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Bioorganic & Medicinal Chemistry 14 (2006) 5651–5665
Bioorganic & Medicinal Chemistry

Regiocontrolled synthesis and HIV inhibitory activity of unsymmetrical binaphthoquinone and trimeric naphthoquinone derivatives of conocurvone

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Received 24 February 2006; revised 10 April 2006; accepted 13 April 2006
Available online 5 June 2006

Abstract—Unsymmetrical biquinone and trimeric quinone derivatives were synthesized using halotriflate-biselectrophilic naphthoquinones through stepwise regioselective quinone substitution chemistry and evaluated for their ability to inhibit the cytopathogenic effects of HIV-1 using an MTT colorimetric assay. Compounds were also screened for their ability to inhibit the activity of HIV-1 integrase in vitro. Pyranylated trimeric quinones and biquinones exhibited both antiviral activity and integrase inhibitory activity. Conocurvone **1** and trimeric quinone **21** were the most potent HIV integrase inhibitors in the series. All of the biquinones showed HIV inhibitory activity. Simple methoxy substituted biquinones did not inhibit HIV-1 integrase.
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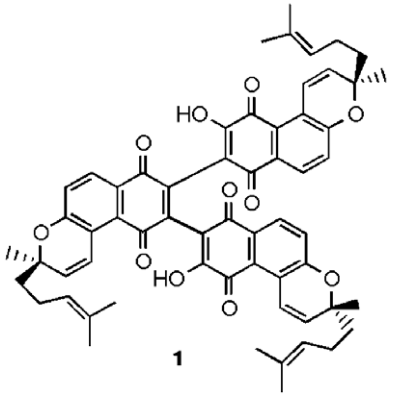
1. Introduction

Biquinones and higher quinone oligomers are a unique group of natural products, which possess a diverse array of biological activities.[1] Their structures are based on two or more quinone units linked together at the quinone double bond. In almost all cases they possess an element of symmetry due to their biosynthetic mechanism of origin, which probably involves oxidative coupling of a common naphthol intermediate in the key step of the oligomerization process.[2] One intriguing member of this class is conocurvone **1** isolated from the Western Australian smoke bush.[3] Conocurvone was shown to inhibit the cytopathogenic effects of HIV-1 in human T-lymphoblastic cells over a broad concentration range (ID_{50} = 0.02 μM; TD_{50} = 50 μM).[3,4] More recently, it was suggested that conocurvone **1** may be a dual inhibitor of both HIV integrase and HIV mediated cell fusion.[5,6]



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Over the past decade, extensive efforts have been made resulting in the discovery of a large number of molecules that can inhibit replication of HIV.[7] An essential step in the HIV life cycle is integration of the viral DNA into

Keywords: Biquinones and trimeric quinones; HIV-1 integrase inhibitor; Regiocontrolled synthesis; Conocurvone.
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0968-0896/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmc.2006.04.034

the host cell genome. The step is catalyzed by the viral enzyme, HIV integrase, which is absolutely required for productive infection[8] and therefore, inhibition of integrase can halt the viral life cycle (for a recent review, see Ref. 9b).[9,10] Integrase catalyzes two separate steps known as 3′-processing (3′-P) and DNA strand transfer (ST). In 3′-processing, integrase removes a dinucleotide next to a conserved cytosine-adenine sequence from each 3′-end of the viral DNA.[9,10] Integrase then attaches the processed 3′-end of the viral DNA to the host cell DNA in the strand transfer reaction.[9–12]

An important result of the structural and biochemical studies on integrase has been the development of practical assays used to identify novel HIV integrase inhibitors.[9,13] These HIV inhibitors not only represent potential chemotherapeutic lead compounds,[7] but as a collection, they are also useful in databases for pharmacophore searching.[14] The most promising inhibitors are proposed to bind to the active site of the integrase enzyme and chelate important metal co-factors such as Mn^{2+}or Mg^{2+}.[9,10]

The ability of hydroxyquinones to bind metal cations[15] and the inhibition of HIV replication by conocurvone[3,4] prompted us to explore whether conocurvone **1** inhibits HIV-1 integrase in vitro. A series of synthetic trimeric quinone and biquinone analogs were also tested for integrase inhibition. In addition, the compounds were evaluated for their ability to inhibit the cytopathogenic effects of HIV-1 using an MTT colorimetric assay.[16]

2. Results and discussion

2.1. Chemistry

Trimeric quinones **21**–**23** (Table 1) were prepared from the corresponding methoxychlorobiquinones as previously described.[17] Pyranylated biquinones **11** and **19** (Table 2) and halohydroxybiquinones (**15a**–**d** and **6b**, Table 3) which lack substituents on the haloquinone unit were prepared in one step by substitution of a halogen in 2,3-dichloro- or 2,3-dibromonaphthoquinone by a hydroxyquinone anion.[17] Synthesis of biquinones (**6e**,**6h**, Table 3) with substituents on the aromatic ring of the haloquinone unit was achieved by regioselective substitution of the triflate group in chlorohydroxyquinone triflates **4e** and **4h** (Scheme 1).

The quinone triflates were prepared starting from readily available quinone 1,4-dipoles, Scheme 1.[18] Reaction with either trimethyl silyl halides or the corresponding hydrohalic acids yielded halohydroxynaphthoquinones **3** in good yields. The chlorohydroxy- and bromohydroxyquinones are most efficiently prepared by reaction of the dipoles **2** with excess HCl or HBr as previously reported.[18] However, reaction of the dipole **2** with excess HI yielded a mixture of 2-hydroxy-3-iodonaphthoquinone **3d** and the corresponding 2-hydroxynaphthoquinone **3a**, which was difficult to separate.[18] Mechanistic studies revealed that HI was functioning as a quinone dehalogenating agent.[19] For example, reaction of the dipole **2a** with equimolar amounts of HI yielded **3d** as a virtually pure product. Furthermore, when the iodohydroxyquinone **3d** was stirred with excess HI in methylene chloride at room temperature a quantitative yield of 2-hydroxynaphthoquinone was obtained. Similar dehalogenations were observed during reaction of **3b** or **3c** with excess HI.

Initial screening of reaction conditions for the triflation of hydroxyquinones was first carried out using lawsone **3a** as a model. Reaction of **3a** with triflic anhydride provided the known[20] *para*-hydroxynaphthoquinone triflate **4a** in 44% yield along with a brilliant red side product which readily decomposed in solution but whose spectral characteristics were consistent with that of the *ortho*-quinone isomer. Triflate **4a** also proved to be unstable requiring storage at −20 °C under nitrogen. In contrast, reaction of halohydroxyquinones **3b**–**i** with 1.2 equiv of triflic anhydride provided only a single regioisomer. Apparently the presence of the halogen atom on the quinone core exerted a strong regiochemical directing effect on the triflation reaction.[21] The robust triflates crystallized as brilliant yellow needles and were air stable at room temperature.

With the triflates in hand, our next objective was to determine if the triflate group would substitute preferentially in the presence of different types of halogens. In addition, the regiochemical effect of electron donating

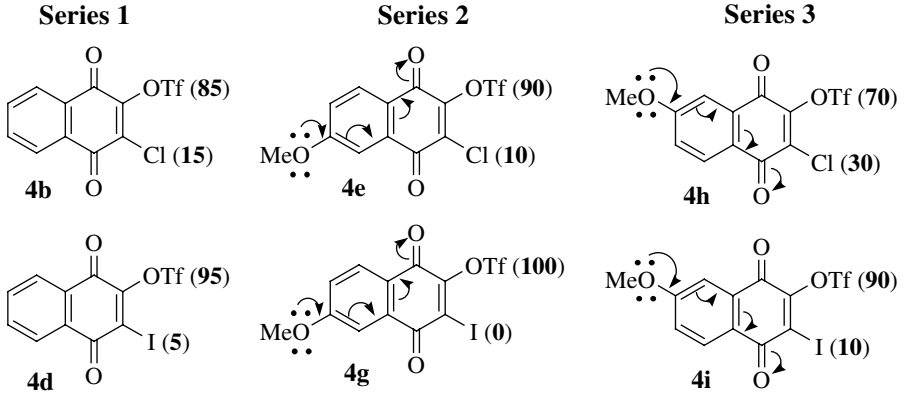
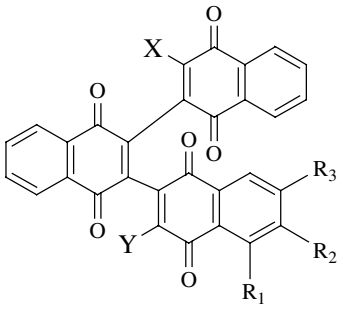
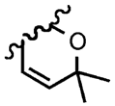


Figure 1. Regioisomeric ratios of triflate versus halogen substitution during biquinone formation as determined by HPLC.

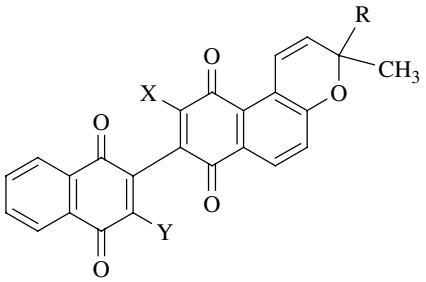
Table 1. Anti-HIV activity of trimeric naphthoquinone derivatives



Compound	X	Y	R_1	R_2	R_3	Integrase inhibition[b] $MnCl_2$		Integrase inhibition[b] $MgCl_2$		MTT assay[a]	
						3′-Processing (μM)	Strand transfer (μM)	3′-Processing (μM)	Strand transfer (μM)	ID_{50} (μM)	TD_{50} (μM)
21[4,17]	OH	OH	H	H	H	22.9 ± 0.2	8.9 ± 4.3	35.5 ± 9.2	29.5 ± 3.5	11 ± 2[c]	28 ± 1[c]
22[d]	OH	OMe	H	H	H	63.9 ± 10.9	55.9 ± 12.2	49.5 ± 4.9	38.0 ± 7.1	43 ± 3[b]	>100[b]
23[d]	OMe	OH	H	H	OMe	59.1 ± 3.5	58.3	45.0 ± 7.1	41.0 ± 5.7	NA[b]	19 ± 1[b]
14	OH	OMe			H	72.2 ± 7.4	72.2 ± 7.4	37.7 ± 4.1	24.3 ± 3.1	6.7 ± 1.2[c]	14 ± 2[c]
1	Conocurvone					>1000[e]	>1000[e]	1.3 ± 0.4	1.4 ± 0.4	0.06 ± 0.00[c]	22 ± 1[c]

[a] MTT assays were conducted as described in Ref. 16.
[b] Values are expressed as means ± SEM of three independent assays.
[c] Values are expressed as means ± SEM of two independent assays.
[d] See Ref. 17.
[e] Conocurvone failed to inhibit integrase in the presence of Mn^{2+} for three out of four integrase preparations. The inhibition observed for the fourth preparation was 10-fold weaker than inhibition in the presence of Mg^{2+}. NA, not active.

