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Synthetic approaches and *in vitro* cytotoxic evaluation of 2-acyl-3-(3,4,5-trimethoxyanilino)-1,4-naphthoquinones

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2-Acyl-1,4-naphthoquinones react with 3,4,5-trimethoxyaniline, under aerobic conditions, to give benzophenanthridinequinone, benzocarbazole and 2-acyl-3-(3,4,5-trimethoxyanilino)-1,4-naphthoquinone derivatives. The formation of the heterocyclic compounds is discussed in terms of the ring closure of C–C Michael type adduct intermediates through two alternative N–C-bond formations. The propensity of the substrates to undergo preferential C–C instead of C–N bond formation and the further heterocyclization of the C–C Michael type adduct intermediates is rationalized by using product stability parameters assessed by DFT calculations. Preliminary results are reported on a convenient access towards 2-acyl-3-(3,4,5-trimethoxyanilino)-1,4-naphthoquinones from 2-acylnaphthoquinones and their cytotoxic activities on cancer cells.

Introduction

Acylated 1,4-quinones are useful precursors of a variety of natural[1–4] and synthetic compounds endowed with a range of biological properties.[5–13] The 2-acyl-1,4-benzo- and 1,4-naphthoquinones exhibit remarkable features in terms of their reactivity with nucleophiles due to the fact that the electrophilic centers at the quinone nucleus and acyl substituent are suitably located, thus enabling reactions with compounds such as arylamines[14–16] azaenamines[17] enaminones[18,19] and 2-aminobenzothiazoles[20] to give rise to a broad variety of quinonoid compounds such as those depicted in Fig. 1.

We have previously reported that a series of 2-acyl-3-anilino-1,4-naphthoquinones possess biological properties such as inhibitors of Hsp90 chaperoning function,[16] cytotoxic actions on cancer cells[21] and DNA-intercalants.[22] These compounds, prepared by amination reaction of 2-acyl-1,4-naphthoquinone with diverse *p*-substituted anilines under aerobic conditions,[21] were originally designed as inhibitors of Hsp90 chaperone and their structures are based on that of the Hsp90 inhibitor, 2-acetyl-3-phenyl-1,4-naphthoquinone (HTS1).[23,24]

As a continuation of our work on biological active 2-acyl-3-arylamino-1,4-naphthoquinones, we are interested to expand the series to new members containing di- and tri-substituted anilino fragments in order to further structure–activity relationships studies. Based on previous results on the synthesis of arylaminoquinones[15,16] we envisaged achieve new members by reaction of 2-acylnaphthoquinones with di- and tri-substituted anilines. As far as we know the only report in the literature regarding the reactivity of 2-acyl-1,4-naphthoquinones with disubstituted anilines was made by Pardo *et al.*,[15] by using 2-acetyl-1,4-naphthoquinone **2a**. According to these authors, the reaction of **2a** with disubstituted anilines take place to give a variety of products derived from 2-acetyl-3-anilino-1,4-naphthoquinone, 2-acetyl-3-aminophenyl-1,4-naphthoquinone

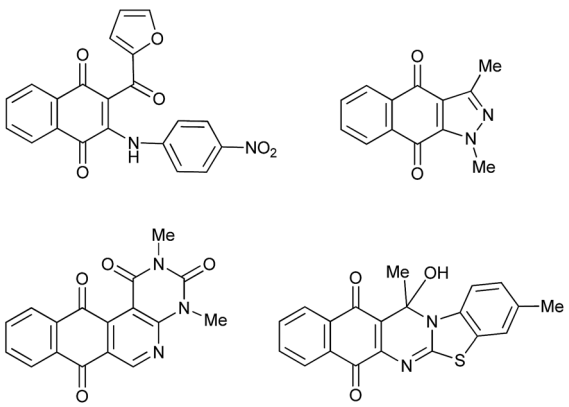

Fig. 1 Quinones prepared from acylquinones and nucleophiles.

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and benzophenanthridine-7,12-quinone. Based on these precedents, it seems to us that the access to 2-acyl-3-anilino-1,4-naphthoquinone derivatives by amination of 2-acyl-1,4-naphthoquinones with di- and tri-substituted anilino groups could have some limitations. To evaluate the feasibility to synthesize 2-acyl-3-anilino-1,4-naphthoquinones, substituted at the anilino group, we examined the behavior of the highly and symmetrically substituted 3,4,5-trimethoxyaniline (TMA) with a representative series of 2-acyl-1,4-naphthoquinones, as reaction models. Herein we report the results of our study, which reveals that TMA exhibits ambident nucleophilic character, acting either as nitrogen or carbon nucleophile with 2-acyl-1,4-naphthoquinones, to give hetero-annulation compounds as the main products, together with the respective amination product named 2-acyl-3-(3,4,5-trimethoxyanilino)-1,4-naphthoquinones. We also report preliminary results on a convenient access to 2-acyl-3-(3,4,5-trimetoxyanilino)-1,4-naphthoquinones from 2-acyl-3-anilino- and 2-acyl-3-(4-nitroanilino)-1,4-naphthoquinones and the *in vitro* cytotoxic activities of the new 2,3-disubstituted-1,4-naphthoquinones on normal fibroblasts and on a panel of three cancer cell lines.

Results and discussion

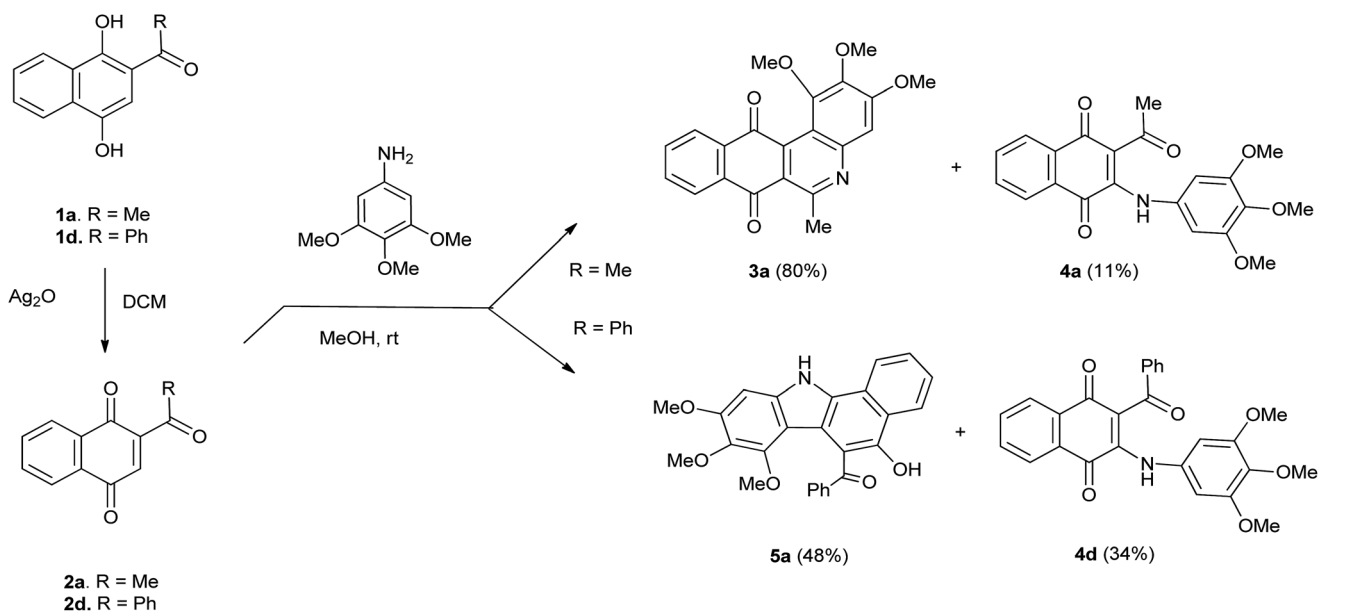
The reactivity of TMA with 2-acetyl-1,4-naphthoquinones **2a** and **2d** were firstly examined. Acylquinone **2a**, prepared by oxidation of acylhydroquinone **1a** with silver(I) oxide, was reacted with TMA in methanol at room temperature for 24 h. Column chromatography of the crude provided benzophenanthridinequinone **3a** in 80% yield together with minor amounts of aminoquinone **4a** (11%; Scheme 1). Interestingly, the reaction of TMA with 2-benzoyl-1,4-naphthoquinone **2d**, prepared from **1d** and conducted under the above mentioned conditions, yielded aminoquinone **4d** (34%) accompanied by a yellow solid substance.

