Efficient regiospecific synthesis of two cytotoxic furonaphthoquinones, 5,7-dimethoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione and 5,6,7-trimethoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione [1]

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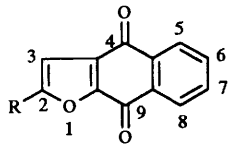
Following the recent isolation of several furonaphthoquinones from two *Latana* species (Verbenaceae), a novel synthetic method for the preparation of two cytotoxic furonaphthoquinones, 5,7-dimethoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione **8a** and 5,6,7-trimethoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione **8b** is described. The title compounds were obtained in high overall yield *via* hydrogen peroxide oxidation of intermediate 4-hydroxy-9-trifluoroacetylnaphthofurans. Both the title compounds exhibit cytotoxic activity towards three mammalian cell lines (2.99–6.6 μmol dm^{-3}).

Following the isolation of two furonaphthoquinones **1** and **2** from *Tabebuia cassinoides* (Lam.) DC (Bignoniaceae)[2] which exhibited activity *in vitro* against the KB epidermoid

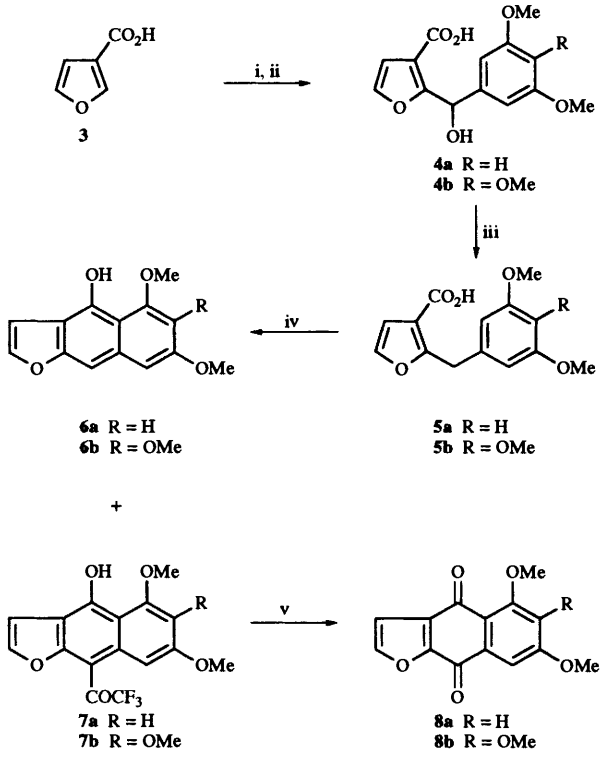


1 R = COMe
2 R = CH(OH)Me

nasopharynx cell line (4.2 and 8.3 μmol dm^{-3}, respectively) a number of methoxy and hydroxy furonaphthoquinones have been isolated from various other *Tabebuia* species.[3–7] The isolation, structural elucidation and cytotoxic activity exhibited by these natural compounds has stimulated subsequent synthetic interest.[8–10] The more recent isolation of several furonaphthoquinones unsubstituted at the 2-position from *Latana achyrantifolia* and *L. camara* (Verbenaceae)[11,12] and the possible antileukaemic nature of these compounds has led us to investigate an efficient synthetic methodology for this pharmacologically interesting ring system. Herein we report the regiospecific synthesis of two furonaphthoquinones, 5,7-dimethoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione **8a** and 5,6,7-trimethoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione **8b**, in 72 and 78% overall yield, respectively.[1]

Results and discussion

Regiospecific metallation of 3-furoic acid **3** at the 2-position using lithium diisopropylamide[13] at −78 °C and subsequent treatment with either 3,5-dimethoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde afforded the hydroxymethylfuroic acids **4a** and **4b** in 81–86% yield. Reduction of **4a** and **4b** with iodotrimethylsilane[14] generated *in situ* gave the benzylfuroic acids **5a** and **5b** respectively, in quantitative yield. Initially it was hoped that mild Friedel–Crafts cyclisation[15] of the acids **5a** and **5b** would give the corresponding 4-hydroxynaphthofurans **6a** and **6b**, which, upon subsequent oxidation, could be converted into the desired furonaphthoquinones **8a** and **8b**. However, when the benzylfuroic acid **5a** was treated with trifluoroacetic anhydride (TFAA) in dichloromethane at 0 °C a mixture of the



