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Synthesis and cytotoxicity of new heterocyclic terpenylnaphthoquinones

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Abstract—Several 2-arylamino-, 2-aryloxy- and 2-arylsulfanyl-6(7)-alkyl-1,4-naphthoquinones (NQ) have been prepared and further transformed into the corresponding heterocyclic-fused naphthoquinones by palladium (II)-catalyzed oxidative cyclization. The compounds synthesized have been evaluated against neoplastic cell lines. The extension of the polycyclic system clearly decreased the cytotoxic potency of the 2-substituted terpenylnaphthoquinones.

1. Introduction

Many planar tri- and tetracyclic quinones have been found to display useful antineoplastic activity, acting as intercalating or alkylating agents. Anthracyclines, mitomycin C and kinamycins are examples of natural and semi-synthetic drugs of this type.[1,2]

The antitumor properties and the mechanism of action of quinone derivatives have been widely studied, and it is known that they can act as topoisomerase inhibitors, via DNA intercalation and reduction of the quinone moiety by oxido-reductases (DT-diaphorase).[3–5] Furthermore, the presence of heteroatoms in the planar structure could contribute to an enhancement of cytotoxicity through enabling hydrogen bonding to DNA.[6]

Additionally, a large number of biologically and pharmacologically active compounds share the 2-phenylnaphthalene structural pattern in a planar conformation, embedded or not in a polycyclic system that seems to be responsible for their bioactivity.[7] Based on these facts, several compounds with a heterocyclic ring attached to a 1,4-naphthoquinone (1,4-NQ) moiety have been designed and evaluated as antineoplastics, such as the benzo[*b*]naphtho[2,3-*d*]furan-6,11-diones and benzo[*b*]carbazole-6,11-diones synthesized by Cheng.[8–10] The same tetracyclic chromophore, benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione, appears in some human telomerase inhibitors.[11] All these facts seemed to justify the design and synthesis of new heterocycle-fused naphthoquinone derivatives.

In the last few years, we have synthesized several monoterpenyl- and diterpenylnaphthoquinones through Diels–Alder addition between natural terpenoids and *p*-benzoquinones.[12] Further transformations on the side chain led to derivatives with IC_{50} values in the μM range against several tumor cell lines. Also, some of them showed a moderate selectivity against P-388 and MEL-28 cell lines. All these results prompted us to study the effect of the introduction of other substituents on the naphthoquinone ring. We had already prepared several derivatives with hydroxyl[13] and amino[14] groups at C-2 and here we describe the preparation and evaluation of new 1,4-NQs bearing fused heterocyclic rings, with the aim of analyzing the influence of the enlargement of the heteropolycyclic system on their cytotoxicity.

2. Results and discussion

2.1. Chemistry

The heterocyclic-fused quinones, which were the objective of this study, are represented by the general

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structure **A** (Scheme 1) and included benzo[*b*]carbazole-6,11-diones, benzo[*b*]naphtho[2,3-*d*]furan-6,11-diones and benzo[*b*]acridine-6,11-diones. Among the various procedures described to obtain this type of heterocyclic quinone, we have chosen the palladium (II)-catalyzed oxidative cyclization of the corresponding diaryl derivatives **B**, because this procedure had provided very good yields in the synthesis of carbazole derivatives from *N*,*N*-diarylamines.[15,16]

The corresponding diarylderivatives **B** can easily be obtained, through addition or substitution reactions, from the monoterpenylnaphthoquinones (MTNQs) **I** and **II**, previously synthesized in our laboratory. We have already used these nucleophilic processes to obtain several 2-aryl(alkyl)amino-MTNQs with potent cytotoxicity.[14] The 1,4-NQs used as starting materials were the naphthoquinone **I** for addition reactions and its chloroderivative **II** for substitution reactions. MTNQs **I** and **II** were obtained by Diels–Alder cycloaddition between α-myrcene and *p*-benzoquinone or 2,5-dichlorobenzoquinone, respectively, as previously reported by us [12–14] (Scheme 2).

The addition of substituted anilines to **I** was performed in methanol at room temperature [17] to produce derivatives **1**(**a**–**d**) and **2**(**a**–**d**) (Scheme 3) in moderate yields (41–60%). In the case of the 2-aminoacetophenones **e** and **f**, the addition was performed in the presence of cerium trichloride.[18] As the starting MTNQs are asymmetrically substituted, all the reaction products consisted of mixtures of the two regioisomers in a 1:1 ratio, although they were separated by column chromatography in quantities sufficient to permit their spectroscopic characterization and biological evaluation. Previous bidimensional NMR experiments performed with similar anilino-1,4-NQs[14], complemented with the experiments done with **2f**, allowed us to establish the location of the alkyl side chain at C-6 for isomer **1**, which was eluted first in the chromatographies, and at C-7 for isomer **2**.

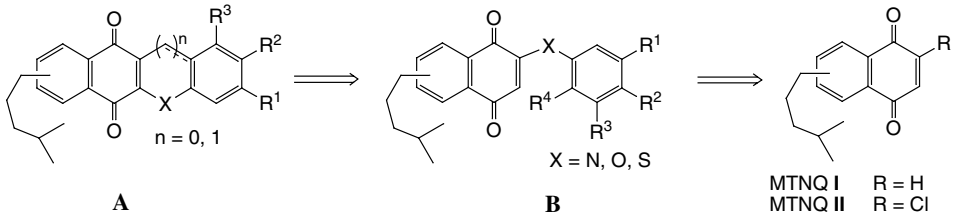
The phenoxy-1,4-NQs **3** and **4** could not be prepared by nucleophilic addition to **I** under a range of reaction conditions; however, they were easily obtained, in acceptable to good yields (30–90%), by nucleophilic substitution from 1,4-NQ **II** in the presence of sodium carbonate in DMSO.

The addition of thiophenols to **I** was performed in ethanol at room temperature to give **5** and **6** in relatively low yields. Attempts to improve these yields led us to treat the NQ **II** with *p*-methylthiophenol and only 1, 4-NQ **7** was isolated, in which both addition and substitution reactions had taken place.

The palladium-mediated cyclization of *N*,*N*-diarylamines was originally reported by Akermark[19] using stoichiometric amounts of the metal, due to consumption of Pd(II) during the oxidative cyclization. Further studies performed by Knolker[20] showed that a catalytic cyclization was feasible by reoxidation of Pd(0) to Pd(II) with cupric acetate. We have applied the latter procedure to anilino-NQs **1**(**a**–**d**) and **2**(**a**–**d**), either as mixtures of regioisomers or separately as indicated in Scheme 4. By this method the benzocarbazolequinones **8** and **9** were obtained in very good yields (88–97%).

