Bioorganic & Medicinal Chemistry 18 (2010) 6442–6450

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Antiviral agents 2. Synthesis of trimeric naphthoquinone analogues of conocurvone and their antiviral evaluation against HIV

Ian T. Crosby [a,*], David G. Bourke [a], Eric D. Jones [a,†], Paula J. de Bruyn [a,‡], David Rhodes [b,†], Nick Vandegraaff [b,†], Susan Cox [b,†], Jonathan A. V. Coates [b,†], Alan D. Robertson [b,§]

[a] Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia
[b] AMRAD Operations Pty Ltd, 576 Swan Street, Richmond, Victoria 3121, Australia

ARTICLE INFO

Article history:
Received 4 May 2010
Revised 25 June 2010
Accepted 30 June 2010
Available online 3 August 2010

Keywords:
Conocurvone
Naphthoquinone trimers
Anti-HIV activity
HIV integrase inhibition

ABSTRACT

The synthesis of a new series of conocurvone analogues is presented that explores the importance of the pyran rings of conocurvone, their degree of unsaturation as well as the role of alkoxy functionalities as pyran ring replacements, for the inhibition of the HIV-1 integrase (IN) enzyme. Difficulties in synthesising a trimeric naphthoquinone where the central quinone bears a *peri*-dihydropyran ring was attributed to distortion of the electrophilic dihaloquinone successfully utilised in the past. Increased electron density could also be a factor in reducing reactivity. The desired central dihydropyran bearing trimeric naphthoquinone was successfully synthesised by using a more reactive bromo-tosyloxyquinone intermediate. A maleimide derivative, where the central quinone between the pendant hydroxyquinones was replaced, was successfully synthesised and although it exhibited comparable enzyme inhibitory activity it had negligible HIV inhibitory cellular activity. Compounds were assessed for activity in both in vitro assays using purified recombinant HIV-1 IN and demonstrated superior or comparable activity to conocurvone derivatives previously reported.

1. Introduction

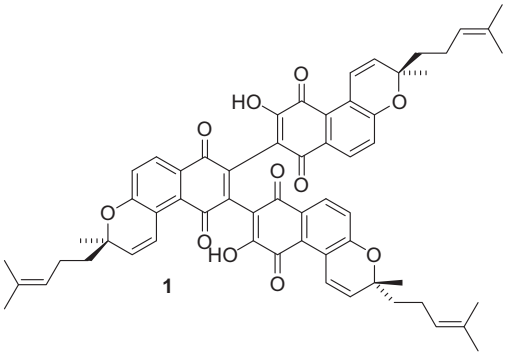
Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS) infection, a pandemic disease that continues to exact a serious toll worldwide.[1] Currently, there are 25 anti-HIV-1 agents approved for clinical use, which target a total of four clinically validated viral proteins (Envelope, Reverse transcriptase, Integrase and Protease) and one cellular target (CCR5).[2] One of two recently FDA-approved drugs, Raltegravir,[3] targets the HIV integrase enzyme, the protein responsible for the integration of newly synthesized HIV-1 DNA into the host cellular chromosome. This process is absolutely required for viral replication and can be recapitulated in vitro using recombinant integrase (IN) enzyme. Such assays were pivotal for the identification and development of integrase strand transfer inhibitors (INSTIs)[4] that culminated in the successful approval of MK-518 (Raltegravir) for clinical use in October 2007. Since the introduction of Raltegravir into the clinical setting, data suggests that while able to reduce viral loads with unprecedented kinetics, this drug appears to have a low genetic barrier to resistance that results in relatively rapid selection of drug-resistant viral species.[1] Thus, there is a clear need to investigate novel scaffolds displaying limited cross-resistance that interfere with HIV-1 IN in a mechanistically different manner to that of Raltegravir and the other INSTIs currently in clinical trials.

Inhibitors of integrase have been identified by HTS campaigns of propriety compound collections[5] and by pharmacophore building and database mining.[6,7] The solved crystal structures of **2** known INSTIs in the prototype foamy virus integrase enzyme[8] is anticipated to increase efforts in structure based design approaches. Screening of natural product libraries against integrase has produced a number of novel inhibitors of integrase[9–11] although none have yet progressed into a clinical development phase. Thus ongoing exploration of the chemical space afforded by natural products[12] continues to be attractive means as identifying new inhibitors. A case in point is conocurvone **1**, which was isolated by the NCI using bioassay guided fraction of extracts from the West Australian Smoke Bush. Conocurvone[13] **1** has been shown to an inhibitor of both HIV integrase and HIV mediated cell fusion.[14,15] We recently reported[16] the synthesis of a series of conocurvone **1** analogues based on the core structure **2**, where the stereochemical complications associated with the chiral quaternary pyran ring carbons had been removed by *gem*-dimethyl substitution. This structural simplification also had the advantage of

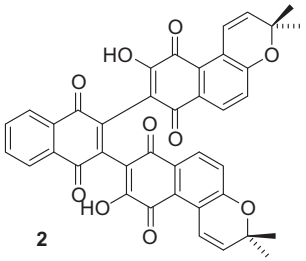
* Corresponding author. Tel.: +61 3 99039536; fax: +61 3 99039582.
E-mail address: ian.crosby@pharm.monash.edu.au (I.T. Crosby).
† Present address: Avexa Ltd, 576 Swan Street, Richmond, Victoria 3121, Australia.
‡ Present address: Davies Collison Cave, 1 Nicholson Street, Melbourne, Victoria 3000, Australia.
§ Present address: Pharmaxis Ltd, 2/10 Rodborough Rd, Frenchs Forest, NSW 2086, Australia.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.06.105

making the compounds less lipophilic. Compound **2** was also found to be well tolerated in rats and fairly well tolerated in beagles but with some signs of minor heptatoxicity being observed which led to the decision that no further animal studies would be undertaken on compound **2**.



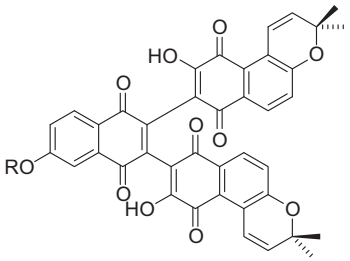
1



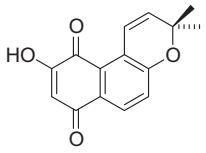
2

This novel class of HIV-inhibitory compounds remains of interest to us due to their unique structure and potential for inhibition of the recently validated clinical target, HIV integrase. Here we present the synthesis of a range of naphthoquinone 'trimers' that explore the importance of the pyran rings, their degree of unsaturation, as well as the role of alkoxy functionalities on anti-integrase and anti-HIV activity.

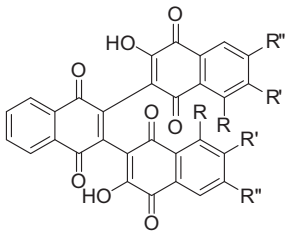
1,4-naphthoquinone, made by alkylation of 6-hydroxy-1,4-naphthoquinone, and 9-hydroxy-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **6** using similar chemistry as for the synthesis of compounds **2–4**.[16] Compounds **7–9** were synthesised from 2,3-dichloro-1,4-naphthoquinone and the corresponding methoxy substituted 2-hydroxy-1,4-naphthoquinones,[17,18] again using similar based promoted condensation.[16]



3 R = Me
4 R = $(CH_2)_2Me$
5 R = $CH(Me)_2$

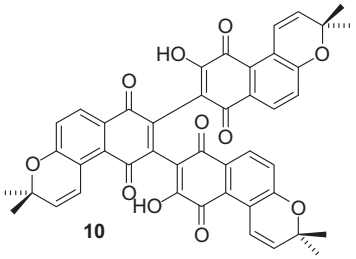


6

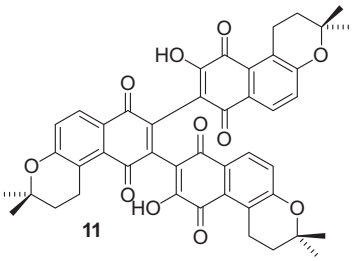


7 R = OMe; R', R'' = H
8 R'' = OMe; R, R' = H
9 R', R'' = OMe; R = H

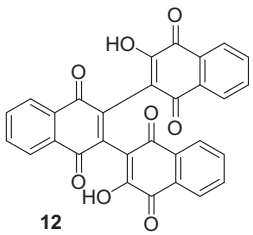
'Symmetrical' trimeric naphthoquinones **10–13** could be synthesised by 'self-trimerisation' of the corresponding monomeric hydroxyquinones by prolonged heating in 1,2-dimethoxyethane in the presence of Hünig's base ($^{i}Pr_2NEt$).



