

Susana M. M. Lopes,^a Mafalda Laranjo,^b Arménio C. Serra,^a
Ana Margarida Abrantes,^{b,c} António M. d'A. Rocha Gonsalves,^a
Maria Filomena Botelho,^{b,c} Ana Matos Beja,^d Manuela Ramos Silva,^d
and Teresa M. V. D. Pinho e Melo^{a,*}

^aDepartment of Chemistry, University of Coimbra, Coimbra 3004-535, Portugal

^bBiophysics/Biomathematics Institute, IBILI, Faculty of Medicine of Coimbra, Coimbra 3000-354, Portugal

^cCenter of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra, Portugal

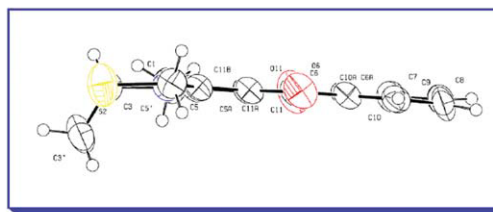
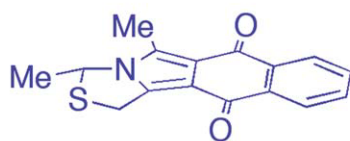
^dDepartment of Physics, University of Coimbra, Coimbra 3004-516, Portugal

*E-mail: tmelo@ci.uc.pt

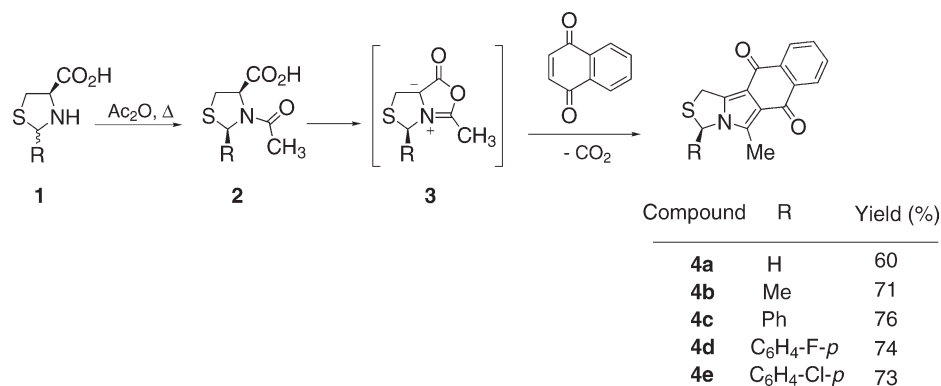
Received October 12, 2009

DOI 10.1002/jhet.396

Published online 21 June 2010 in Wiley InterScience (www.interscience.wiley.com).



Scheme 1



chromophore using 1,4-naphthoquinones as dipolarophiles. The new heterocycles were tested against two cancer cell lines namely A375 melanoma and WiDr colorectal adenocarcinoma.