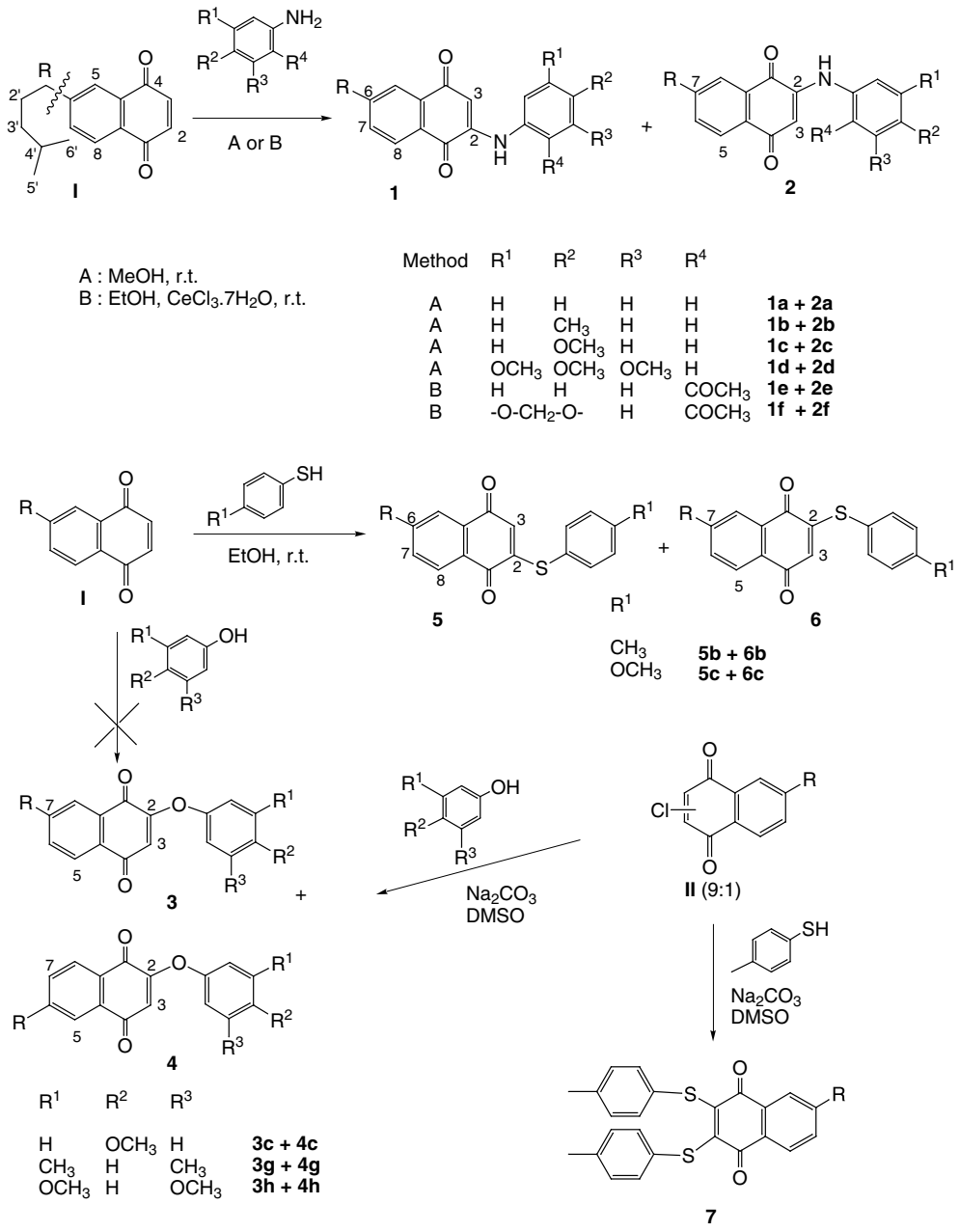
The benzoacridines **10** and **11** were obtained in good yields (85–87%) by cyclization of (**1e** + **2e**) and (**1f** + **2f**) in acidic media.[18] They were mixtures of the two regioisomers, which were separated by chromatographic techniques to allow their individual characterization.

By far, the largest class of heterocyclic quinones are those containing nitrogen,[21] and synthetic procedures for these are very well documented in the literature; however, only a few reports concerning the synthesis of oxygen or sulfur containing heterocyclic quinones[16] have been published. Those dealing with the formation of a fused five-membered heterocyclic ring on the quinone moiety are even less well precedented, and



Scheme 1. Retrosynthetic scheme to the heterocyclic naphthoquinones.

Scheme 2. Synthesis of monoterpenylquinones **I** and **II**.



Scheme 3. Preparation of 6(7)-alkyl-2-arylamino/aryloxy/arylsulfanyl-1,4-naphthoquinones **1**–**7**.

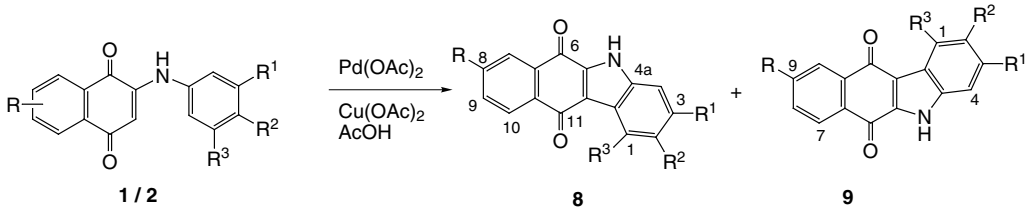
syntheses are commonly based on the use of 1,4-NQs doubly substituted at C-2 and C-3 with halogen atoms.[8,10,11] Having several monosubstituted 1,4-NQs in hand, we applied the same oxidative coupling/cyclization process, described above for the carbazole derivatives, to obtain the corresponding furan analogues.

The use of palladium acetate in the presence of cupric acetate did not yield the expected cyclized products, but the use of palladium acetate alone and in excess (4 equiv) led to the corresponding desired furane-fused quinones **12** and **13**, although in relative low yields (see Scheme 5).

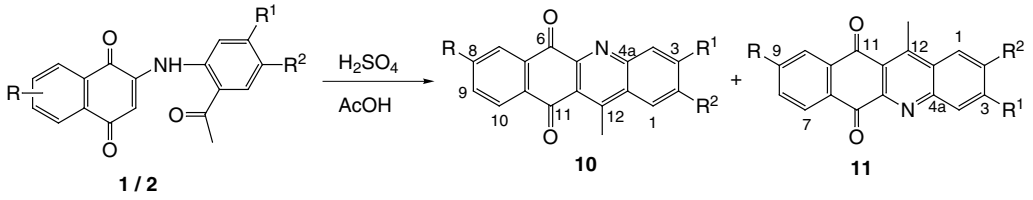
In contrast to the Pd-catalyzed preparation of indoles and benzofurans, the application of these methodologies to the synthesis of benzothiophenes has been much less explored and only a few examples of intramolecular Heck reactions have been reported.[22] In fact, when the same two procedures described above were applied to the phenylthio-1,4-NQs **5/6**, only very complex reaction products were obtained, probably due to the thiophilicity of the palladium species and the 'poisoning effects' of the sulfur atom of thiophenes and benzothiophenes on the catalyst.[15]

2.2. Biological evaluation

Most of the compounds prepared were evaluated in vitro to establish their cytotoxicity against the following tumoural cell lines: MDA-MB-231 breast cancer, A-549 lung carcinoma and HT-29 colon adenocarcinoma.

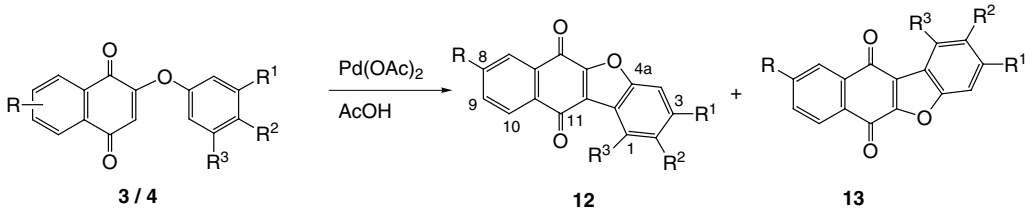


Substrates	R^1	R^2	R^3	Products
1c + 2c (2:1)	H	OCH_3	H	**8c + 9c**
1d + 2d (1:2)	OCH_3	OCH_3	OCH_3	**8d + 9d**
1a	H	H	H	**9a**
2a	H	H	H	**8a**
1b	H	CH_3	H	**9b**
2b	H	CH_3	H	**8b**
1c	H	OCH_3	H	**9c**
2c	H	OCH_3	H	**8c**
1d	OCH_3	OCH_3	OCH_3	**9d**



Substrates	R^1	R^2	Products
1e + 2e (1:1)	H	H	**10e + 11e**
1f + 2f (1:1)	$-O-CH_2-O-$		**10f + 11f**

Scheme 4. Preparation of benzocarbazolequinones **8** and **9**, and benzoacridines **10** and **11**.



