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ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY · SEPTEMBER 2003

Impact Factor: 2.79 · DOI: 10.1016/S0968-0896(03)00310-9 · Source: PubMed

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BIOORGANIC & MEDICINAL CHEMISTRY

New Benzo[*g*]isoquinoline-5,10-diones and Dihydrothieno [2,3-*b*]naphtho-4,9-dione Derivatives: Synthesis and Biological Evaluation as Potential Antitumoral Agents

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Received 3 January 2003; accepted 8 May 2003

Abstract—Novel antitumoral agents with quinonic structure were synthesized and evaluated for their in vitro cytotoxic activities. This study examines the cytotoxic activities of several aryl benzo[*g*]isoquinoline-5,10-dione derivatives and a number of aminoacyl dihydrothieno[2,3-*b*]naphtho-4,9-dione (DTNQ) derivatives containing amino acids in position 3 of the ring system. Compound **6** showed remarkable cytotoxic activity at submicromolar concentration not only against several human leukaemia and solid tumour cell lines, but also toward sensitive and resistant human cell lines.

Introduction

Quinone-containing antitumoral drugs such as doxorubicin, and mitoxantrone (Fig. 1) have been established as one of the most effective classes of anticancer agents in clinical use today with broad application in the treatment of several leukaemia and lymphomas as well as in combination chemotherapy of solid tumours.[1] However, toxic dose-related side effects such as myelosuppresion and cardiotoxicity, limited their clinical application.[2,3] The potent anticancer activity as well as toxic effects described for these compounds are normally ascribed, at least, to two main mechanisms: one, which is associated with protein, involves trapping of a protein enzyme–DNA cleavable intermediate, whereas the other, a non-protein-associated mechanism, is related to redox cycling of the quinone moiety, which produces damaging free-radical species.[4–7]

In searching for agents with an improved pharmacokinetic properties, potency or spectrum and lower side effects, a large number of quinone derivatives and related compounds have been prepared and several of these have shown promise in clinical studies.[8] Regarding structural and chemical modifications of this system, one of the most interesting modifications has been the introduction of heteroatoms (N, S) into different positions of the chromophore through incorporation of one or more of five or six-members heterocyclic ring to the basic quinone system.[9–11] These bioisosteres would clearly differ from their carbocyclic counterparts in their interaction with DNA and enzymes, as well as in their reduction potential.

We have recently reported[12] an easy and versatile method, in a one step reaction, directed towards the synthesis of two new cyclic quinone derivatives, that is, the benzo[*g*]isoquinoline-5,10-dione (**2**) (structurally related to aza-anthracene quinones) and the dihydrothieno[2,3-*b*]naphtho-4,9-dione (DTNQ, **3**) moiety (a novel α,α′-disubstituted amino ester).

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doi:10.1016/S0968-0896(03)00310-9

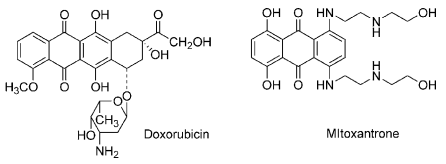


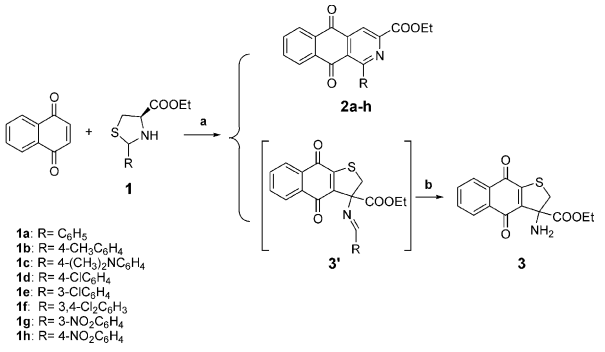
Figure 1.

These compounds, prepared by cycloaddition reactions between naphthoquinone and aryl thiazolidine derivatives (Scheme 1), would clearly retain the planarity, spatial, and electronic characteristics required for molecular recognition at the cellular level, which seem determine the antitumoral activity described for structurally related analogues.[9,11]

This study examines the cytotoxic activities of several aryl benzo[*g*]isoquinoline-5,10-dione derivatives and a number of aminoacyl DTNQ derivatives containing amino acids in position 3 of the ring system. The incorporation of the planar amide group would overall lead to more effective target-binding affinity.[13] In our case, the amino acids Glycine, β-Alanine and Gly-Gly were placed as they are nonchiral and conformationally rather flexible, then L-Phe, D-Phe, and L-Ala were incorporated, as suitable representatives for apolar, chiral and restricted residues, which could affect the affinity with potential target.