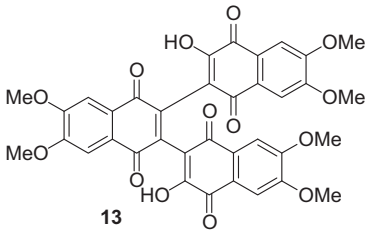
10



11



12



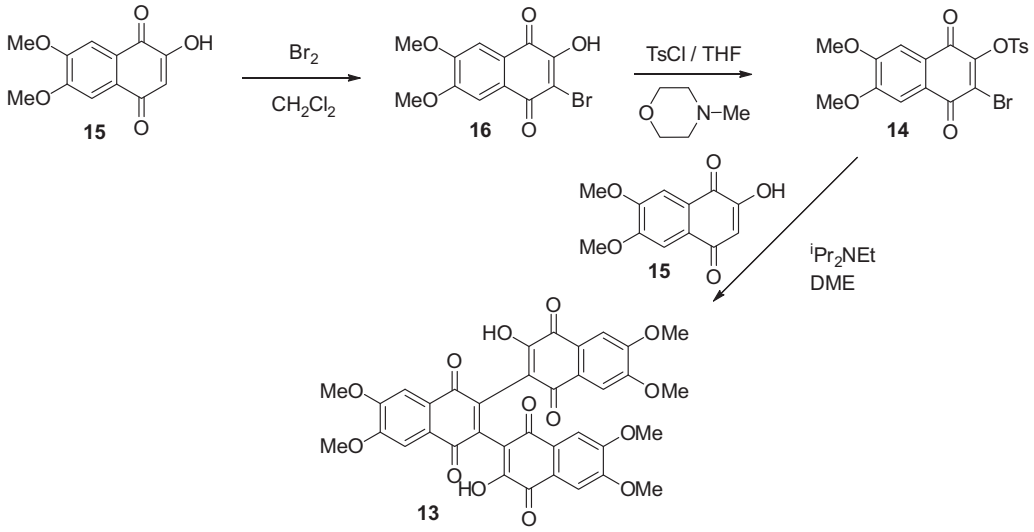
13

2. Results and discussion

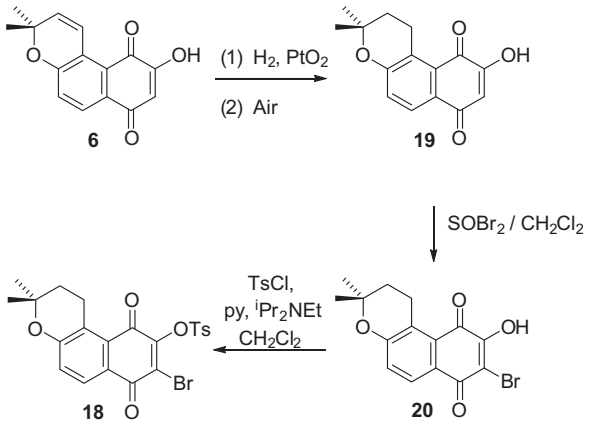
2.1. Chemistry

Compounds **2–4** were synthesised as previously reported.[16] Compound **5** was synthesised from 2,3-dichloro-6-isopropoxy-

Compound **13** was initially isolated as a self-trimerisation by-product from the synthesis of **9** in a reaction of 2,3-dichloro-1,4-naphthoquinone with 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone. For characterisation purposes, **13** was synthesised using the more reactive 2-bromo-3-(4′-toluenesulfonyloxy)-6,7-dimethoxy-1,4-naphthoquinone **14**, as the corresponding dichloronaph-



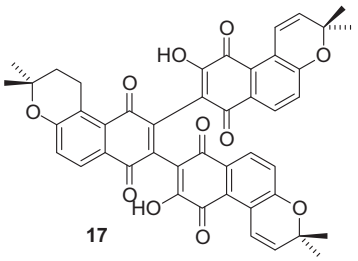
Scheme 1.



Scheme 2.

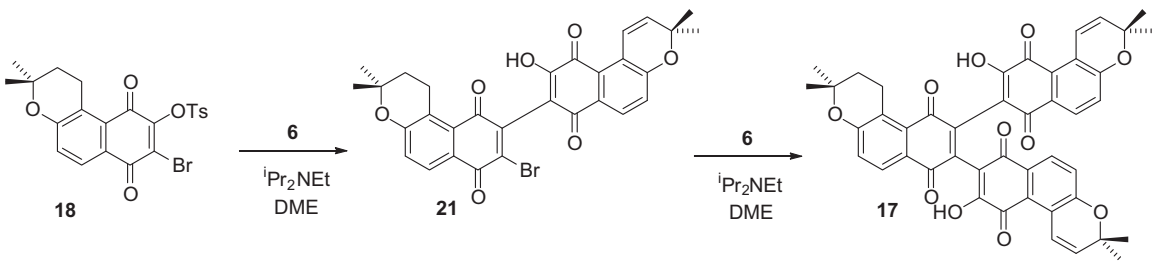
thoquinone gave a complex mixtures of products possibly due to its lower reactivity resulting from the increased electron density due to the two methoxy groups. Compound **13** was made by reaction of 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone **15**[19] with the reactive 2-bromo-3-(4′-toluenesulfonyloxy)-6,7-dimethoxy-1,4-naphthoquinone **14** (Scheme 1). 2-Bromo-3-(4′-toluenesulfonyloxy)-6,7-dimethoxy-1,4-naphthoquinone **14** was synthesised from 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone **15**[19] by bromination to **16** followed by tosylation to afford **14** (Scheme 1).

A compound where the central quinone was substituted with a pyran ring but had a different type of pendant naphthoquinone, such as **17**, also proved to be more difficult to synthesise and a slightly modified strategy was adopted.

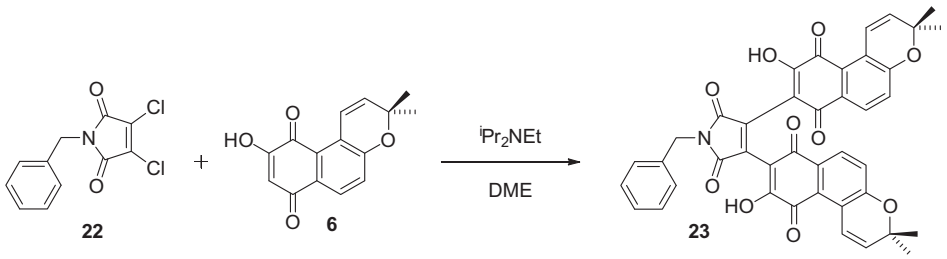


It was thought that the *peri*-dihydropyran ring was distorting the adjacent carbonyl, thereby reducing the reactivity of the quinone as well as increasing the electron density of the naphthoquinone. In order to increase the reactivity, and to allow for chemoselection of the central quinone, 8-bromo-9-tosyloxy-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **18** was utilised. This was synthesised from 9-hydroxy-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **6** by hydrogenation to **19**,[16] brominating to give **20** and finally tosylation to afford **18** (Scheme 2).

The bromo-tosyloxy compound **18** proved to be more reactive with the tosyloxy group preferentially undergoing substitution in the first base catalysed condensation reaction with a 9-hydroxy-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **6** to afford the bromodimer **21**, respectively (Scheme 3). Condensation with a second molecule of the hydroxyquinone **6** gave the trimeric compound **17** (Scheme 3), although self-trimerisation of the hydroxyquinone **6** gave a complex reaction mixture and the reaction often ceased before all of the bromodimer **21** had been consumed. The purification of



Scheme 3.



Scheme 4.

Table 1
Anti-HIV and integrase data for newly synthesized trimeric naphthoquinones

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	IC_{50}[c] (3′)	IC_{50}[d] (3′-ST)
2	0.029	10	2.5	2.0
3	0.027	10	2.3	2.5
4	0.009	1	2.8	5.0
5	0.24	>20	3.0	>100
10	0.39	>20	1.7	3.0
11	ND	ND	ND	9.0
17	0.47	>20	1.8	6.0
23	>20	ND	4.5	4

[a] Antiviral activity was calculated following measurement of P24 levels in culture supernatants on day 5 of a spreading replication assay performed in the presence of serial dilutions of compound using a commercially available kit (Vironostika HIV-1 Antigen kit, Biomerieux). Spreading replication assays were performed at an MOI of 0.001 in CEM cells using HIV-1_{Rf} strain.

[b] Cytotoxicity assays were performed in CEM cells using the commercially available ATPlite kit according to the manufacturers protocol (Perkin Elmer).

[c] 3′-Processing assays (3′) were performed as previously described[11] and assess the ability of recombinant HIV-1 integrase to catalyse the strand transfer reaction only.

[d] 3′-Strand transfer (3′ST) assays were performed as previously described[11] and assess the ability of recombinant HIV-1 integrase to catalyse both the 3′processing and strand transfer reactions.

these reaction mixtures was effected by a combination of flash chromatography followed by preparative HPLC.

