Synthesis of new pyrido[4,3- *g* and 3,4-*g*]quinoline-5,10-dione and dihydrothieno[2,3-*g* and 3,2-*g*]quinoline-4,9-dione derivatives and preliminary evaluation of cytotoxic activity

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Abstract
Several pyrido[4,3-*g* and 3,4-*g*]quinoline-5,10-dione and dihydrothieno[2,3-*g* and 3,2-*g*]quinoline-4,9-dione derivatives were synthesized and evaluated for their potential cytotoxic properties. A number of these compounds exhibited significant *in vitro* antiproliferative activity at submicromolar concentration in a preliminary evaluation for their cytotoxic activity using the MT-4 cell line. These structures represent potential scaffolds in discovery of new agents with antitumoral activity and the synthetic strategy developed could be used to prepare libraries of new derivatives by combinatorial chemistry.

Keywords: Cytotoxic agents, quinolinedione derivatives, MT-4 cell line

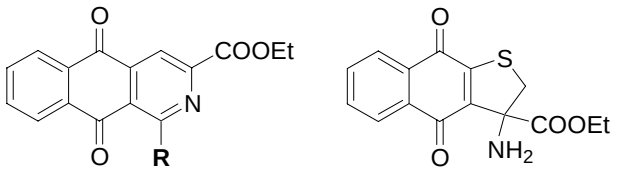
Introduction

The quinone nucleus is an important structural moiety in a number of complex chemotherapeutic agents target the DNA, playing an important role in determining their biological activities. Doxorubicin and mitoxantrone are representative of this class and are widely used in the treatment of several leukaemia and lymphomas as well as in combination chemotherapy of solid tumors.[1]

The importance of this class of antitumour agents has stimulated a number of studies, aimed to developing new agents that retain the core quinonic moiety yet exhibit different spectra of potency, together with reduced overall toxicity.[2] In this sense, the introduction of heteroatom (N, S) into different positions of the basic quinone system, has been one of approach adopted in

order to increase their therapeutic index. [3-5] These bioisosteres retain the planarity, spatial, and electronic characteristics required for molecular recognition at the cellular level and would clearly differ from their carbocyclic counterparts in their interaction with specific targets as well as in their reduction potential.

In this sense, we have recently reported a versatile method directed towards the synthesis of new cyclic quinone derivatives (Figure 1), that is, the benzo[*g*]isoquinoline-5,10-dione (I) and the dihydrothieno[2,3-*b*]naphtho-4,9-dione (II, DTNQ) derivatives which have presented evidence for their *in vitro* antitumour activity.[6,7]



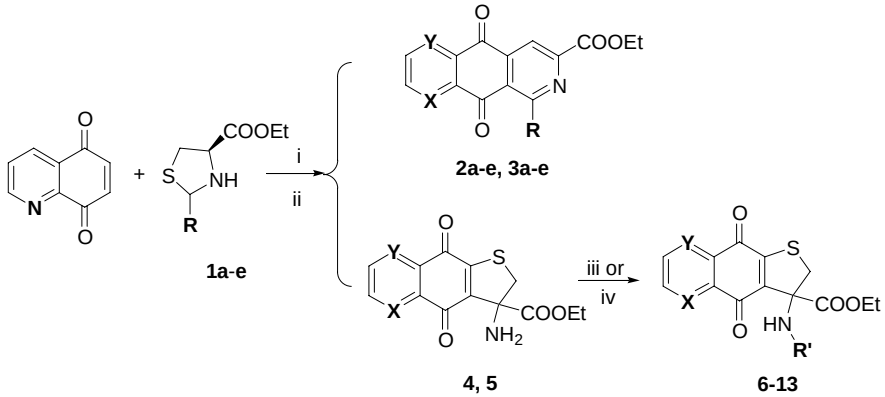
I II

Figure 1. Structures of compounds I and II.

In order to extend the synthetic scope of this method, we planned to synthesize new pyrido[4,3-*g* and 3,4-*g*]quinoline-5,10-dione and dihydrothiopheno[2,3-*g* and 3,2-*g*]quinoline-4,9-dione derivatives (DTQQ) considered as I and II aza analogues. These compounds and several acyl-DTQQ derivatives were evaluated *in vitro* for their cytotoxic activity against MT-4 cell line.

Results and Discussion

The compounds presented in this study were obtained by a cycloaddition reaction between the corresponding thiazolidine derivatives **1a-e** and quinoline-5,8-dione, using silver carbonate and DBU as base, following the method that we previously described [6] (Scheme 1).



Scheme 1. General synthetic method. Reagents: (i), Ag_2CO_3, DBU, CH_3CN; (ii) HCl/H_2O; (iii) Aryl-COCl, TEA, THF; (iv) a: Boc-L-Phe, HBTU, HOBt, DIEA, DMF; b: HCl/diethyl ether solution; c: Phenylisocyanate, CH_2Cl_2, Δ.

After acid hydrolysis and classic work-up, the corresponding pyrido[4,3-*g*]quinoline-5,10-dione (**2a-e)** and pyrido[3,4-*g*]quinoline-5,10-dione **(3a-e)** derivatives were obtained as regioisomeric mixture in different ratios (1-1.3:1), with yields varying from 20-45%. The regioisomeric mixtures **2c/3c** and **2d/3d** were chromatographically resolved whilst the compounds **2a/3a**, **2b/3b** and **2e/3e** were purified and tested as mixture of regioisomers. The ^{1}H NMR analysis of the **2** and **3** derivatives showed differences in the chemical shifts of 4-H and 6 or 9-H protons between the two regioisomers. The 4-H proton of regioisomers **2** was observed low field shifted of 0.11-0.25 ppm compared to corresponding signal for the compounds **3**. On the other hand, the 6-H proton of regioisomers **2** was high shifted of 0.12 ppm respect to 9-H of the compounds **3**. Proof of structures were made by 2D ^{1}H ^{13}C NMR HMBC correlation performed on **2d** and **3d.** (Figure 2).