Scheme 1 *Reagents and conditions:* i, Lithium diisopropylamide, tetrahydrofuran; ii, 3,5-dimethoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde, −78 to 25 °C; iii, sodium iodide, chlorotrimethylsilane, acetonitrile; iv, trifluoroacetic anhydride, dichloromethane; v, hydrogen peroxide, aqueous sodium hydroxide–sodium carbonate, 40 °C

phenols **6a** and **7a** was obtained in a ratio of 40:60 (from ^1H NMR). The 4-hydroxy-9-trifluoroacetylnaphthofuran **7a** was formed presumably by either direct acylation of the highly activated 9-position, or *via* Fries rearrangement of an intermediate trifluoroacetoxy ester of **6a**. As neither the formation nor presence of the trifluoroacetoxy ester of **6a** could be detected by ^1H NMR, direct acylation is the more likely route to **7a**. Mixtures of the phenols **6b** and **7b** were also

Table 1 Effect of compounds **8a** and **8b** on the growth of KB epidermoid nasopharynx, K562 human leukaemia and P388 lymphocytic leukaemia cell lines. Values shown are the concentrations μmol dm^{-3} required to cause 50% inhibition in cell growth

Compound	KB	K562	P388
8a	6.2	3.11	3.35
8b	6.6	4.54	2.99

obtained from the corresponding treatment of the acid **5b** with TFAA.

The formation of products analogous to the phenols **7a** and **7b** is, however, not unknown as other attempted syntheses of furonaphthoquinones have been thwarted by acylation at the 9-position,[9,10] and indeed this side-reaction appears to be inherent to highly activated ring systems. Various attempts to oxidise the phenols **6a** and **6b** with chromium trioxide,[8,15] Fremy's salt[6] and O_2–Triton B[10] failed to give any of the corresponding quinones **8a** and **8b**. Given the inability to isolate either of the quinones **8a** and **8b** from the oxidation of the corresponding phenols, and since the trifluoroacetylphenols **7a** and **7b** were obtained as the sole products when the acids **5a** and **5b** were treated with trifluoroacetic anhydride at room temperature, a method of converting **7a** and **7b** into the desired furonaphthoquinones **8a** and **8b** was sought.

Treatment of the phenols **7a** and **7b** with hydrogen peroxide in aqueous base[16] afforded the desired furonaphthoquinones **8a** and **8b** in quantitative yield. The phenols **7a** and **7b** probably undergo Baeyer–Villiger type oxidation to a 4-hydroxy-9-trifluoroacetoxynaphthofuran intermediate, with subsequent hydrolysis to the corresponding diphenols, and finally autoxidation to the desired furonaphthoquinones **8a** and **8b**.

The two furonaphthoquinones **8a** and **8b** were tested for cytotoxicity against KB epidermoid nasopharynx, K562 human leukaemia and P388 lymphocytic leukaemia cell lines and showed good cytotoxicity (Table 1, ID_{50} 2.99–6.6 μmol dm^{-3}). The naturally occurring cytotoxic furonaphthoquinones **1** and **2** have either an acetyl or hydroxyethyl group on the 2-position. The two quinones **8a** and **8b** synthesised here possess no functionality at the 2-position and yet still show strong cytotoxic activity.

In conclusion, a new method for the formation of the furonaphthoquinone ring system has been established. This route overcomes the problem of acylation at the 9-position of the tricyclic system and should allow the preparation of other furonaphthoquinones which have hitherto been frustrated by this type of acylation. Further studies into the synthesis and cytotoxic nature of furonaphthoquinones, with and without substitution at the 2-position, are in progress.

