Unprecedented synthesis of a novel amino quinone ring system *via* oxidative decarboxylation of quinone-based α,α-amino esters†

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An unusual and efficient method for the synthesis of new quinone-based amine and its derivatives from the corresponding α,α-amino ester is described. The procedure involves the quinone-based system's oxidative decarboxylation *via* hydride transfer throughout basic hydrolysis. This synthetic method provides, with good yields, rapid access to new potentially cytotoxic quinones.

Introduction

Extensive research has been devoted to the development of new potential anticancer agents related to anthracyclines (daunorubicin and doxorubicin) and anthracenediones (mitoxantrone) with improved pharmacokinetic properties, potency or spectrum and lower side effects.[1,2] In this regard and in connection with an ongoing research program aimed at discovering new quinone-based derivatives as potential cytotoxic agents, we have developed four different series of compounds containing a planar ring system, the 3-amino-3-(ethoxycarbonyl)-2,3-dihydrothieno[2,3-*b*]naphtho-4,9-dione system (**1**, DTNQ).[3] In fact, the functionalisation at N-3 position of this α,α-disubtituted amino ester with different acyl-derivatives (amino acids, phosgene, chloroacetyl chloride) gave the appropriate intermediate for the formation of pseudodipeptide (series **I**)[4] or spirohydantoins (series **II**)[5] or spirodiketopiperazine (series **III** and **IV**)[6] derivatives.

Some of these derivatives belonging to series **II** (R_2 = aminoalkyl), **III** (R_1 = thioalkyl or cycloalkyl), and **IV** (R_2 = aminoalkyl) showed remarkable cytotoxic activity against several human solid tumor and doxorubicin- and *cis*-platinum-resistant human cell lines.[5,6]

According to these findings and with the aim to closely examine the structure–activity relationships (SARs) of this compound class we have recently reported convenient methods for the synthesis of the aza analogues 3-amino-3-(ethoxycarbonyl)-2,3-dihydrothieno[2,3-*g* and 3,2-*g*] quinoline-4,9-dione (**2a**, **2b**, DTQQs, Fig. 1) which showed interesting cytotoxic activity.[7]

In view of the importance of quinone based system in the development of anticancer agents, we considered investigating new reactions that allow a more effective exploration of chemical space defined by this biologically active class of compounds. In this paper we present an efficient synthesis of a new and fully aromatic quinone-based amine system, the 3-aminonaphtho[2,3-*b*]thiophene-4,9-dione **3** (TNQ), that presents a more planar structure respect to DTNQ system and maintains the functional amine group.

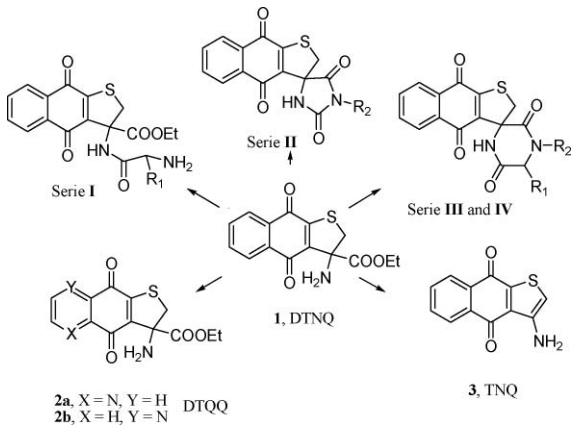

Fig. 1 Structures of the DTNQ derivatives.

Results and discussion

The access to this quinone-based system was first planned *via* direct dealkoxycarbonylation reaction by thermolysis using NaCN, NaCl or LiCl and water in dipolar aprotic solvents such as DMSO and DMF (Scheme 1, *via* A).[8] Instead, when these conditions were applied to the DTNQ (**1**), the formation of the corresponding derivative **3** was not observed. (See Table 1, entries 1, 2, and 3).

A second approach, involving hydrolysis and successive decarboxylation of the corresponding α,α-disubstituted amino acid, was taken in consideration (*via* B).[9]

Interestingly, during the first hydrolysis step of **1** in ethanol using 2 M $NaOH_{aq}$ as the base, we observed the formation of a complex mixture from which a blue colored product was isolated in 10% yield (Table 1, entry 4). The mass of the new compound in ES-MS (*m*/*z* 229.02) corresponded to the same molecular formula as that of decarboxylated derivative **3**. With respect to starting DTNQ the ^{1}H NMR spectrum in CD_3OD of compound **3** showed the