The IR spectrum of this product reveals the presence of O–H, N–H and C=O bands at $\nu_{máx}$ 3382, 2935 and 1673 cm^{-1}. The ^1H NMR spectrum shows hydroxyl and amino proton signals at δ 9.66 (s) and 9.38 (br.s). In the aromatic region it was observed the aromatic pattern signals of two phenyl fragments and a proton signal at δ 6.71 (s). The ^{13}C NMR spectrum displays the characteristic signal of a carbonyl group at δ 198.1 and the mass spectrum shows the molecular ion $[M^+]$ peak at m/z = 428.14814. These data are in agreement with the structure of benzocarbazole **5a** for the new product, isolated in 48% yield (Scheme 1).

Based on evidences reported in literature on the use of Lewis acids to promote the oxidative amination reaction of quinones with arylamines,[24] we examined their influence on the reaction of TMA with quinones **2a** and **2d**, seeking to facilitate the formation of aminoquinones **4a** and **4d** with respect to the respective heterocycles **3a** and **5a**. The assays, performed in the presence of $CeCl_3 \cdot 7H_2O$ and $InBr_3$, demonstrate that these Lewis acids do not show significant changes in the products ratio relative to those obtained in their absence.

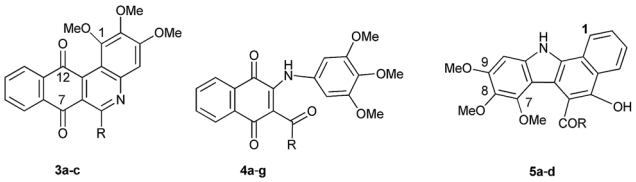
To get more information about these preliminary reactivity assays, we examined the scope of the reaction of TMA with 2-alkanoyl- and 2-aroyl-1,4-naphthoquinone **2b**, **2c**, **2e–g**. These quinones, prepared from their corresponding acylhydroquinones **1b**, **1c**, **1e–g**, were reacted with TMA under the standard conditions and the results of the assays are summarized in Table 1.

The data in Table 1 suggest that the reactions of TMA with 2-acylnaphthoquinones take place by a stepwise process that involves the formation of carbon–carbon (C–C) and carbon–nitrogen (C–N) bonds to produce benzophenanthridines **3**, 2-acyl-3-trimethoxyanilinonaphthoquinones **4** and benzocarbazoles **5**. Our results are in agreement with those reported by Pardo *et al.*,[15]



Scheme 1 Reaction of trimethoxyaniline with acylquinones **2a** and **2d**.

Table 1 Products 3, 4 and 5 arising from the reaction of TMA with acylquinones 2a–g



R	Quinone	Product 3[a] (yield %)	Product 4[a] (yield %)	Product 5[a] (yield %)
Methyl	**2a**	**3a** (80)	**4a** (11)	—
Propyl	**2b**	**3b** (47)	**4b** (40)	—
Heptyl	**2c**	**3c** (59)	**4c** (23)	—
Phenyl	**2d**	—	**4d** (34)	**5a** (48)
Fur-2-yl	**2e**	—	**4e** (45)	**5b** (53)
Thien-2-yl	**2f**	—	**4f** (45)	**5c** (51)
Thien-3-yl	**2g**	—	**4g** (27)	**5d** (58)

[a] Isolated by column chromatography.

on the reactions of acetylnaphthoquinone **2a** with disubstituted anilines in terms of the formation of compounds **3** and **4**. However, the formation of benzocarbazoles **5** constitutes a new and interesting precedent on the reaction of acylnaphthoquinones with substituted anilines. The formation of benzocarbazoles **5** is a new example of the Nenitzescu reaction of 1,4-naphthoquinones with aromatic amines to produce complex 5-hydroxyindole derivatives.[25–27] From these assays, it can also be deduced that the alkyl or aryl nature of the quinone acyl group determines the reaction pathways to the formation of the corresponding benzophenanthridine or benzocarbazole derivatives. The above experiments reveals that TMA behaves both as a carbon and nitrogen nucleophile toward the highly electrophilic acylquinones.

Taking into the account the above results, a reasonable mechanism for the formation of compounds **3**, **4** and **5** from TMA and acylquinones **2a–g** is proposed and illustrated in Scheme 2. Initially, it seems plausible that the attack of the amine to the acylquinones gives rise to C–C and C–N Michael adduct intermediates $\mathbf{I_1}$ and $\mathbf{I_2}$, through two parallel reactions. The aza-Michael intermediate $\mathbf{I_1}$ will evolve to the corresponding amination products **4a–g** by enolization followed by oxidation. Regarding the heterocycles **3a–c** and **5a–d**, it seems plausible that they arise from $\mathbf{I_2}$ (R = alkyl, aroyl), through two alternative cyclodehydration reactions involving amino and carbonyl groups.

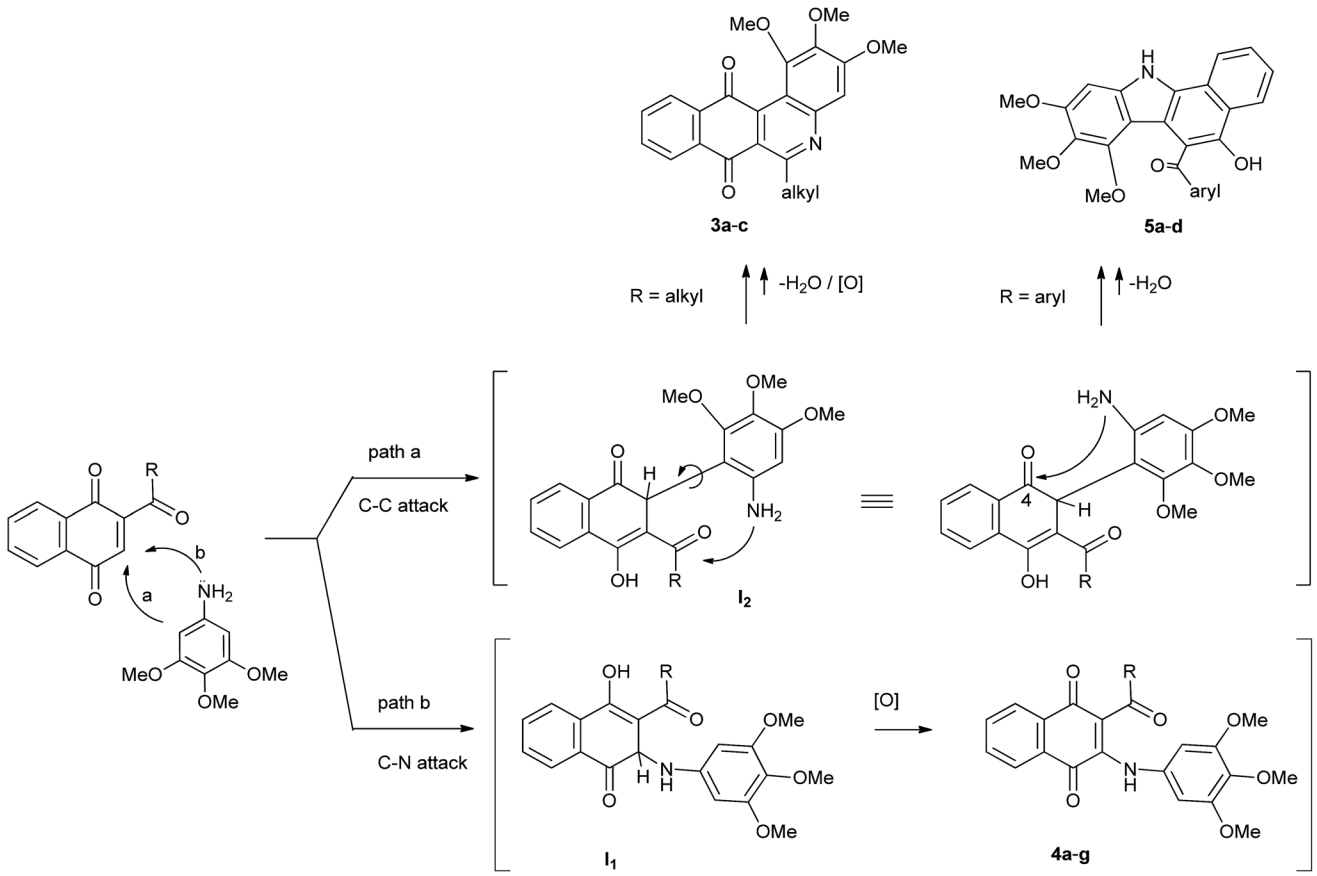
Based on the proposed stepwise mechanism we decided to get theoretical information to rationalize the experimental results. To this end, the energies of the hypothetical intermediates $\mathbf{I_1}$ and $\mathbf{I_2}$, relative to that of **2a,d** + TMA, and the HOMO–LUMO gaps of the products **3**, **4** and **5** were evaluated by using Density Functional Theory (DFT) calculations.[28,29] The analysis of the relative energies of intermediates $\mathbf{I_1}$ and $\mathbf{I_2}$ (R = Me, Ph) indicates that formation of the latter is more favorable than $\mathbf{I_1}$ (Fig. 2). As a consequence, there is a significant selectivity for the nucleophilic attack of TMA, through C-2, to the acylquinones **2a** and **2d** in this stepwise mechanism, in agreement with the experimental outcome.