Experimental

Mps were determined on a hot-stage microscope and are uncorrected. IR spectra were recorded as potassium bromide disks using a Perkin-Elmer 683 Infrared spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC250 spectrometer at 303.3 K in $CDCl_3$ solution, unless stated otherwise. Chemical shifts (ppm) are given downfield of tetramethylsilane. Coupling constants J are given in Hz. Electron impact mass spectra were determined on a VG Trio-3 mass spectrometer at an ionisation energy of 70 eV. Organic solutions were dried over magnesium sulphate. Ether refers to diethyl ether.

General procedure for the preparation of hydroxymethylfuroic acids 4a and 4b

To diisopropylamine (7 cm^3, 50 mmol) at −10 °C under nitrogen was added butyllithium (32 cm^3 of a 1.6 mol dm^{-3} solution in hexanes, 50 mmol) with stirring. After 15 min the resulting viscous solution was diluted with tetrahydrofuran (50 cm^3), cooled to −78 °C, and a solution of 3-furoic acid **3** (2.8 g, 25 mmol) in tetrahydrofuran (50 cm^3) was added to it. The solution was stirred at −78 °C for 30 min when a solution of either 3,5-dimethoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde (50 mmol) in tetrahydrofuran (50 cm^3) was added to it. The mixture was then allowed to warm to room temperature over *ca.* 30 min and the resulting solution was diluted with water and washed with ether (2 × 50 cm^3). The aqueous layer was acidified (2 mol dm^{-3} hydrochloric acid) and extracted with ether (3 × 100 cm^{-3}). The combined extracts were washed with brine, dried, and evaporated to yield the crude products as oils which were crystallised from light petroleum (bp 40–60 °C)–ether (2:1).

2-[(3,5-Dimethoxyphenyl)hydroxymethyl]-3-furoic acid 4a. As a white solid (5.6 g, 81%), mp 136 °C (Found: C, 60.6; H, 5.0. $C_{14}H_{14}O_6$ requires C, 60.4; H, 5.1%); ν_{max}/cm^{-1} 3250 (OH) and 1675 (C=O); δ_H(DMSO) 7.61 (1 H, d, J 1.9, 5-H), 6.66 (1 H, d, J 1.9, 4-H), 6.57 (1 H, s, C*H*OH), 6.56 (1 H, s, 4′-H), 6.39 (2 H, s, 2′-H and 6′-H) and 3.71 (6 H, s, 3′-OMe and 5′-OMe); δ_C(DMSO) 164.5, 160.9, 160.4, 144.4, 142.3, 114.2, 110.7, 104.1, 98.7, 65.8 and 55.0.

2-[(3,4,5-Trimethoxyphenyl)hydroxymethyl]-3-furoic acid 4b. As a white solid (6.62 g, 86%), mp 116 °C (Found: C, 58.2; H, 5.1. $C_{15}H_{16}O_7$ requires C, 58.4; H, 5.2%); ν_{max}/cm^{-1} 3480 (OH) and 1670 (C=O); δ_H(DMSO) 7.62 (1 H, d, J 1.9, 5-H), 6.71 (2 H, s, 2′-H and 6′-H), 6.66 (1 H, d, J 1.9, 4-H), 6.38 (1 H, s, C*H*OH), 3.74 (6 H, s, 3′-OMe and 5′-OMe) and 3.64 (3 H, s, 4′-OMe); δ_C(DMSO) 164.6, 161.0, 152.8, 142.3, 137.7, 136.8, 114.1, 110.7, 103.3, 65.9, 60.0 and 55.8.

General procedure for the preparation of benzylfuroic acids 5a and 5b

To a suspension of sodium iodide (4.5 g, 30 mmol) in acetonitrile (20 cm^3) under nitrogen was added chlorotrimethylsilane (3.86 cm^3, 30 mmol) with stirring, followed by a solution of **4a** or **4b** (5 mmol) in acetonitrile (100 cm^3) and the reaction mixture was stirred at room temperature for 5 min. It was then diluted with water (100 cm^3) and extracted with ether (3 × 100 cm^3). The combined extracts were washed with aqueous sodium thiosulfate (2 × 100 cm^3), saturated brine (100 cm^3) and dried. Evaporation gave the crude products which were recrystallised from methanol affording analytically pure samples.

