Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 1691–1701
Bioorganic & Medicinal Chemistry

Antiproliferative effects on human tumor cells and rat aortic smooth muscular cells of 2,3-heteroarylmaleimides and heterofused imides

Nicola Ferri,[b] Egle Maria Beccalli,[a] Alessandro Contini,[a] Alberto Corsini,[b,*] Manuela Antonino,[b] Tiziano Radice,[a] Graziella Pratesi,[c,*] Stella Tinelli,[c] Franco Zunino[c] and Maria Luisa Gelmi[a,*]

[a]*Istituto di Chimica Organica "A. Marchesini", Facoltà di Farmacia, Università degli Studi di Milano, via Venezian 21, 20133 Milano, Italy*
[b]*Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, via Balzaretti 9, 20133 Milano, Italy*
[c]*Dipartimento di Oncologia Sperimentale e Laboratori, Fondazione IRCCS Istituto Nazionale dei Tumori, via Venezian 1, 20133 Milano, Italy*

Received 5 September 2007; revised 31 October 2007; accepted 7 November 2007
Available online 13 November 2007

Abstract—A series of 2,3-heteroarylmaleimides **9** and polyheterocondensed imides **12** were prepared in good yields and short reaction time using a very efficient procedure consisting in the condensation of the corresponding anhydrides and *N*,*N*-diethylethylenediamine and microwave heating. The antiproliferative activity of the novel molecules was tested against human tumor cells (NCI-H460 lung carcinoma) and rat aortic smooth muscle cells (SMCs). The IC_{50} values for the novel molecules ranged from 0.08 to 13.9 μM in SMCs, and from 0.84 to 9 μM in the tumor cell line. The activity profile for compounds **9** and **12** is comparable to that obtained for amonafide in NCI-H460, except for fused imides **12b**,**i** which proved to be about 10-fold more potent. Whereas, in rat SMCs, only the compound **12b** was shown to be 10-fold more potent than amonafide. Instead **12c** is equipotent to amonafide. These results suggest that the extended π-system and the kind of heteroatom are essential in the binding with the molecular target.

1. Introduction

A bibliographic screening reveals that compounds which show antitumoral activity, and in particular molecules able to interact with DNA, are characterized by the presence of an extended π-system and of the imide function. This is the case of alkaloids incorporating the indolylmaleimide nucleus (compound **1**), of amonafide **2**, and of elinafide **3**, and their synthetic analogues (Fig. 1).[1] Compound **1**, as well as the naphthalimide derivatives classified as *mono*-and *bis*-intercalators of DNA, are known to interact with DNA and efficiently inhibit DNA topoisomerases. In particular, the antitumor activity of amonafide seems to be closely related to its ability to stabilize the DNA–intercalator–topoisomerase II ternary complex, while elinafide is a much stronger intercalator of DNA.[2]

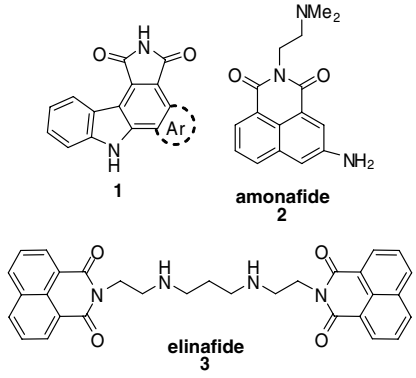


Figure 1. DNA-interacting compounds.

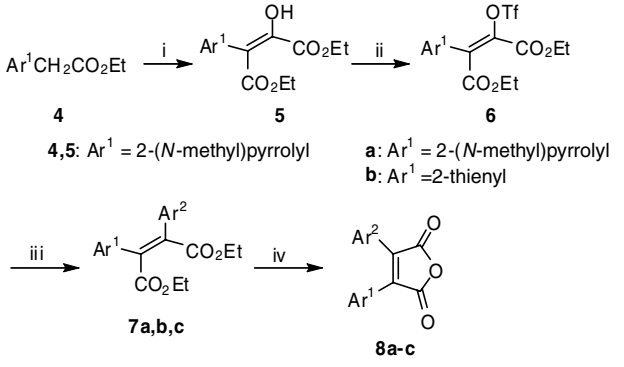
Keywords: 2,3-Heteroarylmaleimides; Polyheterocondensed imides; Antiproliferative activity; NCI-H460; Smooth muscular cells.
* Corresponding authors. Tel.: +39 2 58354481; fax: +39 2 58354476; e-mail: marialuisa.gelmi@unimi.it

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.11.024

DNA topoisomerases are enzymes involved in many DNA functions, particularly in DNA replication and repair.[3] In eukaryotes, the nuclear enzymes known as topoisomerase I (Topo-I) and topoisomerase II (Topo-II) bind to the DNA double coil inducing temporary single (Topo-I) or double strand cuts (Topo-II). These cuts allow for DNA uncoiling during replication, relieving torsional strain. The role of inhibitors of topoisomerases in the oncology field is known since many years and many cytotoxic drugs used in the clinical practice act on Topo-II (e.g., doxorubicin, mitoxanthrone, etoposide), whereas only camptothecins have been developed as inhibitors of Topo-I. Recent studies have reported the ability of topoisomerase inhibitors to interact with proliferation of aortic SMCs,[4] a major event associated to post-angioplasty and in-stent restenosis processes, supporting the potential of this target for a pharmacological approach in cardiovascular diseases.

By combining the structural features of compounds **1** and naphthalimide derivatives, we designed and synthesized two new classes of compounds of general formula **9** (Scheme 2) and **12** (Scheme 3), characterized by (i) the maleimide nucleus, (ii) the functionalization at nitrogen atom with a chain containing a tertiary amino group (iii) the presence of heterocyclic rings. The main difference between the two classes of compounds is related to the planarity of the ring system. In fact, to the first class belong the 2,3-heterocyclic substituted maleimides, to the second the polycondensed heterocyclic systems containing the imide function both characterized by heteroatom diversity.

Several examples of heteroaryl substituted maleimides[5–11] of kind **9**, as well as of heterofused imides of kind **12**,[12–20] were reported in the literature and the synthetic protocol adopted for their preparation in general differs from our methods. Some of the above compounds showed the ability to interfere with cellular proliferation,[11,15,18] and for this reason the antiproliferative activity of the novel molecules belonging to the classes **9** and **12** was tested against human tumor cells (NCI-H460 lung carcinoma) and rat aortic SMCs.



4 **5** **6**

4,5: Ar^1 = 2-(*N*-methyl)pyrrolyl

a: Ar^1 = 2-(*N*-methyl)pyrrolyl
b: Ar^1 =2-thienyl

7a,b,c **8a-c**

7a, 8a: Ar^1 = 2-(*N*-methyl)pyrrolyl,Ar^2 =2-furyl
7b, 8b: Ar^1 = 2-(*N*-methyl)pyrrolyl, Ar^2 =2-thienyl
7c, 8c: Ar^1 = Ar^2 =2-thienyl

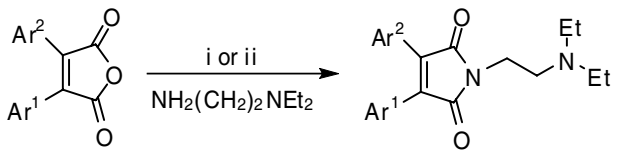
Scheme 1. Synthesis of 2,3-heteroarylmaleic anhydrides **8a–c**. Reagents and conditions: (i) $(CO_2Et)_2$, EtOH, Na, Δ; (ii) CH_2Cl_2, *i*-Pr_2EtN, 0 °C, then $(CF_3SO_2)_2O$; (iii) Ar^2SnBu_3, $(Ph_3P)_4Pd$, LiCl, CuI, THF, Δ; (iv) EtOH, NaOH, H_2O, Δ.

2. Results and discussion

2.1. Synthesis

Recently, a series of symmetric and asymmetric anhydrides **8d–g**[21] (Scheme 2), substituted with heterocyclic rings, were prepared by our group using as key reaction step the Stille reaction. As reported in Scheme 1, the same synthetic protocol was here adopted for the preparation of the new anhydrides **8a–c**. Compound **8a** was prepared starting from the commercially available ethyl (1-methyl-1*H*-pyrrol-2-yl)acetate (**4**) which was first transformed into 2-hydroxy-3-(1-methyl-1*H*-pyrrol-2-yl)but-2-enedioate (**5**) (63%) by condensation with diethyl oxalate in EtOH/EtONa. The hydroxy group of **5** was transformed into triflate (*N*,*N*-diisopropylethylamine/trifluoromethanesulfonic anhydride/CH_2Cl_2/0 °C) and compound **6a** was isolated in 76%. New maleic esters **7a** (92%) and **7b** (91%) were prepared by making to react **6a** with 2-tributyl-2-furylstannane and 2-tributyl-2-thienylstannane, respectively, operating in THF and in the presence of LiCl, CuI, and $Pd(Ph_3P)_4$ at reflux. Instead **7c** (97%) was obtained from **6b**[21] and 2-tributyl-2-thienylstannane. Finally, compounds **7a–c** were transformed into the corresponding anhydrides **8a–c** in EtOH, $NaOH/H_2O$ by reflux.