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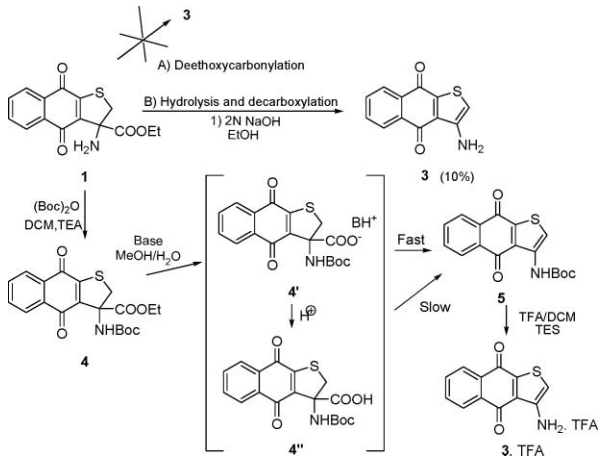
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of compound **3**, **5**, **7a** and **7b**, in addition spectrum of **12**, as sample of acyl derivatives. See DOI: 10.1039/b918898c

Table 1 Oxidative decarboxylation of **1**, **4**, and **4″**derivatives

Entry	Starting DTNQ	Solvent	Salt	Base	*T*/°C	Time/h	Amine Yield (%)
1	**1**	DMSO–H_2O	NaCl	—	160	48	**3** (-)
2	**1**	DMF–H_2O	NaCl	—	130	48	**3** (-)
3	**1**	DMF	LiCl	—	130	24	**3** (-)
4	**1**	EtOH	—	2 M NaOH	25	3	**3** (10)
5	**4**	EtOH	—	2 M NaOH	25	1	**5** (63)
6	**4**	EtOH	—	2 M KOH	25	1	**5** (58)
7	**4**	EtOH	—	2 M LiOH	25	1	**5** (69)
8	**4**	MeOH	—	2 M LiOH	25	0.5	**5** (75)
9	**4**	dry MeOH	—	DBU	25	1	**5** (-)
10	**4**	MeOH	—	DBU	25	1	**5** (10)
11	**4**	MeOH–H_2O	—	DBU	25	0.5	**5** (82)
12	**4**	MeOH–H_2O	—	DBU	reflux	1	**5** (-)
13	**4**	dry pyridine	—	DBU	25	1	**5** (-)
14	**4″**	MeOH	—	—	25	12	**5** (49)
15	**1**	MeOH–H_2O	—	DBU	25	2	**3** (15)
16[a]	**4**	MeOH–H_2O	—	DBU	25	0.5	**5** (80)

[a] The reaction was carried out under inert nitrogen atmosphere using a deoxygenated MeOH–H_2O solution.



Scheme 1 Decarboxylation of the DTNQ system.

disappearance of both the ethyl ester and the AB proton system and the appearance of the corresponding aromatic H-2 proton as single signal at 6.78 ppm. Accordingly, the ^{13}C NMR spectra showed the C-2 and C-3 signals at δ = 118.3 and 127.2 ppm, respectively.[10]

In order to avoid the possible negative influence of amino group in the reaction course, we have performed the reaction starting from N-Boc DTNQ derivative (**4**) obtaining the decarboxylated compound **5** after 1 h in higher yield (50%, entry 5). According to this preliminary result we propose that this unusual decarboxylation reaction goes *via* the hydrolyzed intermediate **4′**.

With the aim to optimize the yield and to shed light on the reaction mechanism, we performed a study of the influence of temperature, bases and solvents on this hydrolysis reaction (Scheme 1, Table 1). All the reactions were conducted from the N-Boc DTNQ derivative (**4**) dissolving it in methanol, ethanol, methanol–water, or pyridine/water and adding dropwise the base (2 M $LiOH_{aq}$, 2 M $NaOH_{aq}$, 2 M KOH_{aq}, or DBU), until depletion of **4**. After the hydrolysis was complete, the dissociated form (**4′**) appeared to be unstable and evolved, spontaneously, toward the formation of 3-(*tert*butyloxy carbonyl)aminonaphtho[2,3-*b*]thiophene-4,9-dione (**5**). The amount of the base used was an excess of 10 eq. in the case of alkali hydroxides or 3 eq. in the case of the DBU. All the other reaction conditions are indicated in Table 1. It is worth noting that the reaction conducted in complete absence of water (entries 9 and 13) did not afford product **5**, while a partial formation of our compound (10%, entry 10) was observed after one hour using a DBU/MeOH solution, probably due to hydroxide generated from water traces in the reaction medium. These results confirmed the reaction course *via* the not isolable hydrolyzed intermediate **4′**. The best conditions were either 2 M LiOH solution in methanol (entry 8) or the combination of DBU and methanol–water (90/10%) at room temperature (entry 11). An increase of temperature reaction, until reflux temperature, leads to a complete degradation within 10 min (entry 12).