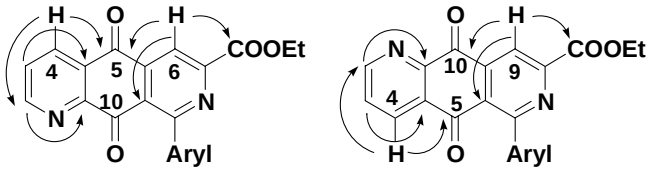


Figure 2. Correlation HMBC for regiosomers **2d** and **3d**.

In these experiments, long-range correlations were observed between the following protons and carbons of **2d**: H-4/C-2, H-4/C-5, H-6/C-5, and H-6/CO ester, while the opposite regioisomer **3d** gave ^{3}J coupling between H-4/C-2, H-4/C-5, H-9/C-10, and H-9/CO ester, so that the connectivities of the quaternary carbons (4a, 5a, 9a, and 10a) in **2** and **3** were clarified.

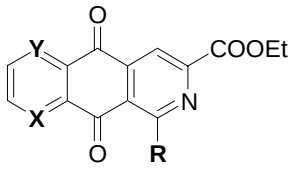
On the other hand, the dihydrothieno[2,3-*g* and 3,2-*g*]quinoline-4,9-dione derivatives **(DTQQs, 4** and **5)** were obtained as 2.2:1 regioisomeric mixture with yields varying from 15-40%, and separated chromatographically. The regiochemistry of compounds **4** and **5** have been assigned by reference to theoretical considerations and by analogy with the work of different authors.[8] Thus, the formation of major compound **4** may be attributed to the electron-withdrawing effect of the quinoline nitrogen atom making the C-8 carbonyl group more electron deficient, with preferential attack of the sulphur at the C-6 position. A series of acyl DTQQs derivatives containing different aromatic moiety, were prepared by coupling of **4** and **5** with both 2-iodo (**6** and **7**) and 3-fluorobenzoyl chloride (**8** and **9**) using TEA as base, and Boc-*L*-Phe amino acid using HBTU/HOBt as coupling agents. After Boc-deprotection, the compounds **10** and **11** were obtained as HCl salts. Subsequently, reaction of these compounds with phenylisothiocyanate gave the *N*-carbamoyl derivatives **12** and **13**, respectively. The structures of all compounds were confirmed from their analytical and spectroscopic data.

In this preliminary study, the compounds were tested *in vitro* for the grow inhibition of MT-4 cell lines, as monitored by the MTT method, [9,10] and the results are reported in Tables 1 and 2.

For comparative purposes, we evaluated the cytotoxic activities of compounds relative to doxorubicine.

As shown in Table 1, the regioisomer mixtures **2,3-b** and **2,3-e** and the compounds **2c**, **3c,** and **3d** exhibited similar activity against of MT-4 cell line at μM range. Only the compounds supporting a chloride atom at position 4 of phenyl ring showed certain specificity in the interaction with the target. In fact, the compound **3d** showed to be 18 times more active compared to the regioisomers **2d**.

Table 1. Cytotoxic Activity of 9-Aryl-7-ethoxycarbonylpyrido[4,3-*g*]quinoline-5,10-dione (**2a-e**) and 6-Aryl-8-ethoxycarbonylpyrido[3,4-*g*]quinoline-5,10-dione (**3a-e**) derivatives



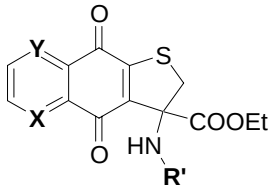
Compound	X	Y	R	Formula	CC_{50}(μM)[a]
Doxo					0.01
2a (3a)	N(CH)	CH(N)	C_6H_5	$C_{21}H_{14}N_2O_4$	36
2b (3b)	N(CH)	CH(N)	4-$CH_3C_6H_4$	$C_{22}H_{16}N_2O_4$	1.3
2c	N	CH	4-$(CH_3)_2NC_6H_4$	$C_{23}H_{19}N_3O_4$	4.4
3c	CH	N	4-$(CH_3)_2NC_6H_4$	$C_{23}H_{19}N_3O_4$	1.7
2d	N	CH	4-ClC_6H_4	$C_{21}H_{13}N_2O_4Cl$	27
3d	CH	N	4-ClC_6H_4	$C_{21}H_{13}N_2O_4Cl$	1.5
2e (3e)	N(CH)	CH(N)	3,4-$Cl_2C_6H_3$	$C_{21}H_{12}N_2O_4Cl_2$	1.3

[a] Compound concentration (μM) required to reduced the viability of mock-infected cells by 50%, as determined by MTT method.

However, more encouraging results were obtained by the DTQQ derivatives. In fact, the regioisomers **4** and **5** showed similar activity at submicromolar concentration (Table 2) and were 4 time more potent that the carbocyclic analogue, (DTNQ, CC_{50}= 1.2 μM).[7]

The incorporation in DTQQs of 2-iodo or 3-fluorobenzoyl residues (**6-9**) conserved practically the activity in the same range, whereas the incorporation of Phe residues (**10**, **11**) significantly reduced the cytotoxicity by 10 and 100 fold, respectively. This activity was recovered with derivatives supporting of phenylthio carbamoyl moiety (**12**, **13**).