Compounds **8a–g** were the starting material for the preparation of disubstituted maleimides **9a–g** obtained by the way of a very efficient protocol consisting in the use of microwave irradiation (MW) (Scheme 2). The reaction conditions were optimized in terms of solvent, temperature, potency of MW, and reaction time. The best protocol allowed to obtain the maleimides **9a–d,f,g** in high yield (80–100%) and short reaction time (15 min), by reaction of an equimolecular amount of anhydride **8a–d,f,g** and *N*,*N*-diethylethylenediamine in



8a-g **9a-g**

8a, 9a: Ar^1 = 2-(*N*-methyl)pyrrolyl, Ar^2 = 2-furyl
8b, 9b: Ar^1 = 2-(*N*-methyl)pyrrolyl, Ar^2 = 2-thienyl
8c, 9c: Ar^1 = Ar^2 = 2-thienyl
8d, 9d: Ar^1 =2-thienyl , Ar^2 = 2-furyl
8e, 9e: Ar^1 = 3-indolyl, Ar^2 = 2-(*N*-methyl)pirrolyl
8f, 9f: Ar^1 = 3-indolyl, Ar^2 = 2-thienyl
8g, 9g: Ar^1 = 2-thienyl, Ar^2 = 2-indolyl

Scheme 2. Synthesis of 2,3-heteroarylmaleimides **9**. Reagents and conditions: (i) **8a–d,f,g**: CH_2Cl_2, MW (450 W, 100 °C); (ii) **8e**: DMF, MW (450 W, 140 °C).

dichloromethane operating at 100 °C and 450 W. Adopting a similar procedure (MW: 450 W, 140 °C), but using DMF as the solvent, compound **9e** (48%) was obtained from **8e**. The ^{1}H NMR spectra of the crude reaction mixtures showed the presence of a single compound in concordance with the expected structure of compounds **9**.

The electrocyclization reaction was not a trivial task and it was performed starting from different reagents and reaction conditions aiming to obtain the polycondensed derivatives. Anhydrides **8c** and **8d** were chosen as models by performing the reaction both in AcOH and in the presence of a stoichiometric amount of $Pd(OAc)_2$ using the traditional heating (reflux) and the MW conditions. In both cases the reaction failed or gave a complex mixture. The photochemical electrocyclization of **8c**,**d** was tested in different solvents such as MeCN, acetone, benzene/I_2, EtOH/I_2, and acetone/I_2. The goal was achieved by using the couple acetone/I_2 and compounds **11c** and **11d** were obtained in 73% and 68% yield (Scheme 3). Compound **11c**, as well as the precursor anhydride **8c**, are known compounds but no data were reported concerning yields and spectroscopic characterization.[22] The reaction failed starting from **8a**,**b** in all reaction conditions tested. Considering the difficulties encountered on the electrocyclization of anhydrides, we turned on the electrocyclization of diethylmaleic esters **7a**–**c** and **7h**[21] which is not operative in thermal conditions but gave positive results using photochemical irradiation. Also in this case, different solvents were tested (acetone, MeCN, MeOH) and CH_2Cl_2 resulted the best one for compounds **7a**–**c**,**h** which were transformed into the corresponding tricyclic ring systems **10a**–**c**,**h** in 45–50% yield (Scheme 3). Instead, compound **10f** (40%) was prepared from **7f**[21] in acetone/I_2 (Scheme 4).

The transformation of **10a**,**b** into the anhydrides **11a** (50%), **11b** (86%) was achieved by a one-pot reaction consisting in a partial hydrolysis of the ester function (EtOH/H_2O/NaOH, 30 min reflux), followed by the treatment of the crude reaction mixture in CH_2Cl_2 with a catalytic amount of *p*-toluenesulfonic acid at reflux. Instead, anhydride **11h** (36%) was formed from **10h** by basic hydrolysis followed by treatment of the bicarboxylic acid with $SOCl_2$ (Scheme 3). The above reaction conditions failed when starting from **10c**,**f**.

Finally, the anhydrides **11a**–**d**,**h** and **11i**[23] were made to react with *N*,*N*-diethylethylenediamine aiming to obtain polycyclic imides **12** (Scheme 3). In the case of **11a**,**d**,**i** the reaction performed in CH_2Cl_2 and microwave heating (650 W, 100 °C, 10 min) gave the corresponding imides **12a**,**d**,**i** in quantitative yield. Instead, **12h** (72%) was obtained from **11h** and the amine in neat conditions (150 °C, 41 h). The reaction failed starting from the anhydrides **11b**,**c** which were unstable in the above reaction conditions. The imides **12b** (38%) and **12c** (93%) were obtained in neat conditions by heating at 150 °C starting from **10b**,**c**. This last procedure was not operative starting from indole derivative **10f**.

2.2. Biological activity

The potential antiproliferative effect of the synthesized compounds of the series **9** and **12** was evaluated, after three days of exposure, in cultured rat aortic SMCs and in the human NCI-H460 lung tumor cell line. The structural analogies between the known compound amonafide and the fused imides **12**, as shown in Figure 2 for compound **12b**, suggest an analogous mechanism for the exerted antiproliferative activity, and for this reason amonafide was included as a reference compound.

All tested compounds affected cell proliferation in a concentration-dependent manner and a representative concentration-dependent effect is reported in Figure 3 for compound **12b**.

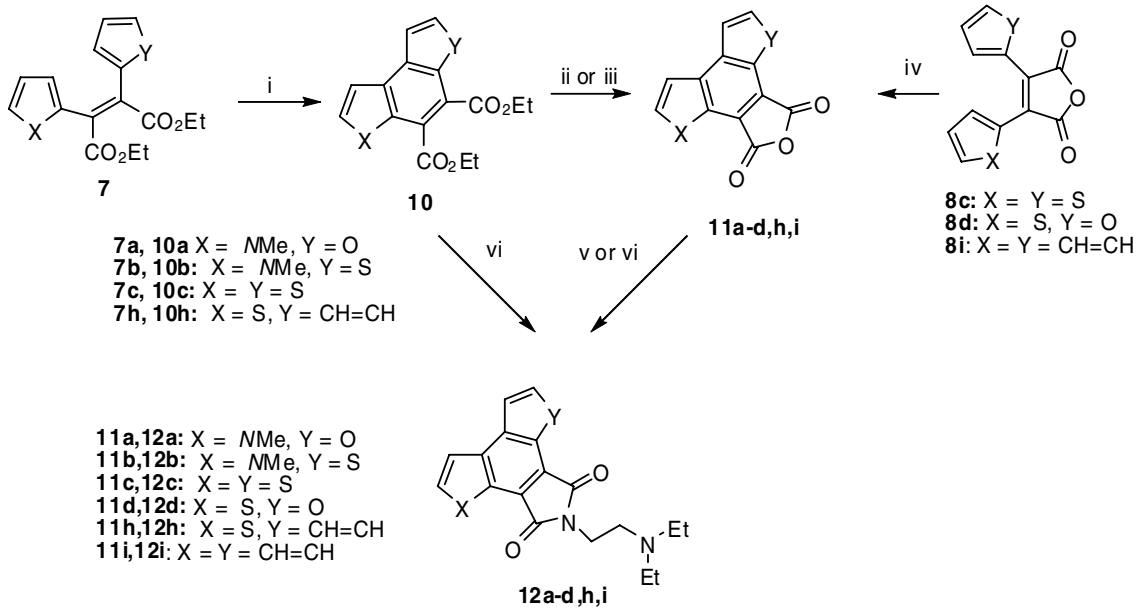
As summarized in Table 1, the IC_{50} values for the novel molecules ranged from 0.08 to 13.9 μM in SMCs, and from 0.84 to 9 μM in the tumor cell line. It should be noted that in NCI-H460 cell line the activity profile for compounds **9b**–**g** and **12a**,**c**,**h** is comparable to that obtained for amonafide, while the fused imides **12b**,**i** proved to be about 10-fold more potent.

Different results have been obtained in rat SMCs, where only the compound **12b** was shown to be 10-fold more potent than amonafide, while compounds **9b**–**g** and **12a**,**h** showed lower activity than amonafide. Finally, compound **12c** was equipotent to amonafide in rat SMCs.

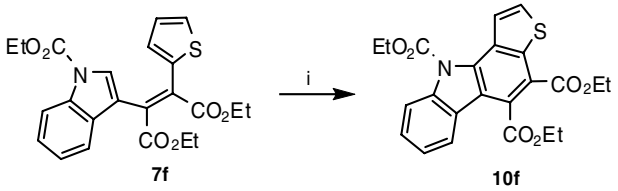
The comparison of biological data of **9a**,**b**,**c** with those of **12a**,**b**,**c**, respectively, suggests that planarity is not essential, however it improves potency, as the heterofused imides **12** are about 10-fold more potent than the corresponding heteroaryl substituted maleimides **9** on both cell lines.

An anomaly is represented by the thiophene-furan substituted compounds **9d** and **12d**, as the former resulted moderately active (IC_{50}s being 2.1 and 8.7 μM on SMCs and tumor cell line, respectively) while the latter was almost inactive.