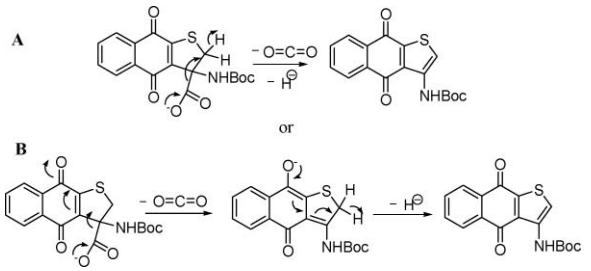
To gain further information on the reaction mechanism, after complete hydrolysis using 2 M $LiOH_{aq}$ as base, we treated immediately the reaction mixture with an aqueous 10% solution of citric acid up to pH 5.[11] Even if more slowly (entry 14), the decarboxylated product was obtained, probably through the non ionic form **4″**, obtained after protonation of **4′**.

Finally, the decarboxylation of DTNQ **1** with DBU and methanol–water (90/10%) at room temperature provided only a slight increase of yield in the formation of **3** (entry 15 *versus* entry 4), confirming its previously hypothesized instability and negative influence of the 3-amino group on reaction outcome in basic media.

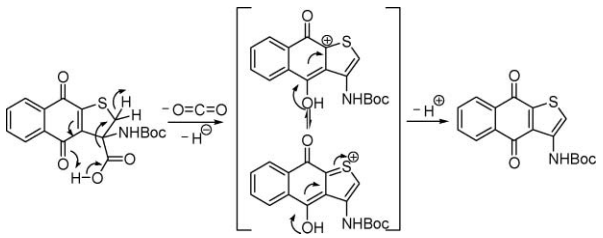
According to these findings, the formation of derivatives **3** and **5** involves a spontaneous oxidative decarboxylation of the quinone system. To the best of our knowledge, no previous examples of this type of reaction performed according the above described conditions were reported.[12]

Moreover, the data are consistent with a double pH dependent mechanism. In basic conditions (Scheme 2), we hypothesized two alternative routes involving A) a concerted elimination of CO_2 and hydride from the ionized form, and B) an initial step of CO_2 elimination that conduce to an electronic movement through the quinone system with subsequent loss of hydride.

As shown in Scheme 3, the non-ionized form could evolve to decarboxylated product through a cyclic transition structure[13]



Scheme 2 Proposed mechanisms of DTNQ decarboxylation in basic condition.



Scheme 3 Proposed mechanisms of decarboxylation in acidic conditions.

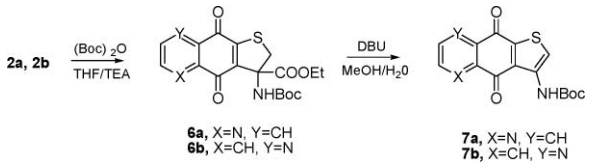
by essentially proton transfer from the carboxylic group to the γ-carbonyl oxygen of quinone system followed by hydride elimination.

Decarboxylation reaction implicates the substrate oxidation *via* hydride transfer to an electrophile[14] partner; we considered that the same DTNQ derivative could be first reduced and stepwise reoxidized by atmospheric oxygen. As a matter of fact Eriksson *et al.* have recently provided spectroscopic and theoretical evidences that some quinone systems could be reduced by NADH *via* hydride transfer.[15]

To evaluate this option, we conducted the reaction in complete absence of oxygen. The comparable reaction outcome (entry 16 *versus* 11), however, indicated that the DTNQ intermediate is not involved in this redox reaction. In addition, the high yield (>50%) of the final compound seems to indicate that the DTNQ naphtoquinone core didn't act as hydride scavenger agent.