Chemistry

The 1-aryl-3-ethoxycarbonylbenzo[*g*]isoquinoline-5,10-dione derivatives, **2**, and the compound 3-amino-3-ethoxycarbonyl-2,3-dihydrothieno[2,3-*b*]naphtho-4,9-dione (DTNQ), (±)**3**, were obtained by cycloaddition reactions between the corresponding thiazolidine derivatives **1a–h** and napthoquinone as we previously described (Scheme 1).[12] Subsequently, the DTNQ derivatives containing L- and D-Phe, Gly, L-Ala, β-Ala and Gly-Gly (**4–9**), were prepared by coupling of DTNQ moiety with the corresponding Boc protected amino acid derivatives using HBTU/HOBt as coupling agents. After Boc-deprotection with HCl or TFA, the resulting products were obtained as racemic or diastereisomeric mixture in high yield (70–75%). The physicochemical properties and purity of the final compounds were assessed by LC–MS, analytical RP-HPLC, ^{1}H NMR, and TLC.



Scheme 1. Reagents and conditions: (a) Ag_2CO_3, DBU, Acetonitrile, rt, 12 h. (b) 1 N HCl, $H_2O/CHCl_3$, 1 h.

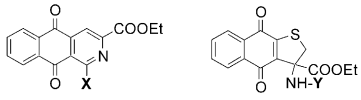
Results and Discussion

The compounds were tested in vitro for the growth inhibition of MT-4 cell and the results are reported in Table 1. As shown in table, the aryl-benzoisoquinolindione derivatives **2b**, **2d**, **2e** and **2f**, inhibited the proliferation of MT-4 cell line at μM concentration. In particular, the most potent compounds of this series were **2d** and **2f**, supporting a chlorine atom at position 3′ and/or 4′ in aryl ring.

Interesting results were obtained by DTNQ derivatives containing one amino acid in position 3 of the ring system. We first tested the DTNQ moiety in racemic (**3**) and in enantiomeric forms (**3a** and **3b**). The enantiomeric forms were separated and resolved as previously described.[12]

The racemic form **3** and the corresponding isomers **3a** and **3b** showed similar activity against MT-4 cell line (CC_{50} = 1.2, 1.3, and 3.2 μM, respectively). Subsequently, we prepared DTNQ analogues containing L-Phe (**4**) and D-Phe (**5**) amino acids starting from compound **3**, the respective diastereisomeric forms of **4** (**4a** and **4b**) and **5** (**5a** and **5b**) were also resolved and tested. The biological results demonstrated that both

Table 1. Antitumor activity of 1-aryl-3-ethoxycarbonylbenzo[*g*]-isoquinoline-5,10-dione derivatives **2a–2h**, and 3-substituted-amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naptho-4,9-dione derivatives **3–9**



Compd	CC_{50} (μM)[a]	X	Y
2a	100	C_6H_5	—
2b	13.0	4-$CH_3C_6H_4$	—
2c	>200	4-$(CH_3)NC_6H_4$	—
2d	8.2	4-ClC_6H_4	—
2e	11.0	3-ClC_6H_4	—
2f	8.4	3,4-diClC_6H_3	—
2g	>200	3-$NO_2C_6H_4$	—
2h	43.0	4-$NO_2C_6H_4$	—
3	1.2	—	H
(+)**3a**	1.3	—	H
(−)**3b**	3.2	—	H
4	3.2	—	L-Phe
4a	3.0	—	L-Phe
4b	3.0	—	L-Phe
5	5.6	—	D-Phe
5a	4.0	—	D-Phe
5b	6.7	—	D-Phe
6	0.06	—	Gly
7	0.08	—	L-Ala
8	0.07	—	β-Ala
9	0.40	—	Gly-Gly

[a]Compound dose required to reduce the viability of mock-infected cells (MT-4) by 50%, as determined by the MTT method.

diastereoisomeric mixtures (**4**, **5**) and diastereisomeric forms (**4a**, **4b, 5a, 5b**) had similar activity and comparable to DTNQ (Table 1). These results suggest that the stereochemistry may not have influence on interaction of these structures with the target. Thus, we decided to prepare and test all other compounds in respective racemic and diastereoisomeric mixtures.

In particular we prepared analogues in which Gly (**6**), L-Ala (**7**), β-Ala (**8**) and the dipeptide Gly-Gly (**9**) were incorporated into DTNQ moiety. Surprisingly, the compounds **6**, **7** and **8** showed a significant increase of activity of almost 20 times compared to lead compound **3** (CC_{50} 0.06, 0.08, 0.07 and 1.2 μM, respectively), while compound **9** showed to be active at 0.4 μM.