To explore the nature of the central linker, the central quinone between the pendant hydroxyquinones was replaced with a maleimide derivative. Accordingly, *N*-benzyl-3,4-dichloro-1*H*-pyrrole-2,5-dione **22** was reacted with the 9-hydroxy-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **6** to afford the maleimide linked analogue **23** (Scheme 4).

2.2. Results and discussion

Previously reported compounds **2–4** showed encouraging low nM potency against HIV infected CEM cells, however we were concerned with emergence of toxicity of this class both in cell culture (see Table 1) and in vivo (data not shown) which could limit their therapeutic utility. In order to explore this series further we embarked on further chemistry examining the nature of the central naphthoquinone resulting in compounds **5, 10, 17** and **23**. Replacing the *n*-propyl group of **4** with *iso*-propyl in **5** shows a loss of antiviral activity, however, this was balanced by less cellular toxicity, interestingly both compounds showed the same level of activity in the 3′ assay but **5** was inactive in the 3′-ST assay.

Reincorporation of the pyran unit back into the central quinone in **10** and a reduced pyran unit in compound **17** afforded compounds of similar activities against HIV as **5**, and once again with diminished cellular toxicity. Of note is compound **23** where the central napthoquinone has been replaced with a maleimide linker. Although this compound retains comparable enzyme activity to **2**–**5** and **10**, anti-HIV activity was abolished.

Table 2
Pharmacokinetics for **17**

Rat pharmacokinetics[a]	
Cl_p (mL/h)	0.44
V_{dss} (mL)	27.7
C_{max} (μg/mL)-IV	50.2
$t_{1/2}$ (h)-IV	45
C_{max} (μg/mL)-PO	3.2
T_{max} (h)	7.5
$t_{1/2}$ (h)-PO	21.7
%*F*	2.6

[a] Average data generated after 5 mg/kg po ($n = 2$ animals/dose) and 2 mg/kg IV ($n = 3$ animals/dose).

Table 3
Anti-HIV and integrase data for newly synthesized trimeric naphthoquinones

Compound	EC_{50} (μM)	CC_{50} (μM)	IC_{50} (3′) (μM)	IC_{50} (3′-ST) (μM)	PSA[a]	MW
17	0.47	>20	1.8	6.0	170.57	750.74
7	>20	ND	ND	13.0	161.34	562.48
8	>20	ND	22.5	10	161.34	562.48
9	>20	ND	62	12	179.8	622.53
12	16	48	56	13	142.88	502.43
13	ND	ND	>150	10	198.26	682.58

[a] PSA calculated using Symyx Draw 3.3.

Armed with a favourable activity profile in **17** the in vivo pharmacokinetic properties of this compound was assessed in rats and the results are summarised in Table 2.

After IV dosing, **17** was slowly absorbed through the stomach with T_{max} of 7.5 h. This could be attributable to the poor aqueous solubility and dissolution of the compound. Given the high molecular weight (750.8) and PSA (173.2) of **17** intestinal permeability was expected to be limiting and comparison of the C_{max} for IV and PO confirms this, ultimately resulting in a low bioavailability (2.6%).

Given these PK results for **17** we turned our attention to modulating the molecular weight and PSA of the class by replacing the pyran containing naphthoquinone arms with smaller substitutions and these results are summarised in Table 3.

We firstly removed all functionality of the trimeric system to give the known compound **12** which showed encouraging antiviral activity with an EC_{50} of 16 μM. For compounds **7–9**, inclusion of methoxy substituents had minimal effect on enzymatic activity compared to **12** but resulted in diminished antiviral activity (all EC_{50} >20 μM), suggesting the pyran rings are required for activity. A comparison of note is the trend of compounds **4**–**7** which both feature the same oxygenation pattern around the trimeric core but vastly different activities against HIV.

In this paper we report a further series of trimeric naphthoquinones and their ability to inhibit HIV and the integrase enzyme.

Although promising anti-HIV data was obtained, the therapeutic use of these compounds could be limited by their low stomach permeability. Although promising anti-HIV data was obtained and that cell based assays indicated that toxicity was lower for some compounds, it was deemed that the poor bioavailability in rats (2.6%) of **17** did not warrant a toxicity study in dogs. Attempts to improve the chemical properties of the series by reducing molecular weight diminished activity. Worth noting is the discordance between enzymatic data and cell based data which suggest inhibition of HIV may not be related to inhibition of integrase. Future publications will explore this further.

3. Experimental

3.1. Chemistry

3.1.1. General experimental

Most of the general experimental has previously described.[16]

HPLC was carried out on a C18 column using mixtures of Solvent A (90% acetonitrile in water) and Solvent B (0.05% aqueous phosphoric acid) detecting at 275 nm. Unless otherwise stated analytical Gradient HPLC used a flow rate of 1.5 mL/min on a C18 column beginning elution with 70% A, 30% B, going to 79% A, 21% B over 15 min, then to 100% A over 3 min and finally 100% A for 8 min. Preparative HPLC was carried out on of solutions of crude compounds filtered through HPLC grade filters (0.22 μm) and then isocratically chromatographed in the specified solvent system at flow rate 5 mL/min, unless otherwise stated.

3.1.2. 2,3-Dichloro-6-isopropyloxy-1,4-naphthoquinone

To a solution of 2,3-dichloro-6-hydroxynaphthoquinone[20] (510 mg, 2.10 mmol) in dichloromethane (30 mL) was added silver(I) oxide (973 mg, 4.20 mmol) and 2-bromopropane (394 μL, 4.20 mmol). The mixture was stirred at room temperature for 19 h, filtered through Celite® and the residue washed with dichloromethane until no further colour was eluted. Removal of the volatiles in vacuo gave a brown residue (497 mg) which was purified by flash chromatography (10% ethyl acetate/hexanes with 1% acetic acid) to afford *2,3-dichloro-6-isopropyloxy-1,4-naphthoquinone* (221 mg, 37%) as yellow microprisms, mp 111–112 °C. ν_{max} 1678 (s), 1578 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.40 (6H, d, *J* 6, $OCH(CH_3)_2$), 4.77 (1H, septet, *J* 6, $OCH(CH_3)_2$), 7.17 (1H, dd, *J* 8, 2, H7), 7.56 (1H, d, *J* 2, H5), 8.12 (1H, d, *J* 8, H8). Calcd for $C_{13}H_{10}Cl_2O_3$: C, 54.8, H, 3.5; found: C, 54.8; H, 3.6.

3.1.3. 9-Hydroxy-8-(3′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″*H*-naphtho[2,1-*b*]pyran-8″-yl)-1,4′-dioxo-6′-isopropyloxy-1′,4′-dihydronaphthalen-2′-yl)-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 5

A mixture of 2,3-dichloro-6-isopropyloxy-1,4-naphthoquinone (112 mg, 0.393 mmol), 9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione[16] **6** (221 mg, 0.864 mmol) and diisopropylethylamine (684 μL, 3.92 mmol) in 1,2-dimethoxyethane (10 mL) was heated at reflux for 70 h under nitrogen. The mixture was cooled, poured into aqueous hydrochloric acid (1 M) and extracted with ethyl acetate and dichloromethane. The combined extracts were evaporated and the residue subjected to flash chromatography in 24% ethyl acetate in hexanes containing 1% acetic acid. The resulting residue (321 mg) gave a mixture of the hydroxyquinone **6** and other unidentified compounds. Further elution of the flash column with 9:1 dichloromethane/methanol containing 1% acetic acid afforded a red/brown solid (70 mg) which was dissolved in 9:1 ethyl acetate/90% aqueous formic acid and filtered through a plug of Celite® to give a dark orange solid (33 mg). This was subjected to preparative HPLC (isocratic 70% A, 30% B) and fractions with a retention time of 7.9 min by analytical HPLC (gradient) were combined and triturated with ethyl acetate and hexanes to afford *9-hydroxy-8-(3′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″H-naphtho[2,1-b]pyran-8″-yl)-1,4′-dioxo-6′-isopropyloxy-1′,4′-dihydronaphthalen-2′-yl)-3,3-dimethyl-3H-naphtho[2,1-b]pyran-7,10-dione* ***5*** (23 mg, 8%) as a orange powder, mp 167–170 °C. ν_{max} 3440 (br w), 2995 (w), 1650 (m), 1645 (m), 1594 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.40 (6H, d, *J* 5, $OCH(CH_3)_2$), 1.48 (12H, s, C–CH_3), 4.77 (1H, m, $OCH(CH_3)_2$), 5.97 (2H, d, *J* 10, H2, H2″), 7.10 (2H, m, H5, H5″), 7.18 (1H, d, *J* 8, H7′), 7.56 (1H, br s, H5′), 7.69 (2H, d, *J* 10, H1, H1″), 7.92 (2H, m, H6, H6″), 8.09 (1H, br d, *J* 8, H8′). ESI-MS (positive, 30 V) 725 (M+H, 32%), 338 (100), 214 (90). HR-ESI-MS (positive) Calcd for $C_{43}H_{33}O_{11}^+$ 725.2023; found 725.2045.

