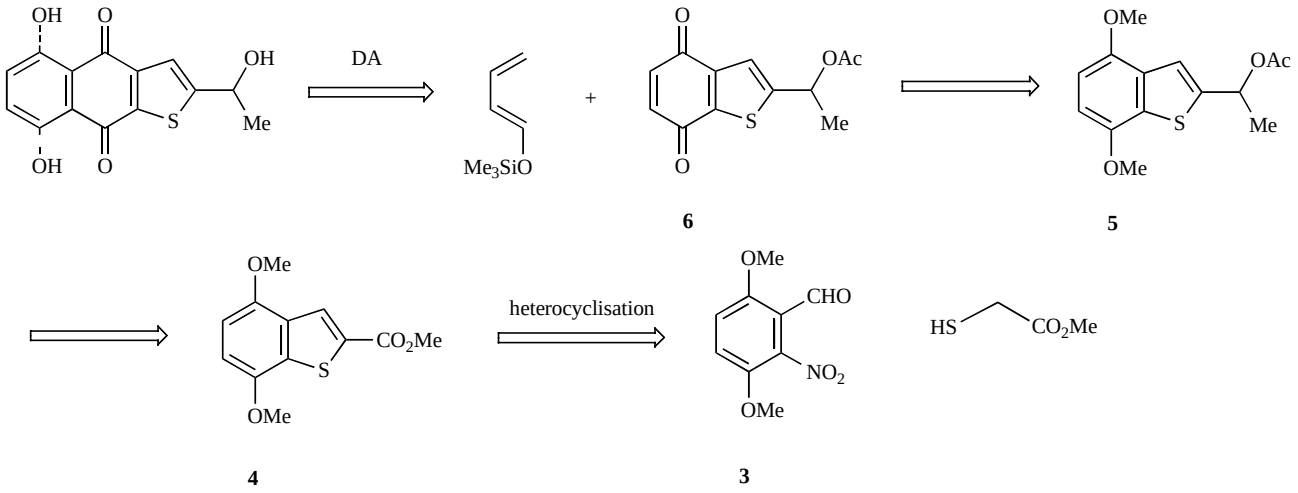
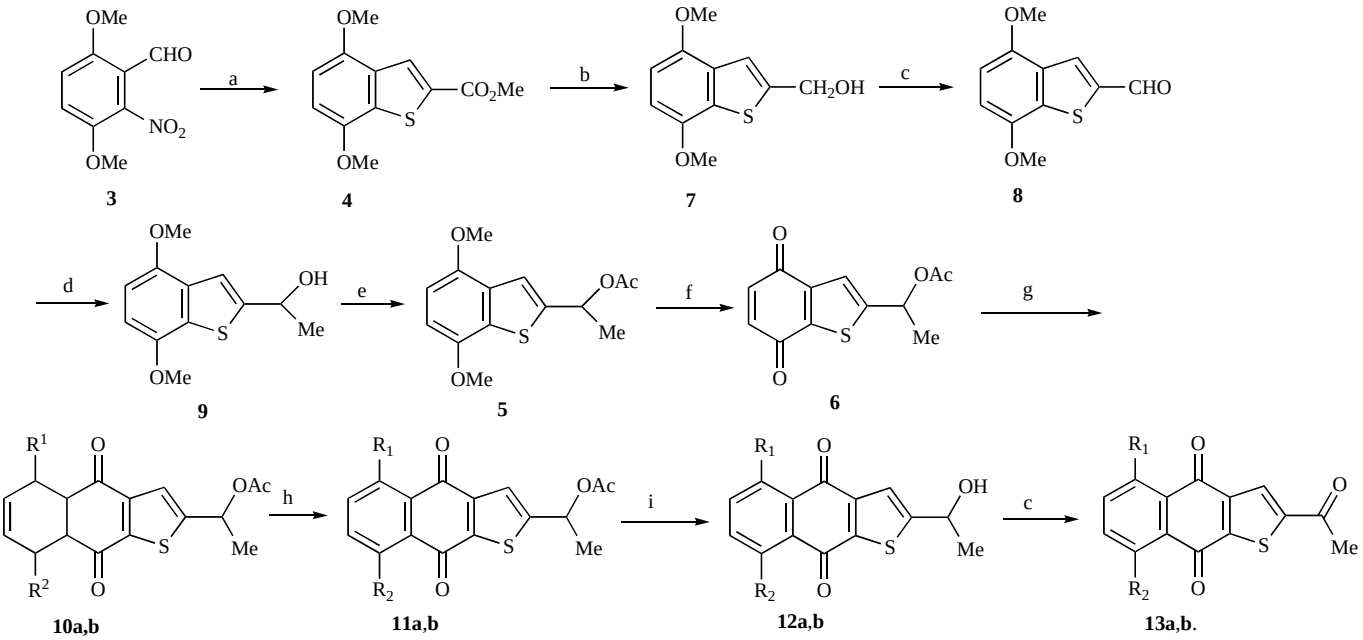