Substrates	R^1	R^2	R^3	Products
3c + 4c (9:1)	H	OCH_3	H	**12c + 13c**
3g + 4g (8:1)	CH_3	H	CH_3	**12g + 13g**
3h + 4h (9:1)	OCH_3	H	OCH_3	**12h + 13h**

Scheme 5. Preparation of benzonaphthofuranequinones **12** and **13**.

A conventional colourimetric assay[23] was set up to estimate the GI_{50} values, i.e., the drug concentration that causes 50% cell growth inhibition after 72 h continuous exposure to the test molecules. The results obtained are shown in Table 1, expressed in μM. From the data some general observations can be made.

The presence of substituents at position C-2 of the 1,4-NQ ring improved the cytotoxicity in comparison with the unsubstituted quinone (**1**, **3**, **4** and **5** vs **I**), as we have already described.[14] However, it is interesting to analyze the different effects observed depending on the regioisomer considered, especially for the arylamino substituents: the 6-alkyl regioisomers **1** were, in general, more potent than the corresponding 7-alkyl derivatives **2**. The observed cytotoxicity also depends upon the substituent on the anilino moiety. Thus, the presence of the acetyl group led to a decrease in cytotoxicity (**e** and **f** vs **c** and **d**), while the presence of electron-donating groups, such as the methoxyl, led to an increase in potency, **1c** being the most potent compound in these series. The aryloxy- and the arylsulfanyl-1,4-NQs kept the cytotoxicity at the same level as the arylamino-1,4-NQs.

Unexpectedly, the heterocyclized compounds turned out to be much less potent than their acyclic analogues. The cyclization to the extended polycyclic quinones led to compounds which were almost inactive at the highest concentration tested, independent of the heteroatom or the size of ring considered.

Table 1. Cytotoxicity data (GI_{50} in μM) for 1,4-NQ derivatives **1**–**12**

Compound	MB-231	A549	HT-29
I		>12.4	>12.4
1a		7.5	2.1
2a		>9.0	>9.0
1b		3.2	3.5
2b		>8.6	>8.6
1c	0.5	0.4	0.6
2c	2.0	6.8	4.7
1d	1.2	1.0	1.3
2d	3.1	3.8	3.3
1e	>26.6	>26.6	>26.6
2e	25.8	18.1	>26.6
1f	>23.9	>23.9	>23.9
2f	>23.9	>23.9	>23.9
3c	4.4	2.0	4.9
3g	2.2	1.9	5.2
3h	1.9	2.5	4.1
5b	1.9	1.5	4.9
6b	3.8	1.9	8.5
6c	2.9	1.7	1.0
7	>20.6	>20.6	>20.6
9a	>30.2	>30.2	>30.2
8b	>29.0	>29.0	>29.0
9b	>29.0	>29.0	>29.0
8c	>27.7	>27.7	>27.7
9c	>27.7	>27.7	>27.7
9d	>23.7	>23.7	>23.7
10e	>28.0	>28.0	>28.0
11e	>28.0	>28.0	>28.0
10f	>27.9	>27.9	>27.9
11f	>27.9	>27.9	>27.9
12c	>27.6	>27.6	>27.6
12h	>25.5	>25.5	>25.5

In summary, we have prepared a number of 6(7)-alkyl-1,4-naphthoquinones substituted at C-2 with arylamino, aryloxy and arylsulfanyl groupings, through nucleophilic processes, either addition or substitution reactions. These substituents at position C-2 of the 1,4-NQ increase the bioactivity of the parent 1,4-NQ **I**. These derivatives were further transformed into the corresponding heterocyclic quinones by palladium (II)-catalyzed oxidative cyclization to give tetracyclic quinones, which were practically inactive against the neoplastic cells tested.

3. Experimental

3.1. Chemistry

IR spectra were obtained on a Nicolet (Impact 410) spectrophotometer in NaCl film. NMR spectra were recorded at 200 MHz for ^{1}H and 50.3 for ^{13}C in deuterochloroform using TMS as internal reference, on a Bruker AC 200. Chemical shift values are expressed in ppm followed by multiplicity and coupling constants (*J*) in Hz. Column chromatography (CC) was performed on silica gel (Merck No 9385). HRMS were run on a VG TS-250 spectrometer working at 70 eV. TLC were carried out on silica gel 60 F_{245} (Merck, 0.25 mm thick). Solvents and reagents were purified by standard procedures as necessary.

3.1.1. 1,4-Naphthoquinones I, II, 1(a,b) and 2(a,b). Compounds **I**, **II**, **1**(**a**,**b**) and **2**(**a**,**b**) were obtained followed the procedures described before.[14]

3.1.2. General procedures for the nucleophilic addition. *Method A*. To a stirred solution of naphthoquinone **I** (0.90 mmol) in methanol (10 mL) was added the corresponding amine (1.10 mmol). The mixture was stirred at room temperature and monitored by TLC until compound **I** disappeared (3–8 days). After removing the solvent, the crude product was purified by column chromatography over silica gel.

Method B. In an open flask, a mixture of naphthoquinone **I** (0.90 mmol), amine (0.90 mmol) and $CeCl_3 \cdot 7$-H_2O (0.40 mmol) in ethanol (10 mL) was stirred at room temperature for several days (10–14 days) until compound **I** disappeared. Then, the solvent was evaporated off and the residue was redissolved in dichloromethane. The organic layer was washed with 2 N HCl and brine, dried over Na_2SO_4, filtered and evaporated until dryness, affording a crude product, which was purified by column chromatography over silica gel.

3.1.3. 2-(*p*-Methoxyanilino)-6-(4-methylpentyl)-1,4-naphthoquinone 1c and 2-(*p*-methoxyanilino)-7-(4-methylpentyl)-1,4-naphthoquinone 2c. Following the method A, treatment of **I** with *p*-anisidine gave after column chromatography (eluent: dichloromethane/ethyl acetate 98:2): (a) 30 mg (9%) of **1c**. IR: 3276, 2954, 2869, 1679, 1588, 1567, 826 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 364.1913, found 364.1879. (b) 86 mg (26%) of mixture of **1c** and **2c**. (c) 19 mg (6%) of **2c**. IR: 3316, 2951, 1668, 1597, 1567, 831 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 364.1913, found 364.1921.

3.1.4. 6-(4-Methylpentyl)-2-(3,4,5-trimethoxyanilino)-1,4-naphthoquinone 1d and 7-(4-methylpentyl)-2-(3,4,5-trimethoxyanilino)-1,4-naphthoquinone 2d. Following the method A, the reaction between **I** and 3,4,5-trimethoxyaniline yielded after chromatographic purification (eluent: dichloromethane/ethyl acetate 96:4 and 9:1): (a) 71 mg (18%) of **1d**. IR: 3318, 2953, 2867, 1671, 1592, 1571, 835 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 424.2124, found 424.2154. (b) 93 mg (24%) of mixture of **1d** and **2d**. (c) 10 mg (3%) of **2d**. IR: 3325, 2953, 2934, 1673, 1594, 1567, 829 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 424.2124, found 424.2173.