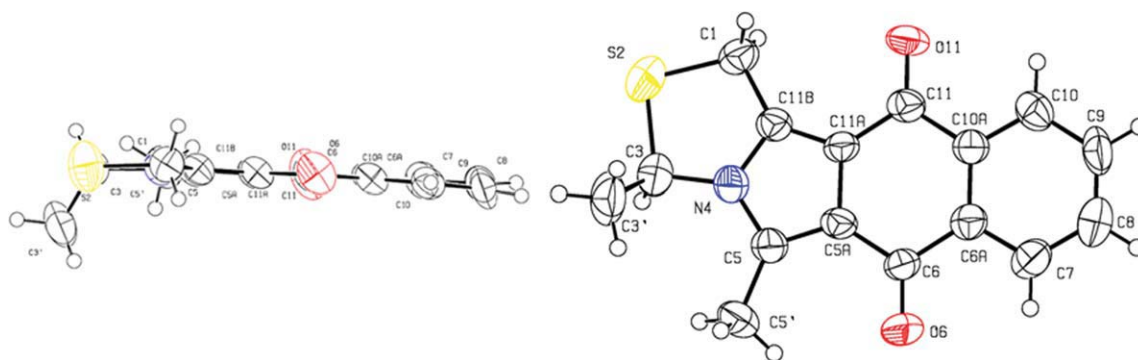
RESULTS AND DISCUSSION

Chemistry. (*R*)-2-Substituted-thiazolidine-4-carboxylic acids **1** was obtained as mixture of the (*2S,4R*) and (*2R,4R*)-diastereoisomers from the reaction of an aldehyde and L-cysteine [20]. The synthesis of the corresponding 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **4** was carried out by heating a solution of the appropriate thiazolidine in acetic anhydride in the presence of 1,4-naphthoquinone. In this process, the thiazolidine undergoes *in situ* acylation followed by cyclodehydration to give a bicyclic münchnone **3**, which reacts further with 1,4-naphthoquinone to afford the corresponding 1,3-dipolar cycloadduct. The benzo[*f*]thiazolo[4,3-*a*]isoindole-6,11(1*H*,3*H*)-diones **4** was obtained in yields ranging from 60 to 76%. It is worth to emphasize that derivatives **4b–4e** were isolated as single enantiomers with *R* configuration (Scheme 1).

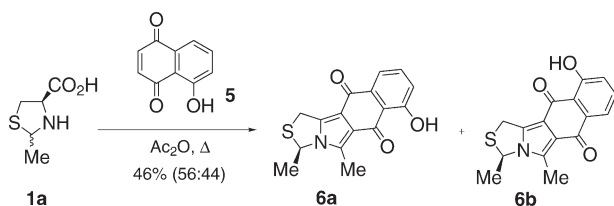
The structure of compound **4b** was established by X-ray crystallography (Fig. 1) determining the absolute

configuration of chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **4b** as being *R*. The compound **4b** crystallizes in the chiral space group *P*3₂, with three symmetry related molecules in the unit cell. The molecules are comprised of four fused rings that are essentially planar. Only the carbon atom C3' deviates significantly from the molecular plane, the C1-S2-C3-C3' torsion angle is 124.1(2)°. In the solid state, due to the lack of conventional donors, only weak C—H...O and C—H... π intermolecular interactions join the molecules in a three-dimensional network.

The selectivity observed can be explained considering that 2-substituted-1,3-thiazolidine-4-carboxylic acids can undergo selective inversion at C-2 through a mechanism involving the opening of the ring with the formation of the corresponding Schiff base. However, the N-acylation of the 2-substituted-1,3-thiazolidine-4-carboxylic acids prevents this epimerization and allows the isolation of pure diastereoisomers [22–26]. Therefore, starting with (*2S,4R*) and (*2R,4R*)-2-substituted-1,3-thiazolidine-4-carboxylic acids mixture **1**, diastereoisomerically pure *N*-acetyl-2-substituted-1,3-thiazolidine-4-carboxylic acids **2** was generated allowing the synthesis of chiral cycloadducts. The chirality of the thiazolidine at C-4 is lost, and the chirality at C-2 is retained.



Scheme 2



Juglone (5-hydroxy-1,4-naphthoquinone) **5** can also be used as dipolarophile in the 1,3-dipolar cycloaddition of the bicyclic münchnone generated from thiazolidine **1a**. However, a mixture of the two possible regioisomers **6a** and **6b** was obtained in 46% overall yield (Scheme 2).

Similar chemistry can be carried to prepare the chiral benzo[*f*]thiazolo[4,3-*a*]isoindole-6,11(1*H*,3*H*)-dione **8**. In this case, D-penicillamine, an α -amino acid with *S* configuration, was condensed with acetaldehyde leading to (4*S*)-2,5,5-trimethyl-1,3-thiazolidine-4-carboxylic acid (**7**) [27]. Therefore, the 1,3-dipolar cycloaddition of the bicyclic münchnone generated from thiazolidine **7** with 1,4-naphthoquinone afforded heterocycle **8** with *S* configuration (Scheme 3).

