Bioorganic & Medicinal Chemistry 19 (2011) 1264–1267

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, anticancer activity, and iron affinity of the *Actinoplanes* metabolite 7,8-dihydroxy-1-methylnaphtho[2,3-*c*]furan-4,9-dione

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ARTICLE INFO

Article history:
Received 6 October 2010
Revised 1 December 2010
Accepted 4 December 2010
Available online 9 December 2010

Keywords:
Isofuranonaphthoquinone
Ortho-lithiation
Anticancer activity
Siderophore

ABSTRACT

The first synthesis of 7,8-dihydroxy-1-methylnaphtho[2,3-*c*]furan-4,9-dione (**1**), an isofuranonaphthoquinone produced by an *Actinoplanes* strain is described. Lactone ring opening of 6-methylfuro [3,4-*c*]furan-1(3*H*)-one (**4**) with *ortho*-lithiated veratrole (**3**), oxidation of product alcohol **5**, and Friedel–Crafts acylation of the resulting aroylcarboxylic acid **7** afforded the mono methyl ether **2** of the target compound. The latter was obtained by demethylation of **2** with BBr_3 in 14% overall yield. While mono ether **2** was distinctly more cytotoxic than catechol **1** against a panel of five cancer cell lines, only the latter showed a siderophore-like binding affinity for Fe(III) with a complex dissociation constant K_D of approximately 10^{-29} M^3 (pM = 25.9).

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1. Introduction

Isofuranonaphthoquinones represent but a small group among the naturally occurring *para*-quinones.[1] Most of them were isolated as secondary metabolites from herbal or fungal sources[2–6] and were found to have moderate antioxidative,[1] antimicrobial,[7,8] or antiplasmodial activity.[1] 7,8-Dihydroxy-1-methylnaphtho[2,3-*c*]furan-4,9-dione (**1**) was the first example to be isolated from a bacterial source, an *Actinoplanes* strain, by Moore and co-workers in 2009 (Fig. 1).[2] While exhibiting no antibacterial activity against *Bacillus subtilis* or *Escherichia coli* it showed an ability to complex Fe(III) and was believed to act as a siderophore and to be utilized by this *Actinoplanes* isolate to sequester iron for growth support.[2] Herein, we report on the first synthesis of **1** via its mono methyl ether **2** and on the cytotoxicities and iron affinities of these two compounds.

2. Results and discussion

2.1. Chemistry

Our synthesis of **1** drew on veratrole (**3**) and 6-methylfuro [3,4-*c*]furan-1(3*H*)-one (**4**) as building blocks. The butyrolactone **4** was accessible by Ag-mediated ring closure of prop-2-ynyl 2-chloro-3-oxobutanoate according to a literature procedure.[9–11] Opening of the lactone ring of **4** by *ortho*-lithiated veratrole led to the primary alcohol **5**, which was oxidized to the corresponding aldehyde **6** using pyridinium dichromate (PDC) and further on to acid **7** with sodium chlorite (Scheme 1).

The carboxylic acid **7** was then converted to its chloride **8** with oxalyl chloride. The crude chloride **8** was cyclized via an intramolecular Friedel–Crafts acylation initiated by an excess of aluminum chloride at room temperature. This reaction proceeded with concomitant loss of the methyl group on the *ortho*-oxygen and afforded the quinone **2** in overall 15% yield. Demethylation of **2** was finally achieved by reaction with boron tribromide (Scheme 2). Compounds **1** and **2** had to be freed of traces of chelated metals by extraction with aqueous Na_2EDTA solution.

2.2. Biological evaluation

2.2.1. MTT-tests

The quinones **1** and **2** were tested for cytotoxicity against cells of human HL-60 leukemia, 518A2 melanoma, HT-29 colon carcinoma, and the multidrug-resistant MCF-7/Top breast and KB-V1/Vbl cervix carcinomas, as well as non-malignant human foreskin fibroblasts (HF) (Table 1). On average, the growth inhibitory effects

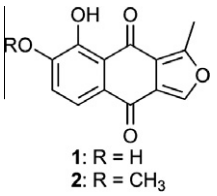

Figure 1. Molecular structures of **1** and **2**.