2-(3,5-Dimethoxybenzyl)-3-furoic acid 5a. As a white solid (1.25 g, 96%), mp 112 °C (Found: C, 64.2; H, 5.15. $C_{14}H_{14}O_5$ requires C, 64.1; H, 5.4%); ν_{max}/cm^{-1} 1680 (C=O); m/z 262 (M^+, 100%), 229 (18), 115 (16) and 51 (19); δ_H(DMSO) 12.72 (1 H, br s, OH), 7.59 (1 H, d, J 1.4, 5-H), 6.66 (1 H, d, J 1.4, 4-H), 6.37 (3 H, m, ArH), 4.24 (2 H, s, CH_2) and 3.69 (6 H, s, 3′-OMe and 5′-OMe); δ_C(DMSO) 164.65, 160.55, 159.4, 142.0, 139.8, 114.2, 110.9, 106.7, 98.1, 55.1 and 32.7.

2-(3,4,5-Trimethoxybenzyl)-3-furoic acid 5b. As a white solid (1.40 g, 96%), mp 109 °C (Found: C, 61.8; H, 5.6. $C_{15}H_{16}O_6$ requires C, 61.6; H, 5.5%); ν_{max}/cm^{-1} 1680 (C=O); m/z 292 (M^+, 100%), 277 (42), 261 (45), 243 (24), 188 (26), 145 (20), 51 (38) and 39 (42); δ_H(DMSO) 7.53 (1 H, d, J 2.1, 5-H), 6.66 (1 H, d, J 2.1, 4-H) 6.54 (2 H, s,2′-H and 6′-H), 4.25 (2 H, s, CH_2), 3.71 (6 H, s, 3′-OMe and 5′-OMe) and 3.62 (3H, s, 4′-OMe); δ_C (DMSO) 164.7, 159.6, 152.9, 141.8, 136.4, 133.2, 114.1, 110.9, 105.9, 59.9, 55.7 and 32.8.

5,7-Dimethoxy-4-hydroxynaphtho[2,3-*b*]furan 6a

To a solution of compound **5a** (1.31 g, 5 mmol) in dichloromethane (25 cm^3) was added trifluoroacetic anhydride (0.71 cm^3, 5 mmol) at 0 °C with stirring and the reaction mixture was stirred for 3 h at this temperature. Water (10 cm^3)

was added to it and the mixture stirred for a further 5 min when the organic phase was separated, washed with brine (10 cm^3) and dried. Evaporation and column chromatography of the residue on silica gel with dichloromethane afforded the title compound as yellow needles (0.47 g, 38%), mp 113 °C; ν_{max}/cm^{-1} 3300 (OH); δ_H 9.63 (1 H, s, OH), 7.52 (1 H, d, *J* 2.5, 2-H), 7.30 (1 H, d, *J* 0.7, 9-H), 6.97 (1 H, dd, *J* 1, 2.5, 3-H), 6.76 (1 H, d, *J* 2.2, 8-H), 6.39 (1 H, d, *J* 2.5, 6-H), 4.06 (3 H, s, OMe) and 3.92 (3 H, s, OMe); δ_C 157.7, 156.8, 156.1, 147.9, 143.5, 135.1, 113.0, 106.5, 103.9, 98.8, 97.6, 95.8, 56.0 and 55.2.

General procedure for the preparation of 4-hydroxy-9-trifluoroacetylnaphtho[2,3-*b*]furans 7a and 7b

To a solution of compound **5a** or **5b** (5 mmol) in dichloromethane (25 cm^3) was added trifluoroacetic anhydride (0.71 cm^3, 5 mmol) at 25 °C with stirring and the reaction mixture was stirred for 6 h. Water (10 cm^3) was added to it and the mixture stirred for a further 5 min when the organic phase was separated, washed with brine (10 cm^3) and dried. Evaporation afforded the crude products which were purified by chromatography on silica gel with dichloromethane. The appropriate fractions were filtered and evaporation afforded the products which were recrystallised from methanol.