Scheme 1. Strategy to prepare kigelinone thiophene-analogues.



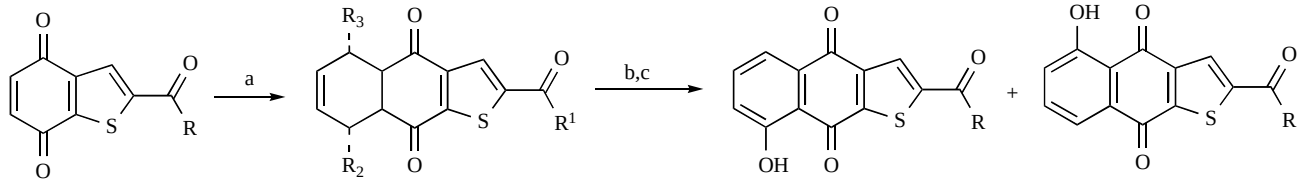
Scheme 2. Synthesis of compounds **13a-b:** substituents: R^1 = H($OSiMe_3$), R^2 = $OSiMe_3$ (H); conditions: a) $HSCH_2CO_2Me$, K_2CO_3, DMF, 60°C; b) $LiAlH_4$, Et_2O, reflux; c) PCC, AcONa, CH_2Cl_2; d) MeMgCl, THF, reflux; e) Ac_2O, 2h, rt; f) CAN, MeCN, H_2O, rt; g) (*E*)-Me_3SiO-CH=CH-CH=CH_2, CH_2Cl_2, 2d, rt; h) HCl, H_2O, rt; i) HCl, H_2O, reflux.

mixture of hydroxynaphthothiophene-quinones **12a**+**12b** in 96% yield [13]. Quinones **12a**/**12b** were converted into the corresponding 2-acetylnaphthothiophenequinones **13a**/**13b** in 95% yield by oxidation with PCC in dichloromethane [14].

Since our efforts to isolate the regioisomers from the corresponding **12a**,**b** and **13a**,**b** mixtures by column and preparative TLC chromatography were unsuccessful, the antitumor evaluation was performed with the isomeric mixtures.

Compounds **18a**,**b** and **19a**,**b** [15], prepared as 70:30 regioisomeric mixtures from benzothiophenequinones **14** [9] and **15**, as shown in Scheme **3**, were also included into the biological screening. The ratio between regioisomers **18a**/**18b** and **19a**/**19b** was established from the signals of the phenolic protons in ^{1}H NMR and the structure of the major regioisomer, in each mixture, on the basis of 2D NMR experiments. Calculation of the HOMO and LUMO eigenvectors of the silyloxydiene and dienophiles **14** and **15** [16] indicates that the regiochemistry of these cycloadditions proceed in accordance with that predicted by FMO theory [17, 18].

Table **1** shows the *in vitro* cytotoxic evaluation of naphthothiophenequinone mixtures **12**, **13**, **18** and **19** against normal MRC-5 human lung fibroblasts and four human tumor cells: AGS gastric adenocarcinoma cell lines, SK-MES-1 lung cancer cells, J82 bladder carcinoma cells, and HL-60 leukemia cells. Data for the etoposide, as positive control, are included for comparison. The results are expressed as IC_{50}-values, that is, as the micromolar concentration of a compound that achieves 50% cellular growth reduction after 72 h of drug exposure.