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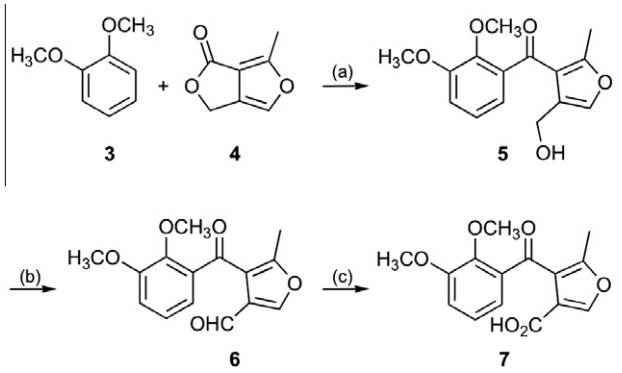
doi:10.1016/j.bmc.2010.12.012

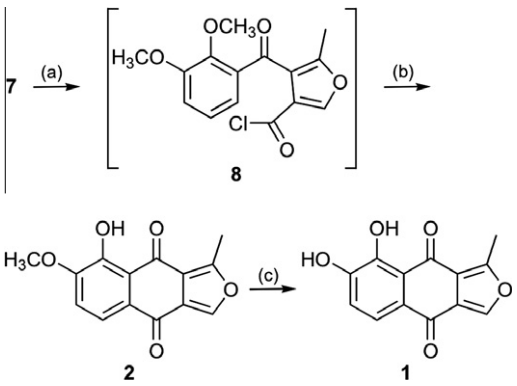


Scheme 1. Reagents and conditions: (a) *n*-BuLi, TMEDA, Et_2O, 0 °C→rt, 16 h, 55%; (b) PDC, CH_2Cl_2, rt, 16 h, 42%; (c) $NaClO_2$, $NaH_2PO_4 \times 2H_2O$, 2-methyl-2-butene, *t*-BuOH, H_2O, rt, 16 h, 93%.

of mono ether **2** were more pronounced than those of the natural catechol **1**. In the resistant cell line MCF-7/Top quinone **2** even surpassed the activity of the clinically established anticancer drug cisplatin (CDDP) and of thymoquinone (TQ), a *para*-benzoquinone occurring in the seed oil of *Nigella sativa* that showed antitumor effects in various in vitro and animal studies.[12–14] Thus quinone **2** might be a promising candidate for further preclinical studies. Whether these efficacy differences between the two test compounds are a consequence of metal complexation or of a lower cellular concentration of **1** when compared with **2** remains to be shown.

2.2.2. Determination of Fe(III) complexation

The natural catechol 1 was analyzed for its ability to complex Fe(III). Figure 2 (top) shows its UV–vis spectrum in DMSO as well as a spectrum obtained upon addition of ferric chloride in 4:1 MeOH/0.1 M aqueous NaOAc buffer solution (pH 7.4). In the latter case, the low energy transition band of 1 at ca. 400 nm was red shifted by more than 50 nm due to an increased conjugative effect when chelate complexes are formed and to a different electronic state of the formal catecholate dianion.[15] The dissociation constant K_D of the presumed octahedral complex Fe(III)(**1**)$_3$ was determined from these spectra to be 1.39 ± 0.26 x 10^{-29} M^3 (pM = 25.9) by competition experiments with the good chelate ligand EDTA. This dissociation constant lies in the range of that of the complex Fe(III)(EDTA) (ca. 5 x 10^{-23} M; pM = 25.1). Hence it is likely that catechol 1 is transported, distributed and stored in a biological context mostly in form of its metal chelate complexes. The UV–vis spectrum of the methyl ether **2** was not sufficiently different from that recorded in the presence of ferric chloride to allow the calculation of a K_D value of a potential iron



Scheme 2. Reagents and conditions: (a) $(COCl)_2$, rt, 16 h; (b) $AlCl_3$, CH_2Cl_2, rt, 16 h, 67% (with respect to **7**); (c) BBr_3, CH_2Cl_2, −78 °C→rt, 16 h, 94%.