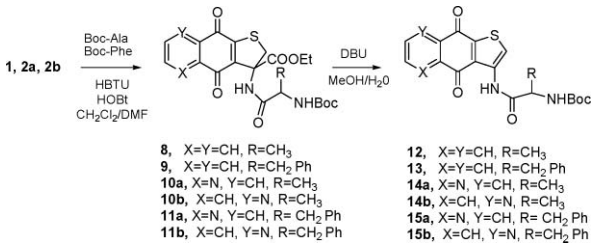
For these reasons we hypothesized that the reaction pathway probably includes a rapid quenching of the hydride by MeOH–H_2O medium. The reaction mechanism, suggested for the amine **3** (TNQ) and **5** formation, justifies both the speediness of reaction and the absence of secondary compounds in solution. After removal of the N-Boc protecting group using 20% TFA in dichloromethane and triethylsilane as scavenger, the amine **3** TNQ was obtained in 92% yield as trifluoroacetate salt. At this point we evaluated the potentiality of our new scaffold as a cytotoxic agent against two cellular lines.[16] This compound showed interesting cytotoxic activity toward the MCF-7 human breast carcinoma (IC_{50} = 3.2 μM) and SW 620 human colon carcinoma cell lines (IC_{50} = 4.0 μM) indicating that the TNQ system could be considered a potential starting point in discovering new and potent cytotoxic agents.

With the aim to assess both the scope of this reaction and its utility as synthetic approach to pharmacologically important thiophenequinone heterocyclics,[17] we performed the synthesis either of new aminoquinones from the conveniently protected DTQQ derivatives **2a** and **2b** (Scheme 4), or of aminoacyl quinone adducts from some DTNQ and DTQQ pseudodipeptide



Scheme 4 Formation of TQQ derivatives **7a** and **7b**.

derivatives (Scheme 5). Thus, treatment of Boc-DTQQ derivatives **6a** and **6b** with DBU in MeOH–H_2O (9/1) solution for 30 min provided the 3-(*tert*butyloxycarbonyl)aminothieno[2,3-*g* and 3,2-*g*]quinoline-4,9-dione derivatives **7a** and **7b** (TQQs) in 81 and 83% yield, respectively. According to these results, the electron withdrawing effect of the quinoline nitrogen atom doesn't seem to play a significant role on the reaction outcome.



Scheme 5 Synthesis of N-acyl decarboxylates.

To further evaluate the worth of this reaction in the preparation of various quinone-containing compounds, we applied the procedure to pseudopeptide derivatives of DTNQ (**1**) or DTQQ (**2a**, **2b**). Decarboxylation of compounds **8**, **9**, **10a**, **10b**, **11a**, and **11b**, obtained by condensation of DTNQ (**1**) or DTQQ (**2a**, **2b**) with Boc-Ala-OH or Boc-Phe-OH according standard procedures,[6a] afforded good yields (45–53% overall yield) of the desired 3-(acyl)aminonaphtho[2,3-*b*]thiophene-4,9-dione (**12**, **13**) or the aza analogues 3-(acyl)aminothieno[2,3-g]quinoline-4,9-dione (**14a**, **15a**) and the 3-(acyl)aminothieno[3,2-*g*]quinoline-4,9-dione (**14b**, **15b**).

Conclusion

In conclusion, the chemistry described here defines an efficient protocol for the synthesis of new quinone-based amine and amide derivatives. This unusual α,α-amino ester oxidative decarboxylation represents a new possibility of molecular diversification of our quinone systems, providing rapid access to TNQ or TQQ moieties, new building blocks for the development of potential antitumoral agents. Studies that include the synthesis and cytotoxic activity of new derivatives are actually in progress.

Experimental

General

Reagents, starting material and solvents were purchased from commercial suppliers and used as received. Analytical TLC was performed on plates coated with a 0.25 mm layer of silica gel 60 F254 Merck and preparative TLC on 20 × 20 cm glass plates coated with a 2 mm layer of silica gel PF254 Merck. Silica gel 60 (300–400 mesh, Merck) was used for flash chromatography. Melting points were measured with a Köfler apparatus and

are uncorrected. Mass spectra were obtained using an ES-MS spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 400 spectrometer operating at 400 and 100 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si and J values are reported.

The 3-amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*b*] naphtho-4,9-dione system (DTNQ) (**1**), the 3-(*N*-*tert*-butyloxy-aminoacyl)aminonaphtho[2,3-b]thiophene-4,9-dione (**8–9**), the 3-(*N*-*tert*-butyloxyaminoacyl) aminothiophene[2,3-g]quinoline-4,9-dione (**10a–11a**) and 3-(*N*-*tert*-butyloxy aminoacyl)amino-thiophene[3,2-g]quinoline-4,9-dione (**10b–11b**) derivatives were synthesized according to the procedure previously described.[3,7]

General procedure for the synthesis of N-protected quinone systems

Synthesis of Boc-DTNQ (**4**), Boc-DTQQ (**6a**, **6b**).

