Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2763–2768

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND CYTOTOXICITY OF ACETYL-4H, 9H-NAPHTHO[2,3-*b*]THIOPHENE-4,9-DIONES

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Received 6 July 1998; accepted 25 August 1998

Abstract: Several new acetyl-4*H*,9*H*-naphtho[2,3-*b*]thiophene-4,9-diones were synthesized and evaluated for in vitro cytotoxicity by NCI against seven cancer cell types. 2,7-Diacetyl naphtho[2,3-*b*]thiophene-4,9-dione (**9**) showed significant cytotoxicity against leukemia cells with log GI_{50} values of –7.61 against SR cells and –7.18 against MOLT-4 cells. 3-Acetyl-naphtho[2,3-*b*]thiophene-4,9-dione (**6**) also demonstrated potent cytotoxicity in the latter cell line with log $GI_{50} < -8$. © 1998 Elsevier Science Ltd. All rights reserved.

Many anthraquinones, including mitoxantrone (**1**), show potent antineoplastic activity.[1] In addition, the natural product 2-acetyl-4*H*,9*H*-naphtho[2,3-*b*]furan-4,9-dione (**2**) isolated from *Tabebuia cassinoids* (Lam.) DC. (Bignoniaceae)[2] demonstrated significant cytotoxicity in the KB cell culture assay (ED_{50} value = 4.2 μM). Thus, the anthraquinone and related furanonaphthoquinone skeletons are important structural features in the synthesis and development of new antitumor agents. Accordingly, a third bioisosteric compound, naphtho[2,3-*b*]thiophene-4,9-dione (**3**)[3] has been evaluated for cytotoxicity against KB cells resulting in an ED_{50} value of 6.5 μM. From this lead compound, we have prepared three mono- (**5–7**) and two di- (**9** and **10**) acetyl substituted derivatives. Herein, we describe their synthesis and cytotoxic evaluation.

Synthesis. Monoacetyl derivatives of 4*H*,9*H*-naphtho[2,3-*b*]thiophene-4,9-dione (**3**) were synthesized as shown below. Compound **3** was first reduced by sodium thiosulfate under alkaline conditions, then dimethyl sulfate was added giving *O*-methylation to the expected 4,9-dimethoxy-naphtho[2,3-*b*]thiophene (**4**).

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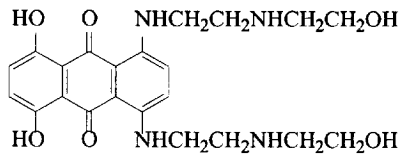
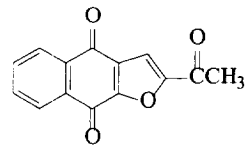
PII: S0960-894X(98)00496-X



1, Mitoxantrone



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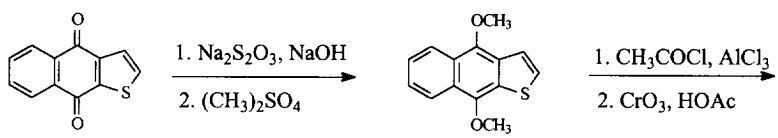
Compound **4** was reacted in a Friedel–Crafts acetylation with an equal molar ratio of acetyl chloride in the presence of $AlCl_3$, and the reaction product was then oxidized by CrO_3. Column chromatography gave three oxidized derivatives: **5** with mp 139–140 °C, **6** with mp 244–245 °C, and **7** with mp 146–147 °C.



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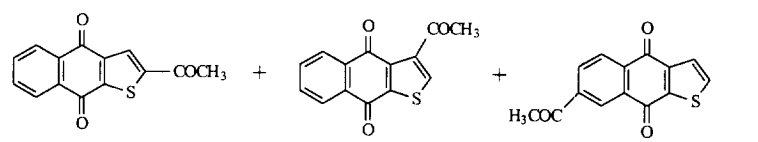
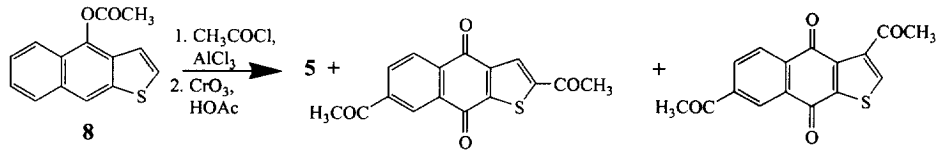


5 6 7

Based on mass spectral [*m/z* (256, M^+)] and elemental analysis data, the molecular formulas of all three products were determined as $C_{14}H_8O_3S$, which suggested positional isomers of monoacetylnaphtho[2,3-*b*]thiophene-4,9-dione. The ^1H NMR and ^{13}C NMR analysis including two dimensional techniques (i.e., ^1H-^1H Cosy, HMQC, and HMBC) led us to assign compound **5** as 2-acetylnaphtho[2,3-*b*]thiophene-4,9-dione; this assignment was confirmed by X-ray crystallographic analysis.