4-Hydroxy-5,7-dimethoxy-9-trifluoroacetylnaphtho[2,3-*b*]-furan 7a. As yellow needles (1.62 g, 95%), mp 168 °C (Found: C, 56.3; H, 3.45; F, 16.7. $C_{16}H_{11}O_5F_3$ requires C, 56.5; H, 3.25; F, 16.75%); ν_{max}/cm^{-1} 3300 (OH), 1670 and 1640 (C=O); *m/z* 340 (M^+, 58%), 271 (100), 256 (21) and 228 (24); δ_H 10.61 (1 H, s, OH), 8.05 (1 H, d, *J* 2.2, 8-H), 7.59 (1 H, d, *J* 2.5, 2-H), 6.97 (1 H, d, *J* 2.2, 3-H), 6.47 (1 H, d, *J* 2.2, 6-H), 4.08 (3 H, s, OMe) and 3.94 (3 H, s, OMe); δ_C 179.4, 161.1, 158.2, 158.0, 156.4, 143.7, 136.4 and 123.8, 119.2, 114.6 and 110.0 (q, *J* 289.3, CF_3), 112.6, 106.6, 103.9, 101.1, 97.4, 97.1, 56.4 and 55.4.

4-Hydroxy-5,6,7-trimethoxy-9-trifluoroacetylnaphtho[2,3-*b*]-furan 7b. As yellow needles (1.76 g, 95%), mp 142–144 °C (Found: C, 55.2; H, 3.6; F, 15.4. $C_{17}H_{13}O_6F_3$ requires C, 55.1; H, 3.5; F, 15.4%); ν_{max}/cm^{-1} 3180 (OH), 1660, 1635 and 1620 (C=O); *m/z* 370 (M^+, 100%), 355 (14), 301 (90), 271 (30), 243 (46) and 144 (20); δ_H 11.18 (1 H, s, OH), 8.35 (1 H, s, 8-H), 7.63 (1 H, d, *J* 2.2, 2-H), 7.02 (1 H, d, *J* 2.2, 3-H), 4.24 (3 H, s, OMe), 4.05 (3 H, s, OMe) and 3.99 (3 H, s, OMe); δ_C 180.0, 157.6, 155.8, 155.5, 149.0, 144.1, 138.6, 131.1 and 123.8, 119.1, 114.5 and 109.9 (q, *J* 289.3, CF_3), 113.1, 108.9, 103.7, 101.5, 100.9, 62.8, 61.2 and 55.9.

General procedure for the preparation of furonaphthoquinones 8a and 8b

To a stirred solution of compound **7a** or **7b** (4 mmol), sodium hydroxide (0.32 g, 8 mmol) and sodium carbonate (0.52 g, 4.8 mmol) in distilled water (24 cm^3) at 40 °C was added hydrogen peroxide (0.38 cm^3, 4.8 mmol, 30% w/w in H_2O) and the mixture was stirred at 40 °C for 24 h. The reaction mixture was acidified (2 mol dm^{-3} hydrochloric acid), extracted with dichloromethane (3 × 50 cm^3) and dried. Evaporation and recrystallisation from methanol afforded the products.

5,7-Dimethoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione 8a. As yellow needles (1.00 g, 97%), mp 242–243 °C (lit.,[17] 243 °C) (Found: C, 64.9; H, 3.95. Calc. for $C_{14}H_{10}O_5$: C, 65.1; H, 3.9%); ν_{max}/cm^{-1} 1660 and 1600 (C=O); *m/z* 258 (M^+, 100%), 241 (20), 229 (56), 227 (19), 212 (18) and 198 (23); δ_H 7.73 (1 H, d, *J* 1.8, 2-H), 7.43 (1 H, d, *J* 2.5, 8-H), 6.96 (1 H, d, *J* 1.8, 3-H), 6.75 (1 H, d, *J* 2.5, 6-H), 4.00 (3 H, s, OMe) and 3.98 (3 H, s, OMe); δ_C 179.0, 172.9, 164.7, 162.6, 150.8, 148.6, 136.9, 132.4, 114.8, 109.1, 104.5, 104.1, 56.4 and 55.9.