Table 1
Inhibitory concentrations IC_{50} (72 h)[a] in μM of **1** and **2** and TQ when applied to cancer and non-malignant cells

	HL-60	HF	518A2	KB-V1/Vbl	HT-29	MCF-7/Top
1	89 ± 11	69 ± 6	84 ± 16	86 ± 10	70 ± 11	>100
2	18 ± 8	31 ± 6	>100	51 ± 12	31 ± 15	16 ± 6
TQ	28 ± 6	33 ± 10	28 ± 9	32 ± 6	47 ± 10	27 ± 6
CDPP	6.2 ± 2.7	91 ± 13	1.2 ± 0.4	8.4 ± 2.2	13 ± 3	32 ± 6

[a] Values are derived from concentration–response curves obtained by measuring the percentual absorbance of viable cells relative to untreated controls (100%) after 72 h exposure of the cells to the test compounds in the MTT assay. Values represent means of four independent experiments ± standard deviation.

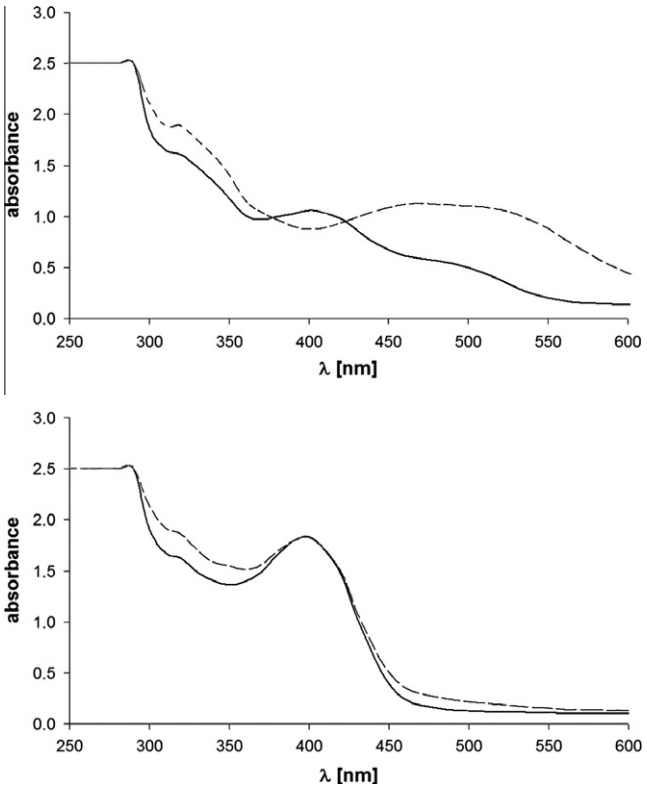


Figure 2. UV–vis spectra of compounds **1** (top) and **2** (bottom) in the absence (—) and in the presence (– –) of ferric chloride.

complex (Fig. 2; bottom). From systematic studies with flavonoids featuring a catecholic site and a β-hydroxyketone one knows that the former is more influential for iron complexation at the physiological pH of 7.4 than the latter.[16,17] Thus compound **2** has presumably formed very little Fe(III) complex under these conditions.

3. Experimental section

3.1. General methods

Melting points were recorded on a Büchi M-565 apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrophotometer equipped with an ATR sampling unit. Nuclear magnetic resonance (NMR) spectra were recorded under conditions as indicated on a Bruker Avance 300 spectrometer. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as internal standard for ^1H and ^{13}C. Mass spectra were recorded using a Varian MAT 311A (EI). Elemental analyses were carried out on a Carlo Erba Strumentazione

Elemental Analyzer Model 1106. For column chromatography Merck silica gel 60 (230–400 mesh) was used. Solvents were dried and distilled (diethyl ether over Na/K, TMEDA over Na and CH_2Cl_2 over CaH_2) and stored under argon. All starting compounds were purchased from the usual sources and used without further purification.

3.2. Syntheses

3.2.1. 4-Hydroxymethyl-2-methyl-3-(2,3-dimethoxybenzoyl)-furan (5)