3.1.5. 2-(2-Acetylanilino)-6-(4-methylpentyl)-1,4-naphthoquinone 1e and 2-(2-acetylanilino)-7-(4-methylpentyl)-1,4-naphthoquinone 2e. Following the method B, the reaction between **I** and 2-aminoacetophenone afforded after column chromatography (eluent: dichloromethane/acetone 98:2): (a) 24 mg (7%) of **1e**. IR: 2953, 2928, 2867, 1672, 1597, 1572, 756 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 376.1913, found 376.1927. (b) 64 mg (19%) of mixture of **1e** and **2e**. (c) 9 mg (3%) of **2e**. IR: 2953, 2926, 2867,

1673, 1610, 1569, 756 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 376.1913, found 376.1892.

3.1.6. 2-(6-Acetylbenzo[1,3]dioxol-5-ylamino)-6-(4-methylpentyl)-1,4-naphthoquinone 1f and 2-(6-acetylbenzo[1,3]dioxol-5-ylamino)-7-(4-methylpentyl)-1,4-naphthoquinone 2f. Application of method B, between **I** and 6-amino-3,4-methylenedioxyacetophenone, yielded after chromatographic purification (eluent: dichloromethane): (a) 20 mg (12%) of **1f**. IR: 2946, 2916, 1673, 1586, 1567, 851 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 420.1811, found 420.1841. (b) 74 mg (43%) of mixture of **1f** and **2f**. (c) 8 mg (5%) of **2f**. IR: 2951, 2918, 2867, 1672, 1587, 1566, 847 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 420.1811, found 420.1844.

3.1.7. 6-(4-Methylpentyl)-2-(*p*-tolylsulfanyl)-1,4-naphthoquinone 5b and 7-(4-methylpentyl)-2-(*p*-tolylsulfanyl)-1,4-naphthoquinone 6b. A solution of **I** (296 mg, 1.22 mmol) and 4-methylthiophenol (110 mg, 0.89 mmol) in ethanol (5 mL) was stirred at room temperature for 12 h. Then, the solvent was evaporated off and the residue was purified by column chromatography over silica gel (eluent: hexane/ethyl acetate 1:1), yielding: (a) 17 mg (4%) of **5b**. IR: 2953, 2927, 2867, 1664, 1599, 1557, 850 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 365.1575, found 365.1602. (b) 148 mg (33%) of mixture of **5b** and **6b**. (c) 8 mg (2%) of **6b**. IR: 2953, 2926, 2867, 1666, 1601, 1558, 834 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 365.1575, found 365.1548.

3.1.8. 2-(4-Methoxyphenylsulfanyl)-6-(4-methylpentyl)-1,4- naphthoquinone 5c and 2-(4-methoxyphenylsulfanyl)-7-(4-methylpentyl)-1,4-naphthoquinone 6c. A mixture of **I** (122 mg, 0.50 mmol) and 4-methoxythiophenol (32 μL, 0.26 mmol) in ethanol (2 mL) was stirred at room temperature for 14 h. Then, the solvent was evaporated off and the residue was purified by column chromatography over silica gel (eluent: hexane/dichloromethane 2:8), affording: (a) 31 mg (16%) of **5c**. IR: 2953, 2933, 2867, 1663, 1598, 1557, 850 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 381.1524, found 381.1538. (b) 19 mg (10%) of mixture of **5c** and **6c**. (c) 5 mg (3%) of **6c**. IR: 2953, 2933, 2867, 1666, 1594, 1560, 835 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 381.1524, found 381.1504.

3.1.9. 2-(4-Methoxyphenoxy)-7-(4-methylpentyl)-1,4-naphthoquinone 3c and 2-(4-methoxyphenoxy)-6-(4-methylpentyl)-1,4-naphthoquinone 4c. *General procedure for nucleophilic substitution.* A 9:1 mixture of **II** (0.55 mmol) in dimethylsulfoxide (2 mL) was treated with the corresponding nucleophile (1.96 mmol) and Na_2CO_3 (2.31 mmol). The reaction was stirred at room temperature and monitored by TLC until mixture **II** disappeared (0.6–1.5 h). Then, ethyl acetate was added and the organic layer was washed with 2 N HCl and brine, dried over Na_2SO_4, filtered and concentrated at vacuum. The crude product was redissolved in dichloromethane, washed with aq 10% NaOH several times and then neutralized with brine, dried and evaporated off. The residue was purified by column chromatography over silica gel.

Following the described general method, the reaction between **II** and *p*-methoxyphenol yielded after chromatographic purification (eluent: hexane/ethyl acetate 8:2): 137 mg (90%) of a 9:1 mixture of **3c** and **4c**. IR: 2953, 2932, 2867, 1682, 1651, 1614, 1504, 845 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 365.1753, found 365.1777.

3.1.10. 2-(3,5-Dimethylphenoxy)-7-(4-methylpentyl)-1,4-naphthoquinone 3g and 2-(3,5-dimethylphenoxy)-6-(4-methylpentyl)-1,4-naphthoquinone 4g. Application of the general method for nucleophilic substitution, between **II** and 3,5-dimethylphenol, afforded by chromatographic purification (eluent: hexane/dichloromethane 1:1): 65 mg (32%) of a 8:1 mixture of **3g** and **4g**. IR: 2953, 2928, 2867, 1682, 1651, 1614, 1468, 849 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 363.1960, found 363.1941.

3.1.11. 2-(3,5-Dimethoxyphenoxy)-7-(4-methylpentyl)-1,4- naphthoquinone 3h and 2-(3,5-dimethoxyphenoxy)-6-(4-methylpentyl)-1,4-naphthoquinone 4h. Following the above described method for nucleophilic substitution, the reaction between **II** and 3,5-dimethoxyphenol afforded by column chromatography (eluent: hexane/dichloromethane 5:95): 68 mg (31%) of a 9:1 mixture of **3h** and **4h**. IR: 2953, 2934, 2867, 1682, 1651, 1588, 1470, 842 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 395.1858, found 395.1854.

3.1.12. 6-(4-Methylpentyl)-2,3-bis-(*p*-tolylsulfanyl)-1,4-naphthoquinone 7. Following the general method for nucleophilic substitution, the reaction between **II** and 4-methylthiophenol afforded by chromatographic purification (eluent: hexane/dichloromethane 6:4): 87 mg (45%) of **7**. IR: 2952, 2924, 2866, 1673, 1662, 1600, 1503, 814 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 487.1765, found 487.1816.

3.1.13. 2-Methoxy-8-(4-methylpentyl)-5H-benzo[*b*]carbazole-6,11- dione 8c and 2-methoxy-9-(4-methylpentyl)-5*H*-benzo[*b*]carbazole-6,11-dione 9c. *General procedure for the preparation of benzocarbazole derivatives.* To a stirred solution of the arylamine (1 equiv) in acetic acid (5 mL) were added copper acetate (II) (2.6 equiv) and palladium acetate (II) (1 equiv). The reaction mixture was stirred at 120 °C for 23 h. To the cooled reaction was added water and ethyl acetate. The organic layer was separated and washed with aq 10% NaOH and brine, dried over Na_2SO_4, filtered and evaporated off yielding the desired product.