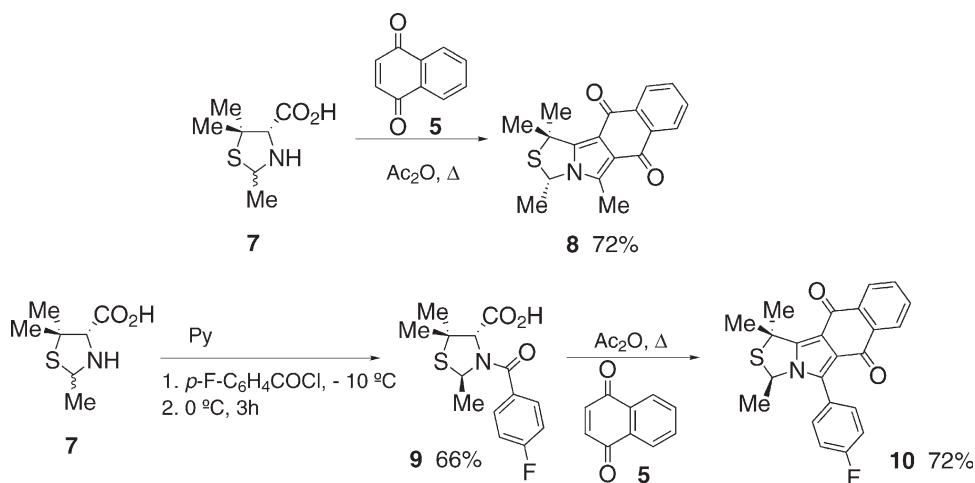
The synthetic strategy to prepare the chiral benzo[*f*]thiazolo[4,3-*a*]isoindole-6,11(1*H*,3*H*)-dione **10** required the synthesis of (2*R*,4*S*)-3-(4-fluorophenylcarbonyl)-2,5,5-trimethylthiazolidine-4-carboxylic acid (**9**) in diastereoisomeric pure form. Thus, the *N*-acylation of the starting thiazolidine **7** was carried out with the 4-fluorobenzoyl chloride following a general procedure previously reported [28,29]. Heating a solution of the heterocycle **9** in acetic anhydride in the presence of 1,4-naphthoquinone afforded the corresponding cycloadduct **10** with *R* configuration (Scheme 3).

Anticancer activity. Studies of the anticancer activity of the new benzo[*f*]thiazolo[4,3-*a*]isoindole-

6,11(1*H*,3*H*)-diones (except compound **4a** due to low solubility) have been carried out against WiDR colorectal adenocarcinoma and A375 melanoma human cancer cell lines. The results of the cell viability using different concentrations of the compounds in cultures of WiDr and A375 cells are presented in Figures 2 and 3. Cells were incubated during 48 h with DMSO solution of the selected compounds, washed, and then cell viability was evaluated by MTT test and compared with control experiments, where the incubation was carried out with only DMSO solution.

Values of cell viability show that the pyrrolo-thiazoles do not show considerable anticancer activity against the two cell lines tested. Nevertheless, the compounds are more active against melanoma cells than against colon adenocarcinoma cells. The comparison of the activity is clearer when the corresponding IC_{50} values (Table 1) calculated from the dose-response curves (Figs. 2 and 3) are analysed. In the case of WiDr cells, with the exception of compound **4b** ($\text{IC}_{50} = 86 \mu\text{M}$), using concentrations of up to $100 \mu\text{M}$, the IC_{50} was not reached. For melanoma cells, with exception of compounds **8** and **6**, the values for IC_{50} allow a comparison of the activity of the different structures. In this case, the anticancer activity order is **4c** > **10,4b,4e** > **4d** > **8,6**. Looking at the results of the two cell lines, it seems that compound **4b** with a methyl groups at positions 3 and 5 is the most active. Curiously, the similar structure **6** with only an additional hydroxyl substituent at the naphthoquinone moiety showed a much lower activity. Relatively to A375 melanoma cells, the pyrrolo-thiazole compounds synthesized are less active than 2-arylthiazolidine compounds described [14]. Also the activities of the pyrrolo-thiazoles are lower than those observed for 4-thiazolidinones for human colon carcinoma, albeit referring to different cell line [12]. The only exception to our results is

Scheme 3



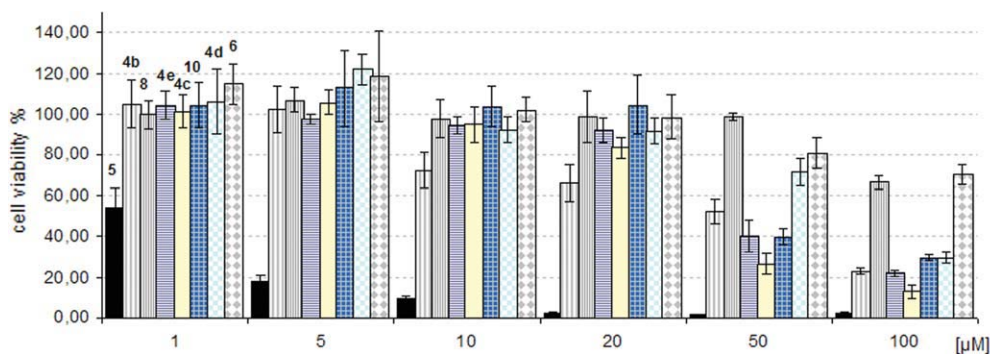


Figure 2. Values of cell viability of tested compounds against A375 melanoma cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the parent quinone, juglone (**5**), which shows potent cytotoxicity against the two cell lines, particularly in the case of the melanoma cells with lower IC_{50} , 1.23 μM for A375 cells and 8.8 μM for WiDr cells.