Table 2. Anti-HIV activity of pyranylated binaphthoquinone derivatives



$R_1 = -CH_2CH_2CH=C(CH_3)_2$
$R_2 = -CH_3$

Compound	X	Y	R	Integrase inhibition[b] $MnCl_2$		Integrase inhibition[b] $MgCl_2$		MTT assay[a]	
				3′-Processing (μM)	Strand transfer (μM)	3′-Processing (μM)	Strand transfer (μM)	ID_{50} (μM)	TD_{50} (μM)
19[d]	OH	Cl	R_1	39.1 ± 8.2	20.7 ± 1.3	29.5 ± 3.5	20.5 ± 0.7	0.4 ± 0.1[b]	2.7 ± 0.1[b]
8b	OH	I	R_1	60.2 ± 0.2	44.3 ± 3.3	43.5 ± 7.8	29.5 ± 0.7	1.7 ± 0.3[c]	4.3 ± 0.8[c]
8a	OH	I	R_2	22.6 ± 5.1	23 ± 8.5	30.9 ± 3.5	20.9 ± 3.5	2.0 ± 0.3[c]	6.8 ± 0.6[c]
11	OH	Cl	R_2	75.7 ± 26.8	41.9 ± 6.7	49.5 ± 8.0	36.3 ± 9.6	2.8 ± 0.4[c]	5.5 ± 0.1[c]
20[d]	OMe	Cl	R_1	>1000	>1000	>1000	>1000	2.3 ± 0.5[b]	5.4 ± 0.2[c]
9	OMe	I	R_2	>1000	>1000	>1000	>1000	2.7 ± 0.3[c]	6.3 ± 0.3[c]
12	OMe	Cl	R_2	[e]	[e]	[e]	[e]	1.8 ± 0.9[c]	3.5 ± 1.9[c]

[a] MTT assays were conducted as described in Ref. 16.
[b] Values are expressed as means ± SEM of three independent assays.
[c] Values are expressed as means ± SEM of three two independent assays.
[d] See Ref. 17.
[e] Not tested.

methoxy groups present on the aromatic ring of the naphthoquinone was also probed. Reaction of the unsubstituted chlorotriflate **4b** with the hydroxyquinone anion **5** yielded biquinone **6b** as the major product (Scheme 1). Similar results were obtained with the iodotriflate **4d** to yield **6d**. In both cases the triflate group underwent regioselective substitution by the hydroxyquinone anion. The approximate regioisomeric ratios (shown in parentheses, Fig. 1) revealed that the nature of the halogen influenced the extent of triflate substitution. For example, when X = I slightly higher regioselectivity was observed compared to when X = Cl.

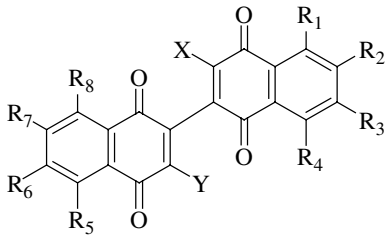
The effect of methoxy group position on regioselectivity was then studied (series 2 and 3, Fig. 1). Reaction of the methoxy substituted chlorotriflate **4h** and iodotriflate **4i** with hydroxyquinone **3a** yielded biquinones **6h** and **6i** as major products. In both cases the reactions proceeded by selective substitution of the triflate group albeit with decreased regioselectivity compared to the corresponding unsubstituted derivatives **4b** and **4d**. Interestingly, the extent of triflate substitution increased in reactions involving **4e** and **4g** demonstrating that the position of the methoxy group does play an important role in controlling regiochemistry. The highest regioselectivity was achieved with iodotriflate **4g** substituted with electron donating methoxy groups at positions where their unpaired electrons could not participate in π-donation to the carbonyl group involved in the conjugate addition step during triflate substitution. All of the biquinones **6b–i** were easily purified from the minor regioisomer by flash chromatography on oxalic acid coated silica gel.[22] Repeated attempts to isolate and characterize the minor regioisomer failed due to facile decomposition.

Pyranylated iodohydroxybiquinones **8** were prepared using the procedure described above with regioisomeric ratios (shown in brackets, Scheme 2). The hydroxybiquinones were methylated with trimethyl oxonium tetrafluoroborate providing biquinone methyl ethers **16a–e** (Table 3)[17] and **9**, **12**, and **20** (Table 2). The hydroxybiquinones were also converted to dichlorobiquinones (**17a–e**) on treatment with oxalyl chloride.[23] Regioselective amination of dichlorobiquinones yielded aminochlorobiquinones **18a–c** and diaminobiquinone **18d**.[23] The unreported pyranylated trimeric quinone **14** (Table 3) was prepared as depicted in Scheme 3 by stepwise substitution of the halogens in dichlorobiquinone **10**.

2.2. Inhibition of HIV-1 integrase catalytic activities

The concentration dependent inhibition of HIV-1 integrase by the compounds presented was tested in an in vitro assay, as illustrated in Figure 2. IC_{50} values were determined for both 3′-processing and strand transfer in the presence of manganese or magnesium cations. All of the trimeric naphthoquinone derivatives shown in Table 1 inhibited integrase 3′-P and ST in the presence of either manganese or magnesium cations. Conocurvone **1**, the most potent derivative, was inhibitory against integrase only in the presence of magnesium (Figs. 2B–D). The best synthetic inhibitor of this study was compound **21**,[4] with an IC_{50} against integrase ST of 8.9 μM (Figs. 2E and F). Inhibition was retained with hydroxy and

Table 3. Anti-HIV activity of simple binaphthoquinone derivatives



Compound	X	Y	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	MTT Assay[a]	
											ID_{50} (μM)	TD_{50} (μM)
15a[e]	OH	Cl	H	H	H	H	H	H	H	H	2.6 ± 0.3	5.3 ± 0.2
15b[e]	OH	Br	H	H	H	H	H	H	H	H	2.3 ± 0.2	5.2 ± 0.4
6a	OH	I	H	H	H	H	H	H	H	H	1.8 ± 0.5	5.0 ± 0.6
15c[e]	OH	Cl	H	OMe	H	H	H	H	H	H	2.4 ± 0.4	5.3 ± 0.2
6b	OH	Cl	H	H	OMe	H	H	H	H	H	2.7 ± 0.2	5.5 ± 0.1
15d[f]	OH	Cl	H	H	H	OMe	H	H	H	H	NA[c]	>100[c]
6e[g]	OH	Cl	H	H	H	H	H	OMe	H	H	5.3[c]	12.0[c]
6h[g]	OH	Cl	H	H	H	H	H	H	OMe	H	6.2[c]	10.0[c]
15e[d,e]	OH	Br	H	H	H	H	H	OMe	H	H	2.0 ± 0.1	5.7 ± 0.2
6d	OH	I	H	H	OMe	H	H	H	H	H	3.7[c]	6.0[c]
6g	OH	I	H	H	H	H	H	OMe	H	H	3.6[c]	7.5[c]
6i	OH	I	H	H	H	H	H	H	OMe	H	4.2[c]	9.8[c]
16a[e]	OMe	Cl	H	H	H	H	H	H	H	H	1.7 ± 0.1[b]	2.8 ± 0.0
16b[e]	OMe	Br	H	H	H	H	H	H	H	H	1.8 ± 0.2[b]	5.4 ± 0.3[b]
16c[e]	OMe	Cl	H	OMe	H	H	H	H	H	H	1.2 ± 0.1[b]	2.9 ± 0.1
16d[e]	OMe	Cl	H	H	OMe	H	H	H	H	H	1.6 ± 0.2[b]	2.5 ± 0.2
16e[d,e]	OMe	Br	H	H	H	H	H	OMe	H	H	1.1[c]	2.8[c]
17a[e]	Cl	Cl	H	H	H	H	H	H	H	H	3.3 ± 0.0	5.4 ± 0.2[b]
17b[f]	Cl	Cl	H	OMe	H	H	H	H	H	H	7.1 ± 0.3[b]	12.0 ± 0.6[b]
17c[f]	Cl	Cl	H	H	OMe	H	H	H	H	H	3.4 ± 0.2	5.7 ± 0.0
17d[f]	Cl	Cl	H	H	H	OMe	H	H	H	H	6.6[c]	11.0[c]
17e[f]	Cl	Cl	H	H	H	OH	H	H	H	H	NA[c]	5.7[c]
18a[f]	Cl	NH_2	H	H	H	H	H	H	H	H	3.5 ± 0.3[b]	5.7 ± 0.0[b]
18b[f,g]	Cl	NH_2	H	OMe	H	H	H	H	H	H	2.2[c]	3.6[c]
18c[f]	Cl	NH_2	H	H	H	OMe	H	H	H	H	2.4[c]	5.7[c]
18d[f,g]	NH_2	NH_2	H	H	H	H	H	H	H	H	0.7 ± 0.0	7.9 ± 1.7

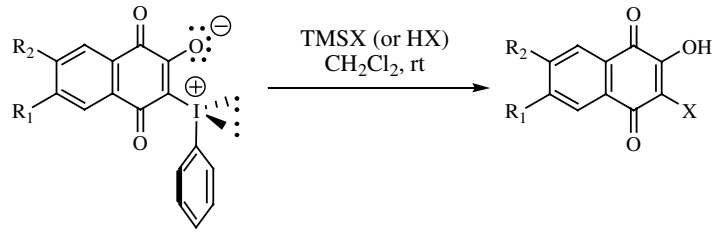
[a] MTT assays were conducted as described in Ref. 16. Values are expressed as the mean ± SEM of three independent assays.
[b] Two assays performed.
[c] One assay performed.
[d] 95:5 mixture of regioisomers.
[e] See Ref. 17.
[f] See Ref. 23. All of these compounds failed to inhibit HIV-1 integrase in vitro.
[g] Four compounds were not tested for integrase inhibition. NA, not active.

methoxy-substitutions at X and Y (compounds **22**, **23**, and **14**, Table 1). The effect on integrase inhibition when both X and Y are methoxy groups is unknown. Pyranylated biquinones inhibited HIV integrase (Table 2), in contrast to the lack of inhibition observed with simple biquinone derivatives (Table 3). The biquinones with X = OH (Table 2, compounds **19**, **8b**,**8a**, and **11**) moderately inhibited integrase 3′-P and ST, with IC_{50} values between 20 and 75 μM. Replacement of X = OH with a methoxy group resulted in a loss of inhibition (compounds **20** and **9**). The halogen (Y) and the size of the R substituents had little effect on integrase inhibition.