Di-*tert*-butyl dicarbonate (1.2 eq.) was added to a solution of DTNQ (**1**) or DTQQ (**2a** or **2b**) (0.2 mmol) and triethylamine (1.2 eq.) in dichloromethane (15 mmol). After 24–48 h at room temperature the solvent was washed with water and dried with Na_2SO_4. The protected derivatives were purified by flash chromatography (FC) using different eluent systems

3-(*tert*-Butyloxycarbonyl)-amino-3-ethoxycarbonyl-2,3-di hydrothieno[2,3-*b*]naphtho-4,9-dione (4). FC: n-hexane–EtOAc (4 : 1 v/v). Orange oil (63 mg, 79%) (Found 403.04 ES-MS. $C_{20}H_{21}NO_6S$ requires 403.10). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.26–1.30 (m, 3 H, CH_3 ester), 1.37 (s, 9H, CH_3 Boc), 3.0–3.82 (m, 2H, H-2), 4.28–4.30 (m, 2H, OCH_2), 6.31 (s, 1H, N*H*Boc), 7.72–7.80 (2H, m, H-6, H-7), 8.05 (1 H, d, J 7.8, H-5), 8.10 (1H, d, $J = 7.7$ Hz, H-8).

3-(*tert*-Butyloxycarbonyl)-amino-3-ethoxycarbonyl-2,3-dihydrothienothieno[2,3-*g*]quinoline-4,9-dione (6a). FC: EtOAc: n-hexane (5 : 1 v/v). Orange oil (56 mg, 70%) (Found 404.22 ES-MS. $C_{19}H_{20}N_2O_6S$ requires 404.11). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.23–1.26 (3 H, m, CH_3 ester) 1.38 (9H, s, CH_3 Boc); 3.82–3.85 (2H, m, CH_2), 4.27–4.30 (2 H, m, OCH_2) 6.31 (1 H, s, N*H*Boc), 7.62–7.65 (1H, m, H-7), 8.40–8.43 (1 H, d, J 9.6, H-8); 8.99–9.01 (1H, d, J 5.6, H-6).

3-(*tert*-Butyloxycarbonyl)-amino-3-ethoxycarbonyl-2,3-dihydro-thieno[3,2-*g*]quinoline-4,9-dione (6b). FC: EtOAc: n-hexane (5 : 1 v/v). Orange oil (54 mg, 67%) (Found 404.19 ES-MS. $C_{19}H_{20}N_2O_6S$ requires 404.11). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.22–1.27 (m, 3 H, CH_3 ester) 1.34 (s, 9H, CH_3 Boc); 3.83–3.84 (m, 2H, CH_2), 4.27–4.30 (m, 2 H, OCH_2) 6.26 (s, 1 H, N*H*Boc), 7.64–7.67 (m, 1H, H-6), 8.35–8.37 (d, $J = 8.0$ Hz, 1 H, H-5); 8.98–9.99 (d, $J = 2.8$ Hz, 1H, H-7).

General procedure for the decarboxylation of quinone-based α,α-amino esters and quinone-based pseudopeptides

Synthesis of Boc-TNQ (**5**), Boc-TQQ (**7a**,**7b**), N-acyl-TNQ (**12**, **13**), and N-acyl-TQQ (**14a**,**14b**, **15a**, and **15b**) derivatives.

DBU (0.6 mmol) was added dropwise to a solution of the corresponding Boc-protected DTNQ (**4**), or DTQQ (**6a** or **6b**), or Boc-pseudopeptide (**8**, **9**, **10a**, **10b**, **11a**, or **11b**) (0.2 mmol) in methanol–water (9 : 1, 10 mL). After 30 min at room temperature the solvents were evaporated and the reaction residues were dissolved in chloroform and washed with water and dried with Na_2SO_4. The title compounds were purified by flash chromatography (FC) using different eluent systems.

3-(*tert*-Butyloxycarbonyl)aminonaphtho[2,3-*b*]thiophene-4,9-dione (5). FC: n-hexane: EtOAc (4 : 1 v/v). Orange solid (54 mg, 82%) mp. 201–202 °C. (Found: C, 61.71; H, 4.34; N, 4.68; S, 10.01%; ES-MS, 329.31. $C_{17}H_{15}NO_4S$ requires C, 61.99; H, 4.59; N, 4.25; S, 9.74%; M 329.37). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.54 (9H, s, CH_3 Boc). 7.78–7.76 (2H, m, H-6 and H-7); 8.09 (1H, s, H-2); 8.24–8.20 (2H, m, H-5 and H-8); 9.43 (1H, s, N*H*); δ_C (100 MHz, $CDCl_3$; Me_4Si) 28.5 (*C*H_3 Boc), 79.2 (*C* Boc); 116.6 (C-2); 127.8 (C-6 and C-7); 129.6 (C-3); 133.7 (C-8a); 134.1 (C-4a); 134.7 (C-5 and C-8); 138.7 (C-3a); 142.8 (C-9a); 152.7; 177.8; and 181.2 (C=O). FAB-MS m/z calc. for $C_{17}H_{15}NO_4S$; found 329.36.