3.2. Synthesis of methoxy substituted 2-hydroxy-1,4-naphthoquinones

The various methoxy substituted were synthesized by reaction of the corresponding methoxy substituted 1-tetralones with oxygen in the presence of potassium *t*-butoxide in *t*-butanol.[17,18]

3.2.1. 2-Hydroxy-5-methoxy-1,4-naphthoquinone

3,4-Dihydro-5-methoxy-1(2*H*)-naphthalenone (2.00 g, 11.48 mmol) afforded 2-hydroxy-5-methoxy-1,4-naphthoquinone (1.10 g, 47%) as fine yellow crystals, mp 170–173 °C (lit.[21] 176–177 °C). ^1H NMR (300 MHz, $CDCl_3$) 3.88 (3H, s, OCH_3), 6.00 (1H, s, H3), 7.53 (1H, d, *J* 8.5, H6), 7.63 (1H, d, *J* 6.5, H8), 7.74 (1H, distorted t, H7), 11.19 (1H, br s, OH). ESI-MS (positive, 30 V) 227 (M+Na, 20%), 205 (M+H, 100%).

3.2.2. 2-Hydroxy-7-methoxy-1,4-naphthoquinone

3,4-Dihydro-7-methoxy-1(2*H*)-naphthalenone (520 mg, 2.8 mmol) gave 2-hydroxy-6-methoxy-1,4-naphthoquinone (214 mg, 36%) as fluffy orange needles, mp 210–213 °C (lit.[18] 214–215 °C). ^1H NMR (300 MHz, $(CD_3)_2SO$) 3.91 (3H, s, OCH_3), 6.07 (1H, s, H3), 7.35 (1H, br d, *J* 9, H6), 4.08 (1H, br s, H8), 7.88 (1H, br d, *J* 9, H5). FAB-MS (3NBA) 205 (M+H).

3.2.3. 2-Hydroxy-6,7-dimethoxy-1,4-naphthoquinone 15

3,4-Dihydro-6,7-dimethoxy-1(2*H*)-naphthalenone (2.00 g, 9.7 mmol) gave 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone **15** (508 mg, 22%) as fine yellow crystals, mp 210 °C (decomp.) (lit.[19] 212 °C (decomp.)). ^1H NMR (300 MHz, $CDCl_3$) 4.02 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 6.24 (1H, s, H3), 7.34 (1H, br s, OH), 7.51, 7.55 (1H, 1H, s, s, H5, H8). ESI-MS (positive, 30 V) 227 (M+Na, 20%), 205 (M+H, 100%). Unreacted starting material (1.31 g, 65.5%) was also recovered.

3.2.4. 2-Hydroxy-3-(3′-(2″-hydroxy-5″-methoxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1″,4″-dihydronaphthalene-2′-yl)-5-methoxy-1,4-naphthoquinone 7

A mixture of 2-hydroxy-5-methoxy-1,4-naphthoquinone (300 mg, 1.46 mmol), 2,3-dichloro-1,4-naphthoquinone (150 mg, 0.66 mmol) and diisopropylethylamine (1.15 mL, 6.60 mmol) in 1,2-dimethoxyethane (15 mL) was heated at reflux for 10 h under nitrogen. The reaction mixture was cooled, poured into hydrochloric acid (1 M) and extracted with ethyl acetate and dichloromethane. The extracts were then combined and washed with aqueous sodium bicarbonate solution (5%, 3 × 15 mL). The combined alkaline extracts were acidified with concentrated hydrochloric acid and extracted with ethyl acetate and dichloromethane. The combined organic extracts were evaporated in vacuo and the residue (440 mg) subjected to flash chromatography using 25% ethyl acetate/hexanes (containing 1% acetic acid) to give a mixture of

2-hydroxy-5-methoxy-1,4-naphthoquinone and other unidentified compounds. Further elution with 9:1 dichloromethane/methanol (containing 1% acetic acid) afforded a red solid (92 mg). This was dissolved in ethyl acetate/90% aqueous formic acid 9:1 and filtered through a plug of Celite® to give a red solid (73 mg) which was subjected to preparative HPLC (70% A–50% A over 60 min, flow rate 5 mL/min). Fractions that had a retention time of 15.1 min by analytical HPLC (30% A, 70% B isocratic) were combined and triturated with ethyl acetate and hexanes to provide *2-hydroxy-3-(3′-(2″-hydroxy-5″-methoxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1″,4″-dihydronaphthalene-2′-yl)-5-methoxy-1,4-naphthoquinone* **7** (30 mg, 8%) as a yellow solid, mp 211–213 °C. ν_{max} 3215 (br w), 1668 (s), 1582 (s), 1276 (br s), 1026 (m) cm^{-1}. ^1H NMR (300 MHz, CD_3OD) 3.90 (6H, s, 2 × OCH_3), 7.39–7.53 (2H, m, H7, H7″), 7.61–7.74 (4H, m, H6, H8, H6″, H8″), 7.82–7.92 (2H, m, H6′, H7′), 8.10–8.20 (2H, m, H5′, H8′). ESI-MS (positive, 30 V) 601 (M+K, 17%), 585 (M+Na, 30%), 563 (M+H, 100%). HR-ESI-MS (positive) Calcd for $C_{30}H_{23}O_8^+$ 563.0973; found 563.0960.

3.2.5. 2-Hydroxy-3-(3′-(2″-hydroxy-7″-methoxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1′,4′-naphthalene-2′-yl)-1,4-dioxo-1,4-dihydronaphthalene 8

Diisopropylethylamine (0.45 mL) was added to a stirred solution of 2-hydroxy-7-methoxy-1,4-naphthoquinone (130 mg) and 2,3-dichloro-1,4-naphthoquinone (60 mg) in 1,2-dimethoxyethane (5 mL). The resulting red solution was heated at reflux for 6 h, allowed to cool, diluted with dichloromethane and washed with aqueous hydrochloric acid (1 M). The organic phase was dried and concentrated, and the resulting residue dissolved in a mixture of dichloromethane (10 mL), acetic anhydride (20 drops) and pyridine (9 drops). After standing for 30 min, water was added and the mixture was vigorously stirred for 15 min. The organic phase was washed with aqueous hydrochloric acid (1 M), dried and concentrated to give a brown residue which was subjected to flash chromatography (30% ethyl acetate in hexanes). The major mobile yellow band afforded 2-acetoxy-3-(3′-(2″-acetoxy-7″-methoxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1′,4′-naphthalene-2′-yl)-7-methoxy-1,4-naphthoquinone (trimeric diacetate intermediate) as a yellow solid (115 mg, 68%), mp 167–171 °C (decomp.). ν_{max} 1784 (m), 1678 (br s), 1660 (s), 1594 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 2.12 (6H, s, 2 × OAc), 3.94 (6H, s, 2 × OMe), 7.24 (2H, dd, *J* 9, 2, H6, H6″), 7.50 (2H, d, *J* 2, H8, H8″), 7.81–7.84 (2H, m, H6′, H7′), 8.04 (2H, d, *J* 9, H5, H5″), 8.17–8.20 (2H, m, H5′, H8′). *m/z* (FAB, 3NBA) 647 (M+H, 7%), 646 (M−H+1, 3), 604 (13), 563 (22), 562 (40), 358 (16).

Ground aluminium chloride (130 mg) was added to a stirred solution of the trimeric diacetate intermediate (42 mg) in dichloromethane (3 mL) and the resulting green mixture stirred at room temperature for 1.5 h. Additional aluminium chloride (50 mg) was added, stirring maintained for a further 45 min, the mixture was diluted with dichloromethane (20 mL) and then washed with aqueous oxalic acid (5%, 2 × 70 mL). The yellow organic phase was washed with water, dried and partially concentrated to a volume of ~5 mL. The solution was heated to boiling and hexanes (1 mL) carefully added before being allowed to stand overnight. The solid which precipitated was collected by filtration and washed with cold dichloromethane to afford the deacetylated trimer **8** as a yellow amorphous solid (27 mg, 75%), mp 238–239 °C (decomp.). ^1H NMR (300 MHz, d_6-DMSO) 3.89 (6H, s, 2 × OMe), 7.29, 7.36 (2H, br d, br d, *J* 8.5, H6, H6″ (isomer a), (isomer b)), 7.38 (2H, br s, H8, H8″), 7.77, 7.89 (2H, d, d, *J* 8.5, H5, H5″ (isomer a), (isomer b)), 7.94–7.97 (2H, m, H6′, H7′), 8.08–8.11 (2H, m, H5′, H8′) [isomer a:b; 1:1.2]. *m/z* FAB-MS (3NBA) 564 (M+H+1, 15%), 563 (M+H, 40), 662 (M+H−1, 61), 561 (M−1, 22), 358 (26). Calcd for $C_{32}H_{18}O_{10}.H_2O$: C, 66.2; H, 3.5; found: C, 66.2; H, 3.7.