In an effort to understand better the biological profile of this series of compounds, we choose one of the most active compounds, that is compound **6**, to test in the panel of human tumour cell lines, using CEM, L1210, SK-MEL-28, MCF7, SK-MES-1 and DU145 cells (Table 2). In this further study we used as reference several antineoplastic drugs. In accordance with the results, this compound showed significant cytotoxic activity not only against several leukaemia cell lines, like CEM and L1210 (IC_{50} = 0.2 and 0.4 μM respectively), but also toward a number of other solid tumour cell lines, SK-MEL-28, MCF7, SK-MES-1, DU145 (IC_{50} range = 0.1–0.5 μM). Finally, since the tumour cells possess different mechanisms which confer resistance to a variety of anticancer agents,[14] we evaluated the cytotoxic activity of the compound **6** against the reference agents doxorubicine (Doxo), vincristine (VCR), and etoposide (VP16) in a panel of sensitive and resistant human cell lines of different origin ($KB_{wt,}$ KB^{MDR}, KB^{7D} and KB^{V20C})[15] (Table 3). It is worth to noting that compound **6** resulted to have a fully inhibitory activity to all these resistant cell lines (IC_{50} range = 0.6–0.9 μM). Further experiments aimed at defining the target and the mechanisms of the inhibitory effect showed by these molecules are in progress.

In conclusion, we report the synthesis and in vitro biological evaluation of new quinone derivatives as potential antitumoral agents. The first results showed that DTNQ-Gly derivative, **6**, was a potent compound characterized by remarkable in vitro antitumoral activity toward several leukaemia, solid tumour, and also resistant human cell lines. These structures represent potential scaffolds in discovery of new agents with antitumoral activity and the simple synthetic strategy developed could be used to perform libraries of new derivatives by combinatorial chemistry.

Table 2. Antitumor activity of 3-(glycyl)amino-3-etoxicarbonyl-2,3-dihydrothieno[2,3-*b*]naphtho-4,9-dione (**6**)

Compd	IC_{50}(μM)[a]						
	MT-4[b]	CEM[c]	L1210[d]	SK-MEL-28[e]	MCF7[f]	SK-MES-1[g]	DU145[h]
6	0.06	0.2	0.4	0.5	0.2	0.1	0.3
Doxo	0.01	0.01	0.2	0.5	0.1	0.07	0.06
VCR	0.0004	0.001	0.003	0.05	0.003	0.005	0.01
VP16	0.08	0.09	0.2	1.1	0.4	0.1	0.1
CAMPTO	ND	ND	ND	0.1	0.04	0.01	0.01
CisPt	ND	ND	ND	> 100	> 100	> 100	> 100

[a]Compound concentration required to reduce cell proliferation by 50% as determined by the MTT method, under condition allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (±SD) for three independent determinations.
[b]$CD4^+$ human T-cells containing an integrated HTLV-1 genome.
[c]$CD4^+$ human acute T-lymphoblastic leukaemia.
[d]Murine T-leukaemic cells.
[e]Human skin melanoma.
[f]Human breast adenocarcinome.
[g]Human lung squamous carcinoma.
[h]Human prostate carcinome. Doxorubicine (Doxo), vincristine (VCR), etoposide (VP16), camptothecin (CAMPTO), cisplatinun (CisPt).

Table 3. Effect of **6** on the proliferation of wild-type and drug-resistant KB cells

Compd	IC_{50}(μM)[a]			
	KB_{wt}[b]	KB^{MDR}[c]	KB^{7D}[d]	KB^{V20C}[e]
6	0.4±0.1	0.6±0.3	0.9±0.4	0.8±0.1
Doxo	0.03±0.02	1.8±0.01	2.8±0.2	0.4±0.1
VCR	0.006±0.001	0.7±0.2	0.05±0.01	0.2±0.09
VP16	0.6±0.1	20±0.01	20±0.05	6.0±1.7

[a]Compound concentration required to reduce cell proliferation by 50% as determined by the MTT method, under condition allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (±SD) for three independent determinations.
[b]KB human nasopharyngeal carcinoma.
[c]KB subclones passaged in the presence of doxorubicin (Doxo) 0.09 μM.
[d]KB subclones passaged in the presence of etoposide (VP16) 7 μM.
[e]KB subclones passaged in the presence of vincristine (VCR) 0.02 μM.

Experimental

General. 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 preparative TLC on 20×20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} Merck. Silica gel 60 (300–400 mesh), Merck, was used for flash chromatography. Melting points were taken on a Kofler apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer-241 MC polarimeter. ^{1}H NMR spectra were recorded with a Bruker-500 spectrometer, operating at 500 MHz. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si and *J* values are reported in Hertz (Hz). The ethyl 2(*S*,*R*)-(aryl)-1,3-thiazolidine-4(*S*)-carboxylate derivatives **1a–h** were prepared following the procedure reported[16] and were used directly in the next step without further purification.