Additionally, calculations of the HOMO–LUMO gaps of products **3a**, **4a**, **4d** and **5a** indicate that formation of **3a** (3.16 eV) and **5a** (3.27 eV) are energetically more favorable than that of the corresponding amination products **4a** (3.06 eV) and **4d** (2.91 eV). These theoretical results are in good agreement with the experimental evidences in terms that the C–C bond formation appears as a more favorable process than C–N bond formation.

Regarding the second step of the proposed mechanism for the reaction of TMA with acylquinones **2a–g**, this involves the 5- and 6-*endo*-trig closures of intermediates $\mathbf{I_2}$ (R = alkyl or aryl) to give the respective heterocycles **3** and **5** (Scheme 2). The behavior of intermediates $\mathbf{I_2}$ (R = alkyl or aryl) to undergo these two alternative cyclization processes probably is controlled by the electrophilic capacity of carbonyl groups of the acyl group and that located at the 4-position.

Although the direct amination of TMA with acylnaphthoquinones does not offer a convenient synthetic approach to 2-acyl-3-(3,4,5-trimethoxyanilino)-1,4-naphthoquinones **4**, it would appear that these compounds may be accessed, albeit more circuitously, by transamination reaction of TMA with 2-acyl-3-anilino-1,4-naphthoquinones. The possibility to apply this strategy to the synthesis of quinones **4** is based on literature precedents on transamination reaction involved in the synthesis of 2,l-benzisoxazolequinones from 2,5-dianilino-3-acetyl-1,4-benzoquinone and hydroxylamine.[30] To check the viability of the designed strategy (Scheme in Table 2), the reactions of TMA with 2-acetyl-3-anilino-, 2-butyroyl-3-anilino- and 2-benzoyl-3-anilino-1,4-naphthoquinone **6a–c** were examined. Precursors **6a–c** were prepared from 2-acyl-1,4-naphthoquinone **2a**, **2b**, **2d** and aniline. The transamination assays, performed in refluxing methanol, produced the expected substitution products **4a**, **4b** and **4d** (Table 2).

Trying to improve the access to compound **4d**, we envisaged that the replacement of the aniline by nitroaniline group in **6c**, as in **6d**, would facilitate the transamination reaction due to the greater ability of the nitroaniline than aniline as leaving group. The required precursor **6d** was prepared in 80% yield from 2-



Scheme 2 Plausible mechanism for the reaction of TMA with acylquinones **2a–g**.

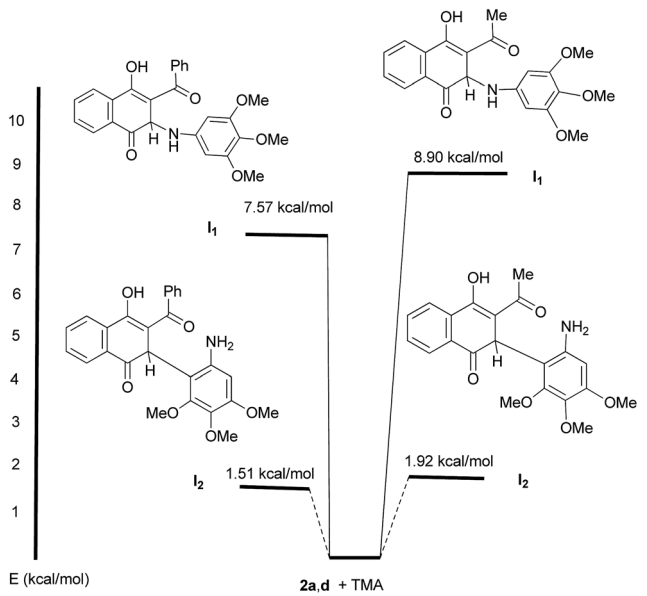
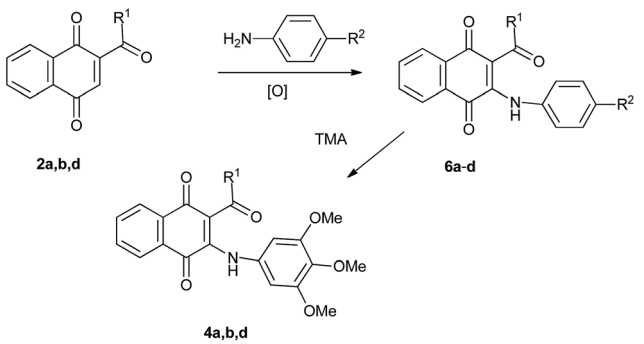


Fig. 2 Energies of intermediates I_1 and I_2 related to those of **2a,d** + TMA.

benzoylnaphthoquinone **2d** and *p*-nitroaniline. Further reaction of TMA with **6d**, in refluxing methanol, yielded the expected product **4d** (24 h) albeit in 42% yield. Optimal results in terms of

Table 2 Synthesis of **4a,b,d** from quinones **6a–d** and TMA



Precursor	Product	R^1	R^2	Time (h)	Yield[a] (%)
6a	**4a**	Me	H	25	94
6b	**4b**	Pr	H	11	75
6c	**4d**	Ph	H	61	31
6d	**4d**	Ph	NO_2	24	42
6c	**4d**	Ph	H	3:30[b]	78
6d	**4d**	Ph	NO_2	0:17[b]	80

[a] Isolated by column chromatography. [b] In refluxing ethanol.

Table 3 *In vitro* cytotoxic activity of **4a–g** on T24 (bladder), DU-145 (prostate) and MCF-7 (breast) cancer cell lines and non-tumor fibroblasts (NIH 3T3)

$IC_{50} \pm SEM^a$ (μM)				
No	T24	DU-145	MCF-7	NIH 3T3
4a	39.99 ± 2.44	27.01 ± 1.36	15.50 ± 0.73	>100
4b	18.03 ± 0.24	12.53 ± 0.44	10.16 ± 0.78	>100
4c	>100	34.86 ± 1.65	45.26 ± 2.77	>100
4d	11.64 ± 0.88	8.12 ± 0.65	7.53 ± 0.54	14.14 ± 1.85
4e	6.53 ± 0.95	9.09 ± 1.99	11.12 ± 1.81	8.89 ± 1.51
4f	9.94 ± 1.14	11.36 ± 1.04	12.95 ± 1.45	114.7 ± 17.4
4g	9.42 ± 1.41	11.04 ± 0.90	11.62 ± 1.66	7.00 ± 2.04
D[b]	0.65 ± 0.07	0.42 ± 0.03	0.33 ± 0.05	0.50 ± 0.02

[a] Data represent mean average values ± SEM for three separate experiments. [b] D = doxorubicin.

the preparation of compound **4d** were achieved by performing the transamination reaction of TMA with **6c** and **6d** in refluxing ethanol that provided, after short reaction times, compound **4d** in 78 and 80% yield, respectively (Table 2).