Interestingly, none of the maleimides **9** resulted significantly selective toward a particular cell type and the whole series showed potencies of the same magnitude. On the other hand, the pyrrole-thiophene and the *bis*-thiophene fused imides **12b** and **12c**, as well as amonafide, proved to be about 10-fold more potent toward the primary SMCs than against the tumor cells. Moreover, the former compound resulted 10-fold more potent than **12c** and amonafide on both cell lines. Again, concerning the activity against SMCs, compound **12b** resulted about 100-fold more potent than the close analogues **12h**, where the pyrrole moiety is replaced by a phenyl, and **12a**, where the thiophene ring is replaced by a furan. Such observations suggest that the thiophene and pyrrole rings improve, while the furan heterocycle reduces, the activity of such a class of compounds. Interestingly, the phenanthrene derivative



Scheme 3. Synthesis of heterofused imides **12**. Reagents and conditions: (i) hν, CH_2Cl_2; (ii) EtOH, NaOH, H_2O, Δ, then CH_2Cl_2, *p*-TsA, reflux; (iii) EtOH, NaOH, H_2O, then H_3O^+, then $SOCl_2$ Δ; (iv) hν, acetone, I_2; (v) **11a**,**d**,**i**: $NH_2(CH_2)_2NEt_2$, CH_2Cl_2, MW (650 W, 100 °C); (vi) **10b**,**c**,**11h**: $NH_2(CH_2)_2NEt_2$, neat, 150 °C.



Scheme 4. Compound **10f**. Reagent: (i) hν, acetone/I_2.

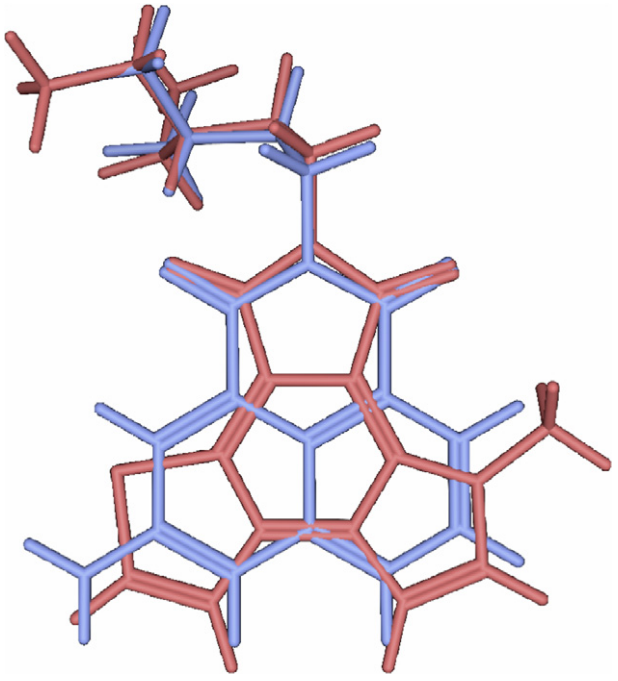


Figure 2. Flexible superposition of compound **12b** (red) and Amonafide (blue).

12i, where both heterocycles are replaced by phenyl rings, resulted as potent as **12c** on SMCs but no selectivity was observed. In order to gain a better insight into the relevance of physico-chemical properties upon activity several molecular descriptors were computed with the MOE software package and evaluated against activity. Unfortunately correlation matrices did not show any relevant correlation, except with parameters related with lipophilicity such as log *P*(o/w) and solvation energy (see Table 1). This is not particularly surprising, as a correlation between lipophilicity and anticancer activity of cyclic imides was already reported.[24] Indeed, concerning SMC, the best linear correlation was found between the logarithm of the inverse of the IC_{50} (log1/c) with an R^2 of 0.27 that was raised to 0.76 by removing compounds **12b**,**d**,**h** which behaved as outliers. Concerning NCI-H460 activity both log *P*(o/w) and solvation energy provided a decidedly worse linear correlation, as the best results obtained by correlating log1/c with log *P*(o/w) provided an R^2 of 0.21 that increased to 0.48 by removing the outlier compounds **12b, d**. From the above data we can conclude that lipophilicity is important for activity, but other parameters such as receptor interactions should be responsible for the loss of activity observed for compound **12d** as well as the potency of compound **12b**.

With the aim to evaluate if the antiproliferative effects of these compounds are a consequence of their DNA intercalating activities, the two most potent compounds of series **12** (**12b** and **12c**) and their respective analogues of series **9** (**9b** and **9c**) were tested. Since amonafide has been shown to stabilize the DNA–intercalator–topoisomerase II ternary complex, while elinafide is a much stronger intercalator of DNA, the latest was used as positive control.[2]

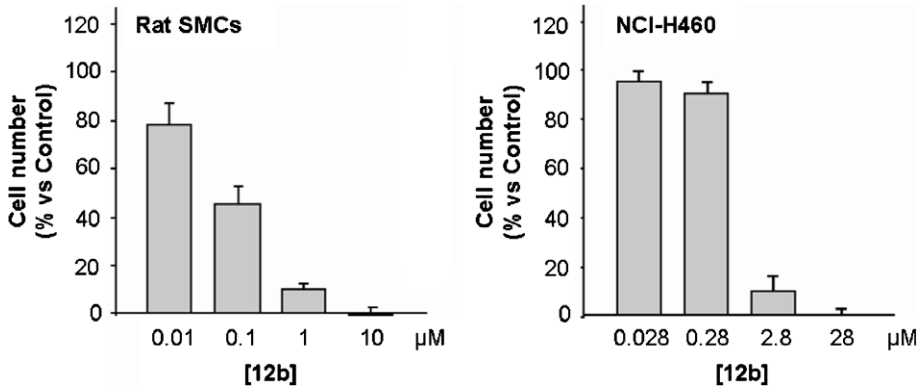


Figure 3. Concentration-dependent antiproliferative effect of compound **12b** on rat aortic SMCs and human tumor cell line NCI-H460.

Table 1. Antiproliferative effects of compounds **9** and **12** in rat aortic SMCs and human tumor cell line NCI-H460

Compound	IC_{50} (μM) SMCs	IC_{50} (μM) NCI-H460	Log *P*(o/w)	E_{sol}
Amonafide	0.48	7.8	1.6	−56.5
9a	N.A.[a]	>30	0.9	−66.7
9b	12.1	9.0	1.7	−65.7
9c	3.9	8.9	2.9	−59.1
9d	2.1	8.7	2.1	−60.3
9e	3.6	8.5	2.9	−61.6
9f	1.0	1.9	3.8	−60.2
9g	5.3	8.4	3.8	−65.6
12a	5.3	8.6	1.7	−58.9
12b	0.08	0.84	2.5	−57.8
12c	0.53	4.8	3.8	−56.5
12d	>30	>30	3.0	−57.8
12h	13.9	7.1	3.9	−56.1
12i	0.68	0.9	4.1	−55.6

[a] Not assessed.

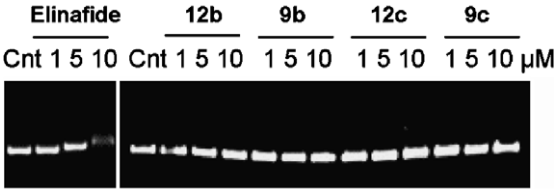


Figure 4. DNA intercalating effect of compounds **12b**,**9b**,**12c** and **9c**.

Incubation of these compounds, at concentrations ranging from 1 to 10 μM, with plasmid DNA did not alter the DNA electrophoretic mobility on agarose gel, indicating the inability to directly interact with DNA (Fig. 4). Under the same experimental conditions elinafide efficiently interacted with plasmid DNA by reducing its electrophoretic mobility.

3. Conclusions

In conclusion, two different classes of new compounds, that is, 2,3-heteroarylmaleimides **9** and polyheterocondensed imides **12**, which differ from extended π-system were efficiently prepared using microwave technique. Their antiproliferative activity was evaluated on human NCI-H460 lung carcinoma cells and rat aortic SMCs. Polyheterocondensed imide **12b** displays the highest potency with respect to other compounds and to amonafide, thus suggesting that the extended π-system and the kind of heteroatom are essential in the binding with the molecular target. Moreover, the DNA intercalating assay indicated that these compounds, in contrast to the *bis*-intercalating agent elinafide, do not directly interact with DNA. These results are supported by the notion that *bis*-intercalating agents have greater affinity for DNA than mono-intercalating agents.[25] Future studies will be performed in order to determine the biological properties of the dimers of these newly described series of molecules and their potential action against topoisomerases.

4. Experimental

4.1. Computational methods

All calculations were performed with the MOE software package version 2006.08.[28] Flexible alignments were performed by applying the method proposed by Feher, Labute and co-workers[29] and accepting the default settings. Molecular descriptors were computed with the QuaSAR-descriptor module implemented in MOE.

4.2. General

NMR spectra were recorded at 500 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts, relative to TMS as internal standard, are given in δ values. *J* are given in Hz. TLC: ready-to-use silica gel plates. Column chromatography: silica gel [Kieselgel 60–70 230 ASTM (Merck)] with the eluant indicated. Compound **4** is commercially available. Triflate **6b**,[21] diethyl 2,3-diheteroaryl-but-2-enedioates **7f**,**7h**,[21] and anhydrides **8d**–**g**[21] and **11i**[23] are known compounds.