Table 2. Cytotoxic Activity of 3-Amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*g*] quinoline-4,9-dione (**4**, **6, 8**, **10**, **12**) and 3-Amino-3-ethoxycarbonyl-2,3-dihydrothieno[3,2-*g*]quinoline-4,9-dione (**5**, **7**, **9**, **11**, **13**) derivatives



4-13

Compound	X	Y	R'	Formula	$CC_{50}(\mu M)$[a]
Doxo					0.01
4	N	CH	H	$C_{14}H_{12}N_2O_4S$	0.3
5	CH	N	H	$C_{14}H_{12}N_2O_4S$	0.3
6	N	CH	2-I-C_6H_4CO	$C_{21}H_{15}N_2O_5SI$	0.6
7	CH	N	2-I-C_6H_4CO	$C_{21}H_{15}N_2O_5SI$	0.8
8	N	CH	3-F-C_6H_4CO	$C_{21}H_{15}N_2O_5SF$	0.6
9	CH	N	3-F-C_6H_4CO	$C_{21}H_{15}N_2O_5SF$	0.9
10	N	CH	*L*-Phe.HCl	$C_{23}H_{22}N_3O_5SCl$	3
11	CH	N	*L*-Phe.HCl	$C_{23}H_{22}N_3O_5SCl$	28
12	N	CH	C_6H_5NHCS-*L*-Phe	$C_{30}H_{26}N_4O_5S_2$	0.8
13	CH	N	C_6H_5NHCS-*L*-Phe	$C_{30}H_{26}N_4O_5S_2$	2

[a] Compound concentration (μM) required to reduced the viability of mock-infected cells by 50%, as determined by MTT method.

Although the compounds here reported are less active compared to doxorubicine, the dihydrothieno[2,3-*g* and 3,2-*g*]quinoline-4,9-dione (DTQQ) derivatives represent a potential starting point in discovering of new antitumoral agents. In particular, the synthetic method developed to produce these compounds could be used to perform libraries by combinatorial approach to optimize the activity of the DTQQ derivatives. In fact, the results obtained indicate that it is possible to design analogues that could be more effective on tumoral cells by introducing of appropriate structural modifications on dihydrothiophene ring.

In conclusion, we report the synthesis and *in vitro* biological evaluation of new quinone derivatives as potential cytotoxic agents. The first results confirm the validity of our synthetic method providing practical access to quinone-based derivatives of intense current interest in antitumoral therapy. Further experiments aimed at defining the target and the mechanisms of the inhibitory effect showed by these molecules are in progress and the results will be reported in a forthcoming paper.

Experimental Section

General Procedures. Abbreviations used follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* 247, (1972) , 977-983. The following additional abbreviations are used: Boc, *tert*-butyloxycarbonyl; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; FAB-MS, fast-atom bombardment mass spectrometry; HOBt, *N*-hydroxybenzotriazole; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluoro-phosphate; FC, flash chromatography; DBU, 1,8-diazabicyclo [5.4.0]undec-7-one.

Reagents, starting material and solvents were purchased from commercial suppliers and used as received. Analytical TLC was performed on a 0.25 mm layer of silica gel 60 F_{254} Merck and silica gel 60 (300-400 mesh), Merck, was used for flash chromatography. Melting points were taken on a Kofler apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker-500 spectrometer, operating at 500 and 125 MHz respectively. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si and *J* values are reported in Herz (Hz). Mass spectra were obtained using a FAB-MS spectrometer. IR spectra were measured on a Nicolet Avatar 360 FT-IR; values are expressed in wavenumbers (cm^{-1}).

The ethyl 2(S,R)-(aryl)-1,3-thiazolidine-4(S)-carboxylate derivatives **1a-e** and the quinoline-5,8-dione were prepared following the procedure reported in literature,[11,12] and were used directly in the next step without further purification.

Chemistry

General procedure for the synthesis of compounds 2a-e/3a-e, 4 and 5

According to the procedure previously described[6] the corresponding thiazolidine (compounds **1a-e**) (1-3 mmol) was dissolved in dry acetonitrile (20-50 mL) and quinoline-5,8-dione (2 eq.), silver carbonate (1 eq. respect to thiazolidine) and a solution of DBU (0.2 eq respect to thiazolidine) in acetonitrile were added. After 12 h at room temperature, diethyl ether was added, the mixture was filtered and the solvent was evaporated. The reaction mixture was dissolved in chloroform and treated with 1N HCl solution (20-30 mL) for 1 h. Then, chloroform and water were added, the organic phase was washed with 1N HCl (2 x 25 mL), water (2 x 25 mL) and dried with anhydrous Na_2SO_4. Removal of the solvent and flash chromatography (FC) of the residues yielded the corresponding Diels-Alder adduct (compounds **2a-e** and **3a-e**). The collected acidic aqueous phases were neutralized with 10% $NaHCO_3$ solution and the free amines **4** and **5** (**DTQQs**) were extracted with chloroform (3 x 25 mL) and purified with FC using a gradient of 0-30% acetone in ethyl acetate.