The successful results achieved on these preliminary transamination assays are of great interest to developed a new and selective access to 2-acyl-3-anilino-1,4-naphthoquinone derivatives, by transamination of 2-acyl-3-anilino-1,4-naphthoquinones with highly substituted anilines.

Quinones **4a–g** were evaluated for their *in vitro* cytotoxic activity on non-tumor fibroblasts and on a panel of three human-derived tumor cell lines, using the conventional MTT (microculture tetrazolium reduction) assay.[31] The data in Table 3 show that most of the quinones have similar cytotoxicity against the three cancer cell lines, with IC_{50} values around 10 μM. Compound **4a** shows a rather low activity whereas quinone **4c** should be considered as devoid of any cytotoxicity. It is noteworthy that doxorubicin is more potent compared to most active naphthoquinone derivatives, but doxorubicin was also active against non-tumor cells, indicating a lack of selective effect. Table 3 also shows that compounds **4d**, **4e** and **4g** did not show a selective effect, killing both normal and cancer cells. Interestingly, compounds **4a**, **4b** and **4f**, because of their extremely low toxicity against non-tumor fibroblasts, show a high selectivity upon cancer cells.

Experimental

1.1. General remarks

All solvents and reagents were purchased from different companies such as Aldrich and Merck and were used as supplied. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. The IR spectra were recorded on an FT IR Bruker spectrophotometer, model vector 22, using KBr disks, and the wave numbers are given in cm^{-1}. ^1H NMR spectra were recorded on Bruker Avance-400 instrument and Bruker Ultrashield-300 in deuterochloroform ($CDCl_3$). ^{13}C NMR spectra were obtained in $CDCl_3$ at 100 and 75 MHz. Bidimensional NMR techniques and DEPT were used for signal assignment. Chemical shifts are expressed in ppm downfield relative to tetramethylsilane and the coupling constants (J) are reported in Hertz. The HRMS spectra were obtained on a Thermo Finnigan spectrometer, model MAT 95XP and LTQ-Orbitrap mass spectrometer (Thermo-Fisher Scientific) with the analysis performed using an APCI source operated in positive mode. Silica gel Merck 60 (70–230 mesh) was used for preparative column chromatography and TLC aluminum foil 60F254 for analytical TL. The acylhydroquinones **1a–g**, used as precursors of quinones **2a–d**, were prepared by solar photoacylation of 1,4-naphthoquinone with the respective aldehydes, according to a previously reported procedure.[32]

1.2. Reaction of TMA with acylnaphthoquinones 2a,g. General procedure

Suspension of acylnaphthohydroquinone **1a,g** (1 equiv.), Ag_2O (2.6 equiv.), anhydrous $MgSO_4$ (500 mg), and dichloromethane (15 mL) were left with stirring at rt for 30 min. The mixtures were filtered and the solvents were removed under reduced pressure. The residues were dissolved in methanol, 3,4,5-trimethoxyaniline (2 equiv.) was added with stirring and, the resulting solutions were left at rt for 24 h. The solvents were evaporated under reduced pressure and the residues were column chromatographied over silica gel (8 : 2 petroleum ether/EtOAc) to yield the corresponding heterocycles **3a–c**, **5a–d** and acylaminoquinones **4a–g**.

1,2,3-Trimethoxy-6-methylbenzo[*j*]phenanthridine-7,12-dione (3a) and 2-acetyl-3-((3,4,5-trimethoxyphenyl)amino)naphthalene-1,4-dione (4a). These compounds were produced following the general procedure from **1a** (200 mg, 0.99 mmol), Ag_2O (596 mg, 2.57 mmol), dry magnesium sulfate (0.5 g) and TMA (363 mg, 1.98 mmol). Compound **3a** (287 mg, 0.79 mmol, 80%), yellow solid, mp: 168–170 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 1676 (C=O), 1240 (C–O–C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.19 (m, 1H, 8-H), 8.04 (m, 1H, 11-H), 7.77 (m, 2H, 9-H + 10-H), 7.24 (s, 1H, 4-H), 4.07 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.97 (s, 3H, OMe), 3.13 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.2, 183.4, 158.8, 158.4, 149.0, 148.2, 144.4, 142.9, 135.4, 133.7, 133.6, 133.5, 126.8, 125.4, 123.3, 113.3, 103.8, 61.3, 61.1, 56.4, 27.1. HRMS (APCI): $[M + H]^+$ calcd for $C_{21}H_{17}NO_5$: 364.11850; found 364.11729. Compound **4a** (42 mg, 0.11 mmol, 11%), red solid, mp: 188–188.5 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3093 (NH), 1633 (C=O), 1593 (C=O), 1124 (C–O–C); ^1H NMR (400 MHz, $CDCl_3$): δ 12.40 (s, 1H, NH), 8.18 (d, 1H, J = 7.6 Hz, 5-H), 7.93 (d, 1H, J = 7.6 Hz, 8-H), 7.80 (t, 1H, J = 7.2 Hz, 7-H), 7.66 (t, 1H, J = 7.2 Hz, 6 H), 6.36 (s, 2H, 2′-H + 6′-H), 3.88 (s, 3H, OMe), 3.83 (s, 6H, OMe), 2.67 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.9, 182.5, 181.8, 153.9 (2 × C), 137.4, 135.7 (2 × C), 135.3, 133.8, 133.1 (2 × C), 131.5, 127.1, 126.4, 102.5 (2 × C), 61.4, 56.6 (2 × C), 33.5. HRMS (APCI): $[M + H]^+$ calcd for $C_{21}H_{19}NO_6$: 382.12907; found 382.12827.

1,2,3-Trimethoxy-6-propylbenzo[*j*]-phenanthridine-7,12-dione (3b) and 2-butanoyl-3-((3,4,5-trimethoxyphenyl)amino) naphthalene-1,4-dione (4b). These compounds were produced following the general procedure from **1b** (200 mg, 0.87 mmol), Ag_2O (524 mg, 2.26 mmol), dry magnesium sulfate (0.5 g) and TMA (319 mg, 1.74 mmol). Compound **3b** (160 mg, 0.41 mmol, 47%), yellow solid, mp: 145–146 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 2944 (C–H

Ar), 1679 (C=O), 1251 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (m, 1H, 8-H), 8.03 (m, 1H, 11-H), 7.77 (m, 2H, 9-H + 10-H), 7.26 (s, 1H, 4-H), 4.07 (s, 6H, 2 × OMe), 3.96 (s, 3H, OMe), 3.49 (dd, 2H, J = 7.8, 7.9 Hz, 1′-H), 1.82 (m, 2H, 2′-H), 1.13 (t, 3H, J = 7.3 Hz, 3′-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.5, 183.2, 162.1, 158.8, 148.9, 148.2, 144.9, 142.8, 135.3, 133.7, 133.6, 133.5, 126.8, 125.2, 123.0, 113.2, 103.9, 61.3, 61.0, 56.4, 40.7, 22.9, 14.4. HRMS (APCI): $[M + H]^+$ calcd for $C_{23}H_{21}NO_5$: 392.14980 found 392.14866. Compound **4b** (142 mg, 0.35 mmol, 40%), red solid, mp: 173–174 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3071 (N–H), 2962 (C–H Ar), 1633 (C=O), 1122 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.35 (bs, 1H, NH), 8.16 (d, 1H, J = 7.7 Hz, 5-H), 7.96 (d, 1H, J = 7.6 Hz, 8-H), 7.79 (dd, 1H, J = 7.6, 7.5 Hz, 7-H), 7.66 (dd, 1H, J = 7.6, 7.5 Hz, 6-H), 6.34 (s, 2H, 2′-H + 6′-H), 3.86 (s, 3H, OMe), 3.82 (s, 6H, 2 × OMe), 2.93 (t, 2H, J = 7.3 Hz, CH_3–CH_2–CH_2–CO), 1.56 (m, 2H, CH_3–CH_2–CH_2–CO), 0.91 (t, 3H, J = 7.4 Hz, CH_3–CH_2–CH_2–CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.6, 182.3, 181.4, 153.6 (2 × C), 136.9, 135.3, 134.5, 133.3, 132.6, 130.8, 126.6, 126.0, 114.6, 113.2, 102.2 (2 × C), 61.0, 56.2 (2 × C), 46.8, 17.4, 13.8. HRMS (APCI): $[M + H]^+$ calcd for $C_{23}H_{23}NO_6$: 410.16037; found 410.15896.