A 2:1 mixture of **1c** and **2c** was treated following the above described method, affording after column chromatography (eluent: dichloromethane/ethyl acetate 95:5): (a) 24 mg (28%) of **8c**. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd

Table 2. ^{1}H NMR ($CDCl_3$-TMS, δ ppm, (J Hz)) data for NQs **1**–**13**

H	**1a**	**2a**	**1b**	**2b**	**1c**	**2c**	**1d**
3	6.38 s	6.40 s	6.32 s	6.31 s	6.19 s	6.19 s	6.29 s
5	7.89 d (1.8)	8.01 d (8.0)	7.90 d (1.8)	7.99 d (7.8)	7.89 d (1.8)	8.00 d (7.8)	7.87 d (1.8)
6		7.55 dd (8.0; 1.8)		7.54 dd (7.8; 1.8)		7.55 dd (7.8; 1.8)	
7	7.42 dd (7.8; 1.8)		7.44 dd (7.7; 1.8)		7.43 dd (7.8; 1.8)		7.42 dd (7.7; 1.8)
8	7.98 d (7.8)	7.91 d (1.8)	8.00 d (7.7)	7.90 d (1.8)	7.99 d (7.8)	7.91 d (1.8)	7.97 d (7.7)
1′	2.69 t (7.7)	2.72 t (7.7)	2.70 t (7.7)	2.70 t (7.7)	2.69 t (7.7)	2.71 t (7.7)	2.68 t (7.7)
2′	1.64 m	1.66 m	1.66 m	1.66 m	1.65 m	1.64 m	1.64 m
3′	1.22 m	1.22 m	1.24 m	1.25 m	1.23 m	1.23 m	1.20 m
4′	1.53 m	1.52 m	1.54 m	1.55 m	1.56 m	1.56 m	1.51 m
5′, 6′	0.86 d (6.6)	0.89 d (6.6)	0.87 d (6.6)	0.88 d (6.6)	0.86 d (6.6)	0.88 d (6.6)	0.85 d (6.6)
Others	7.14-7.43 m	7.16-7.45 m	7.20 d (8.4)	7.21 d (8.6)	7.18 d (8.8)	7.20 d (9.1)	6.47 s
			7.14 d (8.4)	7.14 d (8.6)	6.92 d (8.8)	6.94 d (9.1)	3.84 s
			2.35 s	2.35 s	3.81 s	3.83 s	3.83 s
H	**2d**	**1e**	**2e**	**1f**	**2f**	**3c**	**4c**
3	6.32 s	6.70 s	6.71 s	6.59 s	6.59 s	5.91 s	5.92 s
5	8.02 d (7.7)	7.89 d (1.8)	8.00 d (8.0)	7.89 d (1.6)	8.00 d (7.9)	7.96 d (7.7)	7.86 d (1.8)
6	7.57 dd (7.7; 1.8)		7.55 dd (8.0; 1.8)		7.55 dd (7.9; 1.7)	7.55 dd (7.7; 1.8)	
7		7.47 dd (7.9; 1.8)		7.47 dd (7.7; 1.6)			—
8	7.92 d (1.8)	8.06 d (7.9)	7.96 d (1.8)	8.05 d (7.7)	7.96 d (1.7)	7.96 d (1.8)	8.10 d (8.0)
1′	2.72 t (7.7)	2.71 t (7.7)	2.72 t (7.7)	2.71 t (7.7)	2.72 t (7.7)	2.74 t (7.7)	2.74 t (7.7)
2′	1.67 m	1.65 m	1.64 m	1.66 m	1.68 m	1.68 m	1.68 m
3′	1.24 m	1.22 m	1.23 m	1.22 m	1.24 m	1.23 m	1.23 m
4′	1.54 m	1.57 m	1.55 m	1.54 m	1.57 m	1.56 s	1.56 s
5′, 6′	0.89 d (6.6)	0.87 d (6.6)	0.88 d (6.6)	0.87 d (6.6)	0.87 d (6.6)	0.88 d (6.6)	0.87 d (6.6)
Others	6.49 s	7.15-7.64 m	7.16-7.65 m	7.32 s	7.32 s	7.05 d (9.1)	7.05 d (9.1)
	3.87 s	2.68 s	2.69 s	7.11 s	7.11 s	6.95 d (9.1)	6.95 d (9.1)
	3.86 s			2.58 s	2.59 s	3.83 s	3.84 s
				6.07 s	6.08 s		
H	**3g**	**4g**	**3h**	**4h**	**5b**	**6b**	**5c**
3	5.93 s	5.93 s	6.01 s	6.01 s	6.08 s	6.08 s	6.06 s
5	7.95 d (8.0)	7.58 sa	7.93 d (7.9)	7.84 d (1.8)	7.83 d (1.8)	7.93 d (8.0)	7.82 d (1.8)
6	7.45 dd (8.0; 1.8)		7.53 dd (7.9; 1.8)			7.53 dd (8.0; 1.8)	
7		7.54 m		7.51 dd (8.0; 1.8)	7.50 dd (7.9; 1.8)		7.49 dd (8.0; 1.8)
8	7.98 d (1.8)	8.08 d (8.0)	7.95 d (1.8)	8.07 d (8.0)	8.04 d (7.9)	7.93 d (1.8)	8.03 d (8.0)
1′	2.73 t (7.7)	2.73 t (7.7)	2.71 t (7.7)	2.70 t (7.3)	2.71 t (7.7)	2.72 t (7.7)	2.70 t (7.7)
2′	1.68 m	1.68 m	1.65 m	1.65 m	1.66 m	1.66 m	1.65 m
3′	1.23 m	1.23 m	1.22 m	1.22 m	1.22 m	1.23 m	1.20 m
4′	1.54 s	1.54 s	1.56 s	1.56 s	1.56 m	1.55 s	1.52 s
5′, 6′	0.88 d (6.6)	0.86 d (6.6)	0.86 d (6.6)	0.85 d (6.6)	0.87 d (6.6)	0.88 d (6.6)	0.86 d (6.6)
Others	6.72 sa	6.72 sa	6.27 d (2.2)	6.27 d (2.2)	7.41 d (8.0)	7.41 d (8.0)	7.43 d (8.8)
	6.91 sa	6.91 sa	6.36 d (2.2)	6.36 d (2.2)	7.30 d (8.0)	7.29 d (8.0)	7.00 d (8.8)
	2.32 s	2.32 s	3.76 s	3.76 s	2.42 s	2.42 s	3.86 s

H	6c	7	H	8a	9a	8b	9b
3	6.06 s		1	8.39 d (7.3)	8.17 d (8.0)	8.18 sa	8.15 sa
5	7.93 d (8.0)	7.79 sa	2	7.33-7.56 m	7.33 m		
6	7.53 dd (8.0; 1.8)		3	7.33-7.56 m	7.42 m	7.26 dd (8.6; 1.8)	7.23 d (8.0)
7		7.46 d (7.8)	4	7.33-7.56 m	7.57 d (8.0)	7.42 d (8.6)	7.43 m
8	7.93 d (1.8)	7.88 d (7.8)	7	7.97 sa	7.96 d (8.0)	7.96 d (1.8)	8.03 m
1′	2.72 t (7.7)	2.66 t (7.7)	8		7.35 dd (8.0; 1.7)		7.43 m
2′	1.67 m	1.62 m	9	7.33-7.56 m		7.54 dd (8.0; 1.8)	
3′	1.24 m	1.21 m	10	8.14 d (8.0)	7.87 d (1.7)	8.14 d (8.0)	8.02 m
4′	1.55 s	1.49 s	1′	2.72 t (7.7)	2.70 t (7.6)	2.73 t (7.7)	2.73 t (7.7)
5′, 6′	0.88 d (6.6)	0.87 d (6.6)	2′	1.68 m	1.62 m	1.70 m	1.69 m
Others	7.44 d (8.8)	7.30 d (8.0)	3′	1.25 m	1.17 m	1.26 m	1.26 m
	7.01 d (8.8)	7.11 d (8.0)	4′	1.54 m	1.51 m	1.58 m	1.56 m
	3.87 s	2.34 s	5′, 6′	0.89 d (6.2)	0.84 d (6.5)	0.89 d (6.6)	0.89 d (6.6)
			Others			2.50 s	2.49 s