9-Phenyl-7-ethoxycarbonylpyrido[4,3-*g*]quinoline-5,10-dione and 6-phenyl-8-ethoxy-carbonylpyrido[3,4-*g*]quinoline-5,10-dione (2a and 3a). The crude product was purified by FC using 70:30 EtOAc-hexane as eluent. Yellow solid (49%), m.p.: 205-208 °C.. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.47-1.53 (t, C*H*$_3$ ester), 4.45-4.51 (q, C*H*$_2$ ester), 7.50-7.52, 7.50-7.56, and 7.65-7.67 (m, 5H, aryl), 7.84-7.88 (m, 3-H) 8.55-8.57 (d, 4-H, **3a**, *J* = 8.4 Hz,), 8.62-8.64 (d, 4-

H, **2a**, *J* = 8.4 Hz), 8.73 (s, 6-H, **2a**), 8.85 (s, 9-H, **3a**), 9.03-9.05 (m, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.8 and 14.0 (*C*H_3 ester) 61.7 and 61.8 (*C*H_2 ester), 119.8 and 121.0 (6 and 9), 126.9 (9a), 126.8 and 127.1 (2', 4' and 6'), 128.3 and 128.5 (3), 128.6 and 128.8 (3' and 5'), 129.4 (4a), 135.7 (4), 137.1 (1'), 141.2 and 141.4 (5a), 149.2 (10a), 151.7 and 152.0 (7 and 8), 156.3 (2), 160.9 and 161.2 (6 and 9), 163.5, 181.5, 181.9 and 182.1 (C=O). FABMS m/z calcd for $C_{21}H_{14}O_4N_2$ 358.01, found 357.77 and 358.02.

9-(4'-Methyl)phenyl-7-ethoxycarbonylpyrido[4,3-*g*]quinoline-5,10-dione and **6-(4'-methyl)phenyl-8-ethoxycarbonylpyrido[3,4-*g*]quinoline-5,10-dione (2b and 3b).** The crude product was purified by FC using 70:30 EtOAc-hexane as eluent. Yellow solid (37%), m.p.: 202-209 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46-1.50 (t, C*H*$_3$ ester), 2.43 and 2.45 (s, C*H*$_3$), 4.47-4.50 (q, C*H*$_2$ ester), 6.74-6.78 and 7.58-7.65 (m, aryl), 7.77-7.80 (m, 3-H), 8.53-8.55 (d, 4-H, **3b,** *J* = 8.4 Hz), 8.63-8.65 (d, 4-H, **2b**, *J* = 8.4 Hz), 8.79 (s, 6-H, **2b**), 8.89 (s, 9-H, **3b**), 9.12-9.14 (m, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.8 and 14.0 (*C*H_3 ester), 24.9 and 25.6 (*C*H_3), 60.8 and 61.3 (*C*H_2 ester), 118.4 and 119.1 (6 and 9), 126.9 and 127.1 (2' and 6'), 126.7 and 126.9 (9a), 128.4 (3), 129.4 and 129.9 (4a), 129.5 and 129.8 (3' and 5'), 135.3 and 135.6 (4), 136.3 and 136.7 (1' and 4'), 141.3 and 142.5 (5a), 149.2 and 149.5 (10a), 151.7 and 153.4 (7 and 8), 156.3 and 156.5 (2), 160.8 and 161.2 (6 and 9), 162.7, 163.3, 180.3, 181.8 (C=O). FABMS m/z calcd for $C_{22}H_{16}O_4N_2$ 372.1, found 371.98.

9-[4'-(*N*-Dimethyl)amino]phenyl-7-ethoxycarbonylpyrido[4,3-*g*]quinoline-5,10-dione (2c). The crude product was purified by FC using 85:15 EtOAc-hexane as eluent. Yellow solid (16%), m.p.: 249-251 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.41-1.47 (t, 3H, C*H*$_3$ ester), 3.05 (s, N-(C*H*$_3$)$_2$), 4.50-4.54 (q, C*H*$_2$ ester), 7.29-7.31 (d, aryl, *J* = 8.2 Hz), 7.51-7.53 (d, aryl, *J* = 8.2 Hz), 7.76-7.79 (m, 3-H), 8.61-8.63 (d, 4-H, *J* = 8.4 Hz), 8.73 (s, 6-H), 9.14-9.15 (d, 2-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ14.2 (*C*H_3 ester), 40.1 (N-*C*H_3), 62.2 (*C*H_2 ester), 112.2 (3' and 5'), 118.7 (7), 126.8 and 127.8 (2' and 6'), 128.3 (3), 129.2 (1'), 129.4 (4a), 135.7 (4), 138.2 (4'), 141.4 (5a), 149.2 (10a), 151.7 (7), 156.3 (2), 161.8 (9), 164.0, 181.6, and 182.1 (C=O). FABMS m/z calcd for $C_{23}H_{19}O_4N_3$ 401.03, found 400.95.

6-[4'-(*N*-Dimethyl)amino]phenyl-8-ethoxycarbonylpyrido[3,4-*g*]quinoline-5,10-dione (3c). The crude product was purified by FC using 85:15 EtOAc-hexane as eluent. Yellow solid (17%), m.p.: 246-247 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.41-1.47 (t, C*H*$_3$ ester), 3.03 (s, N-(C*H*$_3$)$_2$), 4.50-4.54 (q, C*H*$_2$ ester), 7.27-7.32 (m, aryl), 7.76-7.79 (m, 3-H), 8.55-8.57 (d, 4-H, *J* = 8.4 Hz), 8.82 (s, 9-H), 9.14-9.15 (d, 2-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.2 (*C*H_3 ester), 40.3 (N-*C*H_3), 61.8 (*C*H_2 ester), 111.7 (3' and 5'), 119.8 (9), 126.3 (9a), 128.0 (2' and 6'), 128.3 (3), 129.1 (1'), 129.5 (4a), 135.2 (4'), 135.7 (4), 141.4 (4a), 146.8 (10a), 151.7 (8), 156.3 (2), 160.0 (6), 163.5, 180.3, and 181.1 (C=O). FABMS m/z calcd for $C_{23}H_{19}O_4N_3$ 401.03, found 401.15.