3-(*tert*-Butyloxycarbonyl)aminothieno[2,3-*g*]quinoline-4,9-dione (7a). FC: EtOAc: n-hexane (1 : 1 v/v). Orange solid (54 mg, 81%), m.p. 165–167 °C (Found: C, 58.27; H, 4.01; N, 8.17; S, 10.09%; ES-MS 330.26. $C_{16}H_{14}N_2O_4S$ requires C, 58.17; H, 4.27; N, 8.48; S, 9.71%; M, 330.07). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.48 (9 H, s, CH_3 Boc), 7.72–7.73 (1 H, m, H-7), 8.21 (1H, s, H-2), 8.57–8.59 (1 H, d, J 7.6 Hz, H-8), 9.07–9.08 (1 H, d, J 4.4, H-6), 9.42 (1H, s, N*H*). δ_C (100 MHz, $CDCl_3$) 28.5 (*C*H_3 Boc), 80.2 (*C* Boc); 110.1 (C-2), 127.0 (C-7); 129.6 (C-8a), 131.2 (C-3), 134.3 (C-8), 142.9 (C-3a) 147.2 (C-4a), 150.1 (C-9a), 154.7 (C-6), 161.1, 176.9 and 179.0 (C=O).

3-(*tert*-Butyloxycarbonyl)aminothieno[3,2-*g*]quinoline-4,9-dione (7b). FC: EtOAc: n-hexane (1 : 1 v/v). Orange solid (55 mg, 83%), m.p. 172–173 °C (Found: C, 58.38; H, 4.31; N, 8.01; S, 10.0%; ES-MS, 330.18. $C_{16}H_{14}N_2O_4S$ requires C, 58.17; H, 4.27; N, 8.48; S, 9.71%, M, 330.07). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.48 (s, 9 H, CH_3 Boc), 7.71–7.74 (m, 1 H, H-6), 8.19 (s, 1H, H-2), 8.55–8.57 (d, $J = 7.6$ Hz, 1 H, H-5), 9.07–9.08 (d, $J = 4.4$ Hz, 1 H, H-7) 9.32 (s, 1H, N*H*). δ_C (100 MHz, $CDCl_3$) 28.0 (*C*H_3 Boc), 80.1 (*C* Boc); 111.0 (C-2), 126.9 (C-7); 129.5 (C-4a), 131.2 (C-3), 134.3 (C-5), 142.9 (C-3a) 147.2 (C-8a), 150.1 (C-9a), 154.7 (C-6), 161.0, 177.6 and 179.7 (C=O).

3-(*N*-*tert*-Butyloxyalanyl)aminonaphtho[2,3-*b*]thiophene-4,9-dione (12). FC: n-hexane: EtOAc (3 : 2 v/v). Orange solid (64 mg, 80%), m.p. 189–190 °C (Found: C, 60.39; H, 4.81; N, 6.92; S, 8,42%; ES-MS, 400.25. $C_{20}H_{20}N_2O_5S$ requires C, 59.99; H, 5.03; N, 7.00; S, 8.01%; M, 400.11). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.51 (9 H, s, CH_3 Boc), 1.53 (3 H, d, β-CH_3), 4.41–4.43 (1 H, m, α-CH), 5.07 (1 H, s, N*H*), 7.77–7.78 (2 H, m, H-6 and H-7), 8.21–8.25 (2 H, m, H-5 and H-8), 8.47 (1H, s, H-2), 10.82 (1H, s, N*H*). δ_C (100 MHz, $CDCl_3$; Me_4Si) 18.8 (β-*C*H_3), 28.5 (*C*H_3 Boc), 49.5 (α-CH), 79.8 (*C* Boc); 116.8 (C-2); 127.8 (C-6 and C-7); 129.6 (C-3); 133.7 (C-8a); 134.1 (C-4a); 134.7 (C-5 and C-8); 138.7 (C-3a); 142.8 (C-9a); 153.7, 173.1, 177.8, and 181.2 (C=O).