H	8c	9c	8d	9d	12c/13c	12g/13g
1	7.70 d (2.6)	7.72 d (2.5)			7.64 d (2.7)	
2						7.29 sa
3	7.03 dd (9.1; 2.6)	7.07 dd (9.1; 2.5)			7.14 dd (9.1; 2.7)	
4	7.40 d (9.1)	7.42 d (9.1)	6.81 s	6.76 s	7.55 d (9.1)	7.06 sa
7	7.89 d (1.8)	8.02 d (8.0)	7.88 d (1.8)	7.87 d (7.8)	8.03 d (1.8)/8.13 d (7.7)	8.02 d (1.8)/8.12 d (8.0)
8		7.45 dd (8.0; 1.8)		7.36 dd (7.8; 1.6)	–/7.56 m	– / 7.54 dd (8.0; 1.8)
9	7.50 dd (8.0; 1.8)		7.50 dd (8.0; 1.8)		7.56 dd (7.8; 1.8) /–	7.56 dd (8.0; 1.8)/–
10	8.09 d (8.0)	8.00 d (1.8)	8.15 d (8.0)	8.00 d (1.6)	8.10 d (7.8)/7.99 d (1.8)	8.12 d (8.0)/8.02 d (1.8)
1′	2.71 t (7.7)	2.73 t (7.7)	2.66 t (7.7)	2.66 t (7.7)	2.74 t (7.7)	2.74 t (7.7)
2′	1.66 m	1.66 m	1.66 m	1.64 m	1.70 m	1.70 m
3′	1.26 m	1.23 m	1.26 m	1.22 m	1.25 m	1.25 m
4′	1.57 m	1.58 m	1.50 m	1.55 m	1.57 m	1.55 m
5′, 6′	0.90 d (6.6)	0.90 d (6.6)	0.88 d (6.6)	0.87 d (6.6)	0.89 d (6.6)	0.89 d (6.6)
Others	3.90 s	3.92 s	4.08 s	4.07 s	3.92 s	2.47 s[a]
			3.94 s	3.94 s		2.92 s[a]
			3.91 s	3.85 s		

H	12h/13h	10e	11e	10f	11f
1		8.46 dd (8.6; 1.5)	8.45 dd (8.4; 1.5)	7.68 s	7.61 s
2	6.73 d (1.8)	7.91 ddd (8.6; 6.9; 1.5)	7.90 ddd (8.4; 6.9; 1.5)		
3		7.77 ddd (8.6; 6.9; 1.5)	7.77 ddd (8.4; 6.9; 1.5)		
4	6.45 d (1.8)	8.37 dd (8.6; 1.5)	8.37 m	7.51 s	7.43 s
7	8.00 d (1.8)/8.10 d (7.7)	8.21 d (1.8)	8.32 d (8.0)	8.15 d (1.8)	8.23 d (7.8)
8	—/7.54 m		7.62 dd (8.0; 1.8)		7.56 d (7.8)
9	7.54 dd (7.9; 1.8)/—	7.66 dd (8.0; 1.8)		7.62 dd (8.0; 1.8)	
10	8.12 d (7.9)/8.02 d (1.2)	8.24 d (8.0)	8.12 d (1.8)	8.18 d (8.0)	8.03 sa
1′	2.73 t (7.7)	2.79 t (7.7)	2.79 t (7.7)	2.77 t (7.7)	2.75 t (7.7)
2′	1.70 m	1.70 m	1.70 m	1.71 m	1.68 m
3′	1.26 m	1.26 m	1.27 m	1.26 m	1.26 m
4′	1.50 m	1.56 m	1.58 m	1.54 m	1.50 m
5′, 6′	0.88 d (6.6)	0.89 d (6.6)	0.89 d (6.6)	0.89 d (6.6)	0.88 d (6.6)
Others	3.88 s[a]	3.31 s	3.30 s	3.14 s	3.07 s
	4.03 s[a]			6.21 s	6.17 s

[a] Exchangeable assignments.

Table 3. ^{13}C NMR ($CDCl_3$-TMS, δ ppm) data for NQs **1–13**

C	1a	2a	1b	2b	1c	2c	1d	2d	1e	2e	1f	2f	3c	4c
1	181.7	182.4	181.9	182.5	181.9	182.6	181.7	182.4	181.5	182.1	181.4	182.0	180.3	179.8
2	144.8	144.7	145.2	145.0	145.8	145.7	145.2	145.2	144.1	144.0	144.2	144.1	146.0	150.6
3	103.2	103.5	102.9	103.0	102.3	102.5	103.2	103.5	106.3	106.5	106.0	106.2	113.0	113.0
4	184.3	184.2	184.3	184.0	184.2	184.0	184.2	184.1	185.0	184.8	184.8	184.5	184.9	185.3
4a	133.3	131.2	133.4	131.3	133.5	131.4	133.3	131.2	—	130.8	132.9	130.8	131.0	131.9
5	126.1	126.4	126.1	126.3	126.1	126.4	126.1	126.4	125.9	126.3	125.9	126.2	126.3	125.9
6	151.3	135.1	151.3	135.0	151.3	135.1	151.4	135.1	151.0	134.8	151.0	134.8	134.4	149.1
7	132.4	148.2	132.3	148.1	132.3	148.1	132.4	148.3	134.2	148.6	132.7	148.5	149.4	133.5
8	126.9	126.4	126.9	126.3	126.8	126.4	126.8	126.5	127.2	126.7	127.2	126.7	126.4	126.9
8a	128.3	130.4	128.3	130.2	128.4	130.5	128.2	130.4	128.6	130.6	128.6	130.6	129.8	129.0
1′	36.6	36.2	36.7	36.2	36.7	36.3	36.7	36.3	36.7	36.3	36.7	36.3	36.2	36.2
2′	28.8	28.9	28.8	28.9	28.8	28.9	28.8	28.9	28.8	28.9	28.8	28.9	28.8	28.8
3′	38.5	38.5	38.6	38.5	38.6	38.5	38.5	38.6	38.6	38.5	38.6	38.5	38.4	38.4
4′	27.8	27.9	27.9	27.9	27.9	27.9	27.9	28.0	27.9	27.9	27.9	27.9	27.8	27.8
5′, 6′	22.6	22.6	22.6	22.6	22.6	22.7	22.6	22.6	22.6	22.6	22.6	22.6	22.5	22.5
1″	137.6	137.7	135.5	135.5	130.2	130.2	136.0	—	140.4	140.5	137.7	137.8	157.7	157.7
2″	129.7	129.7	130.2	130.2	124.8	124.9	100.7	100.8	125.7	125.7	119.3	119.3	121.9	121.9
3″	122.6	122.6	122.7	122.7	114.9	115.0	153.9	154.1	132.3	132.4	110.7	110.7	115.2	115.2
4″	125.5	125.5	134.9	135.0	157.7	157.7	133.3	133.4	132.8	134.2	143.1	143.1	160.9	161.0
5″	122.6	122.6	122.7	122.7	114.9	115.0	153.9	154.1	122.8	122.7	152.3	152.3	115.2	115.2
6″	129.7	129.7	130.2	130.2	124.8	124.9	100.7	100.8	120.6	120.7	101.4	101.4	121.9	121.9
CH_3			21.0	21.0										
OCH_3					55.6	55.6	56.3	55.4					55.6	55.6
							61.0	61.1						
CH_3CO									201.3	201.4	199.3	199.3		
									28.5	28.6	28.6	28.7		
OCH_2O											102.5	102.5		