General procedure for the synthesis of the 1-aryl-3 ethoxycarbonylbenzo[*g*]isoquinoline-5,10-dione derivatives (2a–h) and 3-amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione (3)

According to the procedure previously described,[2] the corresponding thiazolidine **1a–h** (1–3 mmol) was dissolved in dry acetonitrile (20–50 mL) and naphthoquinone (2 equiv), silver carbonate (1 equiv respect to thiazolidine) and a solution of DBU (0.2 equiv 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 residue of the reaction was dissolved in chloroform and treated with 1 N HCl solution (20–30 mL) for 1 h. Then, chloroform and water were added, the organic phase was washed with 1 N HCl (2×25 mL), water (2×25 mL) and dried with Na_2SO_4 anhydrous. Removal of the solvent and flash chromatography of the residues, using $CHCl_3$ as eluent, yielded, in each case, the corresponding Diels–Alder adducts **2a–h**. In the other hand, the collected acid aqueous phases were neutralized with 10% $NaHCO_3$ solution and the free amine **3** (DTNQ) was extracted with chloroform. Flash chromatography using a gradient of 0–30% ethyl ether in $CHCl_3$ gave compound **3** as a orange oil: 11% from **1a**, 20% from **1b**, 50% from **1c**, 45% from **1d**, 38% from **1e**, 45% from **1f**, 47% from **1g** and 45% from **1h**. ^{1}H NMR (500 MHz, $CDCl_3$) δ, 1.25–1.28 (m, 3H, C*H*$_3$ ester) 3.26–3.29 (d, 1H, 2-H, $J_{2,2'}$ = 12.3 Hz), 3.82–3.85 (d, 1H, 2′-H), 4.27–4.31 (m, 2H, CH_2 ester), 7.69–7.76 (m, 2H, 6-H and 7-H) 8.04–8.06 (d, 1H, 8-H or 5-H), 8.09–8.11 (d, 1H, 5-H or 8-H). MS [M^+] calcd for $C_{15}H_{13}O_4NS$: 303.3, found: 304.5.

The following substituted benzo[*g*]isoquinoline-5,10-dione were prepared by the general method above.

1 - Phenyl - 3 - ethoxycarbonylbenzo[g]isoquinoline - 5,10-dione (2a). Yellow solid (49%), mp 218–219 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45–1.48 (t, 3H, C*H*$_3$ ester), 4.45–4.52 (q, 2H, C*H*$_2$ ester), 7.05–7.55 (m, 5H, aryl), 7.83–7.86 (m, 2H, 7-H and 8-H), 8.17–8.19 and 8.32–8.34 (dd, 2H, 6-H and 9-H), 8.85 (s, 1H, 4-H). MS [M^+] calcd for $C_{22}H_{15}O_4N$: 357.9, found: 358.3.

1 - (4′ - Methyl)phenyl - 3 - ethoxycarbonylbenzo[g]isoquinoline-5,10-dione (2b). Yellow solid (37%), mp 263–265 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40–1.42 (t, 3H, C*H*$_3$ ester), 2.45 (s, 3H, p-CH_3), 4.49–4.52 (q, 2H, C*H*$_2$ ester), 7.28–7.30 (m, 2H, aryl), 7.45–7.48 (m, 2H, aryl), 7.83–7.85 (m, 2H, 7-H and 8-H), 8.18–8.20 and 8.31–8.33 (dd, 2H, 6-H and 9-H), 8.81 (s, 1H, 4-H). MS [M^+] calcd for $C_{23}H_{17}O_4N$: 371.3, found: 371.7.

1-[4′-(*N*-Dimethyl)amino]phenyl - 3ethoxycarbonylbenzo[g]-isoquinoline-5,10-dione (2c). Yellow crystalline solid (10%), mp 243–244 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.38–1.41 (t, 3H, C*H*$_3$ ester), 2.98 (s, 6H, N-(C*H*$_3$)$_2$), 4.42–4.56 (q, 2H, C*H*$_2$ ester), 6.69–6.71 (d, 2H, aryl), 7.50–7.52 (d, 2H, aryl) 7.76–7.80 (m, 2H, 7-H and 8-H), 8.14–8.16 and 8.22–8.24 (dd, 2H, 6-H and 9-H), 8.60 (s, 1H, 4-H). MS [M^+] calcd for $C_{24}H_{20}O_4N_2$: 400.1, found: 400.8.

1-(4′-Chloro)phenyl-3-ethoxycarbonylbenzo[g]isoquinoline-5,10-dione (2d). Yellow solid (15%), mp 217–219 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45–1.48 (t, 3H, C*H*$_3$ ester), 4.51–4.55 (q, 2H, C*H*$_2$ ester), 7.46–7.51 (dd, 4H, aryl), 7.85–7.88 (m, 2H, 7-H and 8-H), 8.18–8.20 and 8.33–8.35 (dd, 2H, 6-H and 9-H), 8.86 (s, 1H, 4-H). MS [M^+] calcd for $C_{22}H_{14}O_4NCl$: 391.5, found: 392.1.

1 - (3′ - Chloro)phenyl - 3 - ethoxycarbonylbenzo[g]isoquinoline-5,10-dione (2e). Yellow solid (25%), mp 214–215 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46–1.49 (t, 3H, C*H*$_3$ ester), 4.52–4.56 (q, 2H, C*H*$_2$ ester), 7.42–7.44 (d, 1H, aryl), 7.48–7.51 (m, 2H, aryl), 7.54 (s, 1H, aryl), 7.84–7.87 (m, 2H, 7-H and 8-H), 8.17–8.19 and 8.33–8.35 (dd, 2H, 6-H and 9-H), 8.88 (s, 1H, 4-H). MS [M^+] calcd for $C_{22}H_{14}O_4NCl$: 391.5, found: 392.3.