Assignments of the ^1H NMR and ^{13}C NMR spectra of products **6** and **7** were made by comparison with the spectra of compound **3** and by 2D-NMR techniques including ^1H-^1H Cosy, HMQC, and HMBC. From the above data, we concluded that products **6** and **7** were the 3-acetyl and 7-acetyl derivatives, respectively, of **3**.

For the synthesis of diacetyl derivatives of **3**, 4-acetoxynaphtho[2,3-*b*]thiophene (**8**) (prepared by literature methods)[3c,4] was reacted with excess acetyl chloride and $AlCl_3$, and the resulting reaction products were oxidized with CrO_3. The monoacetylated **5** was isolated by column chromatography together with two new products: **9** with mp 202–204 °C and **10** with mp 180–182 °C. Both compounds were diacetylated as seen from their molecular formula ($C_{16}H_{10}O_4S$) obtained by mass spectroscopy [*m/z* (298, M^+)] and elemental analysis.



8 9 10

The structures of compounds **9** and **10** were identified by comparing their ^1H NMR and ^{13}C NMR spectral data with those of the monoacetylnaphtho[2,3-*b*]thiophene-4,9-diones (**5–7**). We concluded that **9** is 2,7-diacetyl naphtho[2,3-*b*]thiophene-4,9-dione and **10** is 3,7-diacetyl naphtho[2,3-*b*]thiophene-4,9-dione.

Results and Discussion. The unsubstituted (**3**), two mono-acetyl (**5** and **6**), and two di-acetyl (**9** and **10**) derivatives were tested first for cytotoxicity in a KB cell assay. The parent compound **3** and the 3-acetyl derivative **6** showed similar potency in this assay with ED_{50} values of 6.5 and 5 μM, respectively. The potency increased (ED_{50} ~ 1.5 μM) when acetyl groups were added at the 2-position (**5**) or the 2- and 7-positions (**9**). Compound **10** with 3,7-disubstitution was the most potent in this assay with an ED_{50} value of 0.45 μM.

Compounds **5**, **6**, and **9** were also tested in NCI's in vitro human tumor cell line assay; the data from seven cancer types are shown in Table 1. All three compounds were active against all cell lines with similar mean log GI_{50} values ranging from –5.67 to –6.15. (GI_{50} is the molar concentration causing 50% cell growth inhibition; compounds with log $GI_{50} < -4$ are considered active.) The activity order roughly paralleled that in the KB cell assay with compound **9** showing greater activity, especially against HCT-15 colon and MCF breast cancer cell lines. Compound **9** was quite active against leukemia cell lines with log GI_{50} values of –7.61 against the SR and –7.18 against MOLT-4 cells; compound **6** also showed significant cytotoxicity in the latter cell line (log $GI_{50} <$ –8).

In summary, acetyl derivatives of 4*H*,9*H*-naphtho[2,3-*b*]thiophene-4,9-dione show promise as lead compounds for the further development of anticancer agents. Synthesis and cytotoxic evaluation of additional derivatives of this nucleus will be reported in a subsequent article.

Synthetic Methods:

General experimental procedures. All melting points are uncorrected. IR spectra were recorded on Shimadzu IR 440 and Nicolet Impact 400 FT-IR spectrophotometers as KBr pellets. NMR spectra were obtained on Bruker ARX3OO FT-NMR and Varian VXR-300 FT-NMR spectrometers. MS were measured with HP 5995 GC-MS and JEOL JMS-Hx 110 instruments. Elemental analyses were performed by National Cheng Kung University and National Chung Hsing University, Taiwan. Flash column chromatography was performed on silica gel (mesh 25–150 μm). Precoated silica gel plates (Kieselgel 60 F_{254} 0.25 mm, Merck) were used for TLC analysis.

Table 1. Inhibition of In Vitro Cancer Cell Lines by Compounds **5**, **6**, and **9**.