2.3. HIV inhibitory assay

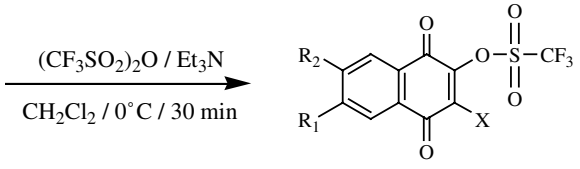
All of the compounds listed in Tables 1–3 were evaluated for in vitro anti-HIV activity in the CEM-T_4 cytoprotection assay.[16] Conocurvone **1** displayed antiviral over a broad concentration range in our assay. However, the simplified trimeric quinone **21**,[4] which lacked the pyran rings was less active and displayed increased cytotoxicity compared to conocurvone **1**. The monomethyl ether **22** was less active than the dihydroxy derivative **21**.[4] Incorporation of a methoxy group on the aromatic ring in **23** resulted in complete loss of in vitro anti-HIV activity as determined through repeated antiviral assays. Comparison of the activity of **21** and **22** to conocurvone **1** revealed significantly decreased activity. Fusion of a pyran ring to **22** yielded trimeric quinone **14** with only a slight increase in anti-HIV activity.

Comparison of the ID_{50} and TD_{50} values of hydroxybiquinones (Table 3) revealed no significant difference between the methoxy group substituted quinones **6b**,**d**,**e**,**g**–**i** and **15c**,**e**, unsubstituted derivatives **15a**,**b**

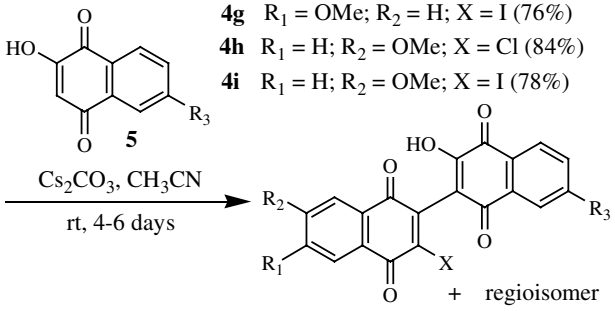


2a $R_1 = R_2 = H$ (88%)
2b $R_1 = OMe$; $R_2 = H$ (95%)
2c $R_1 = H$; $R_2 = OMe$ (72%)

3a $R_1 = R_2 = X = H$
3b $R_1 = R_2 = H$; X = Cl (76%)
3c $R_1 = R_2 = H$; X = Br (75%)
3d $R_1 = R_2 = H$; X = I (76%)
3e $R_1 = OMe$; $R_2 = H$; X = Cl (73%)
3f $R_1 = OMe$; $R_2 = H$; X = Br (78%)
3g $R_1 = OMe$; $R_2 = H$; X = I (79%)
3h $R_1 = H$; $R_2 = OMe$; X = Cl (93%)
3i $R_1 = H$; $R_2 = OMe$; X = I (73%)



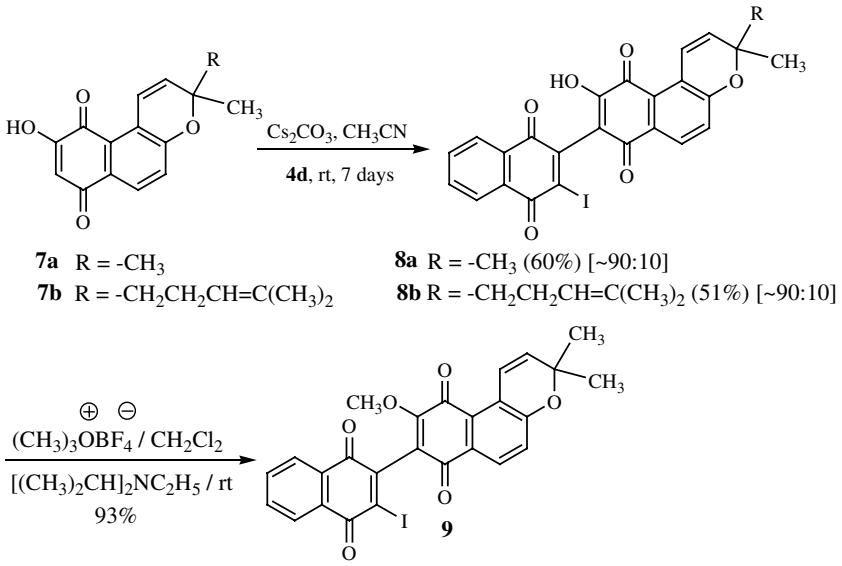
4a $R_1 = R_2 = X = H$ (44%)
4b $R_1 = R_2 = H$; X = Cl (85%)
4c $R_1 = R_2 = H$; X = Br (85%)
4d $R_1 = R_2 = H$; X = I (77%)
4e $R_1 = OMe$; $R_2 = H$; X = Cl (77%)
4f $R_1 = OMe$; $R_2 = H$; X = Br (71%)
4g $R_1 = OMe$; $R_2 = H$; X = I (76%)
4h $R_1 = H$; $R_2 = OMe$; X = Cl (84%)
4i $R_1 = H$; $R_2 = OMe$; X = I (78%)



6a $R_1 = R_2 = R_3 = H$; X = I (72%)
6b $R_1 = R_2 = H$; $R_3 = OMe$; X = Cl (56%)
6d $R_1 = R_2 = H$; $R_3 = OMe$; X = I (67%)
6e $R_2 = R_3 = H$; $R_1 = OMe$; X = Cl (67%)
6g $R_2 = R_3 = H$; $R_1 = OMe$; X = I (83%)
6h $R_1 = R_3 = H$; $R_2 = OMe$; X = Cl (47%)
6i $R_1 = R_3 = H$; $R_2 = OMe$; X = I (60%)

Scheme 1. Regiocontrolled synthesis of unsymmetrical binaphthoquinones.

and **6a**, biquinone methyl ethers **16a**–**e**, or dichlorobiquinones **17a**–**d**. Biquinones **15d** and **17e** which possessed a *peri*-methoxy or *peri*-hydroxy group on one of the quinone units lacked activity but possessed similar cytotoxicity to the other compounds. The diaminobiquinone **18d** was one of the most potent derivatives in the simple biquinone series. The presence of a fused pyran ring on the aromatic ring of biquinone **19** produced slightly improved anti-HIV activity ($ID_{50} = 0.4 \pm 0.1$ μM) by comparison to the unsubstituted derivative **15a** ($ID_{50} = 2.6 \pm 0.3$ μM, Tables 2 and 3). However, the incorporation of the pyran ring had little effect on the toxicity. The length of the aliphatic chain on the pyran ring in **19** (vs **11**), the nature of the halogen atom in **8a** and **11** and formation of the biquinone methyl ethers **9**, **12**, and **20** (Table 2) had little influence.



Scheme 2. Regiocontrolled synthesis of pyranylated iodobinaphthoquinones.

3. Conclusions

In conclusion, a series of unsymmetrical biquinone and trimeric quinone derivatives has been synthesized and evaluated for their ability to inhibit HIV-1 integrase and the cytopathogenic effects of HIV in cells. We have demonstrated that the triflate group undergoes regioselective substitution in halohydroxyquinone triflates to form unsymmetrical halohydroxybiquinones. The position of the methoxy group and the nature of the halogen atom influenced the regioselectivity. Conocurvone **1** displays potent HIV-inhibitory activity, while exhibiting low cytotoxicity in non-infected cells. Conocurvone also inhibits the HIV-1 integrase 3′-P and ST reactions in vitro with greater selectivity for the ST reactions. Another interesting characteristic of conocurvone **1** is its selectivity for integrase inhibition in the presence of magnesium. Prior studies suggested that the ability of a compound to inhibit integrase in the presence of magnesium correlates with antiviral activity (e.g., the diketo acids).[9,24] Hence integrase inhibition in the presence of magnesium, which is the case for conocurvone **1**, is a positive attribute of drugs with potential for therapeutic development.[9,24]

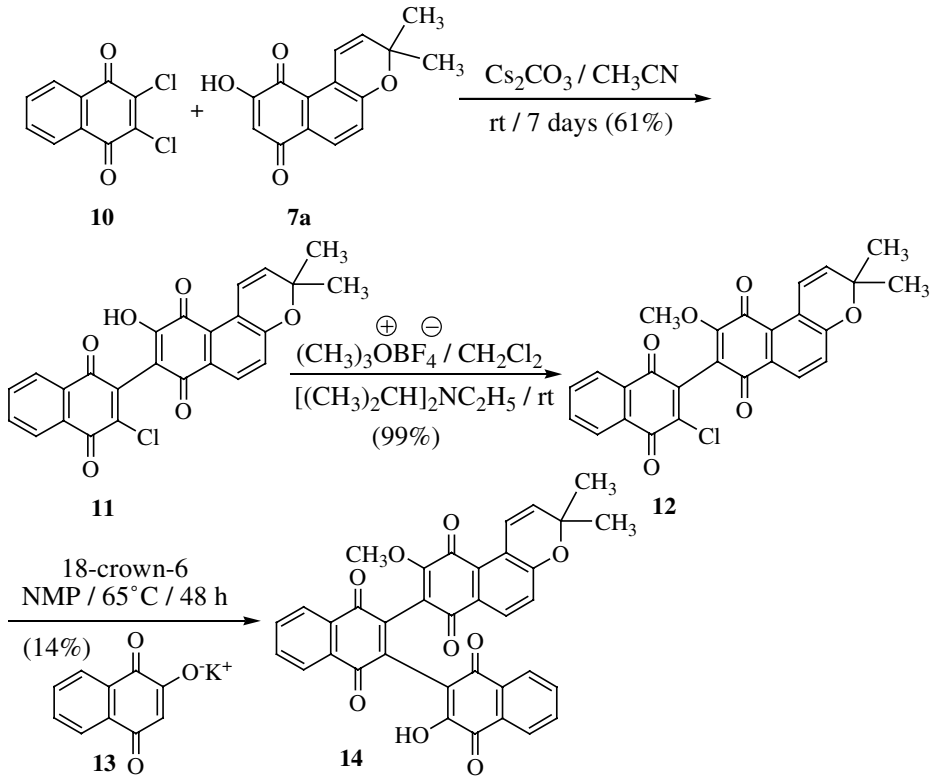
All of the synthetic trimeric quinones (Table 1) possessed HIV and integrase inhibitory activity but were less potent than conocurvone **1**. The simplest triquinone, compound **21**, retained anti-HIV and anti-integrase activities despite the lack of the pyran ring and pyranyl aliphatic chain present in conocurvone. While the full conocurvone structure is required for potent inhibition of HIV, the simple structure of compound **21** is sufficient for integrase inhibition.

In contrast to the trimeric quinones, the biquinones (Tables 2 and 3) require both a hydroxy (=X) and pyranyl group for anti-integrase and anti-HIV activity. The antiviral biquinone derivatives in Table 3 failed to inhibit integrase even when a hydroxy (X = OH) group was present. Addition of the pyranyl group resulted in integrase inhibition. Furthermore, comparison of compounds **19** and **8a**–**20** and **9** (Table 2) shows that the hydroxy (Y = OH) group is required for integrase inhibition. Therefore, our current hypothesis is that the pyranyl group is required to correctly position the hydroxy group for integrase inhibition. It is not known which portion(s) of the pyranyl structure is responsible for positioning, but the nature of the aliphatic chain appears to have no effect. Our studies with the biquinones and trimeric quinones indicate that the natural derivative conocurvone **1** is the most effective anti-integrase and anti-HIV drug, and that a complex substructure is required for potent anti-HIV activity. It is possible that conocurvone **1** and related derivatives modulate more than one physiologic pathway in HIV, as suggested earlier.[5] Further studies are in progress to elucidate the roles of the hydroxy and pyranyl groups of conocurvone derivatives as well as determine their in vivo mechanism of action.