1-(3′,4′-Dichloro)phenyl-3-ethoxycarbonylbenzo[g]isoquinoline-5,10-dione (2f). Yellow solid (20%), mp 271–273 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46–1.49 (t, 3H, C*H*$_3$ ester), 4.52–4.56 (q, 2H, C*H*$_2$ ester), 7.34–7.36 (d, 1H, aryl), 7.54–7.56 (d, 1H, aryl), 7.66 (s, 1H, aryl), 7.84–7.87 (m, 2H, 7-H and 8-H), 8.23–8.25 and 8.33–8.35 (dd, 2H, 6-H and 9-H), 8.89 (s, 1H, 4-H). MS [M^+] calcd for $C_{22}H_{13}O_4NCl_2$: 425.8, found: 426.3.

1-(3′-Nitro)phenyl-3-ethoxycarbonylbenzo[g]isoquinoline-5,10-dione (2g). Yellow solid (18%), mp 230–231 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46–1.49 (t, 3H, C*H*$_3$ ester), 4.51–4.56 (q, 2H, C*H*$_2$ ester), 7.65–7.68 (m, 2H, aryl), 7.80–7.82 (d, 1H, aryl), 7.83 (s, 1H, aryl), 7.85–7.88 (m, 2H, 7-H and 8-H), 8.16–8.18 and 8.38–8.40 (dd, 2H, 6-H and 9-H), 8.94 (s, 1H, 4-H). MS [M^+] calcd for $C_{22}H_{14}O_4N_2$: 402.9, found: 403.6.

1-(4′-Nitro)phenyl-3-ethoxycarbonylbenzo[g]isoquinoline-5,10-dione (2h). Yellow solid (15%), mp 235–236 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46–1.49 (t, 3H, C*H*$_3$ ester), 4.51–4.56 (q, 2H, C*H*$_2$ ester), 7.66–7.69 (m, 2H, aryl), 7.80–7.82 (d, 2H, aryl), 7.86–7.89 (m, 2H, 7-H and 8-H), 8.18–8.20 and 8.38–8.40 (dd, 2H, 6-H and 9-H),

8.91 (s, 1H, 4-H). MS [M^+] calcd for $C_{22}H_{14}O_4N_2$: 402.9, found: 403.7.

General procedure for the synthesis of the 3-(*tert*-butoxycarbonylaminoacyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione derivatives (4′-9′)

The compound **3** (1–2 mmol) was dissolved in (3:1) THF/DMF (30 mL) and the corresponding amino acid (Boc-L-Phe, Boc-D-Phe, Boc-Gly, Boc-L-Ala, Boc-β-Ala or Boc-Gly-Gly) (1 equiv), *O*-Benzotriazole-1-yl-*N*,*N*,*N*′,*N*′-tetramethylisouronium hexafluoro phosphate (HBTU, 1.2 equiv), hydroxybenzotriazol (HOBt, 1.2 equiv) and diisopropylethylamine (DIEA, 2.4 equiv) were added successively to that solution; stirring was continued at room temperature for 48–72 h. Afterwards, the solvents were evaporated, the residue was dissolved in $CHCl_3$, washed successively with 10% citric acid (2×25 mL), 10% $NaHCO_3$ (2×25 mL), H_2O (25 mL), dried over Na_2SO_4 and evaporated. Flash chromatography of the residues with 20–50% gradients of EtOAc in *n*-hexane yielded in each case, the (1:1) diasteroisomeric mixtures **4′**–**9′**.

3-[*N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtha-4,9-dione (4′). Yellow solid (55% yield) mp. 204–206. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25–1.28 (m, 3H, CH_3 ester), 1.39 (s, 9H, Boc), 2.95–3.04 (m, 2H, β-H), 3.56–3.59, 3.66–3.68 and 3.78–3.80 (ddd, 2H, 2,2′-H), 4.24-4.30 (m, 2H, CH_2 ester), 4.39–4.40 and 5.00–5.01 (m, 1H, α-H), 6.78 (s, 1H, NH), 7.13–7.23 (m, 5H, aryl), 7.71 (s, 1H, NH), 7.76–7.79 (m, 2H, 6-H and 7-H), 7.99–8.01 (d, 1H, 8-H or 5H), 8.09–8.11 (d, 1H, 5-H or 8-H).