14. R = OMe
15. R = H

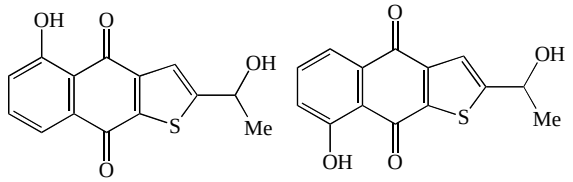
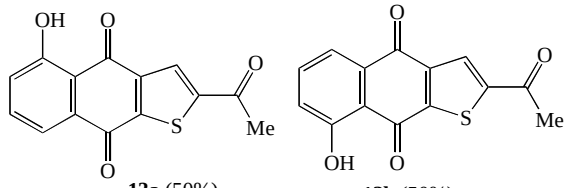
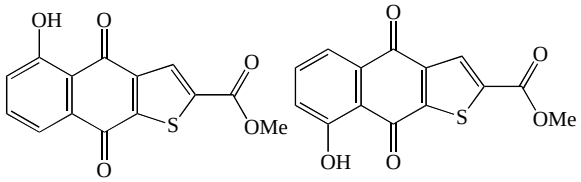
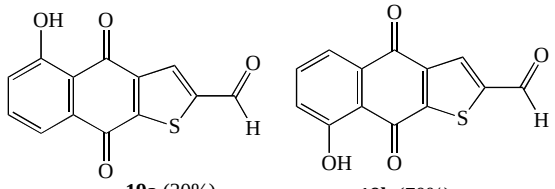
16a. R^1 = OMe, R^2 = $OSiMe_3$, R^3 = H
16b. R^1 = OMe, R^2 = H, R^3 = $OSiMe_3$
17a. R^1 = R^3 =H, R^2 = $OSiMe_3$
17b. R^1 = R^2 = H, R^3 = $OSiMe_3$

18a. R = OMe (70%)
19a. R = H (70%)

18b. R = OMe (30%)
19b. R = H (30%)

Scheme 3. Preparation of compounds **18** and **19**: a) (*E*)-1-Me_3SiO-CH=CH-CH=CH_2, CH_2Cl_2, 2d, rt; b) HCl, H_2O, rt; c) PCC, AcONa, CH_2Cl_2.

Table 1. ***In Vitro*** **Antitumor Activity of Naphthothiophenequinones 12, 13, 18 and 19**

Structure, N° (% in the mixture)	IC_{50} (μM)[a]				
	MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]	HL-60[f]
 **12a** (50%) **12b** (50%)	4.9 ± 0.2	2.7 ±0.1	3.0 ± 0.2	1.6 ± 0.05	10.9 ± 0.7
 **13a** (50%) **13b** (50%)	8.1 ± 0.4	2.6 ± 0.2	2.0 ± 0.2	1.3 ± 0.07	46.9 ± 2.5
 **18a** (30%) **18b** (70%)	10.0 ± 0.6	5.1 ± 0.3	1.8 ± 0.08	1.1 ± 0.06	42.7 ± 2.2
 **19a** (30%) **19b** (70%)	5.9 ± 0.3	1.2 ± 0.07	3.1 ± 0.2	2.2 ± 0.2	15.5 ± 0.9
etoposide	3.9 ± 0.3	0.36 ± 0.1	2.5 ± 0.1	2.8 ± 0.2	0.80 ± 0.1

[a]Data represent mean values (±SEM) for six independent determination; [b]human lung fibroblasts cells; [c]human gastric adenocarcinoma cell line; [d]human lung cancer cell line; [e]human bladder carcinoma cell line; [f]human leukemia cell line.

Comparison of the IC_{50} values obtained with compounds **12**, **13**, **18** and **19** against human lung fibroblast cells indicates that these thiophene analogs are less cytotoxic than the antitumor drug etoposide. The **12** and **13** mixtures, which contain equal amount of their regioisomers, displayed comparable antitumor activity on the cell lines, except for HL-60 leukemia cells.

It should be noted that among the evaluated naphthotiophenequinones, compounds **18a**+**18b** showed the highest antitumor potencies and selectivities against SK-MES-1 human lung cancer and J82 bladder carcinoma cell lines.