A solution of veratrole (**3**) (1.00 g, 7.24 mmol) and TMEDA (1.09 mL, 7.24 mmol) in diethyl ether (50 mL) was cooled to 0 °C and treated dropwise with *n*-BuLi (2.89 mL, 7.24 mmol, 2.5 M in hexane). Stirring was continued at room temperature for 1 h. The mixture was cooled to 0 °C and **4** (1.00 g, 7.24 mmol) was added. After stirring at room temperature overnight it was quenched with a saturated aqueous solution of NH_4Cl and extracted with diethyl ether. The combined organic layers were washed with 1 M HCl and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 6:1) to yield **5** (1.10 g, 3.98 mmol, 55%) as a colorless oil; R_f 0.35 (cyclohexane/ethyl acetate 1:1); ν_{max}/cm^{-1} 3442, 2938, 1634, 1578, 1475, 1425, 1266, 1001, 754; δ_H (300 MHz; $CDCl_3$) 1.91 (3H, s, CH_3), 3.77 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 4.54 (2H, s, CH_2), 6.84 (1H, dd, ^3J = 7.5 Hz, ^4J = 1.7 Hz, H^{ar}), 7.02 (1H, dd, ^3J = 8.3 Hz, ^4J = 1.7 Hz, H^{ar}), 7.12 (1H, dd, ^3J = 7.5 Hz, ^3J = 8.3 Hz, H^{ar}), 7.26 (1H, s, CH); δ_C (75 MHz; $CDCl_3$) 14.2, 55.5, 55.9, 61.9, 114.5, 119.4, 122.8, 124.5, 125.9, 135.6, 138.6, 146.3, 152.9, 161.9, 193.0; *m/z* (EI, 70 eV) 276 (M^+, 46%), 245 (100%), 215 (15%), 165 (52%), 137 (65%), 77 (18%), 43 (29%). Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.2; H, 5.8. Found: C, 65.1; H, 5.6.

3.2.2. 3-Formyl-4-(2,3-dimethoxybenzoyl)-5-methylfuran (6)

Alcohol **5** (1.27 g, 4.59 mmol) and PDC (3.46 g, 9.19 mmol) were dissolved in CH_2Cl_2 (30 mL) and stirred at room temperature overnight. The solution was diluted with diethyl ether, filtered over a pad of Floresil® and silica gel and evaporated. The residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 4:1). Yield: 530 mg (1.93 mmol, 42%) of a colorless oil; R_f 0.52 (cyclohexane/ethyl acetate 1:1); ν_{max}/cm^{-1} 2939, 1683, 1649, 1547, 1475, 1424, 1311, 1264, 1078, 999, 751; δ_H (300 MHz; $CDCl_3$) 2.07 (3H, s, CH_3), 3.67 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 6.87 (1H, dd, ^3J = 7.3 Hz, ^4J = 1.9 Hz, H^{ar}), 6.97 (1H, dd, ^3J = 8.2 Hz, J = 1.9 Hz, H^{ar}), 7.02 (1H, dd, ^3J = 7.3 Hz, ^3J = 8.2 Hz, H^{ar}), 7.82 (1H, s, CH), 9.90 (1H, s, CHO); δ_C (75 MHz; $CDCl_3$) 13.3, 55.7, 61.5, 115.0, 119.7, 120.9, 124.2, 126.9, 134.8, 140.2, 145.9, 152.7, 160.1, 186.1, 190.2; *m/z* (EI, 70 eV) 274 (M^+, 96%), 243 (100%), 199 (17%), 165 (58%), 137 (34%), 107 (38%), 77 (50%), 43 (62%). Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.7; H, 5.2. Found: C, 65.6; H, 5.2.

3.2.3. 4-(2,3-Dimethoxybenzoyl)-5-methylfuran-3-carboxylic acid (7)

Aldehyde **6** (530 mg, 1.93 mmol) and 2-methyl-2-butene (7 mL) were dissolved in *tert*-Butanol (50 mL) and H_2O (10 mL) and $NaClO_2$ (1.64 g, 18.16 mmol) and $NaH_2PO_4 \times 2H_2O$ (2.10 g, 13.51 mmol) were added. The mixture was stirred at room temperature overnight and then all volatiles were evaporated. The remainder was dissolved in ethyl acetate (100 mL) and washed with H_2O and brine. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The crude product was crystallized from ethyl acetate/pentane 1:9 at −20 °C to yield 521 mg (1.79 mmol, 93%) of a bright yellow solid; R_f 0.10 (cyclohexane/ethyl acetate 1:1); mp 133 °C; ν_{max}/cm^{-1} 2583, 1719, 1598, 1578, 1447, 1320, 1265, 1230, 1127, 998, 842, 752; δ_H (300 MHz; $CDCl_3$) 1.94 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 6.83 (1H, dd, ^3J = 7.5 Hz, ^4J = 1.6 Hz, H^{ar}), 7.07 (1H, dd, ^3J = 8.3 Hz, ^4J = 1.6 Hz, H^{ar}), 7.15 (1H, dd, ^3J = 7.5 Hz, ^3J = 8.3 Hz, H^{ar}), 8.10 (1H, s, CH); δ_C (75 MHz; $CDCl_3$) 14.6, 55.9, 61.8, 115.3, 118.7, 119.5, 119.8, 124.9, 133.5, 145.9, 150.1, 152.9, 162.1, 165.3, 195.4; *m/z* (EI, 70 eV) 289 (M^+−1, 86%), 240 (41%), 187 (17%), 165 (100%), 122 (34%), 107 (44%), 43 (32%). Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.1; H, 4.9. Found: C, 61.8; H, 4.6.