C	3g	4g	3h	4h	5b	6b	5c	6c	7	C	8a	9a	8b	9b
1	180.4	179.9	180.2	179.6	182.2	182.2	182.2	182.2	179.1	1	123.9	122.3	123.0	123.0
2	152.7	152.7	154.3	150.7	157.3	156.9	161.5	161.6	148.1[a]	2	124.4	123.8	134.2	134.3
3	113.3	113.3	113.6	113.6	128.1	128.3	128.0	128.3	148.5[a]	3	127.6	126.8	129.5	129.5
4	185.2	185.6	185.0	185.4	182.5	182.7	182.5	182.8	178.7	4	112.9	113.8	112.2	112.7
4a	131.2	132.0	131.1	131.9	132.4	131.9	132.4	131.9	132.9	4a	136.9	137.3	135.9	136.3
5	126.4	126.0	126.4	126.0	126.4	126.7	126.4	126.7	126.9	5a	137.7	138.1	136.7	136.9
6	134.5	150.7	134.5	149.0	150.7	135.7	150.6	134.5	149.8	6	178.7	177.4	178.4	178.4
7	149.5	133.6	149.6	133.6	133.5	149.4	133.4	149.4	133.8	6a	132.7	130.5	132.7	130.8
8	126.6	127.0	126.5	127.0	127.2	126.8	127.1	126.8	127.4	7	126.3	126.2	126.1	126.6
8a	130.0	129.1	129.9	129.0	129.8	130.3	129.8	130.4	130.8	8	148.8	132.9	148.6	132.7
1′	36.3	36.5	36.3	36.3	36.6	36.4	36.5	36.4	36.3	9	134.3	149.5	134.0	150.4
2′	28.9	28.9	28.8	28.8	28.8	28.9	28.8	28.9	28.8	10	127.2	125.8	127.0	126.9
3′	38.5	38.5	38.4	38.4	38.5	38.5	38.5	38.5	38.5	10a	132.9	134.1	132.6	134.9
4′	27.9	27.9	27.8	27.8	27.9	27.9	27.9	27.9	27.9	11	181.3	180.4	181.2	181.5
5′, 6′	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	11a	119.1	123.9	114.0	125.1
1″	160.6	160.8	160.2	160.3	124.0	124.0	117.7	117.8	130.4	11b	124.8	117.3	125.0	118.5
2″	118.6	118.6	99.5	99.5	135.7	135.7	137.2	137.3	131.4	1′	36.3	35.4	36.2	36.6
3″	140.4	140.4	161.8	161.8	131.2	131.2	116.0	116.1	130.0	2′	28.9	28.2	28.7	28.9
4″	128.2	128.2	98.6	98.6	141.0	141.0	157.7	157.3	137.9	3′	38.6	37.9	38.5	38.7
5″	140.4	140.4	161.8	161.8	131.2	131.2	116.0	116.1	130.0	4′	28.0	27.2	27.8	28.0
6″	118.6	118.6	99.5	99.5	135.7	135.7	137.2	137.3	131.4	5′, 6′	22.7	22.4	22.5	22.7
CH_3	21.3	21.3			21.5	21.5			21.3	CH_3			21.5	21.7
OCH_3			55.6	55.6			55.5	55.6						

C	8c	9c	8d	9d	12c/13c	12g	12h/13h	C	10e	11e	10f	11f
1	102.9	102.9	148.7	148.6	113.7	135.6	156.3	1	132.4	132.4	107.8	107.6
2	157.6	157.7	140.9	140.9	158.4/155.4	129.1	97.3	2	132.4	132.4	148.9	148.8
3	119.7	119.6	155.5	155.4	120.2	140.8	159.2	3	129.5	129.4	153.0	152.9
4	114.0	114.1	90.7	90.8	104.1	110.1	88.3	4	125.5	125.5	100.7	100.5
4a	133.2	134.9	136.5	136.0	151.7/154.1	157.5	163.4	4a	129.9	129.9	128.0	127.7
5a	136.7	136.9	136.5	136.5	—	—	—	5a	151.8	151.9	150.8	150.7
6	178.5	178.2	178.2	177.9	175.4	176.1	175.2/174.8	6	182.6	182.1	153.0	182.0
6a	132.7	130.9	133.1	130.1	132.6/130.5	131.9	131.9	6a	135.1	135.5	133.2	131.1
7	126.2	126.6	125.5	125.7	126.8	126.3	126.2	7	127.2	127.2	127.0	127.0
8	148.7	132.8	148.3	132.4	150.1/133.9	149.8	149.6/133.6	8	150.3	134.4	150.0	134.1
9	134.1	150.4	134.2	150.5	134.2/150.5	134.3	134.0/150.2	9	135.1	151.1	134.9	150.7

Table 3 (*continued*)

C	8c	9c	8d	9d	12c/13c	12g	12h/13h	C	10e	11e	10f	11f
10	127.0	126.8	127.7	127.5	127.1/127.3	127.6	127.6/127.3	10	127.9	127.9	127.7	127.6
10a	132.9	134.9	132.3	135.2	131.3/128.9	131.6	131.6	10a	133.4	131.5	133.2	135.1
11	181.2	181.5	179.2	179.4	181.7/182.1	180.7	179.8	11	185.1	185.6	185.2	185.5
11a	118.6	132.8	114.1	119.7	122.1	120.3	—	11a	148.6	148.7	147.0	146.8
11b	125.7	125.3	119.8	114.1	124.2	125.7	—	12	125.5	125.5	124.4	124.5
1′	36.3	36.6	36.3	36.6	36.5	36.4	36.4	12a	148.6	148.7	147.9	147.7
2′	28.8	28.8	28.9	28.8	28.9	28.9	28.9	1′	36.5	36.7	36.4	36.6
3′	38.6	38.6	38.6	38.6	38.6	38.6	38.6	2′	28.9	28.9	28.9	28.8
4′	28.0	28.0	27.9	27.9	28.0	28.0	28.0	3′	38.6	38.6	38.6	38.5
5′, 6′	22.7	22.7	22.6	22.6	22.6	22.6	22.6	4′	28.0	28.0	27.9	27.9
OCH_3	55.8	55.8	62.4	62.4	56.1		56.0[a]	5′, 6′	22.6	22.6	22.6	22.6
			61.6	61.5			56.3[a]	CH_3	16.7	16.7	16.9	16.9
			56.0	56.0				OCH_2O			102.8	102.7
CH_3						21.9[a]						
						22.4[a]						

[a] Exchangeable assignments.

362.1756, found 362.1736. (b) 56 mg (66%) of **9c**. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 362.1756, found 362.1773.

Application of the cyclization method to compound **1c** gave 28 mg of **9c** (97%) and from **2c** afforded **8c** (88%).