1,2,3-Trimethoxy-6-heptylbenzo[*j*]phenanthridine-7,12-diona (3c) and 2-octanoyl-3-((3,4,5-trimethoxyphenyl)amino)naphthalene-1,4-dione (4c). These compounds were produced following the general procedure from **1c** (200 mg, 0.70 mmol), Ag_2O (422 mg, 1.82 mmol), dry magnesium sulfate (0.5 g) and TMA (257 mg, 1.4 mmol). Compound **3c** (184 mg, 0.4 mmol, 59%), yellow solid, mp: 100–101 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 2927 (C–H Ar), 1679 (C=O), 1247 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (m, 1H, 8-H), 8.02 (m, 1H, 11-H), 7.76 (m, 2H, H-9 + 10-H), 7.24 (s, 1H, 4-H), 4.06 (s, 6H, 2 × OMe), 3.95 (s, 3H, OMe), 3.49 (dd, 2H, J = 7.9, 7.9 Hz, 1′-H), 1.77 (m, 2H, 2′-H), 1.53 (m, 2H, 3′-H), 1.38 (m, 2H, 4′-H), 1.31 (m, 4H, 5′-H + 6′-H), 0.88 (t, 3H, J = 6.6 Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.6, 183.2, 162.4, 158.8, 148.9, 148.3, 144.9, 142.8, 135.3, 133.8, 133.6, 133.5, 126.9, 125.2, 123.0, 113.2, 103.9, 61.3, 61.0, 56.4, 38.9, 31.9, 30.0, 29.7, 29.3, 22.7, 14.1. HRMS (APCI): $[M + H]^+$ calcd for $C_{27}H_{29}NO_5$: 448.21240; found 448.21101. Compound **4c** (75 mg, 0.16 mmol, 23%), red solid, mp: 103–104 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3070 (NH), 2927 (C–H Ar), 1633 (C=O), 1122 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.34 (bs, 1H, NH), 8.16 (d, 1H, J = 7.7 Hz, 5-H), 7.97 (d, 1H, J = 7.6 Hz, 8-H), 7.80 (dd, 1H, J = 8.0, 7.6 Hz, 7-H), 7.66 (dd, 1H, J = 8.0, 7.7 Hz, 6-H), 6.34 (s, 2H, 2′-H + 6′-H), 3.86 (s, 3H, OMe), 3.82 (s, 6H, 2 × OMe), 2.94 (t, 2H, J = 7.5 Hz, CH_3–$(CH_2)_5$–CH_2–CO), 1.50 (m, 2H, CH_3–$(CH_2)_4$–CH_2–CH_2–CO), 1.31 (m, 8H, CH_3–CH_2–CH_2–CH_2–CH_2–CH_2–$(CH_2)_2$–CO), 0.91 (t, 3H, J = 7.5 Hz, CH_3–$(CH_2)_6$–CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.8, 182.3, 181.4, 167.7, 153.5, 135.3, 134.5, 133.3, 132.6, 132.4, 130.8, 130.7, 128.7, 126.7, 126.0, 102.1 (2 × C), 61.0, 56.1 (2 × C), 44.9, 31.7, 29.2, 24.1, 23.0, 22.6, 10.9. HRMS (APCI): $[M + H]^+$ calcd for $C_{27}H_{31}NO_6$: 466.22297; found 466.22138.

(5-Hydroxy-7,8,9-trimethoxy-11*H*-benzo[*a*]carbazol-6-yl)(phenyl)-methanone (5a) and 2-benzoyl-3-((3,4,5-trimethoxyphenyl) amino)naphthalene-1,4-dione (4d). These compounds were produced following the general procedure from **1d** (200 mg, 0.76 mmol), Ag_2O (458 mg, 1.98 mmol), dry magnesium sulfate (0.5 g) and TMA (2.78 mg, 1.52 mmol). Compound **5a** (155 mg, 0.36 mmol, 48%), yellow solid, mp: 258–259 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3382 (O–H), 3059 (N–H), 2935 (C–H Ar), 1673 (C=O), 1122 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.66 (s, 1H, OH), 9.38 (bs, 1H, NH), 8.49 (d, 1H, J = 8.3 Hz, 1-H), 8.08 (d, 1H, J = 8.2 Hz, 4-H), 7.68 (d, 2H, J = 7.6 Hz, 2′-H), 7.67 (dd, 1H, J = 8.3, 7.3 Hz, 3-H), 7.53 (dd, 1H, J = 8.3, 7.3 Hz, 2-H), 7.31 (dd, 1H, J = 7.6, 7.3 Hz, 4′-H), 7.19 (dd, 2H, J = 7.6, 7.6 Hz, 3′-H), 6.71 (s, 1H, 10-H), 3.88 (s, 3H, OMe), 3.59 (s, 3H, OMe), 3.48 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.1, 153.0, 149.6, 146.8, 139.6, 135.6, 135.4, 131.8 (2 × C), 129.4 (2 × C), 128.3 (2 × C), 127.7 (2 × C), 125.1, 124.8, 123.5, 123.4, 120.1, 113.9, 111.3, 88.9, 61.2, 59.5, 56.1. HRMS (APCI): $[M + H]^+$ calcd for $C_{26}H_{21}NO_5$: 428.14980; found 428.14814. Compound **4d** (134 mg, 0.30 mmol, 34%), red solid, mp: 213–214 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3259 (N–H), 2929 (C–H Ar), 1682 (C=O), 1130 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, 1H, J = 8.1 Hz, 5-H), 8.15 (d, 1H, J = 8.1 Hz, 8-H), 7.82 (t, 1H, J = 7.8 Hz, 3′- or 5′-H), 7.73 (t, 2H, J = 7.7 Hz, 6-H + 7-H), 7.60 (d, 2H, J = 7.7 Hz, 2′-H + 6′-H), 7.45 (t, 1H, J = 7.7 Hz, 5′- or 3′-H), 7.31 (m, 2H, –NH + 4′-H), 6.02 (s, 2H, 2″-H + 6″-H), 3.76 (s, 3H, OMe), 3.53 (s, 6H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.7, 182.7, 182.3, 153.4 (2 × C), 143.6, 137.8, 137.2, 135.9, 133.4, 133.3, 133.1, 132.7, 130.2, 129.2 (2 × C), 128.7 (2 × C), 127.2, 126.9, 113.6, 104.2 (2 × C), 61.2, 56.1 (2 × C). HRMS (APCI): $[M + H]^+$ calcd for $C_{26}H_{21}NO_6$: 444.14472; found 444.14310.