3.2.4. 8-Hydroxy-7-methoxy-1-methylnaphtho[2,3-*c*]furan-4,9-dione (2)

A solution of acid **7** (139 mg, 0.45 mmol) in oxalyl chloride (3.84 mL, 44.8 mmol) was stirred at room temperature overnight and then evaporated to dryness. The residue was redissolved in CH_2Cl_2 (20 mL) and treated with $AlCl_3$ (300 mg, 2.25 mmol). The mixture was stirred at room temperature overnight and then quenched with ice. It was diluted with 1 M HCl and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography (Silica Gel 60, cyclohexane/ethyl acetate 4:1), dissolved in ethyl acetate (30 mL) and extracted with 0.05 M aqueous solution of Na_2EDTA to yield 97 mg (0.37 mmol, 83%) of a yellow solid. R_f 0.52 (cyclohexane/ethyl acetate 1:1); mp 192 °C (decomp.); ν_{max}/cm^{-1} 3443, 2924, 2850, 1670, 1560, 1456, 1309, 1274, 1254, 1223, 1051, 786; δ_H (300 MHz; $CDCl_3$) 2.74 (3H, s, CH_3), 3.98 (3H, s, OCH_3), 7.10 (1H, d, ^3J = 8.5 Hz, H^{ar}), 7.78 (1H, d, ^3J = 8.5 Hz, H^{ar}), 7.97 (1H, s, CH), 13.22 (1H, s, OH); δ_C (75 MHz; $CDCl_3$) 14.0, 56.4, 115.3, 117.0, 117.9, 120.7, 123.6, 127.6, 143.3, 153.3, 153.9, 160.5, 178.3, 187.0; *m/z* (EI, 70 eV) 258 (M^+, 100%), 229 (51%), 212 (13%), 184 (5%), 131 (6%), 103 (6%), 79 (11%), 43 (23%). Anal. Calcd for $C_{14}H_{10}O_5$: C, 65.1; H, 3.9. Found: C, 64.9; H, 3.8.

3.2.5. 7,8-Dihydroxy-1-methylnaphtho[2,3-*c*]furan-4,9-dione (1)

A solution of methyl ether **2** (40 mg, 0.16 mmol) in CH_2Cl_2 (15 mL) was cooled to −78 °C, treated dropwise with BBr_3 (0.46 mL, 0.46 mmol, 1 M in CH_2Cl_2), and was then allowed to warm to room temperature overnight. Water was slowly added and the mixture was extracted with ethyl acetate. The combined organic layers were extracted with 1 M NaOH. The resulting aqueous layers were acidified to pH 1 with concd HCl and extracted with CH_2Cl_2. The combined extracts were washed with a 0.05 M aqueous solution of Na_2EDTA, dried over anhydrous Na_2SO_4 and evaporated to dryness to yield 35 mg (0.14 mmol, 89%) of an amorphous yellow solid. R_f 0.42 (cyclohexane/ethyl acetate 1:1); ν_{max}/cm^{-1} 3392, 2926, 2855, 1737, 1668, 1633, 1603, 1567, 1453, 1302, 1255, 1107, 1067, 1014, 805; δ_H (300 MHz; DMSO-d_6) 2.73 (3H, s, CH_3), 7.20 (1H, d, ^3J = 8.3 Hz, H^{ar}), 7.62 (1H, d, ^3J = 8.3 Hz, H^{ar}), 8.54 (1H, s, CH), 10.78 (1H, s, OH), 12.92 (1H, s, OH); δ_C (125.75 MHz; DMSO-d_6) 13.8, 116.4, 118.1, 120.4, 120.7, 122.9, 126.2, 144.7, 151.4, 152.6, 160.4, 177.5, 186.9; *m/z* (EI, 70 eV) 244 (M^+, 100%), 215 (34%), 187 (10%), 160 (18%), 131 (14%), 108 (100%), 103 (100%), 77 (22%), 63 (11%), 51 (20%), 43 (32%).