3.1.14. 8-(4-Methylpentyl)-1,2,3-trimethoxy-5*H*-benzo[*b*]-carbazole-6,11-dione 8d and 9-(4-methylpentyl)-1,2,3-trimethoxy-5*H*-benzo[*b*]carbazole-6,11-dione 9d. A 1:2 mixture of **1d** and **2d** was treated following the above described method, affording after chromatographic purification (eluent: dichloromethane/ethyl acetate 8:2): (a) 18 mg (33%) of **8d**. IR: 3236, 2951, 2867, 1646, 1597, 1510, 842 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 422.1967, found 422.1923. (b) 5 mg (9%) of **9d**. IR: 3235, 2954, 2851, 1659, 1597, 1511, 807 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 422.1967, found 422.1937.

Application of the method to compound **1d** yielded **9d** (94%).

3.1.15. 9-(4-Methylpentyl)-5*H*-benzo[*b*]carbazole-6,11-dione 9a. Compound **1a** was treated following the above described method yielding 25 mg (94%) of **9a**. IR: 3222, 2952, 2923, 2855, 1650, 1593, 1530, 745 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 332.1650, found 332.1692.

3.1.16. 8-(4-Methylpentyl)-5*H*-benzo[*b*]carbazole-6,11-dione 8a. Compound **2a** was treated following the above described method affording 16 mg (94%) of **8a**. IR: 3231, 2952, 2922, 2859, 1649, 1595, 1529, 744 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 332.1650, found 332.1603.

3.1.17. 2-Methyl-9-(4-methylpentyl)-5*H*-benzo[*b*]carbazole-6,11-dione 9b. Compound **1b** was treated following the above described method yielding 33 mg (94%) of **9b**. IR: 3255, 2952, 2923, 2852, 1641, 1594, 1530, 734 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 346.1807, found 346.1842.

3.1.18. 2-Methyl-8-(4-methylpentyl)-5*H*-benzo[*b*]carbazole-6,11-dione 8b. Compound **2b** was treated following the above described method affording 16 mg (94%) of **8b**. IR: 3270, 2950, 2932, 1652, 1596, 1530, 803 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 346.1807, found 346.1795.

3.1.19. 12-Methyl-8-(4-methylpentyl)-benzo[*b*]acridine-6,11-dione 10e and 12-methyl-9-(4-methylpentyl)-benzo[*b*]acridine-6,11-dione 11e. *General procedure for the preparation of benzoacridine derivatives.* A solution of the arylamine (0.18 mmol) in acetic acid (4 mL) was treated with conc. sulfuric acid (0.60 mL). The reaction mixture was stirred at 120 °C for 5 min. To the cooled reaction were added water and aq 30% NH_4OH until basic pH, and extracted with dichloromethane. The organic layer was separated and washed with brine, dried over Na_2SO_4, filtered and evaporated off. The crude product was purified by column chromatography over silica gel.

A 1:1 mixture of **1e** and **2e** was treated following the described method, affording after chromatographic purification (eluent: dichloromethane/ethyl acetate 95:5): (a) 4 mg (6%) of **10e**. IR: 2952, 2931, 2867, 1683, 1661, 1599, 763 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 358.1807, found 358.1843. (b) 42 mg (66%) of mixture of **10e** and **11e**. (c) 8 mg (13%) of **11e**. IR: 2952, 2930, 2867, 1688, 1661, 1600, 761 cm^{-1}. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 358.1807, found 358.1822.

3.1.20. 12-Methyl-8-(4-methylpentyl)-benzo[*b*][1,3]dioxole[4,5-*i*]acridine-6,11-dione 10f and 12-methyl-9-(4-methylpentyl)-benzo[*b*][1,3]dioxole[4,5-*i*]acridine-6,11-dione 11f. A 1:1 mixture of **1f** and **2f** was treated following the described method, affording after column chro-

matography (eluent: dichloromethane/ethyl acetate 9:1): (a) 14 mg (19%) of **10f**. IR: 2953, 2928, 2867, 1681, 1600, 1463, 857 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 402.1705, found 402.1678. (b) 18 mg (25%) of mixture of **10f** and **11f**. (c) 31 mg (43%) of **11f**. IR: 2953, 2926, 2867, 1682, 1661, 1601, 859 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 402.1705, found 402.1759.

3.1.21. 2-Methoxy-8-(4-methylpentyl)-benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione 12c and 2-methoxy-9-(4-methylpentyl)-benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione 13c. *General procedure for the preparation of benzofurane derivatives.* To a solution of the aryloxi-derivative (1 equiv) in acetic acid (4 mL) was added palladium acetate (II) (4 equiv). The reaction mixture was stirred at 125 °C and monitored by TLC until the phenoxy-derivative disappeared (18-60 h). To the cooled reaction mixture were added water and ethyl acetate. The organic layer was separated and washed with aq 10% NaOH and brine, dried over Na_2SO_4, filtered and evaporated off. The crude product was purified by column chromatography over silica gel.

A 9:1 mixture of **3c** and **4c** (0.35 mmol) was treated following the above described method, yielding after chromatographic purification (eluent: dichloromethane): 16 mg (13%) of a 9:1 mixture of **12c** and **13c**. IR: 2952, 2929, 2866, 1674, 1599, 1488, 842 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 363.1596, found 363.1595.

3.1.22. 1,3-Dimethyl-8-(4-methylpentyl)-benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione 12g and 1,3-dimethyl-9-(4-methylpentyl)-benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione 13g. A 8:1 mixture of **3g** and **4g** (0.07 mmol) was treated following the above described method, affording after chromatographic purification (eluent: hexane/dichloromethane 1:1): (a) 5 mg (20%) of **12g**. IR: 2952, 2929, 2865, 1672, 1599, 1488, 845 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 393.1702, found 393.1665. (b) 7 mg (28%) of mixture of **12g** and **13g**.

3.1.23. 1,3-Dimethoxy-8-(4-methylpentyl)-benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione 12h and 1,3-dimethoxy-9-(4-methylpentyl)-benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione 13h. A 9:1 mixture of **3h** and **4h** (0.09 mmol) was treated following the above described method, yielding after column chromatography (eluent: dichloromethane): 16 mg (45%) of a 9:1 mixture of **12h** and **13h**. IR: 2952, 2930, 2866, 1675, 1598, 1452, 815 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3. HRMS (FAB-POSI, M+1) calcd 393.1702, found 393.1665.

3.2. Bioactivity: Cell growth inhibition assay

A colourimetric assay using sulforhodamine B (SRB) has been adapted for a quantitative measurement of cell growth and viability, following a previously described method.[23] Cells were seeded in 96-well microtitre plates, at 5×10^3 cells per well in aliquots of 195 μL of RPMI medium, and they were allowed to attach to the plate surface by growing in a drug-free medium for 18 h. Afterwards, samples were added in aliquots of 5 μL (dissolved in DMSO/H_2O, 3:7). After 72 h exposure, the antitumor effect was measured by the SRB methodology: cells were fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates were washed with deionised water and dried; 100 μL of SRB solution (0.4% wt/vol in 1% acetic acid) was added to each microtitre well and incubated for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried and bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analyses were generated automatically by the LIMS implementation. Using control OD values (C), test OD values (T) and time zero OD values (T_0), the drug concentration that caused a 50% growth inhibition (GI_{50}value) was calculated from the equation: $100 \times [(T - T_0)/(C - T_0)] = 50$.

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