3.2.6. 2-Hydroxy-3-(3′-(2″-hydroxy-6″,7″-dimethoxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1′,4′-dihydronaphthalene-2′-yl)-6,7-dimethoxy-1,4-naphthoquinone 9

A mixture of 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone (750 mg, 3.20 mmol), 2,3-dichloro-1,4-naphthoquinone (727 mg, 3.20 mmol) and diisopropylethylamine (5.6 mL, 32 mmol) in 1,2-dimethoxyethane (75 mL) was stirred at room temperature for 23 h under nitrogen. A further portion of 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone (750 mg, 3.20 mmol) was added and the mixture heated under reflux for an additional 24 h. The reaction was then cooled, poured into hydrochloric acid (1 M) and extracted with dichloromethane (2 × 200 mL). The combined extracts were washed with aqueous sodium bicarbonate (5%, 9 × 250 mL) during which water was added to break up the emulsions that formed. The alkaline extracts were combined, carefully acidified with concentrated hydrochloric acid and extracted with chloroform (2 × 500 mL). The combined extracts were dried and concentrated in vacuo to provide a brown solid (1.84 g) which was 80% **9** by analytical HPLC. This residue was dissolved in dichloromethane containing 1% acetic acid (25 mL) and the solution subjected to flash chromatography. Elution with dichloromethane containing 1% acetic acid (7.0 L) removed unreacted 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone, the dimer intermediate and other unidentified compounds. Elution with chloroform containing 1% acetic acid (5.0 L) afforded the crude product, as a red solid (1.4 g, 70%). A small sample (126 mg) was subjected to preparative HPLC (isocratic 70% A, 30% B). Fractions that had a retention time of 10.5 min by analytical HPLC (gradient) were combined to give *2-hydroxy-3-(3′-(2″-hydroxy-6″,7″-dimethoxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1′,4′-dihydronaphthalene-2′-yl)-6,7-dimethoxy-1,4-naphthoquinone* **9**, as a red solid, mp 215 °C. ν_{max} 3325 (br m), 1668 (br s), 1578 (s), 1510 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 3.97 (3H, s, OCH_3), 4.00 (6H, s, 2 × OCH_3), 4.04 (3H, s, OCH_3), 7.45 (2H, br s, H5, H5″ or H8, H8″ (isomers a and b)), 7.55, 7.60 (2H, s, H5, H5″ or H8, H8″, isomer b, isomer a), 7.75–7.84 (2H, m, H6′, H7′), 8.15–8.24 (2H, m, H5′, H8′) [isomers a:b; 5.2:1]. ESI-MS (positive, 30 V) 623 (M+H, 10%), 400 (100), 215 (55). Calcd for $C_{34}H_{22}O_{12}.H_2O$: C, 63.8; H, 3.8; found: C, 63.5; H, 3.7. HR-ESI-MS (positive) Calcd for $C_{34}H_{23}O_{12}^+$ 623.1184; found 623.1202.

3.2.7. 9-Hydroxy-8-(8′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″*H*-naphtho[2,1-*b*]pyran-8″-yl)-3′,3′-dimethyl-7′,10′-dioxo-,7′,10′-dihydro-3′*H*-naphtho[2,1-*b*]pyran-9′-yl)-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 10

A solution of 9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione[16] **6** (500 mg, 1.95 mmol) and diisopropylethylamine (342 μL, 1.96 mmol) in 1,2-dimethoxyethane (10 mL) was heated under reflux under nitrogen for 2.5 days whilst monitoring the reaction by HPLC indicated the significant formation of trimeric products. The reaction was then cooled, aqueous hydrochloric acid (1 M) added and the mixture extracted with ethyl acetate until the extracts were colourless. The combined extracts were washed with aqueous sodium hydrogen carbonate (5%) until the washings were colourless. The organic phase was then evaporated to dryness to leave a residue (593 mg) which was purified by flash chromatography (eluting sequentially with 500 mL of 40% ethyl acetate in hexanes, ethyl acetate, 10% methanol in dichloromethane and finally methanol; all containing 1% acetic acid). The fractions that contained trimeric material (rt = 10.0 min, gradient) (280 mg) were subjected to isocratic preparative HPLC (70% A, 30% B, rt = 15.0 min) to give *9-hydroxy-8-(8′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″H-naphtho[2,1-b]pyran-8″-yl)-3′,3′-dimethyl-7′,10′-dioxo-7′,10′-dihydro-3′H-naphtho[2,1-b]pyran-9′-yl)-3,3-dimethyl-3H-naphtho[2,1-b]pyran-7,10-dione* ***10*** (14 mg, 3%), mp 205–211 °C (decomp.). λ_{max} (log ε) 223, 275, 293 sh, 401 nm (4.64,

4.40, 4.38, 3.97). ν_{max} 3420 (br m), 1655 (m), 1650 (m), 1564 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.48 (18H, s, C–CH_3), 5.88 (1H, d, *J* 10, H2′), 5.96 (2H, d, *J* 10, H2, H2″), 7.02–7.15 (3H, m, H5, H5′, H5″), 7.64–7.72 (3H, m, H1, H1′, H1″), 7.91–8.06 (3H, m, H6, H6′, H6″). ESI-MS (positive, 100 V) 750 (M+H+1, 22%), 749 (M+H, 46), 559 (48), 388 (100), 145 (63), 129 (49), 117 (68), 112 (54). HR-ESI-MS (positive) Calcd for $C_{45}H_{33}O_{11}^+$ 749.2017; found 749.2009.

3.2.8. 9-Hydroxy-8-(8′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-1″,2″,7″,10″-tetrahydro-3″*H*-naphtho[2,1-*b*]pyran-8″-yl)-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′*H*-naphtho[2,1-*b*]-pyran-9′-yl)-1,2-dihydro-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 11

A mixture of 9-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **19**[16] (500 mg, 1.94 mmol) and diisopropylethylamine (0.337 mL, 1.94 mmol) in 1,2-dimethoxyethane (10 mL) was heated at reflux for 70 h under nitrogen. The reaction mixture was cooled, poured into aqueous hydrochloric acid (1 M) and extracted with ethyl acetate and dichloromethane. The extracts were combined and the volatiles removed in vacuo. The resulting residue was dissolved in ethyl acetate (20 mL), washed with aqueous sodium bicarbonate (5%, 3 × 20 mL). The organic phase was separated, dried and volatiles removed in vacuo. The resulting residue (452 mg) was subjected to flash chromatography in 25% ethyl acetate in hexanes (containing 1% acetic acid) to give starting material **19** (44 mg) and other unidentified compounds (191 mg). Further elution with 9:1 dichloromethane/methanol (containing 1% acetic acid) followed by 1% acetic acid in methanol afforded a red solid (420 mg). This solid was dissolved in ethyl acetate/90% aqueous formic acid (9:1) and filtered through Celite®, to give a dark orange solid (190 mg) which was subjected to preparative HPLC (isocratic 70% A, 30% B). Fractions that had a retention time of 11.3 min by analytical HPLC (gradient) were combined and triturated with ethyl acetate and hexanes to afford *9-hydroxy-8-(8′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-1″,2″,7″,10″-tetrahydro-3″H-naphtho[2,1-b]pyran-8″-yl)-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′H-naphtho[2,1-b]pyran-9′-yl)-1,2-dihydro-3,3-dimethyl-3H-naphtho[2,1-b]pyran-7,10-dione* ***11*** (22 mg, 1.5%) as a yellow powder, mp 210–215 °C. ν_{max} 3375 (br w), 1658 (br m), 1470 (br m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.27, 1.29, 1.39 (6H, 6H, 6H, s, s, s, 3 × $(CH_3)_2$), 1.43–1.70 (2H, m, 2 × OH), 1.73–1.90 (6H, m, H2, H2′, H2″), 3.08–3.20 (6H, m, H1, H1′, H1″), 7.00–7.21 (3H, m, H5, H5′, H5″), 7.88–7.98, 8.03 (3H, m, d, *J* 8.5, H6, H6′, H6″, isomers b and c, isomer a [isomers a:b:c; 4:1:1]. ESI-MS (positive, 30 V) 755 (M+H, 7%), 491 (7), 361 (26), 360 (100). HR-ESI-MS (positive) Calcd for $C_{45}H_{39}O_{11}^+$ 755.2587; found 755.2461.