Cell Line	Cytotoxicity log GI_{50} (M)[a,b]		
	5	**6**	**9**
Leukemia			
CCRF-CEM	–6.00	–5.81	–6.74
MOLT-4	–5.69	< –8.00	–7.18
SR	-	-	–7.61
Non-Small Cell Lung Cancer			
NCI-H23	–6.35	–5.98	–6.22
NCI-H460	–6.34	–5.88	–6.44
NCI-H552	–6.39	–5.76	–6.65
Colon Cancer			
HCT-15	–6.15	–4.76	–7.39
SW-620	–6.34	–5.66	–6.69
CNS Cancer			
SF-539	–6.12	–5.69	–6.45
SNB-19	–6.30	–5.75	–5.87
Melanoma			
LOX IMVI	–6.46	–5.63	–6.66
SK-MEL-5	–6.46	–6.77	–6.76
Ovarian Cancer			
IGROV1	–5.75	–5.61	–6.37
OVCAR-3	–6.30	–5.45	–6.48
Breast Cancer			
MCF 7	–5.77	–5.68	–6.50
MCF 7/ ADR-RES	–5.72	–5.54	–6.49
MDA-MB-435	–6.47	–6.49	–6.75
MDA-N	–5.92	–6.03	–6.50
Mean Value[c]	–5.71	–5.67	–6.15

[a]Data obtained from NCI's in vitro disease-oriented tumor cells screen. [b]Data are an average of at least two testings. [c]Mean value over all cell lines tested.

2-Acetyl- (5), 3-Acetyl- (6), and 7-Acetyl-naphtho[2,3-*b*]thiophene-4,9-dione (7). Dimethyl sulfate (7.6 mL, 60 mmol) was added dropwise to a stirred suspension of **3** (1.0 g, 47 mmol), water (40 mL), sodium thiosulfate (10.0 g, 63.3 mmol) and NaOH (3.5 g, 87.5 mmol) maintained at 60 °C. The mixture was allowed to stir at 60 °C for 4 h, then cooled to room temperature, and extracted with $CHCl_3$. The organic layer was washed with

water, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give 1.5 g of the crude intermediate 4,9-dimethoxynaphtho[2,3-*b*]thiophene (**4**).

To **4** (1.5 g) in 1,2-dichloroethane (120 mL) were added acetyl chloride (4.3 mL, 55 mmol) and $AlCl_3$ (7.3 g, 55 mmol). The resulting mixture was stirred at 5 ± 2 °C for 4 h and then poured into ice water and acidified with conc. HCl. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and evaporated. CrO_3 (0.5 g, 50 mmol) and HOAc (12 mL) were added to the residue. The mixture was stirred at room temperature for 8 h, and then *i*-PrOH (30 mL) was added, and the resulting solution extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$, dried over anhydrous $MgSO_4$ and evaporated. Column chromatography (silica gel, $CHCl_3$) gave compounds **5, 6**, and **7** in 20, 11, and 9% yields, respectively.

Compound **5**, yellow needles from $CHCl_3$-EtOH (mp 139–140 °C). R_f value = 0.15, $CHCl_3$. IR (KBr) 1650, 1680 (C=O) cm^{-1}. ^{1}HNMR ($CDCl_3$) δ 2.68 (s, 3H, CH_3), 7.77 (dt, *J* = 2.0, 7.2 Hz, 1H, H-6), 7.80 (dt, *J* = 2.0, 7.2 Hz, 1H, H-7), 7.91 (s, 1H, H-3), 8.21(dd, *J* = 2.0, 7.2 Hz, 1H, H-8), 8.22 (dd, *J* = 2.0, 7.2 Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$) δ 30.6 (CH_3), 126.9 (C-5), 127.7 (C-8), 132.8 (C-4a), 133.5 (C-8a), 133.9 (C-6), 134.3 (C-7), 134.8 (C-3), 139.1 (C-3a), 144.2 (C-2), 147.2 (C-9a), 178.1 (C-4), 179.0 (C-9), 197.3 (C-2-C=O). MS *m/z* (256, M^+). Anal. calcd for $C_{14}H_8O_3S$: C, 65.62; H, 3.15; Found: C, 65.53; H, 3.13%.

Compound **6** (mp 244–245 °C). R_f value = 0.26, $CHCl_3$. IR (KBr) 1650, 1680 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3H, CH_3), 7.78 (dt, *J* = 2.0, 7.2 Hz, 1H, H-6), 7.80 (dt, *J* = 2.0, 7.2 Hz, 1H, H-7), 8.18 (s, 1H, H-2), 8.20 (dd, *J* = 2.0, 7.2 Hz, 1H, H-8), 8.22 (dd, *J* = 2.0, 7.2 Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$) δ 26.9 (CH_3), 126.8 (C-5), 127.6 (C-8), 129.7 (C-2), 132.8 (C-4a), 133.4 (C-8a), 133.8 (C-6), 134.4 (C-7), 142.9 (C-3), 148.9 (C-3a), 150.1 (C-9a), 179.2 (C-9), 179.3 (C-4), 190.5 (C-3-C=O). MS *m/z* (256, M^+). Anal. calcd for $C_{14}H_8O_3S$: C, 65.62; H, 3.15. Found: C, 65.56; H, 3.11%.