5,6,7-Trimethoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione 8b. As yellow needles (1.14 g, 99%) mp 166 °C (Found: C, 62.25; H, 4.3. $C_{15}H_{12}O_6$ requires C, 62.5; H, 4.2%); ν_{max}/cm^{-1} 1680 and 1665 (C=O); *m/z* 288 (M^+, 100%), 273 (62), 245 (18), 230 (23), 215 (50), 187 (27) and 159 (45); δ_H 7.73 (1 H, d, *J* 1.9, 2-H), 7.65 (1 H, s, 8-H), 6.97 (1 H, d, *J* 1.9, 3-H), 4.05 (3 H, s, OMe), 3.99 (3 H, s, OMe) and 3.98 (3 H, s, OMe); δ_C 179.2, 172.6, 157.1, 155.2, 151.1, 148.4, 148.1, 131.8, 129.2, 120.3, 108.9, 106.9, 61.5, 61.2 and 56.4.

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References

1 De Montfort University (V. H. Pavlidis and P. J. Perry), BP Appl. 9419235.8/1994.
2 D. G. I. Kingston and M. M. Rao, *Planta Med.*, 1980, **39**, 230.
3 M. M. Rao and D. G. I. Kingston, *J. Nat. Prod.*, 1982, **45**, 600.
4 B. Kreher, H. Lotter, G. A. Cordell and H. Wagner, *Planta Med.*, 1988, **6**, 562.
5 C. L. Zani, A. B. de Oliveira and G. G. de Oliveira, *Phytochemistry*, 1991, **30**, 2379.
6 Y. Fujimoto, T. Eguchi, C. Murasaki, Y. Ohashi, K. Kakinuma, H. Takagaki, M. Abe, K. Inazawa, K. Yamazaki, N. Ikekawa, O. Yoshikawa and T. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2323.
7 A. B. de Oliveira, D. S. Raslan, G. G. de Oliveira and J. G. S. Maia, *Phytochemistry*, 1993, **34**, 1409.
8 C. C. Lopes, R. S. C. Lopes, A. V. Pinto and P. R. R. Costa, *J. Heterocycl. Chem.*, 1984, **21**, 621.
9 C. L. Zani, A. B. de Oliveira and V. Snieckus, *Tetrahedron Lett.*, 1987, **28**, 6561.
10 C. C. Lopes, E. L. S. Lima, A. J. Monteiro and P. R. R. Costa, *Synth. Commun.*, 1988, **18**, 1731.
11 X. A. Dominguez, R. Franco, G. Garcia Cano, F. Ma. Consue lo, X. A. S. Dominguez Jr. and L. M. de la Pena, *Planta Med.*, 1983, **49**, 63.
12 C. Abeygunawardena, V. Kumar, D. S. Marshall, R. H. Thomson and D. B. M. Wickramaratne, *Phytochemistry*, 1991, **30**, 941.
13 D. W. Knight and A. P. Nott, *J. Chem. Soc., Perkin Trans. 1.*, 1981, 1125.
14 P. J. Perry, V. H. Pavlidis and I. G. C. Coutts, unpublished work.
15 S. O. de Silva, M. Watanabe and V. Snieckus, *J. Org. Chem.*, 1979, **44**, 4802.
16 M. B. Hocking, *Can. J. Chem.*, 1973, **51**, 2384.
17 C. Rivalle, E. Bisagne and J. Andre-Louisfert, *Tetrahedron*, 1974, **30**, 3193.
18 J. M. Edmondson, L. S. Armstrong and A. O. Martinez, *J. Tissue Culture Methods*, 1988, **11**(1), 15.

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