4. Experimental

4.1. Materials

Anhydrous solvents and reagents were purchased from Aldrich and used as received. All NMR spectra were recorded on a Bruker Avance™ 500 MHz (or 300 MHz) NMR spectrometer in $CDCl_3$ with $CHCl_3$ as the internal reference. IR spectra were recorded on a Nicolet Nexus 470 FT-IR in KBr pellets or neat as thin films on NaCl plates. MS were measured under fast atom bombardment (FAB) or electron impact (EI) conditions at the University of Notre Dame Mass Spectrometry Facility. Melting points were taken in open capillary tubes and are uncorrected. Analytic thin-layer chromatography (TLC) was performed on commercial



Scheme 3. Regiocontrolled synthesis of a pyranylated trimeric quinone.

Uniplate silica gel plates (Analtech), 250 μm thickness, with fluorescent indicator (F-254). Preparative thin-layer chromatography was performed on commercial Uniplate silica gel plates (Analtech), 1000 μm thickness, with fluorescent indicator (F-254). Flash chromatography was carried out using 35–70 μm silica gel purchased from Acros. Oxalic acid coated silica gel was prepared as described previously.[22]

4.2. Synthetic procedures

4.2.1. 2-Hydroxy-3-iodo-1,4-naphthoquinone (3d). *Method A.* General procedure. Into an oven dried 50 mL flask, equipped with a magnetic stirrer and under three cycles of vacuum/nitrogen was added the dipole (1 mmol) followed by addition of 20 mL of dry CH_2Cl_2 and 1–2 mmol of TMSX. The suspension was stirred at room temperature for 16–20 h during which time a solution formed. The solvent was removed on a rotatory evaporator and the residue dried under high vacuum for 3 h. The addition of 2–6 mL of cold (0 °C) 50% Et_2O in hexanes to the residue followed by vigorous mixing with a spatula yielded a precipitate, which was filtered and dried to yield the desired halohydroxyquinone **3**.

Reaction of the dipole **2a** (0.243 g, 0.646 mmol) with TMSI (0.130 mL, 0.182 g, 0.913 mmol), as described above, yielded a red colored solution, which was poured into ice water. The organic layer washed with cold water to remove traces of HI. The organic layer was dried ($MgSO_4$), filtered, and evaporated to yield a yellow-orange residue. Precipitation from 50% Et_2O/hexanes, as described above, yielded 0.147 g (76%) of **3d** as an orange-brown solid.

Method B. To a stirred solution of the dipole **2a** (2.68 g, 7.14 mmol) in 100 mL CH_2Cl_2 was added 50% HI (1.28 mL, 0.959 g, 7.5 mmol). The dark orange suspension was stirred at rt for 3 h during which time a dark red solution formed. The solution was poured into 100 mL CH_2Cl_2 and the organic layer washed with water (pH ~4–5) and 1% $Na_2S_2O_3$ to remove traces of I_2. The resulting orange solution was dried ($MgSO_4$), filtered and evaporated to yield a yellow-orange residue. Precipitation with 50% Et_2O/hexanes, as described above, yielded 1.66 g (77%) of the quinone **3d** as an orange-brown solid: mp = 179–180 °C ($CHCl_3$), lit. mp = 177–179 °C,[17] R_f = 0.19 (50% EtOAc in hexanes); ^1H NMR (300 MHz, $CDCl_3$): δ 7.72–7.80 (m, 2H), 8.03 (s, 1H), 8.13–8.16 (m, 1H), 8.19–8.22 (m, 1H); IR (film) 3231 (br s), 1665, 1621, 1574, 1454, 1256 cm^{-1}.

4.2.2. 3-Iodo-2-hydroxy-6-methoxy-1,4-naphthoquinone (3g). Reaction of the dipole **2b** (0.4031 g, 0.990 mmol) with TMSI (0.260 mL, 0.365 g, 1.826 mmol), as described above for the preparation of **3d**, yielded 0.257 g (79%) of the quinone **3g**: mp = 189–191 °C ($CHCl_3$); R_f = 0.23 (EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ 3.98 (s, H), 7.18 (dd, J = 8.7, 2.7 Hz, 1H), 7.65 (d, J= 2.7 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H), 8.16 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.4, 90.1, 112.7, 119.7, 121.9, 129.8, 134.4, 160.0, 165.4, 176.1, 179.1; IR (film): 3259 (br s), 1652, 1616, 1251, 1124 cm^{-1}; Anal. Calcd for $C_{11}H_7IO_4$: C, 40.03; H, 2.14. Found: C, 39.80; H, 2.18.

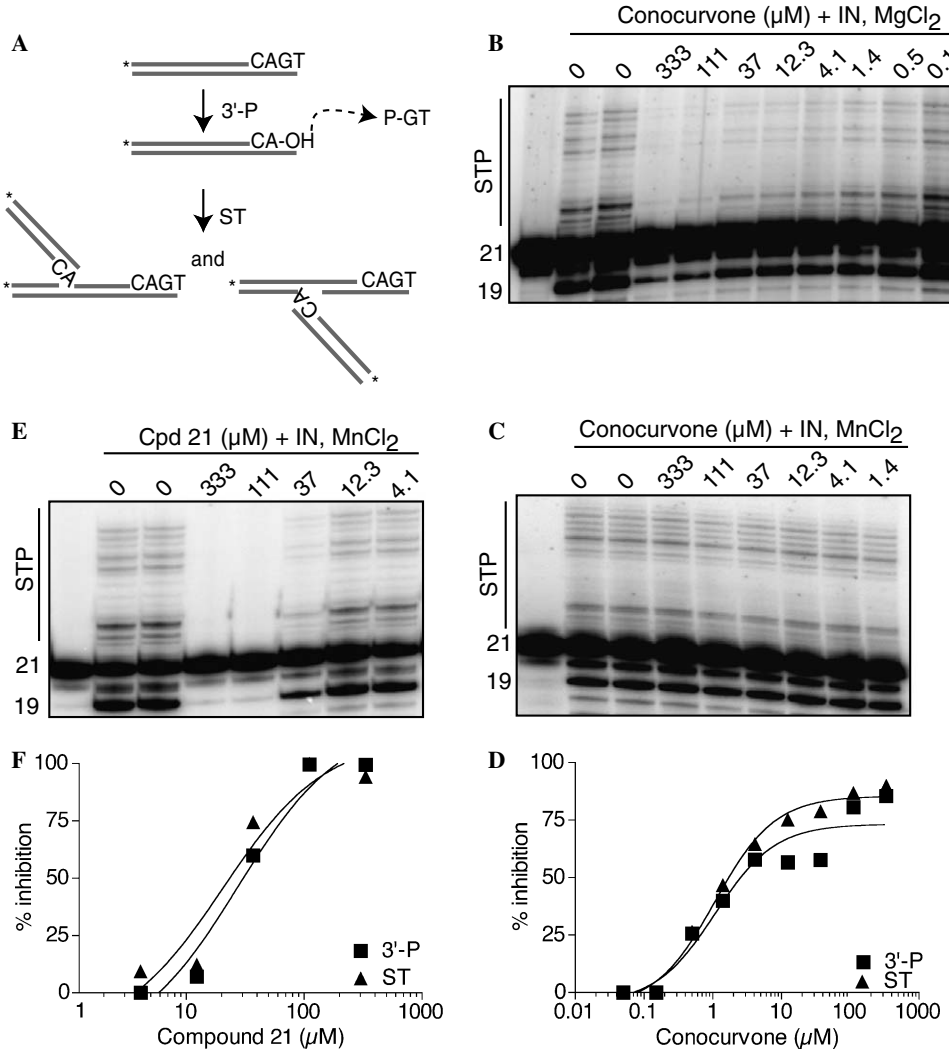


Figure 2. Inhibition of HIV-1 integrase by trimeric naphthoquinone **21** and conocurvone. (A) Schematic diagram of in vitro integrase assay. Integrase is reacted with a [5′-^{32}P] labeled (*) duplex oligonucleotide corresponding to the final 21 nucleotides of the HIV-1 U5 long terminal repeat. First, the 5′-GT-3′ terminal dinucleotide is released by endonucleolytic cleavage, resulting in a 19mer oligonucleotide product (3′-processing, 3′-P). Second, the 3′-ends are covalently inserted into another oligonucleotide duplex at multiple positions (strand transfer, ST), resulting in the ladder of bands migrating slower than the 21mer. (B) and (C) Concentration-dependent inhibition of integrase by conocurvone in the presence of magnesium and manganese. (E) Concentration-dependent inhibition of integrase by compound **21** in the presence of manganese. Results with magnesium were similar (not shown). The micromolar concentrations of inhibitors are above each gel lane. STP indicates strand transfer products. (D) and (F) Graphical representation of integrase 3′-P (squares) and ST (triangles) inhibition by conocurvone and compound **21**. IC_{50} values were determined by fit of the data with a sigmoidal dose–response curve.

4.2.3. 3-Chloro-2-hydroxy-7-methoxy-1,4-naphthoquinone (3h). To a vigorously stirred suspension of the dipole **2c** (1.00 g, 2.46 mmol) in 25 mL of $CHCl_3$ (25 mL) under N_2 at rt, was injected 2.05 mL of concd HCl (24.6 mmol). The brilliant yellow suspension was stirred for 48 h at rt during which time a yellow solution formed. The solution was poured into water and the organic layer washed with water and then extracted with 10% aqueous $NaHCO_3$. The dark red aqueous layer was washed with ether and then acidified in a spacious Erlenmeyer flask with concentrated HCl to pH 2 (litmus). The yellow suspension was extracted with chloroform and the organic layer, dried ($MgSO_4$), filtered, and evaporated to yield 0.55 g (93%) of the quinone **3h** as a yellow solid: mp = 221–227 °C, R_f = 0.29 (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.96 (s, 3H), 7.24–7.26 (dd, J = 8.7 Hz, 2.7 Hz, 1H), 7.52 (s, 1H), 7.57 (d, J = 2.7 Hz, 1H), 8.13–8.15 (d, J = 8.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 56.0, 110.9, 119.0, 121.1, 125.3, 129.9, 130.6, 152.6, 163.9, 177.0, 179.6; IR (KBr) 3177 (br s), 2846, 1681, 1635, 1591, 1485, 1435, 1354, 1203, 1145, 1104, 1035, 1013 cm^{-1}; Anal. Calcd for $C_{11}H_7ClO_4$: C, 55.37; H, 2.96. Found: C, 55.11; H, 3.01.