3.2.9. 2-Hydroxy-3-(3′-(2″-hydroxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1′,4′-dihydronaphthalene-2′-yl)-1,4-naphthoquinone 12[22]

A mixture of 2-hydroxy-1,4-naphthoquinone (253 mg, 1.45 mmol), 2,3-dichloro-1,4-naphthoquinone (150 mg, 0.66 mmol) and diisopropylethylamine (1.15 mL, 6.60 mmol) in 1,2-dimethoxyethane (15 mL) was heated at reflux under nitrogen for 14 h. The reaction mixture was cooled, poured into hydrochloric acid (1 M) and extracted with ethyl acetate and dichloromethane. The extracts were combined and the volatiles removed in vacuo. Flash chromatography (25% ethyl acetate/hexanes containing 1% acetic acid) of the resulting residue (406 mg) gave a mixture of 2-hydroxy-1,4-naphthoquinone and other unidentified compounds. Further elution of the flash column with 10% methanol in dichloromethane (containing 1% acetic acid) afforded a red solid (145 mg) which was dissolved in ethyl acetate/90% aqueous formic acid (9:1) and filtered through a plug of Celite® to give a red solid (84 mg). This was subjected to preparative HPLC (isocratic 60% A, 40% B). Fractions that had a retention time of 9.9 min by analytical HPLC (gradient) were combined, triturated with ethyl acetate and hexanes to provide 2-hydroxy-3-(3′-(2″-hydroxy-1″,4″-dioxo-1″,4″-dihydronaphthalene-3″-yl)-1′,4′-dioxo-1′,4′-dihydronaphthalene-2′-yl)-1,4-naphthoquinone[22] **12** (14 mg, 4%) as a brown/orange solid, mp 245 °C. ν_{max} 3300 (br w), 1674 (s), 1640 (s), 1592 (m) cm^{-1}. ^1H NMR (300 MHz, CD_3OD) 7.65–7.83 (m, 7 ArH), 7.83–7.94 (m, 3 ArH), 7.96–8.09 (m, 3 ArH), 8.11–8.20 (m, 1 ArH). ESI-MS (positive, 30 V) 601 (M+K, 17%), 585 (M+Na, 30%), 503 (M+H, 100%). These data is in good agreement with that for **12** in the NCI patent for **12**.[22]

3.2.10. 2-Bromo-3-hydroxy-6,7-dimethoxy-1,4-naphthoquinone 16

A solution of bromine (77 mg, 0.48 mmol) in dry dichloromethane (1 mL) was added dropwise to a cooled (−5 °C), stirred solution of 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone[19] **15** (100 mg, 0.43 mmol) in dry dichloromethane (14 mL) and glacial acetic acid (1 drop) under nitrogen. After 15 min stirring at room temperature the volatiles were removed in vacuo to afford, as red micro crystals, 2-bromo-3-hydroxy-6,7-dimethoxy-1,4-naphthoquinone **16** (133 mg, 100%), mp 275–277 °C. ν_{max} 3348 (br m), 1658 (m), 1648 (m), 1632 (m) cm^{-1}. ^1H NMR (300 MHz, $(CD_3)_2SO$) 3.92, 3.93 (3H, 3H, s, s, 2 × OCH_3), 7.43, 7.45 (1H, 1H, s, s, H5, H8). ESI-MS (positive, 30 V) 315 (M[^{81}Br]+H, 95%), 313 (M[^{79}Br]+H, 100), 301 (44), 149 (50). This compound was used without further purification.

3.2.11. 2-Bromo-3-(4′-toluenesulfonyloxy)-6,7-dimethoxy-1,4-naphthoquinone 14

N-Methylmorpholine (67 μL, 0.78 mmol) was added to a stirred solution of 2-hydroxy-3-bromo-6,7-dimethoxy-1,4-naphthoquinone **16** (122 mg, 0.39 mmol) in dry THF (15 mL). After 10 min a solution of 4-toluenesulfonyl chloride (89 mg, 0.47 mmol) in dry THF (2 mL) was added and the reaction was stirred at room temperature for 2 h. A further portion of *N*-methylmorpholine (335 μL, 3.90 mmol) was added then after 4 drop more *N*-methylmorpholine (200 μL, 1.43 mmol) and 4-toluenesulfonyl chloride (20 mg, 0.11 mmol) were added. After 20 h hydrochloric acid (1 M, 10 mL) was added and the products were extracted with ethyl acetate (10 mL) and dichloromethane (10 mL). The combined organic extracts were dried and concentrated in vacuo. The resulting red solid (204 mg) was purified by flash chromatography, eluting with ethyl acetate:hexane (3:7) to afford *2-bromo-3-(4′-toluenesulfonyloxy)-6,7-dimethoxy-1,4-naphthoquinone* ***14*** (175 mg, 96%), mp 220–221 °C. ν_{max} 1672 (s), 1572 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 2.51 (3H, s, $PhCH_3$), 4.02, 4.04 (3H, 3H, s, s, 2 × OCH_3), 7.42 (2H, d, *J* 8, H2′, H6′), 7.53, 7.57 (2H, s, s, H5, H8), 7.98 (2H, d, *J* 8, H3′, H5′). ESI-MS (positive, 30 V) 491 (M[^{81}Br]+Na, 96%), 489 (M[^{79}Br]+Na, 100), 469 (M[^{81}Br]+H, 35), 467 (M[^{79}Br]+H, 33), 143 (42), 135 (65). Calcd for $C_{19}H_{16}{}^{79}BrO_7S^+$ 466.9795; found 466.9814.

3.2.12. 2-Hydroxy-3-(3′-(2″-hydroxy-6″,7″-dimethoxy-1″,4″-dioxo-dihydronaphthalene-3″-yl)-1′,4′-dioxo-6′,7′-dimethoxy-naphthalene-2′-yl)-6,7-dimethoxy-1,4-naphthoquinone 13

A stirred mixture of 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone[19] **15** (80 mg, 0.35 mmol), 2-bromo-3-(4′-toluenesulfonyloxy)-6,7-dimethoxy-1,4-naphthoquinone **14** (75 mg, 0.16 mmol) and diisopropylethylamine (280 μL, 1.6 mmol) in dry 1,2-dimethoxyethane (10 mL) was heated under reflux for 19 h. A second portion of 2-hydroxy-6,7-dimethoxy-1,4-naphthoquinone **15** (80 mg, 0.35 mmol) and diisopropylethylamine (1.0 mL, 5.7 mmol) were then added. After a further 77 h heating under reflux, the mixture was cooled to room temperature and acidified using hydrochloric acid (1 M). The mixture was extracted with ethyl acetate (15 mL) and dichloromethane (15 mL), the extracts combined, dried and evaporated. The resulting brown residue (350 mg) was

dissolved in ethyl acetate (100 mL) and extracted with aqueous sodium bicarbonate. The combined extracts were acidified with concentrated hydrochloric acid and extracted with ethyl acetate (10 mL). The organic phase was dried and concentrated in vacuo and the resulting brown solid (100 mg) was subjected to flash chromatography, eluting with ethyl acetate:acetic acid:hexane (50:1:49) followed by methanol:acetic acid:dichloromethane (10:1:89). Evaporation of the fractions from the final elution afforded a brown solid (16 mg) which was dissolved in ethyl acetate:aqueous formic acid (90%) (9:1) and filtered through Celite®. Removal of the volatiles in vacuo afforded a brown solid (7.6 mg) which was subjected to preparative HPLC (gradient 50% A, 50% B to 100% A over 90 min, 5 mL/min). Fractions that had a retention time of 10.2 min by analytical HPLC were combined to give a yellow-orange solid of *2-hydroxy-3-(3′-(2″-hydroxy-6″,7″-dimethoxy-1″,4″-dioxo-dihydronaphthalene-3″-yl)-1′,4′-dioxo-6′,7′-dimethoxy-naphthalene-2′-yl)-6,7-dimethoxy-1,4-naphthoquinone* ***13*** (4.3 mg, 4%), mp 175 °C. λ_{max} (log ε) 273, 320 nm (4.57, 4.13). ν_{max} 1664 (m), 1578 (s) cm^{-1}. δ (^1H) (300 MHz, $CDCl_3$) 3.97, 3.99, 4.00, 4.03 (all s, 6 × OCH_3 (isomers a and b)), 7.42 (2H, br s, 2 × OH), 7.47 (2H, s, H5′, H8′), 7.55 (4H, s, H5, H5″, H8, H8″ (isomer b)), 7.59, 7.61 (s, s, H5, H5″, H8 and H8″ (isomer a) [isomers a:b; ~2:1]. ESI-MS (positive, 30 V) 683 (M+H, 10%), 391 (40), 279 (55). HR-ESI-MS (positive) Calcd for $C_{36}H_{27}O_{14}^+$ 683.1395; found 683.1395.