4.3. Diethyl 2-hydroxy-3-(1-methyl-1*H*-pyrrol-2-yl)but-2-enedioate 5

To a solution of EtONa, prepared from Na (788 mg, 34.3 mmol) and EtOH (60 mL), ethyl (1-methyl-1*H*-pyrrol-2-yl)acetate **4** (4 mL, 24.5 mmol) and diethyl oxalate

(4.6 mL, 34.3 mmol) were added. The resulting mixture was refluxed for 2 h. After evaporation of the solvent, water (40 mL) was added and the mixture was extracted with Et_2O (2× 30 mL). The aqueous layer was then acidified (9% HCl, 15 mL) and extracted with CH_2Cl_2 (2× 30 mL). The organic phase was dried, filtered and the solvent was evaporated. The residue was purified by chromatography on silica gel (hexane/Et_2O, 5:1) to give pure compound **5** (4.1 g, 63%). Mp 70–72 °C (Et_2O/hexane); IR (Nujol): 3400, 1730, 1650, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 12.80 (s, 1H, exch.), 6.65 (dd, J = 2.5, 1.8 Hz, 1H), 6.04 (dd, J = 3.6, 2.5 Hz, 1H), 5.93 (dd, J = 3.6, 1.8 Hz, 1H), 4.42–4.19 (m, 2H), 4.04 (q, J = 6.9 Hz, 2H), 3.46 (s, 3H), 1.26 (t, J = 6.9 Hz, 3H), 1.05 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ: 163.8, 162.9, 124.0, 123.2, 111.5, 107.6, 99.2 (x2), 62.1 (x2), 34.3, 14.5, 14.1. $C_{13}H_{17}NO_5$ (267.28): Calcd C 58.42, H 6.41, N 5.24. Found: C 58.46, H 6.38, N 5.28.

4.4. Diethyl 2-(*N*-methyl-1*H*-pyrrol-2-yl)-3-(trifluoromethanesulfonyloxy)but-2-enedioate 6a

N,*N*-diisopropylethylamine (6.2 mL, 36 mmol) was added to a solution of **5** (6.4 g, 24 mmol) in CH_2Cl_2 (120 mL). The stirred reaction mixture was cooled to 0 °C, whereupon a solution of trifluoromethanesulfonic anhydride (5.2 mL, 31 mmol) in CH_2Cl_2 (10 mL) was dropped. After 10 min at 0 °C, the mixture was washed with water (2× 50 mL). The organic phase was dried, filtered and the solvent was evaporated. The residue was purified by chromatography on silica gel (hexane/Et_2O 5:1) to give pure compound **6a** (7.3 g, 76%) as an oil. IR (Nujol): 1737, 1613 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.82 (dd, J = 2.9, 1.8 Hz, 1H), 6.53 (dd, J = 4.0, 1.8 Hz, 1H), 6.22 (dd, J = 4.0, 2.9 Hz, 1H), 4.35 (q, J = 7.3 Hz, 4H), 3.59 (s, 3H), 1.36 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 164.2, 160.0, 133.7, 132.0, 128.6, 122.1, 118.3 (q, J = 320 Hz), 116.0, 110.0, 63.1, 62.8, 35.5, 13.9 (x2). $C_{14}H_{16}F_3NO_7S$ (399.34): Calcd C 42.11, H 4.04, N 3.51. Found: C 42.00, H 4.09, N 3.47.

4.5. General procedure for the preparation of diethyl 2,3-diheteroaryl-but-2-enedioate 7a–c

To a solution of appropriate triflate **6a**,**b** (2 mmol) in anhydrous THF (40 mL), LiCl (254 mg, 6 mmol), CuI (190 mg, 1 mmol), $Pd(Ph_3P)_4$ (46 mg, 0.04 mmol), and the heteroaryltributylstannane (3 mmol; **6a**: 2-tributyl-2-furylstannane or 2-tributyl-2-thienylstannane; **6b**: 2-tributyl-2-thienylstannane) were added in sequence. The reaction mixture was heated under reflux for 2.5 h, the solvent was evaporated, and the residue was purified by chromatography on silica gel (**7a**: hexane/Et_2O, 8:1; **7b**: hexane/CH_2Cl_2, 10:1; **7c**: hexane/CH_2Cl_2, 5:1) to give pure compound **7**.

4.5.1. Diethyl 2-(furan-2-yl)-3-(*N*-methyl-1*H*-pyrrol-2-yl)but-2-enedioate 7a. Yield 92%; oil. IR (Nujol): 1725, 1599 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.42 (d, J = 1.4 Hz, 1H), 6.72 (dd, J = 2.5, 1.8 Hz, 1H), 6.31 (dd, J = 3.6, 1.4 Hz, 1H), 6.20 (dd, J = 3.6, 2.5 Hz, 1H), 6.07 (dd, J = 3.6, 1.8 Hz, 1H), 5.30 (d, J = 4.3 Hz, 1H), 4.44 (q, J = 7.3 Hz, 2H), 4.23 (q, J = 6.9 Hz, 2H), 3.31 (s, 3H), 1.40 (t, J = 7.3 Hz, 3H), 1.25 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ: 166.5, 166.3, 148.8, 144.6, 136.5, 126.3, 123.3, 120.7, 115.2, 112.7, 109.6, 108.6, 62.0, 61.6, 33.8, 14.3, 14.1. $C_{17}H_{19}NO_5$ (317.34): Calcd C 64.34, H 6.03, N 4.41. Found: C 64.27, H 5.99, N 4.35.

4.5.2. Diethyl 2-(*N*-methyl-1*H*-pyrrol-2-yl)-3-(thiophen-2-yl)but-2-enedioate 7b. Yield 91%; oil. IR (Nujol): 1700, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.35 (dd, J = 5.1, 1.1 Hz, 1H), 7.09 (dd, J = 3.8, 1.1 Hz, 1H), 6.94 (dd, J = 5.1, 3.8 Hz, 1H) 6.79 (dd, J = 2.5, 1.8 Hz, 1H), 6.24 (dd, J = 3.6, 2.5 Hz, 1H), 6.12 (dd, J = 3.6, 1.8 Hz, 1H), 4.46 (q, J = 7.3 Hz, 2H), 4.22 (q, J = 6.9 Hz, 2H) 3.31 (s, 3H), 1.41 (t, J = 7.3 Hz, 3H), 1.24 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ: 168.4, 166.6, 142.0, 137.5, 132.6, 132.3, 126.8, 124.9, 124.1, 120.1, 111.3, 109.3, 62.4, 61.9, 34.2, 14.5, 14.4. $C_{17}H_{19}NO_4S$ (333.4): Calcd C 61.24, H 5.74, N 4.20. Found: C 61.20, H 5.72, N 4.18.

4.5.3. Diethyl 2,3-di(thiophen-2-yl)but-2-enedioate 7c. Yield 97%. Mp 65–67 °C (CH_2Cl_2/hexane); IR (Nujol): 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.42 (dd, J = 5.1, 1.1 Hz, 2H), 7.08 (dd, J = 3.6, 1.1 Hz, 2H), 7.01 (dd, J = 5.1, 3.6 Hz, 2H), 4.33 (q, J = 6.9 Hz, 4H), 1.32 (t, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: (167.1, 136.0, 131.7, 131.5, 130.6, 127.4, 62.3, 14.4)x2. $C_{16}H_{16}O_4S_2$ (336.43): Calcd C 57.12, H 4.79. C 57.01, H 4.85.

4.6. General procedure for the preparation of 8a–c

A solution of NaOH (10 mmol) in EtOH (8 mL) and H_2O (3 mL) was added to a solution of diethyl 2,3-diheteroarylmaleates **7a**–**c** (1.1 mmol) in EtOH (20 mL). The mixture was heated to reflux for 30 min. The solvent was then evaporated and the residue acidified with HCl (5%). The solid formed was filtered and washed with EtOH to give pure compound **8a**–**c**.

4.6.1. 4′-(*N*-Methyl-1*H*-pyrrol-2-yl)-[2,3′]bifuranyl-2′,5′-dione 8a. Yield 86%. Mp 127–128 °C (CH_2Cl_2/hexane). Mp 128 °C.[21] ^1H NMR ($CDCl_3$) δ: 7.58 (dd, J = 2.6, 1.8 Hz, 1 H), 7.48 (dd, J = 3.6, 0.7 Hz, 1H), 6.94 (m, 1 H), 6.76 (dd, J = 3.9, 1.7 Hz, 1 H), 6.63 (dd, J = 3.6, 1.8 Hz) 6.33 (dd, J = 3.9, 2.6 Hz, 1 H), 3.54 (s, 3H).

4.6.2. 3-(*N*-Methyl-1*H*-pyrrol-2-yl)-4-(thiophen-2-yl)-furan-2,5-dione 8b. Yield 77%. Mp 145–146 °C (CH_2Cl_2/hexane). Mp 146 °C.[21] ^1H NMR ($CDCl_3$) δ: 7.91 (dd, J = 1.1, 3.9 Hz, 1 H), 7.60 (dd, J = 5.1, 1.1 Hz, 1H), 7.15 (dd, J = 5.1, 3.9 Hz, 1 H), 6.93 (M, 1 H), 6.66 (dd, J = 3.9, 1.6 Hz, 1 H), 6.35 (dd, J = 3.9, 2.6 Hz), 3.46 (s, 3H).

4.6.3. 3,4-(Di-thiophen-2-yl)-furan-2,5-dione 8c. Yield 82%. Mp 108–109 °C (Et_2O); IR (Nujol): 1790, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.97 (dd, J = 4.0, 1.1 Hz, 2H), 7.67 (dd, J = 5.1, 1.1 Hz, 2H), 7.19 (dd, J = 5.1, 3.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 164.7, 133.2, 132.8, 129.2, 128.8, 128.4. $C_{12}H_6O_3S_2$ (262.3): Calcd C 54.95, H 2.31. Found: C 54.90, H 2.34.