4.2.4. 3-Iodo-2-hydroxy-7-methoxy-1,4-naphthoquinone (3i). Reaction of the dipole **2c** (1.40 g, 3.45 mmol) with TMSI (0.840 mL, 1.18 g, 5.87 mmol), as described above for the preparation of **3d**, yielded 0.837 g (73%) of the quinone **3i**: mp = 181–185 °C; R_f = 0.41 (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.83 (s, 3H), 7.04–7.07 (dd, J = 10.8, 3.2 Hz, 1H), 7.41 (d, J = 3.3 Hz, 1H), 7.95–7.97 (d, J = 10.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 55.8, 110.7, 120.3, 124.5, 128.3, 129.9, 131.2, 161.2, 163.5, 177.6, 178.9; IR (KBr): 3255 (br s),

2950, 1677, 1629, 1596, 1487, 1433, 1351, 1262, 1133, 1093, 1039, 1002, 906, 892, 853, 789, 745, 682, 662, 567, 483, 455 cm^{-1}; Anal. Calcd for $C_{11}H_7IO_4$: C, 40.03; H, 2.14. Found: C, 42.72; H, 2.52.

4.2.5. 2-Chloro-3-[(trifluoromethanesulfonyl)oxy]-1,4-naphthoquinone (4b). The hydroxyquinone **3b** (0.60 g, 2.88 mmol) was placed in a 50 mL round-bottomed flask fitted with a T-bore stopcock. After three vacuum and nitrogen cycles 25 mL of anhydrous CH_2Cl_2 was added and the solution cooled to 0 °C. Freshly distilled Et_3N (0.48 mL, 3.46 mmol) was added followed by 0.58 mL of triflic anhydride (0.975 g, 3.46 mmol). The light red reaction mixture was stirred for 10 min at 0 °C and then for 20 min at rt. The mixture was concentrated to 2 mL at reduced pressure and the residue loaded onto a short column of silica and eluted with 20% EtOAc in hexanes. The eluants were concentrated and the crystals which formed were filtered to yield 0.836 g (85%) of the triflate **4b** as green needles: mp = 120–123 °C; R_f = 0.34 (33% ether in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85–7.88 (m, 2H), 8.21–8.23 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 116.4, 120.6, 127.8, 128.1, 129.7, 130.8, 135.4 (2C), 148.8, 175.6, 176.7; IR (KBr) 1677, 1594, 1424, 1203, 1172, 1122 cm^{-1}; MS (EI) *m/z* (relative intensity) 342 [$(M+2)^+$, 3], 340 (M^+, 11), 248 (42), 207 (33), 153 (12), 123 (100), 104 (12), 76 (58); Anal. Calcd for $C_{11}H_4ClF_3O_5S$: C, 38.78; H, 1.18. Found: C, 39.18; H, 1.35.

4.2.6. 2-Bromo-3-[(trifluoromethanesulfonyl)oxy]-1,4-naphthoquinone (4c). Reaction of the hydroxyquinone **3c** (0.252 g, 0.99 mmol) with Et_3N (0.164 mL, 0.120 g, 1.19 mmol) and triflic anhydride (0.199 mL, 0.333 g, 1.18 mmol) as described above for the preparation of **4b**, provided a crude solid which was purified by flash chromatography on silica eluting with 50% EtOAc in hexane to yield 0.329 g (85%) of the triflate **4c** as bright yellow needles: mp = 114–115 °C; R_f = 0.48 (50% ether in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85–7.88 (m, 2H), 8.21–8.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 116.9, 128.0, 128.5, 129.7, 130.8, 131.1, 135.4, 135.5, 151.8, 175.4, 177.0; IR (KBr) 1676, 1592, 1428, 1215, 1168, 1141 cm^{-1}; MS (EI) *m/z* (relative intensity) 386 [$(M+2)^+$, 12], 384 (M^+,12), 292 (10), 241 (100), 169 (55), 167 (55), 104 (37), 76 (82); Anal. Calcd for $C_{11}H_4BrF_3O_5S$: C, 34.31; H, 1.05. Found: C, 34.14; H, 1.01.

4.2.7. 2-Iodo-3-[(trifluoromethanesulfonyl)oxy]-1,4-naphthoquinone (4d). Reaction of the hydroxyquinone **3d** (0.250 g, 0.883 mmol) with Et_3N (0.140 mL, 0.101 g, 1.00 mmol) and triflic anhydride (0.160 mL, 0.268 g, 0.951 mmol) as described above for the preparation of **4b**, provided a crude solid which was purified by flash chromatography on silica eluting with 5% EtOAc in hexane to yield 0.276 g (77%) of the triflate **4d** as yellow needles: mp = 129–131 °C; R_f = 0.24 (5% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (pentd, J = 7.6, 7.2, 2.0, 1.6 Hz, 2H), 8.22–8.23 (m, 1H), 8.24–8.25 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 115.5, 120.1, 128.1, 128.9, 129.9, 130.1, 135.3 (2C), 157.2, 174.5, 178.5; IR (KBr) 1676, 1587, 1420, 1209, 1150 cm^{-1}; MS (EI) *m/z* (relative intensity) 432 (M^+, 54); 340 (9); 241(62); 215(28); 195 (3); 127 (15); 76 (100); 50 (43); Anal. Calcd for $C_{11}H_4IF_3O_5S$: C, 30.57; H, 0.93. Found: C, 30.60; H, 0.90.

4.2.8. 2-Chloro-7-methoxy-3-[(trifluoromethanesulfonyl)-oxy]-1,4-naphthoquinone (4e). Reaction of the hydroxyquinone **3e** (0.081 g, 0.340 mmol) with Et_3N (0.074 mL, 0.054 g, 0.532 mmol) and triflic anhydride (0.058 mL, 0.097 g, 0.345 mmol) as described above for the preparation of **4b**, provided a crude solid which was purified by flash chromatography on silica eluting with 20% EtOAc in hexanes, as described above, to yield 0.096 g (77%) of the triflate **4e** as orange needles: mp = 167–169 °C, R_f = 0.32 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 3H), 7.30 (dd, J = 8.4, 2.7 Hz, 1H), 7.64 (d, J = 2.7 Hz, 1H), 8.15 (d, J = 8.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.5, 112.0, 116.4, 120.6, 121.4, 122.8, 130.3, 133.0, 149.0, 165.3, 174.4, 177.0; IR (film) 1685, 1667, 1421, 1216, 1129, 1021 cm^{-1}; Anal. Calcd for $C_{12}H_6ClF_3O_6S$: C, 38.88; H, 1.63. Found: C, 38.95; H, 1.72.

4.2.9. 2-Bromo-7-methoxy-3-[(trifluoromethanesulfonyl)-oxy]-1,4-naphthoquinone (4f). Reaction of the hydroxyquinone **3f** (0.125 g, 0.444 mmol) with Et_3N (0.075 mL, 0.054 g, 0.539 mmol) and triflic anhydride (0.088 mL, 0.147 g, 0.523 mmol) as described above for the preparation of **4b**, provided a crude solid which was purified by flash chromatography on silica eluting with 33% EtOAc in hexanes, to yield 0.131 g (71%) of the triflate **4f** as orange needles: mp = 169–171 °C, R_f = 0.46 (33% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.99 (s, 3H), 7.30 (dd, J = 6.6, 1.8 Hz, 1H), 7.64 (d, J = 1.8 Hz, 1H), 8.15 (d, J = 6.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.4, 112.4, 116.9, 120.1, 121.4, 122.9, 130.5, 133.0, 152.1, 165.4, 174.2, 177.3; IR (film) 1684, 1670, 1423, 1217, 1170, 1135 cm^{-1}; Anal. Calcd for $C_{12}H_6BrF_3O_6S$: C, 34.71; H, 1.45. Found: C, 34.63; H, 1.56.

4.2.10. 2-Iodo-7-methoxy-3-[(trifluoromethanesulfonyl)-oxy]-1,4-naphthoquinone (4g). The hydroxyquinone **3g** (0.118 g, 0.357 mmol) was treated with Et_3N (0.060 mL, 0.043 g, 0.431 mmol) and triflic anhydride (0.070 mL, 0.117 g, 0.416 mmol) as described above for the preparation of **4b**, providing a crude solid which was purified by flash chromatography on silica eluting with 20% EtOAc in hexanes to yield 0.121 g (76%) of the triflate **4g** as orange needles: mp = 144–146 °C, R_f = 0.40 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.98 (s, 3H), 7.28 (dd, J = 8.4, 2.7 Hz, 1H), 7.62 (d, J = 2.7 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.5, 112.7, 113.6, 116.3, 121.3, 123.0, 130.5, 132.1, 157.4, 165.0, 173.1, 178.4; IR (film) 1678, 1668, 1423, 1218, 1205 cm^{-1}; Anal. Calcd for $C_{12}H_6IF_3O_6S$: C, 31.18; H, 1.30. Found: C, 31.09; H, 1.39.

4.2.11. 2-Chloro-6-methoxy-3-[(trifluoromethanesulfonyl)-oxy]-1,4-naphthoquinone (4h). Reaction of the hydroxyquinone **3h** (0.540 g, 2.26 mmol) with Et_3N (0.375 mL, 0.274 g, 2.71 mmol) and triflic anhydride (0.456 mL,

0.765 g, 2.71 mmol) as described above for the preparation of **4b**, provided a crude solid which was purified by flash chromatography on silica eluting with 5% EtOAc in hexanes to yield 0.705 g (84%) of the triflate **4h** as orange needles: mp = 124–127 °C, R_f = 0.28 (5% EtOAc in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.98 (s, 3H), 7.24–7.26 (dd, J = 8.7 Hz, 2.7 Hz, 1H), 7.62 (d, J = 2.6 Hz, 1H), 8.14–8.15 (d, J = 8.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 56.2, 111.2, 116.5, 119.6, 121.5, 123.0, 131.1, 131.8, 156.7, 165.0, 174.4, 176.9; IR (KBr) 3108, 3027, 2998, 2953, 2851, 1783, 1683, 1619, 1585, 1499, 1463, 1436, 1353, 1309, 1212, 1135, 1090, 1012 cm^{-1}; Anal. Calcd for $C_{12}H_6ClF_3O_6S$: C, 38.88; H, 1.63. Found: C, 39.28; H, 1.68.