3.3. Cell lines and culture conditions

The human HL-60 leukemia cells were obtained from the German Resource Center for Biological Material (DSMZ), Braunschweig, Germany, the human 518A2 melanoma cells were a gift from the Department of Oncology and Hematology of the Martin-Luther-University Halle-Wittenberg, Germany, the human KB-V1/Vbl cervix and MCF-7/Top breast carcinoma cells were obtained from the Institute of Pharmacy of the University of Regensburg, Germany, and the human colon carcinoma cells HT-29 as well as the non-malignant foreskin fibroblasts HF were obtained from

the University Hospital Erlangen, Germany. HL-60 and HT-29 cells were incubated in RPMI (Roswell Park Memorial Institute) Medium 1640 supplemented with 10% FCS (fetal calf serum), 100 IU/mL penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphothericin B, and 250 μg/mL gentamycin (all from Gibco). 518A2, HF, HT-29, and KB-V1/Vbl cells were cultured in DMEM (Dulbecco's modified Eagle medium; Gibco) containing 10% FCS, 100 IU/mL penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphothericin B, and 250 μg/mL gentamycin. MCF-7/Top cells were grown in EMEM (Eagle's minimum essential medium; Sigma–Aldrich) supplemented with 5% FCS, 110 mg/L sodium pyruvate (Gibco), and 2.2 g/L $NaHCO_3$ (Merck). The cells were maintained in a moisture-saturated atmosphere (5% CO_2, 95% humidity) at 37 °C.

3.4. Inhibition of cancer cell growth (MTT assay)

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide; ABCR] was used to identify the metabolic activity of vital cells which are capable of reducing it to a violet formazan. HL-60 cells (5×10^5/mL) and cells (5×10^4/mL) of 518A2, HF, HT-29, KB-V1/Vbl, and MCF-7/Top were seeded and cultured for 24 h in 96-well microplates.[18] Incubation of cells following treatment with the test compounds was continued for 72 h. Blank and solvent controls were treated identically. Then, MTT in phosphate-buffered saline (PBS) was added to a final concentration of 0.05% (HL-60, 518A2, HF) or 0.1% (HT-29, HT-29, KB-V1/Vbl, MCF-7/Top). After 2 h, the precipitate of formazan was solved in dimethyl sulfoxide (DMSO) containing 10% sodium dodecylsulfate (SDS) and 0.6% acetic acid in the case of the suspension cells HL-60. For the adherent cells (518A2, HF, HT-29, KB-V1/Vbl, MCF-7/Top) the microplates were swiftly turned to discard the medium before adding the solvent mixture. The microplates were gently shaken in the dark for 30 min and absorbances at 570 nm and 630 nm (background) were measured with a Tecan F-200 plate reader (Germany). All experiments were carried out in quadruplicate, and the percentage of the viable cells was calculated with Origin 8.1G as the mean ± standard deviation relative to the control (100%).

3.5. Determination of iron complexation

The dissociation constant K_D for the Fe(III) complex of **1** was determined by competition with EDTA at room temperature using a Shimadzu UV 160 A spectrophotometer. Stock solutions of ferric chloride (10 mM) in pure methanol and of compound **1** (10 mM) in DMSO were prepared and diluted to 150 μM and 500 μM, respectively, with 4:1 MeOH/0.1 M NaOAc buffer solution (pH 7.4). The absorbance of the formed complex was measured at 470 nm in the presence and absence of 200 μM EDTA. At this wavelength, absorption of the complex was at peak level, while that of the Fe-EDTA complex was negligible. Absorption of the uncomplexed compound was taken into account. The difference in absorbance was then used to calculate the dissociation constant K_D by the following equation:

$$K_D = K_D(\text{Fe-EDTA})[\text{Fe-EDTA}][\mathbf{1}]^3/[\text{Fe-}(\mathbf{1})_3][\text{EDTA}]$$

K_D(Fe-EDTA) is 5×10^{-23} M, concentrations were determined by using the measured extinction coefficient of quinone **1**. Data were representative of at least two independent experiments.[19,20]

Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra of compounds **1** and **2**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.012.