9-(4'-Chloro)phenyl-7-ethoxycarbonylpyrido[4,3-*g*]quinoline-5,10-dione (2d). The crude product was purified by FC using 70:30 EtOAc-hexane as eluent. Yellow solid (26%), m.p.: 215-216 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45-1.48 (t, C*H*$_3$ ester), 4.52-4.56 (q, C*H*$_2$ ester), 7.43-7.45 (d, aryl, *J* = 8.6 Hz), 7.53-7.55 (d, aryl, *J* = 8.6 Hz), 7.78-7.81 (m, 3-H), 8.64-8.66 (d, 4-H,

J = 8.4 Hz), 8.84 (s, 6-H), 9.15-9.16 (d, 2-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.2 (*C*H_3 ester) 62.7 (*C*H_2 ester), 119.8 (6), 126.9 (9a), 128.2 (3); 128.5 (2' and 6'), 129.4 (4a), 130.7 (3' and 5'), 135.2 (4'), 135.7 (4), 137.4 (1'), 141.4 (5a), 149.2 (10a), 151.7 (7), 156.3 (2), 161.2 (9), 163.5, 181.5, and 182.1 (C=O). IR (KBr cm^{-1}) 3050, 1740, 1665, 1650, 1610, 1600, 1280, 1123, 835. FABMS m/z calcd for $C_{21}H_{13}O_4N_2Cl$ 392.06, found 392.21.

6-(4'-Chloro)phenyl-8-ethoxycarbonylpyrido[3,4-*g*]quinoline-5,10-dione (3d). The crude product was purified by FC using 70:30 EtOAc-hexane as eluent. Yellow solid (20%), m.p.: 208-210 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42-1.47 (t, C*H*$_3$ ester), 4.52-4.57 (q, C*H*$_2$ ester), 7.47-7.49 (d, aryl, *J* = 8.6 Hz), 7.50-7.52 (d, aryl, *J* = 8.6 Hz), 7.80-7.82 (m, 3-H), 8.53-8.55 (d, 4-H, *J* = 8.4 Hz), 8.95 (s, 9-H), 9.17-9.18 (d, 2-H, *J* = 4.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.2 (*C*H_3 ester), 65.9 (*C*H_2 ester), 120.1 (9), 126.9 (9a), 128.3 (3), 128.1 (2' and 6'), 129.1 (4a), 131.5 (3' and 5'), 133.8 (4'), 134.7 (4), 137.8 (1'), 141.3 (5a), 148.8 (10a), 153.7 (8), 156.0 (2), 160.8 (6), 163.1, 181.0, and 181.9 (C=O). IR (KBr cm^{-1}) 3048, 1742, 1665, 1650, 1610, 1601, 1282, 1123, 835. FABMS m/z calcd for $C_{21}H_{13}O_4N_2Cl$ 392.06, found 392.22.

9-(3′,4′-Dichloro)phenyl-7-ethoxycarbonylpyrido[4,3-*g*]quinoline-5,10-dione and 6-(3',4'-Dichloro)phenyl-8-ethoxycarbonylpyrido[3,4-*g*]quinoline-5,10-dione (2e and 3e). The crude products was purified by FC using 65:35 EtOAc-hexane as eluent. Yellow solid (39%), m.p.: 239-242 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43-1.49 (m, C*H*$_3$ ester), 4.53-4.57 (m, C*H*$_2$ ester), 7.64 (s, aryl), 7.67 (s, aryl), 7.27-7.31 and 7.45-7.50 (m, aryl), 7.80-7.83 (m, 3-H), 8.53-8.55 (d, 4-H, **3e,** *J* = 8.4 Hz), 8.65-8.67 (d, 4-H, **2e,** *J* = 8.4 Hz), 8.81 (s, 6-H, **2e**), 8.93 (s, 9-H, **3e**), 9.16-9.18 (m, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8 and 13.9 (*C*H_3 ester), 61.9 and 60.8 (*C*H_2 ester), 118.6 and 119.1 (6 and 9), 126.3 and 127.0 (9a), 126.9 (6'), 127.1 (2'), 128.0 and 128.2 (3), 129.4 and 129.9 (4a), 130.7 and 130.9 (5'), 132.8 (4'), 134.7 (3'), 135.3 and 135.7 (4), 137.9 and 138.4 (1'), 141.3 and 141.4 (5a), 149.2 and 149.5 (10a), 151.7 and 153.4 (7 and 8), 156.3 and 156.4 (2), 159.8 and 161.2 (6 and 9), 163.7, 180.3, 181.7 and 182.0 (C=O). FABMS m/z calcd for $C_{21}H_{12}O_4N_2Cl_2$ 426.02, found 425.89 and 425.91

3-Amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*g*]quinoline-4,9-dione (4). The product was purified by FC using a gradient of 0-30% acetone in EtOAc. Yellow oil (33%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25-1.29 (m, C*H*$_3$ ester) 3.31-3.34 (d, 2-H, $J_{2,2'}$= 12.2 Hz), 3.86-3.88 (d, 2'-H), 4.25-4.31 (m, C*H*$_2$ ester), 7.66-7.64 (m, 7-H), 8.41-8.43 (d, 8-H, *J* = 7.7 Hz), 9.02-9.03 (d, 6-H, *J* = 4.2 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8 (*C*H_3 ester), 43.6 (2), 62.6 (*C*H_2 ester), 72.0 (3), 126.8 (7), 129.1 (8a), 134.2 (8), 142.4 (3a), 148.4 (4a), 154.6 (6), 156.4 (9a), 171.7, 176.8, and 179.5 (C=O). IR (KBr cm^{-1}) 3420, 3061, 2941, 1768, 1660, 1650, 1599, 1250, 1055, 759. FABMS m/z calcd for $C_{14}H_{12}O_4N_2S$ 303.90, found 303.61.