Compound **7** (mp 146–147 °C). R_f value = 0.35, $CHCl_3$. IR (KBr) 1650, 1680 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3), 7.70 (d, *J* = 5.4 Hz, 1H, H-3), 7.80 (d, *J* = 5.4 Hz, 1H, H-2), 8.33 (br.s, 2H, H-5, H-6), 8.74 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 26.8 (CH_3), 126.7 (C-8), 126.8 (C-3), 127.7 (C-5), 132.7 (C-6), 133.7 (C-8a), 134.6 (C-2), 135.8 (C-3a), 140.5 (C-7), 142.7 (C-4a), 145.3 (C-9a), 177.1 (C-4), 178.3 (C-9), 196.4 (C-7-C=O). MS *m/z* (256, M^+). Anal. calcd for $C_{14}H_8O_3S$: C, 65.62; H, 3.15. Found: C, 65.49; H, 3.17%.

2,7-Diacetyl- (9) and 3,7-Diacetyl naphtho[2,3-*b*]thiophene-4,9-dione (10). To a mixture of **6** (2.0 g, 8 mmol) and 28% ammonia water (100 mL), $CuSO_4$ (0.05 g, 0.3 mmol) and Cu powder (5 g, 79 mmol) were added. This mixture was refluxed for 36 h, with 5 mL of 28% ammonia water added every 6 h during the heating. The reaction mixture was filtered while hot, and the filtrate was acidified with conc. HCl and cooled to form a precipitate. Two grams of the precipitate were dissolved in a mixture of HOAc (22 mL) and acetic anhydride (14 mL). To the resulting solution, newly melted ZnO (0.3 g, 3.7 mmol) was added. Then the mixture was refluxed for 1.5 h, and an equal volume of H_2O was added to the hot mixture. This mixture was

cooled and filtered to give a solid material, which was dried and dissolved in $ClCH_2CH_2Cl$ to form reactant solution A.

To reactant solution B containing acetic anhydride (3 mL, 29.4 mmol), $AlCl_3$ (3.5 g, 26.2 mmol) and 1,2-dichloroethane (150 mL), the reactant solution A was added dropwise at 30–40 °C. The mixture was stirred at the same temperature for 4 h. After the solvent was removed in vacuum, the residue was dissolved in $CHCl_3$ (100 mL) and HOAc (2 mL), and CrO_3 (0.24 g, 2.4 mmol) was added. The reaction was carried out at 30 ± 2 °C for 3 h while stirring. Then the reaction mixture was neutralized with 5% $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with saturated NaCl, dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (100/1) to obtain compounds **5** (9% yield), **9** (13% yield), and **10** (14% yield).

Compound **9** (mp 202–204 °C). R_f value = 0.11, $CHCl_3$/MeOH = 100:1. IR (KBr) 1650, 1680 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.68 (s, 3H, 2-$COCH_3$), 2.75 (s, 3H, 7-$COCH_3$), 7.96 (s, 1H, H-3), 8.33 (br.s, 2H, H-5, H-6), 8.70 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 26.0 (C-7-CH_3), 29.6 (C-2-CH_3), 126.5 (C-5), 126.6 (C-8), 131.9 (C-6), 134.5 (C-3, C-8a), 138.2 (C-3a), 140.3 (C-7, C-4a), 143.3 (C-2), 146.2 (C-9a), 176.4 (C-4), 177.3 (C-9), 195.4 (C-7-C=O), 195.9 (C-2-C=O). MS *m/z* (298, M^+). Anal. calcd for $C_{16}H_{10}O_4S$: C, 64.42; H, 3.36. Found: C, 64.38; H, 3.32%.

Compound **10** (mp 180–182 °C). R_f value = 0.18, $CHCl_3$/MeOH = 100/1. IR (KBr) 1650, 1680, 1700 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.70 (s, 3H, 3-$COCH_3$), 2.75 (s, 3H, 7-$COCH_3$), 8.19 (s, 1H, H-2), 8.33 (br.s, 2H, H-5, H-6), 8.74 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 26.9 (C-3-CH_3), 27.0 (C-7-CH_3), 127.4 (C-8), 127.8 (C-5), 129.7 (C-2), 133.0 (C-6), 133.6 (C-8a), 136.0 (C-4a), 141.2 (C-7), 142.9 (C-3), 149.4 (C-3a), 151.0 (C-9a), 177.5 (C-4), 178.5 (C-9), 190.5 (C-3-C=O), 196.4 (C-7-C=O). MS *m/z* (298, M^+). Anal. calcd for $C_{16}H_{10}O_4S$: C, 64.42; H, 3.36. Found: C, 64.45; H, 3.33%.

Acknowledgement. This work was supported by grants from the National Science Council of the Republic of China (NSC 78-0412-B039-04) (S.C.K.) and the U.S. National Cancer Institute (CA 17625) (K.H.L.).

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