3-(*N*-*tert*-Butyloxyphenylalanyl)aminonaphtho[2,3-b] thiophene-4,9-dione (13). FC: n-hexane–EtOAc (4 : 1). Orange solid (73 mg, 76%), m.p. 201–203 °C (Found: C, 65.13; H, 4.88; N, 5.51; S, 7.02%; ES-MS, 476.34. $C_{26}H_{24}N_2O_5S$ requires C, 65.53; H, 5.08; N, 5.88; S, 6.73%; M, 476.14). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.49 (s, 9 H, CH_3 Boc), 2.95–3.05 (2 H, m, β-CH_2), 4.42–4.45 (1 H, m, α-CH), 5.07 (1 H, s, N*H*), 7.12–7.22 (5 H, m, aryl), 7.77–7.78 (2 H, m, H-6 and H-7), 8.21–8.25 (2 H, m, H-5 and H-8), 8.47 (1H, s, H-2), 10.82 (1H, s, N*H*). δ_C (100 MHz, $CDCl_3$; Me_4Si) 28.5 (*C*H_3 Boc),

37.5 (β-*C*H_2), 50.5 (α-CH), 81.2 (*C* Boc); 116.8 (C-2); 127.8 (C-6 and C-7); 125.9, 127.6, 128.3, 128.9 and 137.9 (aryl), 130.6 (C-3); 133.7 (C-8a); 134.1 (C-4a); 134.7 (C-5 and C-8); 138.7 (C-3a); 142.8 (C-9a); 151.9, 174.3, 176.9, and 180.1 (C=O).

3-(*N*-*tert*-Butyloxylalanyl)aminothiophene[2,3-*g*]quinoline-4,9-dione (14a). FC: EtOAc: n-hexane (4 : 1 v/v). Orange solid (67 mg, 83%), m.p. 170 °C (dec) (Found: C, 57.05; H, 4.41; N, 10.00; S, 8.36%; ES-MS, 401.23. $C_{19}H_{19}N_3O_5S$ requires C, 56.85; H, 4.77; N, 10.47; S, 7.99%; M, 401.11). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.58 (s, 9 H, CH_3 Boc), 1.53 (3 H, d, β-CH_3), 4.39–4.41 (1 H, m, α-CH), 5.07 (1 H, s, N*H*), 7.71–7.74 (1 H, m, H-7), 8.57–8.59 (2 H, m, H-2 and H-8), 9.07–9.08 (1 H, m, H-6) 10.73 (1H, s, N*H*). δ_C (100 MHz, $CDCl_3$; Me_4Si) 18.8 (β-*C*H_3), 28.5 (*C*H_3 Boc), 48.3 (α-CH), 81.0 (*C* Boc); 110.6 (C-2), 126.9 (C-7); 129.8 (C-8a), 131.0 (C-3), 133.7 (C-8), 142.6 (C-3a) 147.0 (C-4a), 149.8 (C-9a), 153.5 (C-6), 161.0, 173.6, 177.6, and 179.7 (C=O).

3-(*N*-*tert*-Butyloxylalanyl)aminothiophene[3,2-*g*]quinoline-4,9-dione (14b). FC: EtOAc: n-hexane (4 : 1 v/v). Orange solid (65 mg, 81%), m.p. 169–170 °C (Found: C, 57.01; H, 4.39; N, 10.03; S, 8.39%; ES-MS, 401.30. $C_{19}H_{19}N_3O_5S$ requires C, 56.85; H, 4.77; N, 10.47; S, 7.99%; M, 401.11. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.57–1.55 (m, 12 H, CH_3 Boc and β-CH_3), 4.25–4.29 (m, 1 H, α-CH), 5.05 (s, 1 H, N*H*), 7.72–7.75 (m, 1 H, H-7), 8.45 (s, 1H, H-2), 8.54–8.56 (m, 1 H, H-6), 9.08–9.09 (m, 1 H, H-6) 10.71 (s, 1H, N*H*). δ_C (100 MHz, $CDCl_3$; Me_4Si) 19.8 (β-*C*H_3), 28.7 (*C*H_3 Boc), 50.1 (α-CH), 81.2 (*C* Boc); 110.8 (C-2), 126.3 (C-7); 130.0 (C-4a), 131.1 (C-3), 133.9 (C-5), 143.5 (C-3a) 146.9 (C-8a), 149.9 (C-9a), 154.0 (C-6), 160.7, 172.8, 177.6, and 179.7 (C=O).