3.2.13. 8-Bromo-9-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 20

Bromine (2.1 g, 13.1 mmol) in dry dichloromethane (20 mL). was added dropwise to a cooled (0 °C) solution of 9-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-7,10-dione[16] **19** (3.00 g, 11.6 mmol) in dichloromethane (50 mL) containing 10 drops of glacial acetic acid. The cooling bath was removed and the mixture was stirred at room temperature for 20 min after which the solvent was evaporated in vacuo to afford, as a bright orange solid, *8-bromo-9-hydroxy-3,3-dimethyl-1,2-dihydro-3H-naphtho[2,1-b]pyran-7,10-dione* ***20*** (3.86 g, 99%), mp 201–204 °C. ν_{max} 3328 (br m), 1657 (s), 1642 (s), 1568 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.38 (6H, s, C–CH_3), 1.89 (2H, t, *J* 7, H2), 3.27 (2H, t, *J* 7, H1), 7.12 (1H, d, *J* 8.5, H5), 7.88 (1H, br s, OH), 8.05 (1H, d, *J* 8.5, H6). ESI-MS (positive, 30 V) 377 (M[^{81}Br]+K, 13%), 375 (M[^{79}Br]+K, 13), 356 (M[^{81}Br]+NH_4, 23), 354 (M[^{79}Br]+NH_4, 25), 339 (M[^{81}Br]+H, 100), 337 (M[^{79}Br]+H, 97), 219 (27), 215 (27), 159 (25), 64 (30). Calcd for $C_{15}H_{13}BrO_4$: C, 53.5; H, 3.9; found: C, 53.4; H, 3.9.

3.2.14. 8-Bromo-3,3-dimethyl-9-(4-toluenesulfonyloxy)-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 18

A solution of 8-bromo-9-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **20** (583 mg, 1.71 mmol) in pyridine (4 mL), diisopropylethylamine (4 mL) and dichloromethane (4 mL) was cooled to 0 °C and 4-toluenesulfonyl chloride (350 mg, 1.84 mmol) added. After stirring for 10 min the solution was added to aqueous hydrochloric acid (2 M) and extracted with ethyl acetate. The extract was evaporated and the resulting residue subjected to flash chromatography (10% ethyl acetate in hexanes) to afford *8-bromo-3,3-dimethyl-9-(4-toluenesulfonyloxy)-1,2-dihydro-3H-naphtho[2,1-b]pyran-7,10-dione* ***18*** (709 mg, 84%), mp 168–169.5 °C. ν_{max} 1680 (s), 1668 (m), 1620 (m), 1578 (w), 1566 (w) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.37 (6H, s, C–CH_3), 1.86 (2H, t, *J* 7, H2), 2.49 (3H, s, ArCH_3), 3.27 (2H, t, *J* 7, H1), 7.10 (1H, d, *J* 8.5, H5), 7.40 (2H, d, *J* 8.5, H3′), 7.97 (2H, d, *J* 8.5, 2 × H2′), 8.02 (1H, d, *J* 8.5, H6). ESI-MS (positive, 70 V) 531 (M[^{81}Br]+K, 16%), 529 (M[^{79}Br]+K, 13), 515 (M[^{81}Br]+Na, 17%), 513 (M[^{79}Br]+Na, 17), 493 (M[^{81}Br]+H, 36%), 491 (M[^{79}Br]+H, 35), 337 (25), 336 (100), 155 (42), 149 (23), 91 (39). Calcd for $C_{21}H_{21}BrO_6S$: C, 53.8; H, 3.9; found C, 53.6; H, 3.8.

3.2.15. 8-(8′-Bromo-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′*H*-naphtho[2,1-*b*]pyran-9′-yl)-9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 21

Diisopropylethylamine (2.0 mL, 11.5 mmol) to a solution of 8-bromo-3,3-dimethyl-9-*p*-toluenesulfonyloxy-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **20** (1.10 g, 2.23 mmol) and 9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione[16] **6** (0.575 g, 2.24 mmol) in 1,2-dimethoxyethane (25 mL) under nitrogen and the resulting mixture was heated under reflux overnight. The reaction mixture was cooled, poured into hydrochloric acid (1 M) then ethyl acetate was added and the organic phase was separated. This was washed with aqueous sodium hydrogen carbonate solution (5%), water then dried and the volatiles were removed in vacuo. Flash chromatography (15–50% ethyl acetate in hexanes with 1% acetic acid) of the resulting residue (1.27 g) gave a brown solid which was triturated with ethyl acetate/hexanes to give 8-(8′-bromo-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′*H*-naphtho[2,1-*b*]pyran-9′-yl)-9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **21** (0.64 g, 50%) as an orange solid, mp 285–295 °C (decomp.). ν_{max} 3300 (w), 1664 (s), 1632 (m), 1580 (m), 1562 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.35, 1.37 (3H, 3H, s, s, C′-CH_3), 1.50 (6H, s, C–CH_3), 1.83 (2H, t, *J* 7, H2′), 3.21 (2H, t, *J* 7, H1′), 6.03 (1H, d, *J* 10.5, H2), 7.10, 7.15 (1H, 1H, d, d, *J* 8.5, H5, H5′), 7.80 (1H, d, *J* 10.5, H1), 8.03, 8.07 (1H, 1H, d, d, *J* 8.5, H6, H6′). FAB-MS (3NBA) 578 (M[^{81}Br]+H+1, 12%), 577 (M[^{81}Br]+H, 17), 576 (M[^{79}Br]+H+1, 13), 575 (M[^{79}Br]+H, 14), 497 (23), 496 (46), 495 (39), 481 (26), 213 (38), 187 (35), 165 (82). Calcd for $C_{30}H_{23}BrO_7$: C, 62.6; H, 4.0; found: C, 62.6; H, 4.0.

Evaporation of the trituration filtrate gave more of the dimer **21** (0.145 g, 10%) which was of comparable purity to that of the precipitated material. Acidification of the alkaline wash and subsequent extraction with ethyl acetate afforded 8-bromo-9-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **19** (0.12 g, 16%).

3.2.16. 9-Hydroxy-8-(8′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″*H*-naphtho[2,1-*b*]pyran-8″-yl)-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′*H*-naphtho[2,1-*b*]pyran-9′-yl)-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 17

Diisopropylethylamine (464 μL, 2.66 mmol) was added to a solution of 8-(8′-bromo-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′*H*-naphtho[2,1-*b*]pyran-9′-yl)-9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **21** (620 mg, 1.08 mmol) and 9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione[16] **6** (1.12 g, 4.37 mmol) in 1,2-dimethoxyethane (35 mL) and the resulting mixture was refluxed under nitrogen. The reaction was monitored by HPLC until the ratio of the trimers (rt = 10.0 min, gradient) to dimer (rt = 11.5 min, gradient) was ~5 (6–12 h). When the reaction had reached this stage the mixture was cooled, and then aqueous hydrochloric acid (1 M) and ethyl acetate were added. The organic phase was separated, dried and the volatiles were removed in vacuo. Flash chromatography (25% ethyl acetate in hexanes containing 1% acetic acid) of the resulting residue (1.80 g) gave a mixture of starting materials (0.99 g), bromodimer **21** and monomer **6** in the ratio of 9:2 from which monomer **6** can be isolated by extraction with 5% $NaHCO_3$. Further elution of the flash column using 9:1 dichloromethane:methanol (with 1% acetic acid) afforded a red solid which was dissolved in ethyl acetate containing 10% of a 90% aqueous formic acid solution and filtered through a plug (or short column) of Celite® to give a dark orange solid (644 mg) which was 81% trimers by HPLC. Further elution of the flash column with 1% acetic acid in methanol gave a semi-solid which was dissolved in ethyl acetate containing 10% of a 90% formic acid solution and filtered through a plug of Celite® to give a pale orange solid (194 mg) which was 73% trimers by HPLC.