3-[*N*-(*tert*-Butoxycarbonyl)-D-phenylalanyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione (5′). Yellow solid (51% yield), mp 205–207. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.14–1.19 (m, 3H, CH_3 ester), 1.39 (s, 9H, Boc), 2.98–3.05 (m, 2H, β-H), 3.57–3.60, 3.67–3.70 and 3.78–3.81 (ddd, 2H, 2,2′-H), 4.23–4.33 (m, 2H, CH_2 ester), 4.44–4.45 and 4.95–4.96 (m, 1H, α-H), 6.58 (s, 1H, NH), 7.12–7.23 (m, 5H, aryl), 7.61 (s, 1H, NH), 7.69–7.76 (m, 2H, 6-H and 7-H), 7.98–8.00 (d, 1H, 8-H or 5H), 8.08–8.10 (d, 1H, 5-H or 8-H).

3-[*N*-(*tert*-Butoxycarbonyl)-glycyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione (6′). Yellow foam (60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13–1.27 (m, 3H, CH_3 ester), 1.43 (s, 9H, Boc), 3.71–3.74 (d, 1H, 2-H, $J_{2,2'} = 12.5$ Hz), 3.78–3.81 (d, 1H, 2′-H), 3.93–3.85 (m, 2H, α-H), 4.23–4.33 (m, 2H, CH_2 ester), 5.23 (s, 1H, NH), 7.73–7.75 (m, 2H, 6-H and 7-H), 7.78 (s, 1H, NH), 7.95–7.97 (d, 1H, 8-H or 5-H), 8.04–8.06 (d, 1H, 5-H or 8-H).

3-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione (7′). Yellow oil (59% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.20–1.24 (m, 3H, CH_3 ester), 1.38 (s, 9H, Boc), 3.40–3.43 (m, 3H, CH_3), 3.69–3.72 and 3.77–3.80 (m, 2H, 2,2′-H), 4.07–4.08 and 4.03–4.04 (m, 2H, α-H), 4.21–4.29 (m, 2H, CH_2 ester), 5.23 (s, 1H, NH), 7.67–7.72 (m, 2H, 6-H and 7-H), 7.78 (s, 1H, NH), 7.97–7.99 (d, 1H, 8-H or 5-H), 8.06–8.08 (d, 1H, 5-H or 8-H).

3-[*N*-(*tert*-Butoxycarbonyl)-β-alanyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]-naphtho-4,9-dione (8′). Yellow oil (64% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.21–1.24 (m, 3H, CH_3 ester), 1.37 (s, 9H, Boc), 2.40–2.43 (m, 2H, β-H), 3.31–3.35 (m, 2H, α-H), 3.70–3.73 (d, 1H, 2-H $J_{2,2'} = 12.5$ Hz), 3.77–3.80 (d, 1H, 2′-H), 4.25–4.28 (m, 2H, CH_2 ester), 5.15 (s, 1H, NH), 7.30 (s, 1H, NH), 7.64–7.71 (m, 2H, 6-H and 7-H), 7.96–7.98 (d, 1H, 8-H or 5-H), 8.04–8.06 (d, 1H, 5-H or 8-H).

3-[*N*-(*tert*-Butoxycarbonyl)-glycyl-glycyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione (9′). Yellow foam (61% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.19–1.12 (m, 3H, CH_3 ester), 1.38 (s, 9H, Boc), 3.68–3.71 (d, 1H, 2-H $J_{2,2'} = 12.5$ Hz), 3.76–3.79 (d, 1H, 2′-H), 3.83–3.84 and 3.99–4.00 (m, 4H, α, α′-H), 4.24–4.30 (m, 2H, CH_2 ester), 3.83–3.84 and 3.99–4.00 (m, 4H, α, α′-H), 5.13 (s,1H, NH), 6.66 (s, 1H, NH), 7.60 (s, 1H, NH), 7.66–7.73 (m, 2H, 6-H and 7-H), 7.98–8.00 (d, 1H, 8-H or 5-H), 8.06–8.08 (d, 1H, 5-H or 8-H).

General procedure for the deprotection of the *N*-Boc group

Synthesis of the compounds 4a, 4b, 5a, 5b, 7 and 8. Method A. 3-[*N*-(*tert*-Butoxycarbonyl)-L or D-phenylalanyl or L-alanyl or β-alanyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione (4′, 5′, 7′ or 8′) (0.2–0.3 mmol) were dissolved in saturated EtOAc (HCl) solution (15 mL). After 4–6 h under stirring at room temperature, the solution was neutralizated with triethylamine and the solvents were evaporated to dryness. Flash chromatography of 4, 5 with (90:10:1:1) CH_2Cl_2–MeOH–HAcO–H_2O yielded isolated in each case, in decreasing order of R_f, the diasteroisomers **4a** and **4b** or **5a** and **5b**. All compounds, including the compounds **7** and **8**, were collected as the chloride salts after treatment with HCl(g)/ether saturated solution.