4.7. General procedure for the preparation of maleimides 9a–d,f,g

To a solution of anhydrides **8a**–**d**,**f**,**g** (0.3 mmol) in CH_2Cl_2 (3 mL), *N*,*N*-diethylethylenediamine (0.04 mL, 0.3 mmol) was added. The mixture was irradiated with microwave (100 °C, 450 W) in the presence of a Weflon stirrer (**8a**–**d**,**g**: 15 min; **8f**: 30 min). After cooling at 25 °C, water (5 mL) was added and the organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by crystallization or by silica gel column chromatography (hexane/Et_2O, 1:1) affording pure compounds **9**.

4.7.1. 1-(2-(Diethylamino)ethyl)-3-(furan-2-yl)-4-(*N*-methyl-1*H*-pyrrol-2-yl)-1*H*-pyrrole-2,5-dione 9a. Yield 92%, oil. IR (Nujol): 1760, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.50 (d, J = 1.8 Hz, 1H), 7.37 (d, J = 3.3 Hz, 1H), 6.85 (dd, J = 3.3, 1.8 Hz, 1H), 6.60–6.53 (m, 2H), 6.29 (dd, J = 3.6, 2.5 Hz, 1H), 3.68 (t, J = 7.3 Hz, 2H), 3.44 (s, 3H), 2.66 (t, J = 7.3 Hz, 2H), 2.56 (q, J = 7.3 Hz, 4H), 1.00 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 170.7, 169.6, 145.9, 145.3, 127.5, 123.6, 123.0, 122.8, 116.9, 116.2, 112.8, 109.6, 50.4, 47.5 (x2), 36.6, 35.4, 12.2 (x2). $C_{19}H_{23}N_3O_3$ (341.4): Calcd C 66.84, H 6.79, N 12.31. Found: C 66.76, H 6.84, N 12.27.

4.7.2. 1-(2-(Diethylamino)ethyl)-3-(*N*-methyl-1*H*-pyrrol-2-yl)-4-(thien-2-yl)-1*H*-pyrrole-2,5-dione 9b. Yield 81%, oil. IR (Nujol): 1750, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.70 (dd, J = 3.6, 1.1 Hz, 1H), 7.42 (dd, J = 5.1, 1.1 Hz, 1H), 7.06 (dd, J = 5.1, 3.6 Hz, 1H), 6.85 (dd, J = 2.5, 1.8 Hz, 1H), 6.49 (dd, J = 3.6, 1.8 Hz, 1H), 6.30 (dd, J = 3.6, 2.5 Hz, 1H), 3.70 (t, J = 6.9 Hz, 2H), 3.37 (s, 3H) 2.68 (t, J = 6.9 Hz, 2H), 2.56 (q, J = 7.3 Hz, 4H), 1.01 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 170.8, 170.6, 131.7 (x2), 131.5, 130.5, 128.0, 127.0, 125.1, 121.8, 114.6, 110.0, 50.6, 47.6 (x2), 36.8, 35.5, 12.4 (x2). $C_{19}H_{23}N_3O_2S$ (357.47): Calcd C 63.84, H 6.49, N 11.75. Found: C 63.79, H 6.54, N 11.70.

4.7.3. 1-(2-(Diethylamino)ethyl)-3,4-di(thien-2-yl)-1*H*-pyrrole-2,5-dione 9c. Yield 85%, oil. IR (Nujol): 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.81 (d, J = 3.6 Hz, 2H), 7.56 (dd, J = 5.1, 1.1 Hz, 2H), 7.12 (dd, J = 5.1, 3.6 Hz, 2H), 3.71 (t, J = 7.3 Hz, 2H), 2.69 (t, J = 7.3 Hz, 2H), 2.57 (q, J = 7.3 Hz, 4H), 1.02 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: [(170.3, 131.4, 131.0, 130.0, 127.8, 127.7, 50.3, 47.5) x2] 36.8, 12.1 (x2). $C_{18}H_{20}N_2O_2S_2$ (360.49): Calcd C 59.97, H 5.59, N 7.77. C 59.94, H 5.62, N 7.74.

4.7.4. 1-(2-(Diethylamino)ethyl)-3-(furan-2-yl)-4-(thien-2-yl)-1*H*-pyrrole-2,5-dione 9d. Yield 94%, oil. IR (Nujol): 1750, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 8.14 (dd, J = 3.6, 1.1 Hz, 1H), 7.62–7.65 (m, 2H), 7.50 (d, J = 3.6 Hz, 1H), 7.17 (dd, J = 5.1, 3.6 Hz, 1H), 6.63 (dd, J = 3.6, 1.8 Hz, 1H), 3.71 (t, J = 7.1 Hz, 2H), 2.69 (t, J = 7.1 Hz, 2H), 2.59 (q, J = 7.1 Hz, 4H), 1.02 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 171.1, 169.9, 145.8, 145.0, 133.1, 132.2, 130.8, 127.7, 124.6, 120.3, 118.1, 113.4, 50.5, 47.6 (x2), 36.9, 12.4 (x2). $C_{18}H_{20}N_2O_3S$ (344.43): Calcd C 62.77, H 5.85, N 8.13. Found: C 62.70, H 5.89, N 8.08.

4.7.5. 1-(2-(Diethylamino)ethyl)-3-(1*H*-indol-3-yl)-4-(thien-2-yl)-1*H*-pyrrole-2,5-dione 9f. Yield 95%. Mp 74–75 °C (CH_2Cl_2/hexane). IR (Nujol): 3300, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 9.16 (brs, 1H, exch.), 7.83 (d, J = 2.5 Hz, 1H), 7.46–7.40 (m, 2H), 7.25–7.16 (m, 2H), 7.00–6.93 (m, 2H), 6.80 (d, J = 8.0 Hz, 1H), 3.76 (t, J = 6.9 Hz, 2H), 2.75 (t, J = 6.9 Hz, 2H), 2.62 (q, J = 7.3 Hz, 4H), 1.05 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 172.1, 171.7, 136.8, 131.7, 131.1, 130.3, 129.9, 128.3, 127.3, 125.7, 124.3, 123.2, 122.9, 121.0, 112.1, 105.9, 50.8, 47.5 (x2), 36.7, 12.2 (x2). $C_{22}H_{23}N_3O_2S$ (393.5): Calcd C 67.15, H 5.89, N 10.68. Found: C 67.11, H 5.87, N 10.65.

4.7.6. 1-(2-(Diethylamino)ethyl)-3-(1*H*-indol-2-yl)-4-(thien-2-yl)-1*H*-pyrrole-2,5-dione 9g. Quantitative yield, oil. IR (Nujol): 3300, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 10.0 (brs, 1H, exch.), 7.94 (d, J = 2.9 Hz, 1H), 7.65–7.56 (m, 3H), 7.40 (d, J = 7.7 Hz, 1H), 7.30–7.19 (m, 2H), 7.10 (dd, J = 7.7, 7.5 Hz, 1H), 3.73 (t, J = 7.3 Hz, 2H), 2.70 (t, J = 7.3 Hz, 2H), 2.58 (q, J = 7.3 Hz, 4H), 1.02 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 172.1, 170.4, 137.7, 130.9, 130.8, 130.4, 127.8, 127.7, 127.5, 125.4, 125.3, 124.3, 121.8, 121.0, 111.8, 108.4, 50.4, 47.5 (x2), 36.8, 12.2 (x2). $C_{22}H_{23}N_3O_2S$ (393.5): Calcd C 67.15, H 5.89, N 10.68. Found: C 67.10, H 5.94, N 10.64.

4.8. 1-(2-(Diethylamino)ethyl)-3-(1*H*-indol-3-yl)-4-(*N*-methyl-1*H*- pyrrol-2-yl)-1*H*-pyrrol-2,5-dione 9e

To a solution of **8e** (70 mg, 0.24 mmol) in DMF (3 mL) was added *N*,*N*-diethylethylenediamine (0.05 mL, 0.36 mmol). The mixture was stirred under microwave irradiation (140 °C, 10 min, 450 W). The mixture was then diluted with Et_2O (15 mL) and extracted with brine (4× 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give pure compound **9e** (90 mg, 96%). Mp 109–111 °C (hexane/Et_2O). IR (Nujol): 3470, 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 9.23 (brs, 1H, exch.), 8.05 (d, J = 2.9, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.13 (dd, J = 8.1, 8.0 Hz, 1H), 6.82 (dd, J = 8.1, 6.9 Hz, 1H), 6.63 (dd, J = 2.5, 1.8 Hz, 1H), 6.50 (dd, J = 3.6, 1.8 Hz, 1H), 6.39 (d, J = 8.0 Hz, 1H), 6.26 (dd, J = 3.6, 2.5 Hz, 1H), 3.75 (t, J = 7.3 Hz, 2H), 3.1 (s, 3H), 2.76 (t, J = 7.3 Hz, 2H), 2.65 (q, J = 7.3 Hz, 4H), 1.05 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 171.9, 171.7, 136.3, 130.3, 130.0, 125.8, 125.7, 123.6, 123.2, 122.9, 121.6, 121.4, 114.9, 111.3, 109.8, 107.6, 50.7, 47.4 (x2), 36.5, 35.3, 12.1 (x2). $C_{23}H_{26}N_4O_2$ (390.48): Calcd C 70.75, H 6.71, N 14.35. Found: C 70.74, H 6.73, N 14.33.