Furan-2-yl(5-hydroxy-7,8,9-trimethoxy-11*H*-benzo[*a*]carbazol-6-yl)methanone (5b) and 2-(furan-2-carbonyl)-3-((3,4,5-trimethoxyphenyl)amino)naphthalene-1,4-dione (4e). These compounds were produced following the general procedure from **1e** (200 mg, 0.79 mmol), Ag_2O (476 mg, 2.05 mmol), dry magnesium sulfate (0.5 g) and TMA (289 mg, 1.58 mmol). Compound **5b** (174 mg, 0.42 mmol, 53%), yellow solid, mp: 100–102 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3292 (O–H), 3114 (N–H), 2937 (C–H Ar), 1624 (C=O), 1247 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.25 (s, 1H, OH), 8.63 (bs, 1H, NH), 8.52 (d, 1H, J = 8.4 Hz, 1-H), 8.00 (d, 1H, J = 8.2 Hz, 4-H), 7.70 (dd, 1H, J = 8.2, 7.4 Hz, 3-H), 7.55 (dd, 1H, J = 8.4, 7.4 Hz, 2-H), 7.51 (s, 1H, 4′-H), 6.77 (s, 1H, 10-H), 6.58 (bs, 1H, 2′-H), 6.28 (m, 1H, 3′-H), 3.94 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.67 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 191.6, 154.1, 153.2, 151.9, 147.1, 145.9, 136.0, 135.5, 130.9, 128.9, 128.6, 125.5, 124.9, 123.9, 123.2, 119.9, 118.5, 111.9, 111.7, 110.1, 89.2, 61.2, 60.3, 56.1. HRMS (APCI): $[M + H]^+$ calcd for $C_{24}H_{19}NO_6$: 418.12907; found 418.12785. Compound **4e** (153 mg, 0.35 mmol, 45%), red solid, mp: 204–206 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3384 (NH), 2935 (C–H Ar), 1623 (C=O), 1124 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (dd, 2H, J = 6.7 Hz, 6.7 Hz, 5-H + 8-H), 7.81 (t, 1H, J = 7.6 Hz, 7-H), 7.76 (m, 2H, 3′-H + NH), 7.71 (t, 1H, J = 7.6 Hz, 6-H), 7.14 (s, 2H, 2′-H+ 4′-H), 6.09 (s, 2H, 2″-H + 6″-H), 3.77 (s, 3H, OMe), 3.62 (s, 6H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.7, 182.4, 181.7, 153.0 (2 × C), 143.4, 142.9, 136.6, 135.6, 133.4, 132.9, 132.8, 132.5, 129.7, 126.9, 126.8, 126.6, 125.8, 113.9, 103.4 (2 × C), 60.9, 55.8 (2 × C). HRMS (APCI): $[M + H]^+$ calcd for $C_{24}H_{19}NO_7$: 434.12398; found 434.12301.

(5-Hydroxy-7,8,9-trimethoxy-11*H*-benzo[*a*]carbazol-6-yl)-(thiophen-2-yl)methanone (5c) and 2-(thiophene-2-carbonyl)-3-((3,4,5-trimethoxyphenyl)amino)naphthalene-1,4-dione (4f). These compounds were produced following the general procedure from **1f** (200 mg, 0.74 mmol), Ag_2O (445 mg, 1.92

mmol), dry magnesium sulfate (0.5 g) and TMA (271 mg, 1.48 mmol). Compound **5c** (164 mg, 0.38 mmol, 51%), yellow solid, mp: 256–258 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3400 (O–H), 3325 (N–H), 2960 (C–H Ar), 1678 (C=O), 1257 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.69 (s, 1H, OH), 8.62 (bs, 1H, NH), 8.51 (d, 1H, J = 8.5 Hz, 1-H), 8.02 (d, 1H, J = 8.0 Hz, 4-H), 7.70 (dd, 1H, J = 8.0, 7.2 Hz, 3-H), 7.55 (dd, 1H, J = 8.5, 7.3 Hz, 2-H), 7.51 (d, 1H, J = 4.8 Hz, 4′-H), 7.12 (d, 1H, J = 4.0 Hz, 2′-H), 6.78 (dd, 1H, J = 4.8, 4.0 Hz, 3′-H), 6.77 (s, 1H, 10-H), 3.93 (s, 3H, OMe), 3.71 (s, 3H, OMe), 3.61 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.7, 153.3, 150.3, 147.1, 146.8, 136.0, 135.5, 133.3, 132.9, 128.7, 128.6, 127.4, 125.4, 125.0, 123.6, 123.3, 119.9, 113.5, 111.5, 111.4, 89.0, 61.2, 59.9, 56.1. HRMS (APCI): $[M + H]^+$ calcd for $C_{24}H_{19}NO_5S$: 434.10622; found 434.10483. Compound **4f** (150 mg, 0.33 mmol, 45%), red solid, mp: 172–173 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3437 (O–H), 3294 (N–H), 2937 (C–H Ar), 1639 (C=O), 1130 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (dd, 2H, J = 7.7, 7.7 Hz, 5-H + 8-H), 7.81 (m, 2H, 7-H + 3′-H), 7.71 (t, 1H, J = 7.5 Hz, 6-H), 7.46 (bs, 1H, NH), 6.90 (m, 1H, 2′-H or 4′-H), 6.44 (m, 1H, 4′-H or 2′-H) 6.19 (s, 2H, 2″-H + 6″-H), 3.77 (s, 3H, OMe), 3.65 (s, 6H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.2, 181.4, 180.9, 153.5, 153.2 (2 × C), 145.7, 143.5, 136.9, 135.6, 132.9, 132.7, 132.5, 129.7, 126.7, 126.6, 117.3, 112.8, 112.6, 102.8 (2 × C), 60.9, 55.9 (2 × C). HRMS (APCI): $[M + H]^+$ calcd for $C_{24}H_{19}NO_6S$: 450.10114; found 450.09986.

(5-Hydroxy-7,8,9-trimethoxy-11*H*-benzo[*a*]carbazol-6-yl)-(thiophen-3-yl)methanone (5d) and 2-(thiophene-3-carbonyl)-3-((3,4,5-trimethoxyphenyl)amino)naphthalene-1,4-dione (4g). These compounds were generated following the general procedure from **1g** (200 mg, 0.74 mmol), Ag_2O (446 mg, 1.92 mmol), dry magnesium sulfate (0.5 g) and TMA (271 mg, 1.48 mmol). Compound **5d** (186 mg, 0.43 mmol, 58%), dark yellow solid, mp: 150–152 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3346 (O–H), 3107 (N–H), 2933 (C–H Ar), 1623 (C=O), 1126 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.05 (s, 1H, OH), 8.69 (bs, 1H, NH), 8.52 (d, 1H, J = 8.5 Hz, 1-H), 8.02 (d, 1H, J = 8.0 Hz, 4-H), 7.69 (dd, 1H, J = 8.0, 7.5 Hz, 3-H), 7.58 (d, 1H, J = 5.2 Hz, 4′-H), 7.55 (dd, 1H, J = 8.0, 7.5 Hz, 2-H), 7.46 (m, 1H, 1′-H), 7.14 (dd, 1H, J = 5.2, 3.0 Hz, 3′-H), 6.75 (s, 1H, 10-H), 3.91 (s, 3H, OMe), 3.66 (s, 3H, OMe), 3.60 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 191.4, 153.3, 150.8, 147.0, 144.0, 135.8, 135.5, 132.8, 128.7, 128.5, 127.7, 125.5, 124.9, 124.8, 123.7, 123.3, 119.9, 113.7, 111.7, 111.5, 89.0, 61.2, 59.9, 56.1. HRMS (APCI): $[M + H]^+$ calcd for $C_{24}H_{19}NO_5S$: 434.10622; found 434.10451. Compound **4g** (90 mg, 0.20 mmol, 27%), red solid, mp: 102–103.5 °C. IR (KBr) $\nu_{máx}$ cm^{-1}: 3099 (N–H), 2939 (C–H Ar), 1662 (C=O), 1128 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.15 (dd, 2H, J = 6.5, 6.5 Hz, 5-H + 8-H), 7.81 (m, 2H, 7-H + NH), 7.72 (t, 1H, J = 7.5 Hz, 6-H), 7.55 (d, 1H, J = 4.7 Hz, 2′-H or 3′-H), 7.42 (d, 1H, J = 4.7 Hz, 3′-H or 2′-H), 6.99 (dd, 1H, J = 4.3, 4.0 Hz, 4′-H), 6.12 (s, 2H, 2″-H + 6″-H), 3.79 (s, 3H, OMe), 3.60 (s, 6H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.1, 182.2, 181.5, 153.1 (2 × C), 145.5, 143.1, 136.8, 135.6, 134.5, 133.4, 132.9, 132.8, 132.2, 129.7, 127.6, 126.8, 126.6, 113.3, 103.7 (2 × C), 60.9, 55.9 (2 × C). HRMS (APCI): $[M + H]^+$ calcd for $C_{24}H_{19}NO_6S$: 450.10114; found 450.09948.