3-Amino-3-ethoxycarbonyl-2,3-dihydrothieno[3,2-*g*]quinoline-4,9-dione (5). The product was purified by FC using a gradient of 0-30% acetone in EtOAc. Orange oil (15%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20-1.27 (m, C*H*$_3$ ester), 3.32-3.34 (d, 2-H, $J_{2,2'}$= 12.2 Hz), 3.87-3.89 (d, 2'-H), 4.24-4.31 (m, C*H*$_2$ ester), 7.66-7.69 (m, 6-H), 8.37-8.39 (d, 5-H, *J* = 7.7 Hz,), 8.99-9.00 (d, 7-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9 (*C*H_3 ester), 44.0 (2), 62.6 (*C*H_2 ester), 72.1 (3), 127.5 (6), 129.9 (4a), 134.5 (5), 141.4 (3a), 148.2 (8a), 154.1 (7), 157.2 (9a), 171.9,

177.8, and 178.8 (C=O). IR (KBr cm^{-1}) 3420, 3060, 2950, 1768, 1658, 1599, 1250, 1055, 756. FABMS m/z calcd for $C_{14}H_{12}O_4N_2S$ 303.90, found 303.61.

General procedure for the synthesis of compounds 6-9

A solution of the corresponding 3-amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*g* or 3,2-*g*]quinoline-4,9-dione (**4** or **5**, 300 mg, 1 mmol) in CH_2Cl_2, was treated with of the appropriate 2-iodo or 3-fluorobenzoyl chloride (1.1 eq) and triethylamine (2.2 eq). After 1 h of stirring at room temperature, the solvent was evaporated to dryness. Flash chromatography of the resulting residues with CH_3Cl yielded the following compounds:

3-(2'-Iodophenylcarbonyl)amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*g*]quinoline-4,9-dione (6). Orange oil (410 mg, 78 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.26-1.28 (m, C*H*$_3$ ester) 3.86-3.89 (d, 2-H, $J_{2,\,2'}$= 12.6 Hz), 4.03-4.06 (d, 2'-H), 4.31-4.35 (m, C*H*$_2$ ester), 7.10-7.13 (t, aryl, *J*= 8.1 Hz), 7.35-7.38 (t, aryl, *J*= 8.1 Hz), 7.43-7.44 (d, aryl *J* = 5.1 Hz), 7.62-7.66 (m, 7-H and NH), 7.84-7.86 (d, aryl *J* = 4.3 Hz), 8.42-8.44 (d, 8-H, *J* = 7.7 Hz), 9.00-9.01 (d, 6-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9 (*C*H$_3$ ester), 40.5 (2), 63.6 (*C*H$_2$ ester), 72.5 (3), 92.3 (2'), 126.9 (5'), 128.2 (7), 128.5 (6'), 129.9 (8a), 131.4 (4'), 134.5 (8), 136.5 (1'), 139.9 (3'), 140.5 (3a), 148.6 (4a), 154.7 (6), 159.9 (9a), 168.5, 169.1, 176.6 and 179.5 (C=O). FABMS m/z calcd for $C_{21}H_{15}O_5N_2SI$ 533.97, found 534.16.

3-(2'-Iodophenylcarbonyl)amino-3-ethoxycarbonyl-2,3-dihydrothieno[3,2-*g*]quinoline-4,9-dione (7). Orange oil (430 mg, 81 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24-1.28 (m, C*H*$_3$ ester) 3.88-3.91 (d, 2-H, $J_{2,\,2'}$= 12.6 Hz), 4.02-4.05 (d, 2'-H), 4.31-4.37 (m, C*H*$_2$ ester), 7.11-7.14 (t, aryl, *J*= 8.1 Hz) 7.38-7.44 (m, aryl), 7.58 (s, NH), 7.65-7.68 (dd, 6- H, *J* = 7.7 and 4.3 Hz), 7.85-7.87(d, aryl *J* = 5.1 Hz), 8.36-8.38 (d, 5-H, *J* = 7.7 Hz,), 9.00-9.01 (d, 7-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.7 (*C*H$_3$ ester), 44.0 (2), 63.8 (*C*H$_2$ ester), 72.6 (3), 96.1 (2'), 126.9 (5'), 127.5 (6), 128.2 (6'), 129.4 (4a), 131.6 (4'), 134.5 (5), 136.5 (1'), 140.0 (3'), 140.4 (3a), 148.6 (8a), 154.8 (7), 160.0 (9a), 168.6, 169.2, 177.8 and 178.8 (C=O). FABMS m/z calcd for $C_{21}H_{15}O_5N_2SI$ 533.97, found 533.77.