3-(L-Phenylalanyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione hydrochloride (4a). Yellow solid (98%), mp. 155–157 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25–1.28 (m, 3H, CH_3 ester), 2.66–2.71 and 3.10–3.13 (m, 2H, β-H), 3.60–3.64 (m, 1H, α-H), 3.74–3.77 (d, 1H, 2-H, $J_{2,2'} = 12.5$ Hz), 3.80–3.83 (d, 1H, 2′-H), 4.25–4.32 (m, 2H, CH_2 ester), 7.34–7.26 (m, 5H, aryl), 7.68–7.74 (m, 2H, 6-H and 7-H), 7.99–8.01 (d, 1H, 8-H or 5H), 8.09–8.11 (d, 1H, 5-H or 8-H), 8.85 (s, 1H, NH). $[\alpha]_D^{20} = +18.1°$ (*c* 3.0, MeOH). MS [M^+] calcd for $C_{24}H_{23}O_5N_2SCl$: 486.9. Found: 487.3.

3-(L-Phenylalanyl]amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione hydrochloride (4b). Yellow solid (96%), mp. 160–162. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22–1.25 (m, 3H, CH_3 ester), 2.68–2.73 and 3.24–3.27 (m, 2H, β-H), 3.60–3.64 (m, 1H, α-H), 3.74–3.77 (d, 1H, 2-H, $J_{2,2'} = 12.5$ Hz), 3.81–3.84 (d, 1H, 2′-H), 4.27–4.29 (m, 2H, CH_2 ester), 7.36–7.28 (m, 5H, aryl), 7.63–7.67 (m, 2H, 6-H and 7-H), 7.99–8.01 (d, 1H,

8-H or 5H), 8.08–8.10 (d, 1H, 5-H or 8-H), 8.89 (s, 1H, NH). $[\alpha]_D^{20} = +38.1°$ (*c* 2.5, MeOH). MS $[M^+]$ calcd for $C_{24}H_{23}O_5N_2SCl$: 486.9. Found: 487.5.

3-(D-Phenylalanyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione hydrochloride (5a). Yellow solid (96%), mp. 157–158. ^1H NMR (500 MHz, $CDCl_3$) δ 1.22–1.25 (m, 3H, CH_3 ester), 2.68–2.73 and 3.24–3.27 (m, 2H, β-H), 3.60–3.64 (m, 1H, α-H), 3.74–3.77 (d, 1H, 2-H, $J_{2,2'} = 12.5$ Hz), 3.81–3.84 (d, 1H, 2′-H), 4.27–4.29 (m, 2H, CH_2 ester), 7.36–7.28 (m, 5H, aryl), 7.63–7.67 (m, 2H, 6-H and 7-H), 7.99–8.01 (d, 1H, 8-H or 5H), 8.08–8.10 (d, 1H, 5-H or 8-H), 8.89 (s, 1H, NH). $[\alpha]_D^{20} = -17.8°$ (*c* 1.2, MeOH). MS $[M^+]$ calcd for $C_{24}H_{23}O_5N_2SCl$: 486.9. Found: 487.8.

3-(D-Phenylalanyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione hydrochloride (5b). Yellow solid (96%), mp 161–162. ^1H NMR (500 MHz, $CDCl_3$) δ 1.22–1.25 (m, 3H, CH_3 ester), 2.68–2.73 and 3.24–3.27 (m, 2H, β-H), 3.60–3.64 (m, 1H, α-H), 3.74–3.77 (d, 1H, 2-H, $J_{2,2'} = 12.5$ Hz), 3.81–3.84 (d, 1H, 2′-H), 4.27–4.29 (m, 2H, CH_2 ester), 7.36–7.28 (m, 5H, aryl), 7.63–7.67 (m, 2H, 6-H and 7-H), 7.99–8.01 (d, 1H, 8-H or 5H), 8.08–8.10 (d, 1H, 5-H or 8-H), 8.89 (s, 1H, NH). $[\alpha]_D^{20} = -39.2°$ (*c* 3.0, MeOH). MS $[M^+]$ calcd for $C_{24}H_{23}O_5N_2SCl$: 486.9. Found: 487.7.

3-(L-Alanyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione hydrochloride (7). Yellow foam (86%). ^1H NMR (500 MHz, $CDCl_3$) δ 1.18–1.20 (m, 3H, CH_3 ester), 2.61–2.62 and 2.65–2.66 (m, 3H, CH_3), 3.30–3.32 and 3.34–3.36 (m, 1H, α-H), 3.67–3.70, 3.77–3.80, and 3.79–3.81 (ddd, 2H, 2,2′-H), 4.26–4.29 (m, 2H, CH_2 ester), 7.54–7.61 (m, 2H, 6-H and 7-H), 7.97–7.99 (d, 1H, 8-H or 5H), 8.07–8.09 (d, 1H, 5-H or 8-H), 8.24 (s, 1H, NH). MS $[M^+]$ calcd for $C_{18}H_{19}O_5N_2SCl$: 411.1. Found: 411.7.