3-(*N*-*tert*-Butyloxylphenylalanyl) aminothiophene [2, 3-*g*] quinoline-4,9-dione (15a). FC: EtOAc: n-hexane (3 : 1 v/v). Orange solid (68 mg, 71%), m.p. 190–192 °C (Found: C, 62.31; H, 4.45; N, 8.39; S, 7.04%; ES-MS, 477.23. $C_{25}H_{23}N_3O_5S$ requires C, 62.88; H, 4.85; N, 8.80; S, 6.70%; M, 477.14. δ_H (400 MHz; $CDCl_3$; Me_4Si) δ, 1.58 (s, 9 H, CH_3 Boc), 2.95–3.00 (m, 1 H, β-CH_2), 3.04–3.10 (m, 1 H, β-CH_2), 4.58–4.61 (m, 1 H, α-CH), 6.01 (s, 1 H, N*H*), 7.22–7.31 (m, 5 H, aryl), 7.69–7.72 (m, 1 H, H-7), 8.56–8.59 (m, 2 H, H-2 and H-8), 9.05–9.07 (m, 1 H, H-6), 10.62 (s, 1H, N*H*). δ_C (100 MHz, $CDCl_3$; Me_4Si) 27.1 (*C*H_3 Boc), 33.6 (β-*C*H_2), 51.1 (α-CH), 80.3 (*C* Boc); 111.6 (C-2), 126.1, 127.8, 128.9, and 138.2 (aryl), 126.1(C-7); 129.2 (C-8a), 130.9 (C-3), 133.9 (C-8), 141.9 (C-3a) 146.8 (C-4a), 150.1 (C-9a), 153.6 (C-6), 159.9, 173.0, 176.9, and 179.6 (C=O).

3-(*N*-*tert*-Butyloxylphenylalanyl) aminothiophene [3, 2-*g*] quinoline-4,9-dione (15b). FC: EtOAc: n-hexane (3 : 1 v/v). Orange solid (70 mg, 73%), m.p. 188–190 °C (Found: C, 62.36; H, 4.38; N, 8.47; S, 7.01%; ES-MS, 477.18. $C_{25}H_{23}N_3O_5S$ requires C, 62.88; H, 4.85; N, 8.80; S, 6.70%, M, 477.14). δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.50 (s, 9 H, CH_3 Boc), 2.92–3.01 (m, 1 H, β-CH_2), 3.04–3.07 (m, 1 H, β-CH_2), 4.51–4.58 (m, 1 H, α-CH), 5.94 (s, 1 H, N*H*), 7.26–7.37 (m, 5 H, aryl), 7.68–7.72 (m, 1 H, H-7), 8.53–8.55 (m, 2 H, H-2 and H-8), 9.02–9.03 (m, 1 H, H-6), 10.60 (s, 1H, N*H*). δ_C (100 MHz, $CDCl_3$; Me_4Si) 25.9 (*C*H_3 Boc), 32.3 (β-*C*H_2), 50.9 (α-CH), 81.0 (*C* Boc); 111.1 (C-2), 126.1, 127.6, 127.9, 128.6, and 138.9 (aryl), 126.2 (C-7); 128.9 (C-4a), 131.1 (C-3), 134.0 (C-5), 141.3 (C-3a) 148.0 (C-8a), 149.2 (C-9a), 153.7 (C-6), 161.0, 171.8, 177.5, and 179.9 (C=O).

Cytotoxic activity

The human breast adenocarcinoma MCF-7 and SW 620 colon carcinoma human were obtained from American Type Culture Collection. The cells were grown as monolayers in RPMI-1640 (Life Technologies, Inc.) containing 10% fetal bovine serum (Life Technologies, Inc., NY) and were allowed to attach and recover for another 24 h. Varying concentrations of drugs alone were then added to each well, and the plates were incubated in an atmosphere of 5% CO_2 and 95% air at 37 °C for an additional 24 h; then the plates were washed to remove the drug and incubated for 48 h. At the end of the treatment, cell viability was assessed by the sulforhodamine B (SRB) assay.[16] Data were expressed as %(T/C) (OD of treated cells/OD of control cells) × 100, and the concentration of the test compound causing a 50% inhibition of cell growth (IC50) was calculated from the dose/effect curve for each tested compound. Every assay was performed in triplicate, and the drug IC50 of each cell line was the average of at least three independent experiments.

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Me_4Si) 5.1 (2H, s br, NH_2), 7. 19 (1H, s, H-2); 7.88-7.92 (2H, m, H-6 and H-7); 7.98-8.01 (2H, m, H-5 and H-8). δ_C (100 MHz, $CDCl_3$; Me_4Si) 108.4 (C-2); 120.2 (C-3); 128.2 (C-5 and C-8); 129.7 (C-8a); 130.1 (C-4a); 134.7 (C-6 and C-7); 147.2 (C-3a); 149.7 (C-9a); 178.2, 179.8 (C=O).

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