References and notes

1. Bezabih, M.; Abegaz, B. M.; Dufall, K.; Croft, K.; Skinner-Adams, T.; Davis, T. M. E. *Planta Med.* **2001**, *67*, 340.
2. Zhang, Q.; Peoples, A. J.; Rothfelder, M. T.; Millet, W. P.; Pescatore, B. C.; Ling, L. L.; Moore, C. M. *J. Nat. Prod.* **2009**, *72*, 1213.
3. Abegaz, B. M. *Phytochem. Rev.* **2002**, *1*, 299.
4. Hanumaiah, T.; Rao, G. S. R.; Rao, C. P.; Rao, K. V. J.; Cowe, H. J.; Cox, P. J.; Howie, R. A.; Marshall, D. S.; Thomson, R. H. *Tetrahedron* **1985**, *41*, 635.
5. Koyama, J.; Ogura, T.; Tagahara, K. *Phytochemistry* **1994**, *37*, 1147.
6. Yamamoto, Y.; Kinoshita, Y.; Thor, G. R.; Hasumi, M.; Kinoshita, K.; Koyama, K.; Takahashi, K.; Yoshimura, I. *Phytochemistry* **2002**, *60*, 741.
7. Baker, R. A.; Tatum, J. H.; Nemec, S., Jr. *Mycopathologia* **1990**, *111*, 9.
8. Muleya, V.; Hayeshi, R.; Ranson, H.; Abegaz, B.; Bezabih, M.-T.; Robert, M.; Ngadjui, B. T.; Ngandeu, F.; Mukanganyama, S. *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 391.
9. Mottet, C.; Hamelin, O.; Garavel, G.; Deprés, J.-P.; Greene, A. E. *J. Org. Chem.* **1999**, *64*, 1380.
10. Garanti, L.; Sala, A.; Zecchi, G. *Synthesis* **1975**, 666.
11. Verniest, G.; Padwa, A. *Org. Lett.* **2008**, *10*, 4379.
12. Ivankovic, S.; Stojkovic, R.; Jukic, M.; Milos, M.; Milos, M.; Jurin, M. *Exp. Oncol.* **2006**, *28*, 220.
13. Dandawate, P. R.; Vyas, A. C.; Padhye, S. B.; Singh, M. W.; Baruah, J. B. *Mini-Rev. Med. Chem.* **2010**, *5*, 436.
14. (a) Breyer, S.; Effenberger, K.; Schobert, R. *ChemMedChem* **2009**, *4*, 761; (b) Effenberger, K.; Breyer, S.; Schobert, R. *Chem. Biodiv.* **2010**, *7*, 120; (c) Effenberger, K.; Schobert, R. *Cancer Chemother. Pharmacol.* **2010**. doi:10.1007/s00280-010-1386-x.
15. Vaillancourt, F. H.; Barbosa, C. J.; Spiro, T. G.; Bolin, J. T.; Blades, M. W.; Turner, R. F. B.; Eltis, L. D. *J. Am. Chem. Soc.* **2002**, *124*, 2485.
16. Engelmann, M. D.; Hutcheson, R.; Cheng, I. F. *J. Agric. Food Chem.* **2005**, *53*, 2953.
17. Bodoni, M. E.; Copia, G.; Tapia, R.; Leighton, F.; Herrera, L. *Polyhedron* **1999**, *18*, 2233.
18. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
19. Kaufmann, G. F.; Sartorio, R.; Lee, S.-H.; Rogers, C. J.; Meijler, M. M.; Moss, J. A.; Clapham, B.; Brogan, A. P.; Dickerson, T. J.; Janda, K. D. *PNAS* **2005**, *102*, 309.
20. Wang, J.; Buss, J. L.; Chen, G.; Ponka, P.; Pantopoulos, K. *FEBS Lett.* **2002**, *529*, 309.