These compound along with isomers **13a**+**13b** were less cytotoxic on MRC-5 human lung fibroblasts and more potent than the reference etoposide on SK-MES-1 lung cancer and J82 bladder carcinoma cell lines. Furthermore, the kigelinone thiophene-analogs **12a**,**12b** and their acetyl congeners **13a**,**13b** exhibited less cytotoxic effect and more antitumor potencies against J82 bladder carcinoma cells than etoposide

In conclusion, we have described the synthesis of kigelinone thiophene-analogs from 2- substituted 4,7-dimethoxybenzo[*b*]thiophenes. The reported synthesis involves easily available precursors and an oxidative deprotection, a Diels-Alder, and oxidative aromatization reaction sequence. This approach may be used to make a large number of such thiophene analogs from diverse 2-substituted 4,7-dimethoxybenzo[*b*]-thiophenes. The described biological evaluation provides significant information about the design of novel thiophene-containing quinones as potential antitumor agents based on bioisosteric replacement. Further studies on the preparation of 2-substituted naphthothiophenequinones and isolation of regioisomers **12a** and **12b** *via* derivatization are in progress.

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[11] *Experimental procedure*: A solution of cerium ammonium nitrate (300 mg, 0.55 mmol) in water (10 mL) was dropwise added to a stirred solution of **5** (50 mg, 0.18 mmol) in water (5 mL). The mixture was left for 10 min at rt and then extracted with ethyl acetate (3x15 ml). The organic layer was dried over magnesium sulfate and evaporated under reduced pressure. Column chromatography of the crude over silica gel (CH_2Cl_2) yield quinone **6** (42 mg, 94%) as a yellow solid mp 112-113°C. IR (KBr): ν 1745 (C=O, ester), 1670-1635 (C=O quinone); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.65 (d, 3H, *J* = 6.5 Hz, CHC<u>H</u>$_3$), 2.10 (s, 3H, $COCH_3$), 6.11 (q, 1H, *J* = 6.5 Hz, CH), 6.77 (d, 1H, *J* = 10 Hz, 5- or 6-H), 6.82 (d, 1H, *J* = 10 Hz, 6- or 5-H), 7.41 (s, 1H, 3-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 52.6, 76.4, 133.9, 136.9, 137.9, 139.5, 145.8, 147.0, 162.1, 180.3, 182.1; Anal Calcd for $C_{12}H_{10}O_4S$: C, 57.59; H, 4.03; S, 12.81. Found: C, 57.21; H, 3.99; S, 12.76.

[12] The LUMO eigenvector coefficients (eV) were determined using the semiempirical AM1 method implemented in the PC Spartan Pro Inc package.

[13] Spectral and analytical data for compounds **12a**+**12b**: IR (KBr): ν 3400-3200 (OH), 1660, 1635 (C=O quinone). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.66 (d, 3H, *J* = 6.5 Hz, CH_3), 2.13 (s, 1H, OH), 5.08 (m, 1H, CH), 7.30-7.80 (m, 3H, arom.), 8. 09 (s, 0.5H, 3-H), 8.11 (s, 0.5H, 3-H), 12.07 (s, 0,5H, OH), 12.17 (s, 0,5H, OH); Anal Calcd for $C_{14}H_{10}O_4S$: C, 61.30; H, 3.67; S, 11.69. Found: C, 61.17; H, 3.53; S, 11.76.

[14] Spectral and analytical data for compounds **13a**+**13b**: IR (KBr): 3300 (OH), 1683 (C=O), 1646 (C=O quinone). ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.66 (s, 3H, CH_3), 7.30-7.85 (m, 3H, arom.); 8.29-8.30 (2s br, 2H, 3-H), 12.01(s, 0.5H, OH); 12.15 (s, 0.5H, OH). Anal Calcd for $C_{14}H_8O_4S$: C, 61.76, H, 2.96, S, 11.79. Found: C, 61.56; H, 3.08, S, 11.67.

[15] Spectral and analytical data for compounds **19a**+**19b**: IR (KBr): 1680 (C=O aldehyde), 1645 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ7.30-7.85 (m, 3H, arom.), 8.40 (s, 0.7H, 3-H), 8.42 (s, 0.3H, 3-H), 10.1 (s,1H, CHO), 11.97 (s, 0.7H, OH); 12.13 (s, 0.3H, OH); Anal Calcd for $C_{13}H_6O_4S$: C, 60.46; H, 2.34; S, 12.42. Found: C, 60.01; H, 2.99; S, 12.3.

[16] LUMO eigenvectors (eV): Compound **14**: C-5 = -0.2634; C-6 = 0.2307. Compound **15**: C-5 = -0.3126; C-6 = -0.2291. HOMO eigenvectors (eV): silyloxydiene: C-1 = -0.4744 , C-4 = 0.4929.

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