1.3. Synthesis of 3-acyl-2-arylamino-1,4-naphthoquinones 6a–d. General procedure[21]

Suspension of acylhydroquinones **1a**, **1b**, **1d** (1 equiv.), Ag_2O (2.6 equiv.), anhydrous $MgSO_4$ (500 mg), and dichloromethane (15 mL) were left with stirring at rt after completion of the reaction as indicated by TLC. The mixtures were filtered, the solvent removed under reduced pressure and the residues dissolved in methanol. The required anilines (1.1 equiv.) were added with stirring, and the resulting solutions were left at room temperature after completion of the reaction as indicated by TLC. The solvent was evaporated under reduced pressure and the residues were column chromatographied over silica gel (8 : 2 petroleum ether/EtOAc) to yield the corresponding acylaminoquinones **6a–d**.

3-Acetyl-2-anilino-1,4-naphthoquinone (6a). Prepared in 78% yield (2 h) from **1a** (150 mg, 0.74 mmol), Ag_2O (445 mg, 1.92 mmol), and aniline (75 mg, 0.81 mmol); dark red solid, mp: 135.5–136 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.69 (bs, 1H, NH), 8.10 (d, 1H, J = 7.4 Hz, 5-H), 7.82 (d, 1H, J = 7.5 Hz, 8-H), 7.71 (t, 1H, J = 7.4 Hz, 7-H), 7.56 (t, 1H, J = 7.4 Hz, 6-H), 7.31 (t, 2H, J = 7.3 Hz, 3′- and 5′-H), 7.23 (t, 1H, J = 7.1 Hz, 4′-H), 7.04 (d, 2H, J = 7.5 Hz, 2′-and 6′-H), 2.63 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.9, 182.3, 181.7, 151.6, 139.4, 135.5, 133.6, 132.8, 131.4, 129.4 (2 × C), 127.1, 126.8, 126.3, 124.7 (2 × C), 112.3, 33.3. The spectral data are in agreement to those reported in literature.[21]

2-Anilino-3-butiroyl-1,4-naphthoquinone (6b). Prepared in 71% yield (2.5 h) from **1b** (100 mg, 0.43 mmol), Ag_2O (260 mg, 1.12 mmol), and aniline (44 mg, 0.47 mmol); red solid, mp: 142–142.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.18 (bs, 1H, NH), 8.18 (dd, 1H, J = 7.8, 0.8 Hz, 5-H), 7.94 (dd, 1H, J = 7.7, 0.9 Hz, 8-H), 7.80 (dt, 1H, J = 7.6, 1.4 Hz, 7-H), 7.65 (dt, 1H, J = 7.5, 1.3 Hz, 6-H), 7.39 (m, 2H, 3′- and 5′-H), 7.29 (m, 1H, 4′-H), 7.12 (d, 2H, J = 7.6 Hz, 2′- and 5′-H), 3.05 (d, 2H, J = 7.4 Hz, CO–CH_2), 1.60 (m, 2H, $COCH_2CH_2$), 0.96 (t, 3H, J = 7.4 Hz, 13-$COCH_2CH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 205.1, 182.3, 181.6, 150.5, 139.1, 135.3, 133.4, 132.6, 131.0, 129.2 (2 × C), 126.9, 126.7, 126.1, 124.6 (2 × C), 112.6, 46.7, 17.7, 13.9. The spectral data are in agreement to those reported in literature.[33]

2-Anilino-3-benzoyl-1,4-naphthoquinone (6c). Prepared in 81% yield (3 h) from **1d** (200 mg, 0.76 mmol), Ag_2O (459 mg, 1.98 mmol), and aniline (78 mg, 0.84 mmol), red solid mp: 220.5–222.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (dd, 1H, J = 7.6, 1.2 Hz, 5-H), 8.12 (dd, 1H, J = 7.8, 1.1 Hz, 8-H), 7.90 (bs, 1H, NH), 7.81 (dt, 1H, J = 7.5, 1.4 Hz, 7-H), 7.72 (dt, 1H, J = 7.5, 1.4 Hz, 6-H), 7.55 (d, 2H, J = 8.4 Hz, 3′- and 5′-H anilino), 7.46 (t, 1H, J = 7.4 Hz, 4′-H anilino), 7.29 (m, 3H, 2′- 5′-H anilino), 6.99 (m, 3H, benzoyl), 6.85 (m, 2H, benzoyl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.7, 182.3, 182.1, 143.6, 137.4, 136.7, 135.5, 133.0, 132.9, 132.7, 129.9, 128.8 (4 × C), 128.2 (2 × C), 127.1, 126.7, 126.6, 126.1 (2 × C), 113.5. The spectral data are in agreement to those reported in literature.[33]

2-(4-Nitroanilino)-3-benzoyl-1,4-naphthoquinone (6d). Prepared in 80% yield (48 h) from **1d** (100 mg, 0.38 mmol), Ag_2O (229 mg, 0.99 mmol), *p*-nitroaniline (58 mg, 0.42 mmol); yellow solid, mp: 261–261.5 °C. IR (KBr) ν_{max} cm^{-1}: 3321 (N–H),

1667 (C=O), 1507 (NO_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (dd, 1H, J = 7.6, 1.1 Hz, 5-H), 8.16 (dd, 1H, J = 7.6, 1.1 Hz, 8-H), 8.02 (bs, 1H, NH), 7.87 (m, 3H, 3′-, 5′- and 7-H), 7.78 (dt, 1H, J = 7.5, 1.4 Hz, 6-H), 7.59 (dd, 2H, J = 8.3, 1.2 Hz, benzoyl), 7.54 (t, 1H, J = 7.4 Hz, benzoyl), 7.35 (t, 2H, J = 7.7 Hz, benzoyl), 6.97 (d, 2H, J = 8.9 Hz, 2′-and 6′-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.5, 182.1, 181.8, 145.1, 143.1, 142.5, 136.9, 135.8, 133.9, 133.3, 132.5, 129.8, 128.8 (2 × C), 128.6 (2 × C), 126.9, 126.8, 124.8 (2 × C), 124.5 (2 × C), 115.6. HRMS (ESI): $[M - H]^-$ calcd for $C_{23}H_{14}N_2O_5$: 398.09027; found: 398.08264.

1.4. Synthesis of 3-acyl-2-(3,4,5-trimethoxyphenyl)amino-1,4-naphthoquinones 4a, 4b and 4d. General procedure

Solutions of **6a–d** (1 equiv.), 3,4,5-trimethoxyaniline (1.1 equiv.) and methanol or ethanol (15 mL) were refluxed after completion of the reaction as indicated by TLC (0:13–61 h). The solvents were removed under vacuum and the corresponding products **4a**, **4b** and **4d** were isolated by column chromatography over silica gel (35 : 5 petroleum ether/EtOAc). Table 2 summarizes the results of these experiments.

1.5. Computational details

All the structures have been fully optimized using the Becke-3 for exchange and Lee–Yang–Parr for correlation (B3LYP)[34–37] functional with the standard 6-311G (d,p) basis set in solvent phases. To model the solvent, we used polarizable continuum model (PCM)[38] considering methanol (ε = 32.613). All the stationary states were confirmed by frequency calculations. All the above-mentioned calculations were performed using the Gaussian 09 suite of programs.[39]

1.6. Cytotoxicity assay

Cell Lines and Culture Conditions. Human cancer cell lines T24 (bladder), DU-145 (prostate), MCF-7 (breast) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cultures were maintained at a density of 1–2 × 10^5 cells per ml and the medium was changed at 48 to 72 h intervals. They were cultured in high-glucose Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum, penicillin (100 U per mL), and streptomycin (100 μg mL^{-1}) from Gibco (Grand Island, NY, USA). Balb/3T3 cells (normal mouse fibroblasts) were obtained from the European Collection of Cell Cultures (ECACC, UK) and cultured in the same medium, except that the fetal calf serum was replaced by 10% newborn calf serum. All cultures were kept at 37 °C in 95% air/5% CO_2 at 100% humidity. Phosphate-buffered saline (PBS) was purchased from Gibco. Cells were incubated at the indicated times at 37 °C with or without naphthoquinones **4a–g** at various concentrations.