4.2.12. 2-Iodo-6-methoxy-3-[(trifluoromethanesulfonyl)-oxy]-1,4-naphthoquinone (4i). Reaction of the hydroxyquinone **3i** (0.720 g, 2.18 mmol) with Et_3N (0.363 mL, 0.265 g, 2.62 mmol) and triflic anhydride (0.441 mL, 0.739 g, 2.62 mmol) as described above for the preparation of **4b**, provided a crude solid which was purified by flash chromatography on silica eluting with 5% EtOAc in hexanes, to yield 0.783 g (78%) of the triflate **4i** as a yellow powder: mp = 135–137 °C, R_f = 0.27 (5% EtOAc in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.99 (s, 3H), 7.27–7.30 (dd, J = 10.9 Hz, 3.4 Hz, 1H), 7.62 (d, J = 3.3 Hz, 1H), 8.14–8.16 (d, J = 10.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.2, 111.3, 120.0, 121.5, 123.9, 130.5, 131.7, 137.0, 148.5, 165.2, 175.4, 175.8; IR (KBr) 3076, 3006, 2957, 2852, 1768, 1685, 1663, 1588, 1496, 1468, 1421, 1347, 1302, 1235, 1149, 1132, 1019, 1000 cm^{-1}; Anal. Calcd for $C_{12}H_6IF_3O_6S$: C, 31.18; H, 1.30. Found: C, 31.48; H, 1.45.

4.2.13. 3′-Hydroxy-3-iodo-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (6a). The hydroxyquinone triflate **4d** (0.300 g, 0.69 mmol), 2-hydroxy-1,4-naphthoquinone **3a** (0.120 g, 0.69 mmol) and Cs_2CO_3 (0.450 g, 1.38 mmol) were placed in a 25 mL round bottom flask fitted with a T-bore stopcock. After three vacuum and nitrogen cycles, 10 mL of anhydrous CH_3CN was added, and the suspension stirred at room temperature for 6 days during which time the color changed to a very dark red. The mixture was acidified with concentrated HCl to pH 2 (litmus) forming a yellow-green precipitate. The suspension was poured into 100 mL of water and stirred for 1 hour to dissolve the remaining traces of cesium carbonate. The precipitate was filtered and the product mixture air-dried for 3 days at rt. The product consisted of a 90:10 mixture 3′-hydroxy-3-iodo-2,2′-binaphthalenyl-1,4,1′,4′-tetraone **6a** and 3′-hydroxy-3-[(trifluoromethanesulfonyl)oxy]-2,2′-binaphthalenyl-1,4,1′,4′-tetraone as determined by HPLC analyses of the crude reaction mixture. The molecular weight of the minor regioisomer was determined by GC–MS analysis of the crude reaction mixture. Purification by flash chromatography on oxalic acid coated silica gel[22] with gradual elution from 10% to 50% EtOAc in hexanes yielded 0.226 g (72%) of the iodohydroxybiquinone **6a** as a yellow-orange solid: mp = 213–218 °C, R_f = 0.28 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (s, 1H, br), 7.75–7.81 (m, 3H), 7.84–7.87 (m, 1H), 8.13–8.15 (m, 1H), 8.19–8.20 (m, 2H), 8.22–8.24 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 122.1, 126.5, 126.8, 127.3, 128.2, 129.3, 130.1, 130.5, 131.5, 132.0, 134.4, 135.0, 135.3, 135.7, 150.6, 155.6, 179.28, 179.33, 181.2, 181.7; FT-IR (KBr) 3334 (br s), 3114, 1675, 1660, 1636, 1589, 1480, 1457, 1371, 1333, 1267, 1214, 1160, 1125, 1080, 1040, 1011 cm^{-1}; MS (FAB) *m/z* (relative intensity) 458 [$(M+2H)^+$, 5], 457 [$(M+H)^+$, 3], 391 (40), 330 [$(M-I)^+$, 17], 279 (9), 167 (23), 149 (100), 136 (29); HRMS (FAB) Calcd for $C_{20}H_{10}{}^{127}IO_5$ $(M+H)^+$ was not found but for $C_{20}H_{10}O_5$ $(M-I)^+$ calcd: 330.0528. Found: 330.0527.

4.2.14. 3′-Chloro-3-hydroxy-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (6b). Reaction of the hydroxyquinone triflate **4b** (0.325 g, 0.96 mmol) with hydroxyquinone **5** (0.196 g, 0.96 mmol) and Cs_2CO_3 (0.626 g, 1.92 mmol) for 6 days, according to the general procedure described above for the preparation of **6a**, resulted in an 85:15 mixture of 3′-chloro-3-hydroxy-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone **6b** and 3-hydroxy-7-methoxy-3′-[(trifluoromethanesulfonyl)oxy]-2,2′-binaphthalenyl-1,4,1′,4′-tetraone. Purification by flash chromatography on oxalic acid coated silica gel[22] with gradual elution from 10% to 50% EtOAc in hexanes yielded 0.212 g (56%) of the chlorohydroxybiquinone **6b** as a yellow solid: mp = 212–216 °C, R_f = 0.29 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.88 (s, 3H), 7.10–7.12 (dd, J = 10.7 Hz, 3.1 Hz, 1H), 7.50 (d, J = 3.0 Hz, 1H), 7.70–7.73 (m, 2H), 8.01–8.03 (d, J = 10.9 Hz, 1H), 8.04–8.05 (m, 1H), 8.12–8.14 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.0, 110.7, 115.2, 119.5, 123.4, 127.1, 127.2, 129.1, 131.3, 131.9, 134.0, 134.3, 134.9, 139.8, 145.5, 155.8, 165.1, 177.5, 179.5, 180.4, 181.9; FT-IR (KBr) 3334 (br s), 3085, 2925, 2847, 1675, 1642, 1591, 1499, 1449, 1373, 1274, 1168, 1133, 1102, 1076, 1040, 1013 cm^{-1}; MS (FAB) *m/z* (relative intensity) 395 [$(M+H)^+$, 6], 360 (5), 307 (18), 289 (12), 255 (6), 220 (7), 173 (13), 154 (100), 136 (89), 107(44); HRMS (FAB) calcd for $C_{21}H_{12}{}^{35}ClO_6$ $(M+H)^+$: 395.0322. Found: 395.0333.

4.2.15. 3-Hydroxy-3′-iodo-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (6d). Reaction of the hydroxyquinone triflate **4d** (0.250 g, 0.58 mmol) with hydroxyquinone **5** (0.119 g, 0.58 mmol) and Cs_2CO_3 (0.378 g, 1.16 mmol) for 6 days, according to the general procedure described above for the preparation of **6a**, resulted in a 95:5 mixture of 3-hydroxy-3′-iodo-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone **6d** and 3-hydroxy-7-methoxy-3′-[(trifluoromethanesulfonyl)oxy]-2,2′-binaphthalenyl-1,4,1′,4′-tetraone. Purification by flash chromatography on oxalic acid coated silica gel[22] with gradual elution from 10% to 50% EtOAc in hexanes yielded 0.189 g (67%) of the iodohydroxybiquinone **6d** as an orange-yellow solid: mp = 204–207 °C, R_f = 0.49 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.84 (s, 3H), 7.06–7.09 (dd, J = 10.8 Hz, 3.1 Hz, 1H), 7.45–7.46 (d, J = 3.2 Hz, 1H), 7.61–7.69 (m, 2H), 7.97–7.99 (m, 2H), 8.06–8.08 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.0, 110.6, 119.4, 120.9, 123.5, 127.2, 127.9, 128.4, 129.1, 130.0, 131.7, 133.8, 134.2, 134.8, 150.5, 154.9, 165.0, 178.6, 178.8, 179.7, 181.5; FT-IR (KBr) 3267 (br s), 2924, 2850, 1730, 1672, 1656, 1642, 1590, 1498, 1437, 1371, 1339, 1299, 1268, 1131, 1073, 1011 cm^{-1}; MS

(FAB) *m/z* (relative intensity) 488 [(M+2+H)$^+$, 3], 391 (10), 360 (6), 338 (6), 307 (31), 289 (19), 255 (6), 218 (9), 154 (100), 136 (91), 107 (45); HRMS (FAB) calcd for $C_{21}H_{13}{}^{127}IO_6$ (M+2H)$^+$: 487.9757. Found: 487.9759.

4.2.16. 3-Chloro-3′-hydroxy-6-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (6e). Reaction of the hydroxyquinone triflate **4e** (0.130 g, 0.35 mmol) with hydroxyquinone **3a** (0.061 g, 0.35 mmol) and Cs_2CO_3 (0.228 g, 0.70 mmol) for 6 days, according to the general procedure described above for the preparation of **6a** yielded a 90:10 mixture of 3-chloro-3′-hydroxy-6-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone **6e** and 3′-hydroxy-7-methoxy-3-[(trifluoromethanesulfonyl)oxy]-2,2′-binaphthalenyl-1,4,1′,4′-tetraone. Purification by flash chromatography on oxalic acid coated silica gel[22] with gradual elution from 10% to 50% EtOAc in hexanes yielded 0.092 g (67%) of the chlorohydroxybiquinone **6e** as yellow needles: mp = 297–300 °C, R_f = 0.31 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.99 (s, 3H), 7.24–7.27 (dd, J = 8.7 Hz, 2.6 Hz, 1H), 7.65–7.66 (d, J = 2.6 Hz, 1H), 7.76–7.86 (m, 2H), 8.08–8.10 (d, J = 8.6 Hz, 1H), 8.18–8.20 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.0, 110.8, 115.5, 121.0, 125.2, 126.7, 127.3, 129.3, 129.8, 132.7, 133.3, 133.5, 135.6, 138.8, 145.2, 153.1, 164.3, 177.5, 179.4, 180.7, 181.5; FT-IR 3319 (br s), 3076, 2989, 2950, 2844, 1678, 1653, 1582, 1499, 1457, 1378, 1298, 1244, 1216, 1196, 1135, 1076, 1012 cm^{-1}; MS (FAB) *m/z* (relative intensity) 395 [(M+H)$^+$, 2], 360 (1), 307 (26), 289 (14), 273 (3), 154 (100), 136 (77), 107 (25); HRMS (FAB) calcd for $C_{21}H_{12}{}^{35}ClO_6$ (M+H)$^+$: 395.0322. Found: 395.0331.

4.2.17. 3′-Hydroxy-3-iodo-6-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (6g). Reaction of the hydroxyquinone triflate **4g** (0.200 g, 0.43 mmol) with 2-hydroxynaphthoquinone **3a** (0.075 g, 0.43 mmol) and Cs_2CO_3 (0.280 g, 0.86 mmol) for 6 days, according to the general procedure described above for the preparation of **6a** yielded 0.173 g (83%) of the iodohydroxybiquinone **6g** as a yellow orange solid: mp = 174–176 °C, R_f = 0.47 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.98 (s, 3H), 7.23–7.26 (dd, J = 8.6 Hz, 2.6 Hz, 1H), 7.65–7.66 (d, J = 2.6 Hz, 1H), 7.77–7.87 (m, 2H), 8.07–8.08 (d, J = 8.6 Hz, 1H), 8.19–8.21 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.0, 111.6, 120.9 (2C), 125.1, 126.7, 127.3, 129.4, 129.9, 132.1, 132.7, 133.5, 135.6, 149.6, 151.9, 161.9, 164.1, 177.9, 178.4, 180.9, 181.2; FT-IR (KBr) 3324 (s and br), 2924, 2849, 1734, 1669, 1653, 1587, 1539, 1472, 1374, 1340, 1280, 1230, 1189, 1135, 1068, 1012 cm^{-1}; MS (FAB) *m/z* (relative intensity) 488 [(M+2+H)$^+$, 2], 391 (5), 360 (4), 338 (5), 307 (25), 289 (16), 255 (4), 218 (8), 154 (100), 136 (100), 107 (39); HRMS (FAB) calcd for $C_{21}H_{13}{}^{127}IO_6$ (M+2H)$^+$: 487.9757. Found: 487.9743.