3-(3′-Fluorophenylcarbonyl)amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*g*] quinoline-4,9-dione (8). Orange oil (350 mg, 84 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24-1.28 (m, C*H*$_3$ ester) 3.87-3.90 (d, 2-H, $J_{2,\,2'}$= 12.7 Hz), 3.91-3.94 (d, 2-H), 4.31-4.37 (m, C*H*$_2$ ester), 6.68 (s, aryl), 7.39-7.43 (m, aryl), 7.50-7.52 (d, aryl *J* = 8.0 Hz), 7.56-7.58 (d, aryl, *J* = 6.9 Hz), 7.62-7.65 (m, 7-H and NH), 8.06 (s, NH), 8.41-8.43 (d, 8-H, *J* = 7.6 Hz), 8.97-8.99 (d, 6-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9 (*C*H$_3$ ester), 40.5 (2), 63.6 (*C*H$_2$ ester), 72.5 (3), 92.3 (2'), 126.9 (5'), 128.2 (7), 128.5 (6'), 129.9 (8a), 131.4 (4'), 134.5 (8), 136.5 (1'), 139.9 (3'), 140.5 (3a), 148.6 (4a), 154.7 (6), 159.9 (9a), 168.5, 169.1, 176.6 and 179.5 (C=O). FABMS m/z calcd for $C_{21}H_{15}O_5N_2SF$ 426.07, found 426.11.

3-(3'-Fluorophenylcarbonyl)amino-3-ethoxycarbonyl-2,3-dihydrothieno[3,2-*g*]quinoline-4,9-dione (9). Orange oil (345 mg, 82 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24-1.28 (m, C*H*$_3$ ester) 3.86-3.89 (d, 2-H, $J_{2,\,2'}$= 12.6 Hz), 3.92-3.95 (d, 2-H), 4.31-4.37 (m, C*H*$_2$ ester), 7.11-7.14 (t, aryl, *J* = 8.0 Hz), 7.38-7.44 (m, aryl), 7.85-7.87 (d, aryl *J* = 8.0 Hz), 7.58 (s, NH), 7.65-7.68

(m, 6-H), 8.37-8.39 (d, 5-H, *J* = 7.7 Hz), 8.99-9.00 (d, 7-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8 (*C*H_3 ester), 41.9 (2), 62.7 (*C*H_2 ester), 71.9 (3), 114.2 (2'), 119.0 and 122.3 (4' and 6'), 127.5 (6), 129.1 (4a), 129.5 (5'), 134.8 (5), 135.1 (1'), 141.6 (3a), 148.2 (8a), 155.3 (7), 159.8 (9a), 160.1 (3'), 168.1, 169.2, 177.3 and 179.5 (C=O). FABMS m/z calcd for $C_{21}H_{15}O_5N_2SF$ 426.07, found 426.32.

3-(*L*-Phenylalanyl)amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*g*]quinoline-4,9-dione hydrochloride (10). The compound **4** (300 mg, 1 mmol) was dissolved in DMF (30 mL) and the Boc-*L*-Phe-OH (266 mg, 1 mmol), HBTU (517.6 mg, 1.2 mmol), HOBt (162.2 mg, 1.2 mmol) and DIPEA (2.4 mmol) were added successively to that solution. Stirring was continued at room temperature for 12 h. Afterwards, the solvent was evaporated, the residue was dissolved in $CHCl_3$, washed successively with 10% citric acid (2 x 25 mL), 10% $NaHCO_3$ (2 x 25 mL), H_2O (2 x 25 mL), dried over Na_2SO_4 and evaporated. Flash chromatography of the residue, using 30:70 hexane-EtOAc as eluent, yielded the corresponding 3-[*N*-(*tert*-Butoxycarbonyl)-*L*-phenylalanyl] amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*g*]quinoline-4,9-dione as diasteroisomeric mixture. Successively, the compound was dissolved in saturated EtOAc/(HCl) solution (15 mL) and stirred at room temperature for 4-6 h. The solvent was evaporated and the solid residue recovered was washed with diethyl ether. Yellow solid (230 mg, 47 %), m.p.: 219-221 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22-1.25 (m, C*H*$_3$ ester), 2.68-2.73, 2.98-3.17 and 3.24-3.27 (m, β-H), 3.42-3.50 and 3.58-3.61 (m, α-H), 3.75-3.78 (d, 2-H, *J* = 12.5 Hz), 3.79-3.81 (d, 2'-H), 4.27-4.30 (m, C*H*$_2$ ester), 7.19-7.21 (t, aryl, *J* = 8.6 Hz), 7.29-7.31 (d, aryl, *J* = 8.0 Hz), 7.64-7.67 (m, 7-H), 8.44-8.47 (m, 8-H), 8.90 and 8.96 (NH), 9.01-9.03 (m, 6-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.7 and 13.9 (*C*H_3 ester), 40.2 (β), 41.3 (2), 55.3 and 58.4 (α), 62.8 and 63.1 (*C*H_2 ester), 71.8 and 72.3 (3), 125.7 and 126.0 (4'), 126.5 and 127.1 (2' and 6'), 127.2 (7), 128.3 and 128.5 (3' and 5'), 129.0 and 129.3 (8a), 135.5 (8), 137.6 (1'), 141.9 (3a), 148.3 and 149.9 (4a), 154.7 (6), 159.9and 161.4 (9a), 168.5, 169.1, 170.1, 176.8 and 179.9 (C=O). FABMS m/z calcd for $C_{23}H_{21}O_5N_3S.HCl$ 487.62, found 487.09 and 487.33.