4.9. General procedure for the preparation of compounds 10

A solution of compounds **7a**–**c**,**h** (2 mmol) dissolved in CH_2Cl_2 (90 mL) **7f** (2 mmol) and in acetone (90 mL) in the presence of a catalytic amount of I_2 was irradiated for 8 h in a reactor with an HPK-125W Philips high-pressure mercury vapor lamp in a water-jacketed immersion well. The solvent was evaporated and the residue was purified by chromatography on silica gel (**10a**: CH_2Cl_2/Et_2O, 100:1; **10b**,**10c**,**10f**: hexane/Et_2O, 5:1; **10h**: hexane/CH_2Cl_2, 2:1) to give pure compounds **10**.

4.9.1. Diethyl 6-methyl-6*H*-furo[3,2-*e*]-indole-4,5-dicarboxylate 10a. Yield 46%. Mp 85–86 °C (CH_2Cl_2/hexane); IR (Nujol): 1700, 1581 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.80 (d, J = 1.8 Hz, 1H), 7.20 (d, J = 2.9 Hz, 1H), 7.01 (d, J = 1.8 Hz, 1H), 6.68 (d, J = 3.2 Hz, 1H), 4.5–4.43 (m, 4H), 3.85 (s, 3H), 1.49–1.39 (m, 6H); ^{13}C NMR ($CDCl_3$) δ: 169.0, 165.8, 147.6, 146.3, 134.4, 128.6, 126.4, 121.6, 117.5, 109.0, 105.6, 100.1, 62.3, 61.8, 35.9, 14.7, 14.4. $C_{17}H_{17}NO_5$ (315.32): Calcd C 64.75, H 5.43, N 4.44. Found: C 64.71, H 5.45, N 4.41.

4.9.2. Diethyl 6-methyl-6*H*-thieno[3,2-*e*]indole-4,5-dicarboxylate 10b. Yield 52%. Mp 90–92 °C (Et_2O/hexane); IR (Nujol): 1714, 1587 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.64 (d, J = 1.8 Hz, 2H), 7.23 (d, J = 2.9 Hz, 1H), 6.81 (d, J = 2.9 Hz, 1H), 4.58–4.46 (m, 4H), 3.89 (s, 3H), 1.53–1.40 (m, 6H); ^{13}C NMR ($CDCl_3$) δ: 169.5, 166.5, 134.1, 133.7, 132.2, 130.1, 129.6, 129.0, 121.2, 120.3, 115.7, 100.2, 62.3, 62.2, 35.6, 14.7, 14.4. $C_{17}H_{17}NO_4S$ (331.39): Calcd C 61.61, H 5.17, N 4.23. Found: C 61.57, H 5.15, N 4.19.

4.9.3. Diethyl benzo[1,2-*b*-4,3-*b*′]dithiophene-4,5-dicarboxylate 10c. Yield 54%. Oil; IR (Nujol): 1720 cm^{-1}; ^1H NMR δ: 7.80–7.73 (m, 4H), 4.51 (q, J = 7.3 Hz, 4H), 1.45 (t, J = 7.3 Hz, 6H); ^{13}C NMR δ: [(167.3, 137.0, 136.0, 131.7, 123.9, 121.9, 62.5, 14.5) x2]. $C_{16}H_{14}O_4S_2$ (334.41): Calcd C 57.47, H 4.22. Found: C 57.60, H 4.35.

4.9.4. Ethyl 10-ethoxycarbonyloxy-9a,10-dihydro-5b*H*-thieno[3,2-*a*]carbazole-4,5-dicarboxylate 10f. Yield 40%. Mp 104–106 °C (*i*-Pr_2O/hexane); IR (Nujol): 1750, 1732, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 8.22 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 5.8 Hz, 1H), 7.88 (dd, J = 1.1, 7.3 Hz, 1H), 7.68 (d, J = 5.8 Hz, 1H), 7.53 (dt, J = 1.1, 7.3 Hz, 1H), 7.39 (dt, J = 1.1, 7.3 Hz, 1H), 4.66 (m, 4H), 4.56 (d, J = 7.0 Hz, 2H), 1.50 (m, 9H); ^{13}C NMR δ: 169.0, 165.6, 152.3, 141.1, 139.3, 136.6, 129.3, 128.6, 127.6, 127.3, 124.3, 124.1, 123.7, 121.0, 120.0, 117.8, 115.8, 64.4, 62.3, 62.2, 14.5, 14.4, 14.2. $C_{23}H_{21}NO_6S$ (439.11): Calcd C, 62.86; H, 4.82; N, 3.19. Fund C, 62.80; H, 4.93; N, 3.10.

4.9.5. Diethyl naphthol[2,1-*b*]thiophene-4,5-dicarboxylate 10h. Yield 41%. Mp 118–120 °C (Et_2O/hexane); IR (Nujol): 1710, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 8.39 (d, J = 8.2 Hz, 1H), 8.02 (d, J = 5.4 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.78–7.57 (m, 3H), 4.63–4.49 (m, 4H), 1.54–1.43 (m, 6H); ^{13}C NMR ($CDCl_3$) δ: 169.3, 165.6, 137.9, 135.0, 133.1, 131.0, 130.5, 129.2, 127.6, 127.1, 126.8, 124.1, 121.4, 120.6, 62.5, 62.2, 41.46, 41.40. $C_{18}H_{16}O_4S$ (328.38): Calcd C 65.84, H 4.91. Found: C 65.80, H 4.94.

4.10. General procedure for the preparation of anhydrides 11a,b

A solution of NaOH (1.2 g, 30 mmol) in EtOH (20 mL) and H_2O (10 mL) was added to a solution of **10a**,**b** (3.2 mmol) in EtOH (60 mL). The mixture was refluxed for 1.5 h. The solvent was then evaporated, the residue was acidified with 5% HCl and extracted with CH_2Cl_2 (2× 40 mL). After evaporation of the solvent, the crude residue was dissolved in CH_2Cl_2 (20 mL) and a catalytic amount of *p*TSA was added. The resulting mixture was refluxed for 7 h. The solvent was concentrated, Et_2O was added and a solid was separated, filtered, and washed with Et_2O giving pure compounds **11a**,**b**.

4.10.1. 6-Methyl-6*H*-furo[3,2-*e*]-furo[3,4-*g*]indol-7,9-dione 11a. Yield 50%. Mp 290–309 °C, dec.; IR (KBr): 1827, 1751 cm^{-1}; ^1H NMR (DMSO) δ: 8.40 (d, J = 2.0 Hz, 1H), 7.82 (d, J = 2.9 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.00 (d, J = 2.9 Hz, 1H), 4.26 (s, 3H); ^{13}C NMR (DMSO) δ: 163.2, 162.0, 151.0, 143.1, 137.5, 129.3, 127.97, 127.90, 112.0, 110.0, 106.9, 102.2, 37.8. $C_{13}H_7NO_4$ (241.2): Calcd C 64.73, H 2.93, N 5.81. Found: C 64.67, H 2.99, N 5.77.

4.10.2. 6-Methyl-6*H*-furo[3,4-*g*]thieno[3,2-*e*]indol-7,9-dione 11b. Yield 86%. Mp 280–300 °C, dec.; IR (KBr): 1824, 1763 cm^{-1}; ^1H NMR (DMSO) δ: 8.27 (d, J = 5.4 Hz, 1H), 7.97 (d, J = 5.4 Hz, 1H), 7.81 (d, J = 2.9 Hz, 1H), 7.15 (d, J = 2.9 Hz, 1H), 4.24 (s, 3H); ^{13}C NMR (DMSO) δ: 164.2, 163.1, 139.4, 136.4, 134.9, 130.8, 128.4, 125.8, 122.6, 120.2, 114.3, 102.5, 37.6. $C_{13}H_7NO_3S$ (257.27): Calcd C 60.69, H 2.74, N 5.44. Found: C 60.80, H 2.88, N 5.53.

4.11. General procedure for the preparation of anhydrides 11c,d

A solution of anhydride **8c**,**d** (10 mmol) in acetone (350 mL) was irradiated in presence of a catalytic amount of I_2 for 13 h in an reactor with a HPK-125W Philips high-pressure mercury vapor lamp in a water-jacketed immersion well. The solvent was concenrated and a solid was formed and filtered.

4.11.1. Thieno[3,2-*e*]furo[3,4-*g*]benzothiophene-7,9-dione 11c. Yield 73%. Mp 327–329 °C; IR (Nujol): 1820, 1770 cm^{-1}; ^1H NMR (DMSO) δ: 8.43 (d, J = 5.3 Hz, 2H), 8.27 (d, J = 5.3 Hz, 2H); ^{13}C NMR (DMSO) δ: [(163.5, 141.3, 135.8, 130.1, 123.9, 123.5) x2]; MS: *m*/*z* 260.1 [*APCI*-]; $C_{12}H_4O_3S$ (260.29): Calcd C 55.37, H 41.55. Found: C 55.48, H 41.62.