4.2.18. 3-Chloro-3′-hydroxy-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (6h). Reaction of the hydroxyquinone triflate **4h** (0.400 g, 1.08 mmol) with 2-hydroxynaphthoquinone **3a** (0.188 g, 1.08 mmol) and Cs_2CO_3 (0.704 g, 2.16 mmol) for 7 days, according to the general procedure described above for the preparation of **6a**, yielded a 70:30 mixture of 3-chloro-3′-hydroxy-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone **6h** and 3′-hydroxy-6-methoxy-3-[(trifluoromethanesulfonyl)oxy]-2,2′-binaphthalenyl-1,4,1′,4′-tetraone. Purification by flash chromatography on oxalic acid coated silica gel[22] with gradual elution from 10% to 50% EtOAc in hexanes yielded 0.200 g (47%) of the chlorohydroxybiquinone **6h** as yellow needles: mp = 262–266 °C, R_f = 0.50 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.96 (s, 3H), 7.24–7.27 (dd, J = 8.7 Hz, 2.6 Hz, 1H), 7.57–7.58 (d, J = 2.6 Hz, 1H), 7.77–7.87 (m, 2H), 8.17–8.19 (d, J = 8.7 Hz, 1H), 8.17–8.21 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.0, 110.7, 115.4, 120.7, 124.8, 126.7, 127.3, 129.3, 130.0, 132.7, 133.5, 133.9, 135.6, 138.1, 146.5, 153.1, 164.6, 176.2, 180.4, 180.7, 181.5; FT-IR (KBr) 3337 (br s), 3078, 2978, 2944, 2842, 1673, 1651, 1593, 1495, 1460, 1377, 1339, 1284, 1235, 1167, 1141, 1091, 1041, 1011 cm^{-1}; MS (FAB) *m/z* (relative intensity) 395 [(M+H)$^+$, 11], 391 (20), 360 (9), 307 (62), 289 (48), 273 (9), 154 (100), 136 (89), 107 (70); HRMS (FAB) calcd for $C_{21}H_{12}{}^{35}ClO_6$ (M+H)$^+$: 395.0322. Found: 395.0319.

4.2.19. 3′-Hydroxy-3-iodo-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (6i). Reaction of the hydroxyquinone triflate **4i** (0.400 g, 0.87 mmol) with 2-hydroxynaphthoquinone **3a** (0.152 g, 0.87 mmol) and Cs_2CO_3 (0.567 g, 1.74 mmol) for 7 days, according to the general procedure described above for the preparation of **6a**, resulted in a 90:10 mixture of 3′-hydroxy-3-iodo-7-methoxy-2,2′-binaphthalenyl-1,4,1′,4′-tetraone **6i** and 3′-hydroxy-6-methoxy-3-[(trifluoromethanesulfonyl)oxy]-2,2′-binaphthalenyl-1,4,1′,4′-tetraone. Purification by flash chromatography on oxalic acid coated silica gel[22] with gradual elution from 10% to 50% EtOAc in hexanes yielded 0.254 g (60%) of the iodohydroxybiquinone **6i** as a yellow-orange solid: mp = 199–204 °C, R_f = 0.45 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.96 (s, 3H), 7.20–7.23 (dd, J = 8.7 Hz, 2.7 Hz, 1H), 7.55 (d, J = 2.6 Hz, 1H), 7.77–7.87 (m, 2H), 8.16–8.18 (d, J = 8.7 Hz, 1H), 8.19–8.21 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.0, 110.8, 120.6, 120.8, 123.5, 126.7, 127.3, 129.4, 129.9, 130.8, 132.7, 133.5, 133.7, 135.6, 148.9, 152.0, 164.5, 177.2, 178.9, 180.9, 181.2; FT-IR (KBr) 3327 (br s), 2936, 2840, 1729, 1670, 1643, 1592, 1495, 1458, 1444, 1373, 1337, 1281, 1171, 1135, 1087, 1039, 1013 cm^{-1}; MS (FAB) *m/z* (relative intensity) 488 [(M+2+H)$^+$, 3], 391 (5), 360 (7), 307 (25), 289 (14), 273 (3), 219 (4), 154 (100), 136 (85), 107 (31); HRMS (FAB) calcd for $C_{21}H_{12}{}^{127}IO_6$ (M+H)$^+$: 486.9679. Found: 486.9663.

4.2.20. 9-Hydroxy-8-(3-iodo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3,3-dimethyl-3*H*-benzo[*f*]chromene-7,10-dione (8a). Reaction of hydroxyquinone triflate **4d** (0.700 g, 1.62 mmol) with 9-hydroxy-3,3-dimethyl-3*H*-benzo[*f*]-chromene-7,10-dione **7a**[25] (0.415 g, 1.62 mmol) and Cs_2CO_3 (1.056 g, 3.24 mmol) for 7 days, according to the general procedure described above for the preparation of **6a**, yielded a 90:10 mixture of 9-hydroxy-8-(3-iodo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3,3-dimethyl-3*H*-benzo[*f*]chromene-7,10-dione **8a** and 9-hydroxy-8-(3-[(trifluoromethanesulfonyl)oxy]-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3,3-dimethyl-3*H*-benzo[*f*]chro-

mene-7,10-dione. Purification by flash chromatography on oxalic acid coated silica gel[22] eluting with 10% EtOAc in hexanes, yielded 0.524 g (60%) of the iodohydroxybiquinone **8a** as a red solid: mp = 213–217 °C, R_f = 0.21 (50% EtOAc in hexanes), ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.52 (s, 6H), 6.04–6.06 (d, J = 10.3 Hz, 1H), 7.15–7.17 (d, J = 8.4 Hz, 1H), 7.75–7.81 (m, 4H), 8.04–8.05 (d, J = 8.4 Hz, 1H), 8.12–8.14 (dd, J = 7.2 Hz, 1.67 Hz, 1H), 8.21–8.23 (dd, J = 7.2 Hz, 1.65 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0, 28.0, 119.0, 119.5, 121.9, 122.8, 123.3, 126.8, 127.5, 128.2, 128.5, 128.7, 129.3, 130.1, 131.7, 133.9, 134.3, 136.6, 149.7, 151.9, 158.2, 178.4, 178.8, 180.6, 182.8; FT-IR (KBr) 3318, 2980, 1733, 1656, 1626, 1590, 1562, 1456, 1440, 1380, 1350, 1270, 1162, 1129, 1098, 1075, 1005 cm^{-1}; MS (FAB) *m/z* (relative intensity) 539 [(M+H)$^+$, 7], 422 (12), 397 (12), 307 (11), 289 (9), 252 (10), 213 (5), 154 (100), 136 (76), 107 (28); HRMS (FAB) calcd for $C_{25}H_{16}{}^{127}IO_6$ (M+H)$^+$: 538.9992. Found: 539.0004.

4.2.21. 9-Hydroxy-8-(3-iodo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3-methyl-3-(4-methyl-pent-3-enyl)-3*H*-benzo[*f*]chromene-7,10-dione (8b). Reaction of the hydroxyquinone triflate **4d** (0.821 g, 1.90 mmol) with teretifolione B **7b**[25] (0.616 g, 1.90 mmol) and Cs_2CO_3(1.238 g, 3.80 mmol) for 7 days, according to the general procedure described above for the preparation of **6a**, yielded a 90:10 mixture of 9-hydroxy-8-(3-iodo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3-methyl-3-(4-methyl-pent-3-enyl)-3*H*-benzo[*f*]chromene-7,10-dione **8b** and 9-hydroxy-8-[3-[(trifluoromethanesulfonyl)oxy]-1,4-dioxo-1,4-dihydronaphthalen-2-yl]-3-methyl-3-(4-methyl-pent-3-enyl)-3*H*-benzo[*f*]chromene-7,10-dione. Purification by flash chromatography on oxalic acid coated silica gel[22] eluting with 10% EtOAc in hexanes, yielded 0.587 g (51%) of the iodohydroxybiquinone **8b** as a dark red solid: mp = 93–97 °C, R_f = 0.30 (50% EtOAc in hexanes), ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.48 (s, 3H), 1.58 (s, 3H), 1.66 (d, J = 3.2 Hz, 3H), 1.71–1.76 (m, 2H), 1.80–1.83 (m, 2H), 2.12 (s, broad, 1H), 5.08–5.11 (m, 1H), 6.00 (d, J = 10.5 Hz, 1H), 7.13–7.15 (d, J = 8.5 Hz, 1H), 7.73–7.80 (m, 2H), 7.85–7.87 (d, J = 10.5 Hz, 1H), 8.03–8.04 (d, J = 8.5 Hz, 1H), 8.12–8.14 (m, 1H), 8.21–8.23 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.7, 22.7, 25.6, 26.7, 41.3, 79.6, 119.0, 120.0, 121.8, 122.5, 123.3, 123.4, 126.6, 127.5, 128.2, 128.5, 129.4, 130.1, 131.7, 132.3, 133.9, 134.3, 135.9, 149.7, 151.9, 158.6, 178.4, 178.8, 180.6, 182.8; FT-IR (KBr) 3321, 2966, 2922, 1672, 1645, 1592, 1562, 1460, 1436, 1354, 1264, 1198, 1128, 1075, 1002, 951, 915, 848, 781, 748, 700, 617, 538, 464, 432 cm^{-1}; MS (FAB) *m/z* (relative intensity) 607 [(M+H)$^+$, 20], 523 (26), 480 (16), 397 (76), 307 (24), 289 (16), 220 (9), 165 (11), 154 (100), 136 (97), 107 (45); HRMS (FAB) calcd for $C_{30}H_{24}{}^{127}IO_6$ (M+H)$^+$: 607.0618. Found: 607.0629.