Combining the material obtained as described above from five different reactions that used a total of (2.76 g, 4.80 mmol) of 8-(8′-bromo-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′*H*-naphtho[2,1-*b*]pyran-9′-yl)-9-hydroxy-3,3-dimethyl-3*H*-naphtho-[2,1-*b*]pyran-7,10-dione **17** afforded, after preparative HPLC purification [70% A, 30% B, (analytical retention time 17.8 min)], and a final precipitation by dissolving in a small amount of ethyl acetate and added dropwise to a large volume of hexanes, *9-hydroxy-8-(8′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″H-naphtho[2,1-b]pyran-8″-yl)-3′,3′-dimethyl-7′,10′-dioxo-1′,2′,7′,10′-tetrahydro-3′H-naphtho[2,1-b]pyran-9′-yl)-3,3-dimethyl-3H-naphtho[2,1-b]pyran-7,10-dione* ***17*** (1.008 g, 28%) as a pale orange solid, mp 184–187 °C (decomp.). λ_{max} (log ε) 218, 275, 295 sh, 389 nm (5.17, 4.95, 4.85, 4.52). ν_{max} 3388 (br m), 1658 (s), 1648 (s), 1566 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 1.37, 1.39, 1.46, 1.48, 1.52 (18H, all s, C–CH_3), 1.60 (2H, br s, OH), 1.78–1.90 (2H, m, H2′), 3.28 (2H, t, *J* 7, H1′), 5.97, 5.98 (1H, 1H, d, d, *J* 10.5, H2, H2″), 7.08–7.10, 7.11 and 7.13 (3H, m, d, d, *J* 6.5, H5, H5′, H5″ (isomers b and c) and H5, H5′, H5″ (isomer a)), 7.43–7.68, 7.69 and 7.71 (2H, m, d, d, *J* 10.5, H1, H1″ (isomers b and c) and H1, H1″ (isomer a)), 7.91–8.00, 8.03 (3H, m, app d, H6, H6′, H6″ (isomers b and c) and H6, H6′, H6″ (isomer a)). ESI-MS (positive, 30 V) 753 (M+H+2, 22%), 752 (M+H+1, 53), 751 (M+H, 100), 397 (19), 381 (24). Calcd for $C_{45}H_{34}O_{11}$: C, 72.0; H, 4.6; found C, 71.7; H, 4.9%.

3.2.17. 9-Hydroxy-8-(4′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″*H*-naphtho[2,1-*b*]pyran-8″-yl)1-benzyl-1*H*-pyrrole-2,5-dione)-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione 23

A mixture of 1-benzyl-1*H*-pyrrole-2,5-dione[23] **22** (125 mg, 0.49 mmol), 9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione[16] **6** (250 mg, 0.98 mmol) and diisopropylethylamine (850 μL, 4.9 mmol) in 1,2-dimethoxyethane (10 mL) was heated under reflux for 7.5 h. The mixture was then cooled to room temperature, acidified with hydrochloric acid (1 M) and extracted with ethyl acetate. Evaporation of the extracts in vacuo to gave a red solid (376 mg). Analytical HPLC indicated one product in equal proportions with monomer **6**, suggesting incomplete reaction. A sample of this solid (308 mg), 9-hydroxy-3,3-dimethyl-3*H*-naphtho[2,1-*b*]pyran-7,10-dione **6** (323 mg, 1.68 mmol) and diisopropylethylamine (185 μL, 1.06 mmol) in 1,2-dimethoxyethane (10 mL) was heated under reflux for 56 h. The mixture was then worked up as before to give a red solid (620 mg) which was subjected to flash chromatography in 30–99% ethyl acetate in hexanes (containing 1% acetic acid) followed by 10% methanol in dichloromethane (containing 1% acetic acid). Evaporation of the fractions from the final elution afforded a semi-solid which was subjected to preparative HPLC (isocratic 65% A, 35% B, 4.5 mL/min). Fractions that had a retention time of 7.3 min by analytical HPLC (gradient) were combined to give *9-hydroxy-8-(4′-(9″-hydroxy-3″,3″-dimethyl-7″,10″-dioxo-7″,10″-dihydro-3″H-naphtho[2,1-b]pyran-8″-yl)1-benzyl-1H-pyrrole-2,5-dione)-3,3-dimethyl-3H-naphtho[2,1-b]pyran-7,10-dione* **23** (66 mg, 19%), mp 165–170 °C. ν_{max} 3346 (br m), 1713 (s), 1655 (s), 1560 (m) cm^{-1}. δ (^1H) (300 MHz, $CDCl_3$) 1.47 (6H, s, 2 × $(CH_3)_2$), 4.79 (2H, s, NCH_2), 5.98 (4H, d, *J* 10.5, H2 and H2″), 7.08 (2H, d, *J* 8.5, H5 and H5″), 7.28–7.47 (5H, m, C_6H_5), 7.71 (2H, d, *J* 10.5, H1 and H1″), 7.91 (2H, br s, 2 × OH), 7.95 (2H, d, *J* 8.5, H6 and H6″). ESI-MS (positive, 70 V) 734 (M+K, 22%), 719 (M+Na+1, 46), 718 (M+Na, 100), 696 (M+H, 45), 175 (45), 131 (49), 120 (36), 91 (27). Calcd for $C_{41}H_{29}NO_{10}$: C, 70.8; H, 4.2; N, 2.0; found C, 70.7; H, 4.1; N, 1.8. HR-ESI-MS (positive) Calcd for $C_{41}H_{30}O_{10}^+$ 696.1869; found 696.1894.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.105.

References and notes

1. Dickinson, L.; Khoo, S.; Back, D. *Antiviral Res.* **2010**, *85*, 176.
2. De Clerk, E. *Rev. Med. Virol.* **2009**, *19*, 287.
3. Emery, S.; Winston, A. *Lancet* **2009**, *374*, 764.
4. McColl, D. J.; Chen, X. *Antiviral Res.* **2010**, *85*, 101.
5. Neamati, N.; Turpin, J. A.; Winslow, H. E.; Christensen, J. L.; Williamson, K.; Orr, A.; Rice, W. G.; Pommier, Y.; Garofalo, A.; Brizzi, A.; Campiani, G.; Fiorini, I.; Nacci, V. *J. Med. Chem.* **1999**, *42*, 3334.
6. Deng, J.; Dayam, R.; Al-Mawsawi, L. Q.; Neamati, N. *Curr. Pharm. Des.* **2007**, *13*, 129.
7. Chen, I.-J.; Nouri Neamati, N.; Nicklaus, M. C.; Ann Orrb, A.; Andersonb, L.; Barchi, J. J.; Kelleyb, J. A.; Pommier, Y.; MacKerell, A. D. *Bioorg. Med. Chem.* **2000**, *8*, 2385.
8. Hare, S.; Gupta, S. S.; Valkov, E.; Engelman, A.; Cherepanov, P. *Nature* **2010**, *464*, 232.
9. Singh, S. B.; Zink, D. L.; Heimbach, B.; Genilloud, O.; Teran, A.; Silverman, K. C.; Lingham, R. B.; Peter Felock, P.; Hazuda, D. J. *Org. Lett.* **2002**, *4*, 1123.
10. Yang, S. S.; Cragg, G. M.; Newman, D. J.; Bader, J. P. *J. Nat. Prod.* **2001**, *64*, 265.
11. Ovenden, S. P. B.; Yu, J.; Wan, S. S.; Sberna, G.; Tait, R. M.; Rhodes, D.; Cox, S.; Coates, J.; Walsh, N. G.; Meurer-Grimes, B. M. *Phytochemistry* **2004**, *65*, 3255.
12. Ganesan, A. *Curr. Opin. Chem. Biol.* **2008**, *12*, 1.
13. Decosterd, L. A.; Parsons, I. C.; Gustafson, K. R.; Cardellina, J. H., II; McMahon, J. B.; Cragg, G. M.; Murata, Y.; Pannell, L. K.; Steiner, J. R.; Clardy, J.; Boyd, M. R. *J. Am. Chem. Soc.* **1993**, *115*, 6673.
14. Kearney, M.; Pallansch, L.; Cox, S.; Coates, J.; Buckheit, R. W. J. *Antivir. Ther.* **2000**, *6*, 11.
15. Stagliano, K. W.; Emadi, A.; Lu, Z.; Malinakova, H. C.; Twenter, B.; Yu, M.; Holland, L. E.; Rom, A. M.; Harwood, J. S.; Amin, R.; Johnson, A. A.; Pommier, Y. *Bioorg. Med. Chem.* **2006**, *14*, 5651.
16. Crosby, I. T.; Rose, M. L.; Collis, M. P.; de Bruyn, P. J.; Keep, P. L. C.; Robertson, A. D. *Aust. J. Chem.* **2008**, *61*, 768.
17. Baillie, A. C.; Thomson, R. H. *J. Chem. Soc. (C)* **1966**, 2184.
18. Kasturi, T. R.; Arunachalam, T. *Can. J. Chem.* **1966**, *44*, 1086.
19. Bently, H. R.; Dawson, W.; Pring, F. S. *J. Chem. Soc.* **1952**, 1763.
20. Cameron, D. W.; Feutrill, G. I.; Keep, P. L. C. *Tetrahedron Lett.* **1989**, *30*, 5173.
21. Cooke, R. G.; Segal, R. G. *Aust. J. Sci. Res. Ser. A* **1950**, *3*, 628.
22. Boyd, M. R.; Cardelina, J. H.; Gustafson, K. R.; Decosterd, L. A.; Parsons, I.; Pannell, L.; McMahon, J. B.; Cragg, G. M. WO9417055, 1994.
23. Reddy, P. Y.; Kondo, S.; Toru, T.; Ueno, Y. *J. Org. Chem.* **1997**, *62*, 2652.