1.7. Cellular assays

The cytotoxicity of compounds **4a–g** was assessed by following the reduction of MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) to formazan blue.[30] Briefly, cells were seeded into 96-well plates at a density of 10 000 cells per well for 24 h and then incubated for 48 h with or without the compounds. Cells were then washed twice with warm PBS and incubated with MTT (0.5 mg mL^{-1}) for 2 h at 37 °C. Incubation medium was thereafter discarded and the blue formazan crystals were solubilized by adding 100 μL DMSO per well. The color solution was subsequently read at 550 nm. Results are expressed as % of MTT reduction compared to untreated control conditions. The calculation of IC_{50} values were performed by using GraphPad Prism software (San Diego, CA, USA).

Conclusions

In summary, in the present work we have described results on the synthesis of novel 2-acyl-3-(3,4,5-trimethoxyanilino)-1,4-naphthoquinones of interest as potential anticancer agents. The synthetic approaches were based on direct amination of 2-acyl-1,4-naphthoquinones with TMA and on transamination reactions of TMA with 2-acyl-3-anilino- and 2-acyl-3-(4-nitroanilino)-1,4-naphthoquinones. The results reveal that the reactions of TMA with 2-acyl-1,4-naphthoquinones proceed through N–C and C–C bonding formation to give benzophenanthridinequinone and benzocarbazole derivatives, together with 2-acyl-3-(3,4,5-trimetoxyanilino)-1,4-naphthoquinones as the minor products. A density functional theory analysis performed on the stabilities of intermediates and products in these reactions are in good agreement with the experimental results on these competitive processes. Preliminary results on the reactions of TMA with 2-acyl-3-anilino-1,4-naphthoquinones **6a**, **6b**, **6c** that yield 2-acyl-3-(3,4,5-trimetoxyphenyl)amino-1,4-naphthoquinones **4a** and **4d**, demonstrates the potential use of this strategy for the synthesis of 2-acyl-1,4-naphthoquinones containing highly substituted anilino groups at 3-position. Studies on the scope of the transamination reactions of 2-anilino-3-acyl-1,4-naphthoquinones with substituted anilines are currently underway.

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Notes and references

1 R. H. Thompson, *Naturally Occurring Quinones IV: Recent Advances*, Blackie Academic & Professional, London, UK, 4th edn, 1997.
2 K. Maruyama and Y. Naruta, *Chem. Lett.*, 1977, **8**, 847–850.
3 H. J. Uno, *J. Org. Chem.*, 1986, **51**, 350–358.
4 M. A. Brimble and S. M. Lynds, *J. Chem. Soc., Perkin Trans. 1*, 1994, **5**, 493–496.
5 G. A. Kraus and H. Maeda, *Tetrahedron Lett.*, 1994, **35**, 9189–9190.
6 P. A. Waske, J. Mattay and M. Oelgemöller, *Tetrahedron Lett.*, 2006, **47**, 1329–1332.

7 J. A. Valderrama, D. Pessoa-Mahana, R. A. Tapia, A. Rojas de Arias, H. Nakayama, S. Torres, J. Miret and M. E. Ferreira, *Tetrahedron*, 2001, **57**, 8653–8658.
8 J. A. Valderrama, J. Benites, M. Cortés, D. Pessoa-Mahana, E. Prina and A. Fournet, *Tetrahedron*, 2002, **58**, 881–886.
9 J. A. Valderrama, C. Zamorano, M. F. González, E. Prina and A. Fournet, *Bioorg. Med. Chem.*, 2005, **13**, 4153–4159.
10 J. A. Valderrama, M. F. González, D. Pessoa-Mahana, R. A. Tapia, H. Fillion, F. Pautet, J. A. Rodríguez, C. Theoduloz and G. Schmeda-Hishmann, *Bioorg. Med. Chem.*, 2006, **14**, 5003–5011.
11 J. A. Valderrama and D. Vásquez, *Tetrahedron Lett.*, 2008, **49**, 703–706.
12 D. Vásquez, J. A. Rodríguez, C. Theoduloz, P. Buc and J. A. Valderrama, *Eur. J. Med. Chem.*, 2010, **45**, 5234–5242.
13 V. Delgado, A. Ibacache, C. Theoduloz and J. A. Valderrama, *Molecules*, 2012, **17**, 7042–7056.
14 W. Schäfer, A. Aguado and U. Sezer, *Angew. Chem., Int. Ed.*, 1971, **10**, 406–407.
15 M. Pardo, K. Joos and W. Schäfer, *Liebigs Ann. Chem.*, 1979, **4**, 503–521.
16 D. Ríos, J. Benites, J. A. Valderrama, M. Farias, R. C. Pedrosa, J. Verrax and P. Buc Calderon, *Curr. Top. Med. Chem.*, 2012, **12**, 2094–2102.
17 H. Buff and U. Kuckländer, *Tetrahedron*, 2000, **56**, 5137–5145.
18 G. R. Allen Jr and M. J. Weiss, *J. Org. Chem.*, 1968, **33**, 198–200.
19 J. A. Valderrama, M. F. González, P. Colonelli and D. Vásquez, *Synlett*, 2006, **17**, 2777–2780.
20 J. A. Valderrama, D. Ríos, G. G. Muccioli, P. Buc Calderon, I. Brito and J. Benites, *Tetrahedron Lett.*, 2015, **56**, 5103–5105.
21 D. Ríos, J. Benites, F. Torrejón, C. Theoduloz and J. A. Valderrama, *Med. Chem. Res.*, 2014, **23**, 4149–4155.
22 M. S. Farias, C. T. Pich, N. C. Falcão, K. B. Felipe, M. R. Kviecinski, F. Ourique da Silva, T. M. Fisher, D. Ríos, J. Benites, J. A. Valderrama, P. Buc Calderon and R. Curi Pedrosa, *Mol. Med. Rep.*, 2014, **10**(1), 405–410.
23 M. K. Hadden, S. A. Hill, J. Davenport, R. L. Matts and B. S. Blagg, *Bioorg. Med. Chem.*, 2009, **17**, 634–640.
24 C. D. S. Lisboa, V. G. Santos, B. G. Vaz, N. C. de Lucas, M. N. Eberlin and S. J. Garden, *J. Org. Chem.*, 2011, **76**, 5264–5273 and references cited therein.
25 Sh. A. Patil, R. Patil and D. D. Miller, *Curr. Org. Chem.*, 2008, **12**, 691–717.
26 J.-L. Bernier, J.-P. Henichart, C. Vaccher and R. Houssain, *J. Org. Chem.*, 1980, **45**, 1493–1496.
27 B. Dotzauer, R. Grunert, P. J. Bednarski, H. Lanig, J. Landwehr and R. Troschutz, *Bioorg. Med. Chem.*, 2006, **14**, 7282–7292.
28 P. Hohenberg and W. Kohn, *Phys. Rev. [Sect.] B*, 1964, **136**, 864–879.
29 R. G. Parr and W. Yang, *Density Functional Theory of Atoms and Molecules*, Oxford University Press, New York, USA, 1989.
30 W. Schäfer, H. W. Moore and A. Aguado, *Synthesis*, 1974, 30–32.
31 T. J. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63.
32 J. Benites, D. Ríos, P. Díaz and J. A. Valderrama, *Tetrahedron Lett.*, 2011, **52**, 609–611.
33 K. Kobayashi, M. Suzuki, H. Takeuchi, A. Konishi, H. Sakurai and H. Suginome, *J. Chem. Soc., Perkin Trans. 1*, 1994, **8**, 1099–1104.
34 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652.
35 C. Lee, W. Yang and R. Parr, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 1988, **37**, 785–789.
36 B. Miehlich, A. Savin, H. Stoll and H. Preuss, *Chem. Phys. Lett.*, 1989, **157**, 200–206.
37 S. Vosko, L. Wilk and M. Nusair, *Can. J. Phys.*, 1980, **58**, 1200–1211.
38 J. Tomasi, B. Mennucci and R. Cammi, *Chem. Rev.*, 2005, **105**, 2999–3094.
39 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven Jr J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz and J. D. J. Cioslowski Fox, *Gaussian 09 Revision B.01*, Gaussian, Inc., Wallingford CT, 2010.