4.11.2. Furo[3,4-*g*]thieno[3,2-*e*]benzofuran-7,9-dione 11d. Yield 68%. Mp 314–316 °C; IR (Nujol): 1840, 1750 cm^{-1}; ^1H NMR (DMSO) δ: 8.54 (d, J = 2.1 Hz, 1H), 8.43 (d, J = 5.1 Hz, 1H), 8.07 (d, J = 5.1 Hz, 1H), 7.71 (d, J = 2.1 Hz, 1H); ^{13}C NMR (DMSO) δ: 163.5, 161.2, 151.6, 145.7, 139.8, 136.6, 130.3, 129.3, 122.9, 122.5, 113.5, 107.5. $C_{12}H_4O_4S$ (242.22): Calcd C 59.02, H 1.65. Found: C 58.94, H 1.70.

4.11.3. Benzo[*e*]furo[3,4-*g*]benzothiophene-8,10-dione 11h. A solution of NaOH (252 mg, 6.3 mmol) in EtOH (5 mL) and H_2O (2 mL) was added to a solution of **10h** (232 mg, 0.7 mmol) in EtOH (15 mL). The mixture was refluxed for 30 min. The solvent was then evaporated, the residue was acidified with 5% HCl and extracted with CH_2Cl_2 (2× 15 mL). After evaporation of the solvent, the crude residue was dissolved in $SOCl_2$ (10 mL) and heated to reflux for 4.5 h after which the solvent was evaporated. The residue was washed with

CH_2Cl_2 (2× 20 mL), dried under reduce pressure, taken up with Et_2O, and filtered to give pure compound **11h** (63 mg, 36%). Mp 303–304 °C (CH_2Cl_2). IR (Nujol): 1820, 1770 cm^{-1}; ^1H NMR (DMSO) δ: 8.80–8.71 (m, 2H), 8.53–8.46 (m, 2H), 7.99–7.85 (m, 2H); ^{13}C NMR (DMSO) δ: 164.6, 163.9, 144.3, 136.2, 132.6, 131.3, 130.9, 129.9, 128.9, 128.1, 127.7, 127.5, 125.9, 124.1. $C_{14}H_6O_3S$ (254.26): Calcd C 66.13, H 2.38. C 66.00, H 2.45.

4.12. General procedure for the preparation of imides 12a,d,i

To a solution of anhydrides **11a**,**d**,**i** (0.3 mmol) in CH_2Cl_2 (3 mL) was added *N*,*N*-diethylethylenediamine (0.04 mL, 0.3 mmol). The mixture was stirred under microwave irradiation (100 °C, 650 W, 15 min) in the presence of a Weflon stirrer. The solvent was then evaporated and the residue purified by crystallization giving pure compounds **12** in quantitative yield.

4.12.1. 8-(2-Diethylaminoethyl)-6-methyl-6*H*-pyrrolo[3,4-*g*]furo[3,2-*e*]indol-7,9-dione 12a. Melting point 109–111 °C (Et_2O). IR (KBr): 1756, 1701 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.90 (d, J = 2.2 Hz, 1H), 7.29 (d, J = 2.9 Hz, 1H), 7.05 (d, J = 2.2 Hz, 1H), 6.75 (d, J = 2.9 Hz, 1H), 4.36 (s, 3H), 3.79 (t, J = 7.3 Hz, 2H), 2.74 (t, J = 7.3 Hz, 2H), 2.60 (q, J = 6.9 Hz, 4H), 1.03 (t, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 168.7, 167.4, 148.3, 143.6, 134.5, 128.2, 126.2, 112.6, 111.1, 105.7, 101.1, 50.4, 47.3 (x2), 38.3, 35.9, 12.1 (x2). $C_{19}H_{21}N_3O_3$ (339.39): Calcd C 67.24, H 6.24, N 12.38. Found: C 67.15, H 6.21, N 12.34.

4.12.2. 8-(2-Diethylaminoethyl)-8*H*-pyrrolo[3,4-*g*]thieno[3,2-*e*]benzofuran-7,9-dione 12d. Melting point 100–102 °C (Et_2O). IR (KBr): 1761, 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.98 (d, J = 2.2 Hz, 1H), 7.91 (d, J = 5.4 Hz, 1H), 7.66 (d, J = 5.4 Hz, 1H), 7.19 (d, J = 2.2 Hz, 1H), 3.85 (t, J = 6.9 Hz, 2H), 2.80 (t, J = 6.9 Hz, 2H), 2.64 (q, J = 6.9 Hz, 4H), 1.04 (t, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 168.5, 166.7, 148.9, 146.2, 138.4, 133.8, 129.0, 128.7, 122.9, 121.2, 113.3, 106.2, 50.6, 47.4 (x2), 36.3, 12.1 (x2). $C_{18}H_{18}N_2O_3S$ (342.41): Calcd C 63.14, H 5.30, N 8.18. Found: C 63.09, H 5.34, N 8.14.

4.12.3. 2-(2-Diethylaminoethyl)-phenanthro[9,10,*c*]pyrrol-1,3-dione 12i. Melting point 81–82 °C (Et_2O). IR (KBr): 1755, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 9.13 (dd, J = 7.7, 1.8 Hz, 2H), 8.71 (dd, J = 7.7, 1.8 Hz, 2H), 7.84–7.71 (m, 4H), 3.84 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H), 2.62 (q, J = 6.9 Hz, 4H), 1.03 (t, J = 6.9 Hz, 6H); ^{13}C NMR δ: [(170.3, 133.7, 129.7, 128.7, 127.9, 126.6, 126.0, 123.5)x2], 50.9, 47.7(x2), 36.3, 12.4(x2); MS: *m*/*z* 347.7 [MH^+]. $C_{22}H_{22}N_2O_2$ (346.17): Calcd C 76.28, H, 6.40. Found: C 76.22, H, 6.51.

4.13. 9-(2-Diethylaminoethyl)-9*H*-benzo[*e*]pyrrolo[3,4-*g*]benzothiophene-8,10-dione 12h

A solution of anhydride **11h** (43 mg, 0.17 mmol) in *N*,*N*-diethylethylenediamine (2 mL) was heated in a oven for 1 h at 150 °C. The solution was diluted with CH_2Cl_2 (15 mL) and repeatedly washed with water until pH 5. The organic layer was then dried over anhydrous Na_2SO_4, filtered and the solvent evaporated and the crude reaction mixture was chromatographed (CH_2Cl_2/MeOH, 30:1) to give pure compound **12h** (43 mg, 72%) as an oil. IR (Nujol): 1764, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 9.02 (d, J = 8.0 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 5.4 Hz, 1H), 7.93 (d, J = 5.4 Hz, 1H), 7.75–7.69 (m, 2H), 3.83 (t, J = 7.3 Hz, 2H), 2.77 (t, J = 7.3 Hz, 2H), 2.61 (q, J 6.9 Hz, 4H), 1.03 (t, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 169.9, 168.5, 142.2, 132.7, 131.9, 129.0, 128.7, 128.0, 126.8, 125.9, 125.8, 124.4, 124.1, 122.0, 50.7, 47.5 (x2), 36.2, 12.2 (x2). $C_{20}H_{20}N_2O_2S$ (352.45): Calcd C 68.16, H 5.72, N 7.95. Found: C 68.07, H 5.77, N 7.90.

4.14. Preparation of imide 12b,c

A solution of diethyl dicarboxylates **10b**,**c** (0.3 mmol) in *N*,*N*-diethylethylenediamine (3 mL) was heated in a oven for 12 h at 150 °C. The mixture was diluted with a solution of CH_2Cl_2 (10 mL) and H_2O (10 mL) at room temperature and acidified with HCl 5% to pH 6. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 30:1) to give pure compounds **12b** and **12c**.

4.14.1. 8-(2-Diethylaminoethyl)-6-methyl-6*H*-pyrrolo[3,4-*g*]thieno[3,2-*e*]indol-7,9-dione 12b. Yield 28%. Mp 133–135 °C (CH_2Cl_2/hexane). IR (Nujol): 1748, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.80 (d, J = 5.4 Hz, 1H), 7.65 (d, J = 5.4 Hz, 1H), 7.29 (d, J = 2.9 Hz, 1H), 6.87 (d, J = 2.9 Hz, 1H), 4.36 (s, 3H), 3.82 (t, J = 7.6 Hz, 2H), 2.79 (t, J = 7.6 Hz, 2H), 2.65 (q, J = 7.3 Hz, 4H), 1.07 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 169.3, 168.8, 138.1, 133.7, 132.4, 130.1, 129.1, 125.9, 121.5, 121.3, 114.4, 101.6, 50.5, 47.5 (x2), 38.3, 35.8, 12.0 (x2). $C_{19}H_{21}N_3O_2S$ (355.45): Calcd C 64.20, H 5.95, N 11.82. Found: C 64.16, H 5.98, N 11.80.

4.14.2. 8-(2-Diethylaminoethyl)-8*H*-pyrrolo[3,4-*g*]thieno[3,2-*e*]benzothiophene-7,9-dione 12c. Oil. IR (Nujol): 1760, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.89 (d, J = 5.4 Hz, 2H), 7.80 (d, J = 5.4 Hz, 2H), 3.83 (t, J = 7.3 Hz, 2H), 2.77 (t, J = 7.3 Hz, 2H), 2.61 (q, J = 7.3 Hz, 4H), 1.02 (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: [(168.7, 140.1, 132.9, 130.1, 123.6, 121.9)x2], 50.7, 47.5 (x2), 36.4, 12.2 (x2). $C_{18}H_{18}N_2O_2S_2$ (358.48): Calcd C 60.31, H 5.06, N 7.81. Found: C 60.24, H 5.14, N 7.76.