4.2.22. 8-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-9-hydroxy-3,3-dimethyl-3*H*-benzo[*f*]chromene-7,10-dione (11). Reaction of the hydroxyquinone **7a** (1.00 g, 3.9 mmol), 2,3-dichloro-1,4-naphthoquinone **10** (0.886 g, 3.9 mmol) and cesium carbonate (2.542 g, 7.8 mmol) for 7 days, according to the general procedure described for the preparation of **6a**, yielded 1.537 g (61%) of naphthopyranyl biquinone as a brown solid: mp = 204–208 °C; R_f = 0.21 (50% EtOAc in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (s, 6H), 6.02–6.04 (d, J = 10.3 Hz, 1H), 7.13–7.15 (d, J = 8.44 Hz, 1H), 7.73–7.81 (m, 3H), 8.01–8.03 (d, J = 8.44 Hz, 1H), 8.12–8.14 (m, 1H), 8.19–8.22 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0 (2C), 77.3, 113.6, 119.5, 121.9, 122.8, 123.3, 126.7, 127.3, 127.4, 129.3, 131.4, 131.8, 134.1, 134.4, 136.7, 138.9, 145.8, 153.2, 158.2, 177.4, 180.4, 180.9, 182.6; FT-IR 3298, 2977, 2926, 1679, 1659, 1631, 1590, 1557, 1466, 1436, 1378, 1355, 1277, 1219, 1188, 1163, 1137, 1120, 1006 cm^{-1}; MS (FAB) *m/z* (relative intensity) 449 [(M+2+H)$^+$, 6], 447 [(M+H)$^+$, 10], 411 (6), 397 (5), 307 (11), 213 (10), 154 (100), 136 (72); HRMS (FAB) calcd for $C_{25}H_{16}{}^{35}ClO_6$ (M+H)$^+$: 447.0635. Found: 447.0623.

4.2.23. 8-(3-Iodo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-9-methoxy-3,3-dimethyl-3*H*-benzo[*f*]chromene-7,10-dione (9). The iodohydroxybiquinone **8a** (0.094 g, 0.175 mmol) and trimethyloxonium tetrafluoroborate (0.052 g, 0.35 mmol) were placed in a 50 mL round bottom flask fitted with a T-bore stopcock. After three vacuum and nitrogen cycles, 25 mL of anhydrous CH_2Cl_2 was added and the suspension stirred at rt for 30 min. *N*,*N*-Diisopropylethylamine (0.046 mL, 0.034 g, 0.263 mmol) was added and the dark red solution stirred for 3 h during which time the color changed to dark yellow. The solution was diluted with 60 mL of CH_2Cl_2 and the organic layer washed successively with water (containing three drops of concd HCl) then 10% $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered, and evaporated to yield 0.090 g (93%) of the iodomethoxybiquinone **9** as a brown solid: mp = 90–95 °C; R_f = 0.19 (10% EtOAc in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (s, 6H), 4.09 (s, 3H), 5.97–6.00 (d, J = 13.0 Hz, 1H), 7.09–7.11 (d, J = 10.4 Hz, 1H), 7.66–7.68 (d, J = 12.9 Hz, 1H), 7.74–7.80 (m, 2H), 7.95–7.97 (d, J = 10.6 Hz, 1H), 8.12–8.14 (m, 1H), 8.21–8.23 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0 (2C), 61.1, 77.3 ($CDCl_3$ overlaps), 119.7, 121.2, 121.9, 125.9, 126.2, 127.2, 127.5, 128.1, 128.2, 128.6, 130.2, 131.6, 134.0, 134.3, 135.4, 151.1, 157.0, 158.7, 178.3, 179.0, 181.0, 183.2; FTIR (KBr) 3466, 3398, 2946, 2872, 1670, 1659, 1642, 1592, 1563, 1328, 1320, 1288, 1275, 1266, 1218, 1162, 1119, 1022 cm^{-1}; MS (FAB) *m/z* (relative intensity) 553 [(M+H)$^+$, 14], 537 (10), 425 (16), 411 (20), 391 (29), 307 (10), 289 (9), 279 (7), 220 (11), 149 (100), 136 (65); HRMS (FAB) calcd for $C_{26}H_{18}{}^{127}IO_6$ (M+H)$^+$: 553.0148. Found 553.0136.

4.2.24. 8-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-9-methoxy-3,3-dimethyl-3*H*-benzo[*f*]chromene-7,10-dione (12). Reaction of the chlorohydroxybiquinone **11** (0.500 g, 1.12 mmol), trimethyloxonium tetrafluoroborate (0.414 g, 2.8 mmol) and *N*,*N*-diisopropylethylamine (0.217 g, 0.292 mL, 1.68 mmol) for 5 h according to the general procedure described above for the preparation of **9** yielded 0.510 g (99%) of the chloromethoxybiquinone **12** as a dark red solid: mp = 87–93 °C; R_f = 0.38 (50% EtOAc in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48–1.52 (s, 6H), 4.09 (s, 3H), 5.97–5.99 (d, J = 12.8 Hz, 1H), 7.09–7.11 (d,

J = 10.7 Hz, 1H), 7.65–7.68 (d, J = 12.8 Hz, 1H), 7.74–7.82 (m, 2H), 7.94–7.96 (d, J = 10.7 Hz, 1H), 8.12–8.15 (m, 1H), 8.21–8.24 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0 (2C), 61.1, 77.3 ($CDCl_3$ overlaps), 119.7, 121.2, 121.9, 122.0, 125.9, 126.2, 127.3, 127.4, 128.6, 131.4, 131.8, 134.2, 134.4, 135.4, 140.3, 145.2, 158.2, 158.7, 177.3, 180.6, 181.4, 183.0; FT-IR (KBr) 3071, 2980, 2850, 1677, 1643, 1597, 1562, 1458, 1311, 1278, 1216, 1162, 1119, 1084, 1022 cm^{-1}; MS (FAB) m/z (relative intensity) 463 [$(M+2+H)^+$, 31], 461 [$(M+H)^+$, 57], 445 (13), 425 (21), 245 (14), 213 (32), 167 (26), 149 (100); HRMS (FAB) calcd for $C_{26}H_{18}{}^{35}ClO_6$ $(M+H)^+$: 461.0792. Found: 461.0787.

4.2.25. 3-Hydroxy-3′-(9-methoxy-3,3-dimethyl-7,10-dioxo-7,10-dihydro-3*H*-benzo[*f*]chromen-8-yl)-2,2′-binaphthalenyl-1,4,1′,4′-tetraone (14). The chloromethoxybiquinone **12** (0.220 g, 0.48 mmol), hydroxyquinone anion **13** (0.102 g, 0.48 mmol) and 18-crown-6 (0.127 g, 0.48 mmol) were placed in a 25 mL round bottom flask fitted with a T-bore stopcock. After three vacuum and nitrogen cycles, 15 mL of anhydrous NMP was added. The suspension was stirred at 65 °C for 2 days. The dark red suspension was acidified with concentrated HCl to pH 2 (litmus) and diluted with 100 mL $CHCl_3$. The mixture was washed with 10% K_2CO_3 and the organic layer dried ($MgSO_4$), filtered and evaporated to yield a red solid. Recrystallization from methanol yielded 0.04 g (14%) of an orange solid: mp = 169–173 °C; R_f = 0.26 (EtOAc); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.36 (s, 6H), 3.82 (s, 3H), 5.88–5.91 (d, J = 13.0 Hz, 1H), 7.05–7.08 (d, J = 10.6 Hz, 1H), 7.47–7.55 (m, 3H), 7.61–7.72 (m, 4H), 7.81–7.83 (d, J = 9.4 Hz, 1H), 7.97–7.99 (d, J = 10.5 Hz, 1H), 8.07–8.09 (d, J = 9.4 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.8 (2C), 53.0, 76.4, 120.4, 120.5, 124.1, 124.2, 125.7, 126.8, 128.1, 128.3, 128.5, 128.9, 129.4, 131.3, 131.3, 131.4, 131.8, 132.4, 133.1, 133.2, 134.0, 134.3, 137.0, 137.2, 139.6, 156.8 (2C), 160.4, 177.8, 178.4, 180.3, 183.6, 189.8, 197.9; FT-IR 3439, 2975, 2924, 1669, 1647, 1640, 1586, 1560, 1529, 1457, 1436, 1399, 1363, 1276, 1218, 1146, 1119, 1092, 1019 cm^{-1}; MS (FAB) m/z (relative intensity) 599 [$(M+H)^+$, 26], 539 (20), 338 (19), 255 (78), 154 (60), 109 (100); HRMS (FAB) calcd for $C_{36}H_{23}O_9$ $(M+H)^+$: 599.1342. Found 599.1359.

4.3. HIV-1 integrase assay

The concentration dependent inhibition of test compounds against HIV-1 integrase was measured using an oligonucleotide based in vitro assay (schematically shown in Fig. 2A).[13] The [5′-^{32}P] labeled oligonucleotide duplex (20 nM) derived from the last 21 base pairs of the HIV-1 U5 LTR was mixed with 400 nM HIV-1 integrase in the presence of 7.5 mM $MnCl_2$ or $MgCl_2$, 25 mM MOPS, pH 7.2, and 14.3 mM 2-mercaptoethanol. Inhibitors were then added and the reactions were incubated at 37 °C for 1 h. Reactions were quenched by the addition of formamide-containing gel loading dye and loaded onto 20% (19:1) denaturing polyacrylamide gels. The gels were analyzed using a Molecular Dynamics Phosphorimager (Sunnyvale, CA). IC_{50} values were determined from two or three separate experiments.

4.4. Antiviral assay (MTT method)

The antiviral activity of test compounds against HIV was measured as the inhibition of viral cytopathogenic effect. CEM-T_4 cells used for the assay were maintained in RPMI-1640 culture medium containing 2 mM L-glutamine and 25 mM HEPES, and supplemented with 10% fetal bovine serum, 50 U/mL of penicillin G, and 50 μg/mL streptomycin sulfate. The antiviral assays were performed in 96-well tissue culture plates. Cells were treated with polybrene at a concentration of 2 μg/mL, and 1×10^4 cells were dispensed into each well. Appropriate concentrations of test compounds were prepared in sterile DMSO and diluted with culture medium to the desired concentrations. Each dilution of test compound was added to multiple wells of cells, and the cells were incubated at 37 °C for 1 h. Cells were then infected at a multiplicity of infection of 0.025 $TCID_{50}$/cell by the addition of a diluted stock of the HTLV-IIIB strain of HIV-1. Compounds were tested in triplicate wells per concentration for infected cells and in duplicate wells per concentration for uninfected cells.

Assay plates were incubated at 37 °C in a humidified, 5% CO_2 atmosphere and examined microscopically for toxicity and/or cytopathogenic effect. Once the viral cytopathogenic effect was maximal (the eighth day post-infection), the surviving cells in each test well were quantified using the MTT assay.[16] Cytoprotection and toxicity are reported as the concentration of drug required to inhibit viral-mediated cytopathicity by 50% of infected controls (ID_{50}) and to cause reduction of cell growth by 50% of uninfected controls (TD_{50}), respectively. When a dose-dependent effect for either anti-HIV activity or cytotoxicity was observed, values for the 50% effective dose were calculated using the dose–effect analysis software of Chou and Chou (Elsevier-Biosoft).

Acknowledgments

We are grateful to the National Institutes of Health (Grant No. AI43687) for their generous support of this research. We thank Dr. Jonathan Coates of AMRAD Operations Pty Ltd (Victoria, Australia) for the gift of an authentic sample of conocurvone. CEM-T_4 cells were obtained from Dr. J. P. Jacobs through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. We also thank University of Notre Dame Mass Spectrometry Facility for assistance with low- and high-resolution mass spectra. This research was supported [in part] by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

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