3-(*L*-Phenylalanyl)amino-3-ethoxycarbonyl-2,3-dihydrothieno[3,2-*g*]quinoline-4,9-dione hydrochloride (11). This compound was prepared from **5** (300 mg, 1 mmol) in an analogous way to **10**. Yellow solid (245 mg, 51 %), %), m.p.: 222-224 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.19-1.23 (m, CH_3 ester), 2.60-2.63, 2,79-2.91 and 3.17-3.21 (m, β-H, 2-H, *J* = 12.6 Hz), 3.41-3.47 and 3.59-3.62 (m, α-H), 3.75-3.81 (dd, 2'-H), 4.28-4.32 (m, CH_2 ester), 7.17-7.19 (t, aryl, *J* = 8.6 Hz), 7.21-7.23 (d, aryl, *J* = 8.0 Hz), 7.64-7.67 (m, 6-H), 8.43-8.47 (m, 5-H), 8.87 (s, NH), 8.91 (s, NH), 9.00-9.02 (m, 7-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ13.9 and 14.1 (*C*H_3 ester), 41.2 (β), 40.5 (2), 56.1 and 59.0 (α), 62.0 and 62.9 (*C*H_2 ester), 71.1 (3), 125.7, 126.9 and 127.1 (2', 4', and 6'), 127.5 (6), 128.2 and 128.5 (3' and 5'), 129.2 (4a), 134.9 (5), 137.5 (1'), 141.5 (3a), 148.0 (8a), 154.8 (7), 160.1 (9a), 168.6, 169.8, 176.9 and 179.8 (C=O). FABMS m/z calcd for $C_{23}H_{20}O_5N_3S.HCl$ 487.62, found 486.63 and 486.91.

3-[(*N*-Phenylthiocarbamoyl)-*L*-phenylalanyl]amino-3-ethoxycarbonyl-2,3-dihydro-thieno[2,3-*g*]quinoline-4,9-dione (12). Phenylisothiocyanate (16.2 mg, 0.12 mmol) was added to a solution of **10** (50 mg, 0.1 mmol) in CH_2Cl_2 (20 mL) and triethylamine (0.4 mmol). After 1 h of stirring at reflux temperature, the solvent was evaporated to dryness. Flash chromatography of the resulting residues, using 95:5 CH_3Cl-MeOH, yielded the title compound as a yellow solid (47 mg, 80%), m.p.: 247-248 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.20-1.25 (m, C*H*$_3$ ester), 3.01-3.05 and 3.09-3.13 (m, β-C*H*$_2$), 3.69-3.72 (d, 2-H, *J* = 12.6 Hz), 3.74-3.77 (d, 2'-H), 4.24-4.31 (m, C*H*$_2$ ester), 5.23-5.27 and 5.28-5.31 (m, α -H), 6.33-6.35 and 6.43-6.45 (NH), 6.94- 6.99 (m, aromatic), 7.09-7.14 (m, aromatic), 7.26-7.32 (m, aromatic), 7.63-7.67 (m, 7-H), 7.78 (s, NH), 7.80 (s, NH), 8.28-8.30 and 8.33-8.36 (m, 8-H), 8.97-8.99 (d, 6-H, *J* = 4.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8 and 14.1 (*C*H$_3$ ester), 38.7 (β), 41.3 (2), 59.3 and 60.4 (α), 62.8 and 63.1 (*C*H$_2$ ester), 70.1 and 71.3 (3), 124.5, 125.7, 126.0, 127.1 and 128.0 (aromatic), 127.2 (7), 128.3 and 128.5 (aromatic), 129.0 and 129.3 (8a), 135.5 (8), 136.7 and 139.4 (aromatic), 141.9 (3a), 148.3 and 149.9 (4a), 154.7 (6), 159.9 and 161.4 (9a), 167.3, 168.1, 171.0, 179.3 and 180.9 (C=O), 182.6 (C=S). FABMS m/z calcd for $C_{30}H_{26}O_5N_4S_2$ 586.19, found 586.34.

3-[(*N*-Phenylthiocarbamoyl)-*L*-phenylalanyl]amino-3-ethoxycarbonyl-2,3-dihydro-thieno [3,2-*g*]quinoline-4,9-dione (13). This compound was prepared from **11** (50 mg, 0.1 mmol) in an analogous way to **12**. Yellow solid (50 mg, 87%), m.p.: 236-238 °C . ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20-1.24 (m, C*H*$_3$ ester), 3.00-3.06 and 3.09-3.15 (m, β-C*H*$_2$), 3.68-3.71 (d, 2-H, *J* = 12.6 Hz), 3.76-3.79 (d, 2'-H), 4.24-4.32 (m, C*H*$_2$ ester), 5.20-5.24 (m, α-H), 6.24 (s, NH), 6.42 (s NH), 7.01-7.16 (m, 5H, aromatic), 7.27-7.33 (m, 5H, aromatic), 7.62-7.64 (m, 7-H), 7.73 (s, NH), 7.82 (s, NH), 8.40-8.43 (m, 5-H), 8.99-9.01 (m, 7-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.7 and 13.8 (*C*H$_3$ ester), 41.2 (β), 40.5 (2), 59.1 and 61.0 (α), 62.0 and 62.9 (*C*H$_2$ ester), 71.1 (3), 125.3, 126.8, 127.0, 127.9 (aromatic), 127.5 (6), 128.2 and 128.5 (aromatic), 129.2 (4a), 134.9 (5), 137.5 and 139.1 (aromatic), 141.5 (3a), 148.0 (8a), 154.8 (7), 160.1 and 161.3 (9a), 168.6, 169.8, 176.9 and 179.8 (C=O),183.6 (C=S). FABMS m/z calcd for $C_{30}H_{26}O_5N_4S_2$ 586.19, found 586.03 and 586.20.

Biological Activity Assays

Assays of antiviral and cytotoxic activities were carried out following established procedures. [10, 11] The compounds were dissolved in DMSO at an initial concentration of 200 μM and then were serially diluted in culture medium. Tumour cell growth at each drug concentration was expressed as percentage of untreated controls, and the concentration resulting in 50% (CC_{50}) growth inhibition was determined by linear regression analysis.

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