5. Biological methods

5.1. Cell culture

The human NCI-H460 lung tumor cells and primary rat aortic SMCs were used in this study. SMCs cultured from the intimal-medial layers of aorta of male Sprague–Dawley rats (200–250 g) were grown in monolayers at 37 °C in a humidified atmosphere of 5% CO_2 in MEM supplemented with 10% (v/v) fetal calf serum (FCS), 100 U/ml penicillin, 0.1 mg/mL streptomycin, 20 mM tricine buffer, and 1% (v/v) non-essential amino acid

solution. Cells were used between the fourth and tenth passages.[26] The human NCI-H460 lung carcinoma cells (ATCC HTB 177) were cultured in RPMI-1640 supplemented with 10% FCS. Eagle's MEM and RPMI-1640 were purchased from SIGMA (Milan, Italy), trypsin ethylenediamine tetraacetate, penicillin (10,000 U/mL), streptomycin (10 mg/mL), tricine buffer (1 M, pH 7.4), non-essential amino acid solution (100×), and fetal calf serum (FCS) were purchased from Invitrogen (Carlsbad, CA, USA). Disposable culture flasks and Petri dishes are from Corning Glassworks (Corning, NY).

5.2. Cell proliferation assay

Rat SMCs were seeded at a density of 2×10^5 cells/Petri dish (35 mm) and incubated with MEM supplemented with 10% FCS. Twenty-four hours later, the medium was changed to one containing 0.4% FCS to stop cell growth, and the cultures were incubated for 72 h. At this time (time 0), the medium was replaced with one containing 10% FCS in the presence or absence of known concentrations of the tested compounds, and the incubation was continued for a further 72 h at 37 °C. NCI-H460 cells were seeded at a density of 8×10^4 cells/Petri dish (35 mm), and incubated with RPMI-1640 supplemented with 10% FCS.[27] Twenty-four hours after seeding, cells were exposed to test compound, then harvested 72 h later. Cell proliferation was evaluated by cell counting after trypsinization of the monolayers with use of a Coulter Counter model ZM.[26] All the compounds were dissolved in DMSO prior to dilution, being the final concentration of DMSO at a maximum of 1%. The concentration of compounds required to inhibit 50% of cell proliferation (IC_{50}) was calculated by linear regression analysis of the logarithm of the concentration (in micromoles per liter) versus logit.

5.3. DNA intercalating assay

For the analysis of DNA-compound interaction the supercoiled pRYG DNA was utilized as substrate according to protocol provided by TopoGen Inc. (Columbus, USA). Supercoiled pRYG DNA was incubated in a reaction buffer containing 10 mM Tris–HCl, pH 7.9, 1 mM EDTA, 150 mM NaCl, 0.1% BSA, 0.1 mM spermidine, and 5% glycerol. The tested compound was added at the indicated concentration and after 10 min at 37 °C the reaction was stopped by addition of the stop buffer containing the loading dye (15 sarkosyl, 0.025% bromophenol blue, 5% glycerol). Then the reaction mixture was analyzed on a 1% agarose gel by running at 80 V in TAE buffer (400 mM Tris–base, 10 mM EDTA, 200 mM sodium acetate, pH 8.3). Gels were stained with ethidium bromide and the image acquired with Gel Doc acquisition system and Quantity One software (Bio-Rad).

Acknowledgments

We thank MUR for financial support (Grants COFIN and FIRB). Alessandro Contini is also very grateful to Chemical Computing Group for their scientific support.

References and notes

1. Wang, H. K.; Morris-Natschke, S. L.; Lee, K.-H. *Med. Res. Rev.* **1997**, *17*, 367–425.
2. Braña, M. F.; Gradillas, A.; Gomez, A.; Acero, N.; Llinares, F.; Muñoz-Mingarro, D.; Abradelo, C.; Rey-Stolle, F.; Yuste, M.; Campos, J.; Gallo, M. A.; Espinosa, A. *J. Med. Chem.* **2004**, *47*, 2236–2242.
3. Wang, J. C. *Ann. Rev. Biochem.* **1996**, *65*, 635–692.
4. Brehm, B. R.; Bock, C.; Wesselborg, S.; Pfeiffer, S.; Schuler, S.; Schulze-Osthoff, K. *Biochem. Pharmacol.* **2001**, *61*, 119–127.
5. Dubernet, M.; Caubert, V.; Guillard, J.; Viaud-Massuard, M.-C. *Tetrahedron* **2005**, *61*, 4585–4593.
6. Uchida, K.; Kido, Y.; Yamaguchi, T.; Irie, M. *Bull. Soc. Chim. Jpn.* **1998**, *71*, 1101–1108.
7. Shirinian, V. Z.; Krayushkin, M. M.; Nabatov, B. V.; Kuznetsova, O. Yu.; Shimkin, A. A. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 889–893.
8. Roy, S.; Gribble, G. W. *Org. Lett.* **2006**, *8*, 4975–4977.
9. Faul, M. M.; Grutsch, J. L.; Kobierski, M. E.; Kopach, M. E.; Krumrich, C. A.; Staszak, M. A.; Udodong, U.; Vicenzi, J. T.; Sullivan, K. A. *Tetrahedron* **2003**, *59*, 7215–7229.
10. Brenner, M.; Rexhausen, H.; Steffan, B.; Steglich, W. *Tetrahedron* **1988**, *44*, 2887–2892.
11. Braña, M. F.; Añorbe, L.; Tarrasón, G.; Mitjans, F.; Piulats, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2701–2703.
12. Desarbre, E.; Bergman, J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2009–2016.
13. Zhang, M.; An, H.-Y.; Zhao, B.-G.; Xu, J.-H. *Org. Biomol. Chem.* **2006**, *4*, 33–35.
14. Hodges, L. M.; Spera, M. L.; Moody, M. W.; Harman, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 7117–7127.
15. Sanchez-Martinez, C.; Shih, C.; Faul, M. M.; Zhu, G.; Paal, M.; Somoza, C.; Li, T.; Kumrich, C. A.; Winneroski, L. L.; Xun, Z.; Brooks, H. B.; Patel, B. K. R.; Schultz, R. M.; DeHahn, T. B.; Spencer, C. D.; Watkins, S. A.; Considine, E.; Dempsey, J. A.; Ogg, C. A.; Campbell, R. M.; Anderson, B. A.; Wagner, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3835–3839.
16. Sanchez-Martinez, C.; Faul, M. M.; Shih, C.; Sullivan, K. A.; Grutsch, J. L.; Cooper, J. T.; Kolis, S. P. *J. Org. Chem.* **2003**, *68*, 8008–8014.
17. Braña, M. F.; Cacho, M.; García, M. A.; de Pascual-Teresa, B.; Ramos, A.; Domínguez, M. T.; Pozuelo, J. M.; Abradelo, C.; Rey-Stolle, M. F.; Yuste, M.; Báñez-Coronel, M.; Lacal, J. C. *J. Med. Chem.* **2004**, *47*, 1391–1399.
18. Faul, M. M.; Sullivan, K. A.; Grutsch, J. L.; Winneroski, L. L.; Shih, C.; Sanchez-Martinez, C.; Cooper, J. T. *Tetrahedron Lett.* **2004**, *45*, 1095–1098.
19. Lakatosh, S. A.; Luzikov, Y. N.; Preobrazhenskaya, M. N. *Tetrahedron* **2005**, *61*, 8241–8248.
20. Fröhner, W.; Monse, B.; Braxmeier, T. M.; Casiraghi, L.; Sahagún, H.; Seneci, P. *Org. Lett.* **2005**, *7*, 4573–4576.
21. Beccalli, E. M.; Gelmi, M. L.; Marchesini, A. *Eur. J. Org. Chem.* **1999**, 1421–1426.
22. Fields, E. K.; Behrend, S. J. *J. Org. Chem.* **1990**, *55*, 5165–5170.
23. Jeanes, A.; Adams, R. *J. Am. Chem. Soc.* **1937**, *59*, 2608–2622.
24. Abdel-Aziz, A. A.-M. *Eur. J. Med. Chem.* **2007**, *42*, 614–626.
25. Braña, M. F.; Cacho, M.; Gradillas, A.; de Pascual-Teresa, B.; Ramos, A. *Cur. Pharm. Des.* **2001**, *7*, 1745–1780.

26. Corsini, A.; Mazzotti, M.; Raiteri, M.; Soma, M. R.; Gabbiani, G.; Fumagalli, R.; Paoletti, R. *Atherosclerosis* **1993**, *101*, 117–125.
27. Cassinelli, G.; Lanzi, C.; Petrangolini, G.; Tortoreto, M.; Pratesi, G.; Cuccuru, G.; Laccabue, D.; Supino, R.; Belluco, S.; Favini, E.; Poletti, A.; Zunino, F. *Mol. Cancer Ther.* **2006**, *5*, 2388–2397.
28. MOE software available from Chemical Computing Group Inc., Montreal, Canada, http://www.chemcomp.com.
29. Labute, P.; Williams, C.; Feher, M.; Sourial, E.; Schmidt, J. *J. Med. Chem.* **2001**, *44*, 1483–1490.