3-(β-Alanyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione hydrochloride (8). Yellow foam (95%). ^1H NMR (500 MHz, $CDCl_3$) δ 1.23–1.26 (m, 3H, CH_3 ester), 2.99–3.19 (m, 2H, α-H), 3.38–3.39 (m, 2H, β-H), 3.82–3.85 (d, 1H, 2-H, $J_{2,2''} = 12.5$ Hz), 3.94–3.97 (d, 1H, 2′-H), 4.26–4.32 (m, 2H, CH_2 ester), 7.60–7.74 (m, 2H, 6-H and 7-H), 7.94–7.96 (d, 1H, 8-H or 5H), 8.07–8.09 (d, 1H, 5-H or 8-H), 8.51 (s, 1H, NH). MS $[M^+]$ calcd for $C_{18}H_{19}O_5N_2SCl$: 411.1. Found: 411.8.

Synthesis of 3-(glycyl and glycyl-glycyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno-[2,3-*b*]naphtho-4,9-dione (6 and 9). Method B. TFA (0.5 mL) was added to a solution of Boc-protected compounds **6′** or **9′** (0.1 mmol) in CH_2Cl_2 (5 mL), stirring was continued for 3–4 h at room temperature, the reaction mixture was concentrated to half volume and ether was added. The title compounds as the trifluoroacetate salt, were racolted by filtration.

3-(Glycyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno-[2,3-*b*]naphtho-4,9-dione trifluoroacetate (6). Yellow solid (97%), mp 214–215. ^1H NMR (500 MHz, $CDCl_3$) δ 1.14–1.21 (m, 3H, C*H*$_3$ ester), 3.57–3.54 (d, 1H, 2-H, $J_{2,2''} = 12.4$ Hz), 3.61–3.64 (d, 1H, 2′-H), 3.90–3.92 (m, 2H, α-H), 4.15–4.20 (m, 2H, C*H*$_2$ ester), 7.57–7.67 (m, 2H, 6-H and 7-H), 7.88–7.90 (d, 1H, 8-H or 5-H), 7.97–7.99 (d, 1H, 5-H or 8-H), 8.23 (s, 1H, N*H*). MS $[M^+]$ calcd for $C_{19}H_{17}O_7N_2SF_3$: 475.2. Found: 476.7.

3-(Glycyl-glycyl)amino-3-ethoxycarbonyl-2,3-dihydrothiopheno[2,3-*b*]naphtho-4,9-dione trifluoroacetate (9). Yellow solid (89%) mp. 231–233. ^1H NMR (500 MHz, $CDCl_3$) δ 1.12–1.20 (m, 3H, CH_3 ester), 2.72–2.74 (m, 2H, α-H), 3.46–3.48 (m, 2H, α′-H), 3.67–3.70 (d, 1H, 2-H, $J_{2,2''} = 12.5$ Hz), 3.76–3.79 (d, 1H, 2′-H), 4.15–4.28 (m, 2H, CH_2 ester), 7.12 (s, 1H, NH), 7.57–7.63 (m, 2H, 6-H and 7-H), 7.89–7.91 (d, 1H, 8-H or 5H), 7.98–8.00 (d, 1H, 5-H or 8-H), 8.62 (s, 1H, NH). MS $[M^+]$ calcd for $C_{21}H_{20}O_8N_3SF_3$: 532.3. Found: 532.9.

Biological methods

Compounds were dissolved in DMSO at an initial concentration of 200 μM and then were serially diluted in culture medium.

Cells

Cell lines were from American Type Culture Collection (ATCC). The human nasopharyngeal carcinoma KB cell line and the drug-resistant subclones KB^{MDR}, KB^{7D}, and KB^{V20C} were a generous gift of Prof. Y. C. Cheng, Yale University. H9/IIIb, MT-4, and C8166 (grown in RPMI 1640 containing 10% fetal calf serum (FCS), 100 U/mL penicillin G, and 100 μg/mL streptomycin) cells were used for anti-HIV assays. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect kit (Gibco).

Cytotoxic and antiviral assays

Cytotoxicity of compounds, based on the viability of mock-infected cells, as monitored by the MTT method[17,18] was evaluated in parallel with their antiviral activity. Activity of compounds against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells.

Antitumor assays

Exponentially growing leukaemia and lymphoma cells were resuspended at a density of 1×10^5 cells/mL in RPMI containing serial dilutions of the test drugs. Cell viability was determined after 4 days at 37 °C by the MTT method. Activity against cell lines derived from solid tumours was evaluated in exponentially growing cultures seeded at 5×10^4 cells/mL which were allowed to adhere for 16 h to culture plates before addition of the drugs. Cell viability was determined by the MTT method for 4 days later.

Linear regression analysis

Tumour cell growth at each drug concentration was expressed as percentage of untreated controls, and the concentration resulting in 50% (CC_{50}, IC_{50}) growth inhibition was determined by linear regression analysis.

Acknowledgements

This work was supported by grant from Regione Campania ai sensi della L.R.31 Dicembre 1994, No. 41, art. 3, 1° comma. The NMR spectral data and FAB-Massa were provided by Centro di Ricerca Interdipartimentale di Analisi Strumentale, Università degli Studi di Napoli 'Federico II'. The assistance of the staff is gratefully appreciated.

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