It is evident from the results that the incorporation of a thiazolidine ring to the quinone structure drastically reduce the anticancer activity as can be seen by the observed activity of juglone (**5**) and that of the corresponding 1,3-dipolar cycloadducts **6**. This can be explained by the fact that one important mechanism of action of quinones is related to the oxidation–reduction properties [30], which are probably altered by the introduction of the extra ring in compound **6**. Another plausible explanation for the observed low activity is related to the fact that juglone or quinone derivatives are good Michael acceptors that can react with the thiol group of proteins causing their deactivation as described for Pin 1 isomerase [31]. Our pyrrolo-thiazole compounds without the α,β -unsaturated carbonyl system lost this ability. Nevertheless, the low cytotoxicity of the pyrrolo-thiazole compounds was somewhat unexpected considering the geometry of the molecule (see Fig. 1). Molecular shape of thiazolidinones, characterized by the preferential “butterfly-like” conformation, is particularly important regarding the activity as HIV [8]. However, in the

case of quinones others suggest that planarity is an important factor to achieve biological activity because DNA intercalation is another possible mechanism of action [6]. For anthracene-9,10-diones which interfere with topoisomerase II, the derivatives need to be planar and also need another structural feature, like alkyl amino side chains, to interact with protein as observed for mixoxantrone [32]. In our case, the very planar structure of the benzo[*f*]thiazolo[4,3-*a*]isindole-6,11(1*H*,3*H*)-diones caused by the extended conjugation is not sufficient to allow a high anticancer activity possible because of the lack of this type of side chains. Studies are underway to construct new structures via our synthetic methodology aiming to obtain higher activities.

CONCLUSIONS

Herein, we describe the successful synthesis of new naphthoquinone-containing heterocyclic compounds. Two kinds of chiral benzo[*f*]thiazolo[4,3-*a*]isindole-6,11(1*H*,3*H*)-diones, one derived from 1,4-naphthoquinone and the other from juglone, were prepared in good yield and high stereoselectivity. The new heterocyclic systems are comprised of four fused rings that are

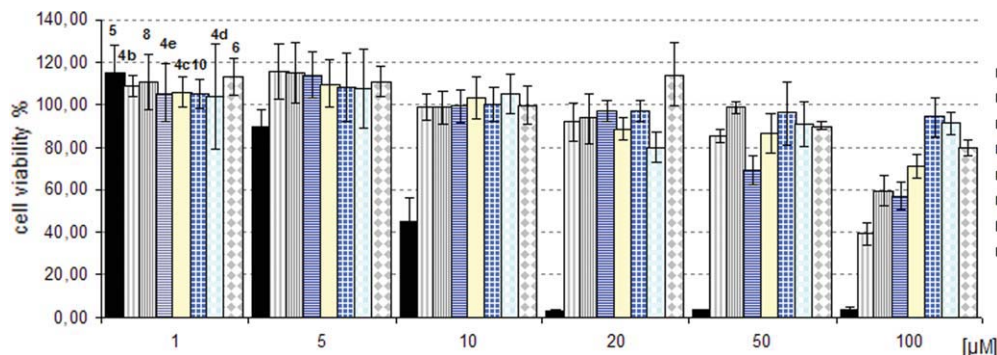
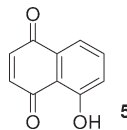
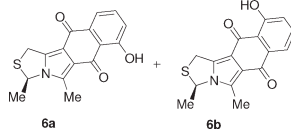
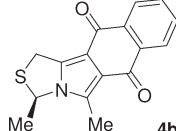
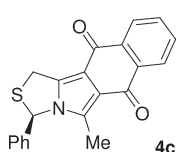
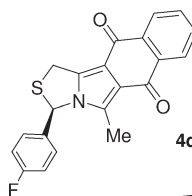
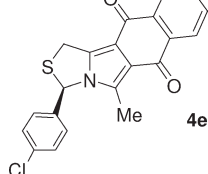
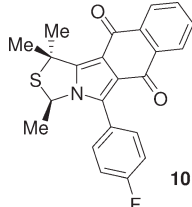
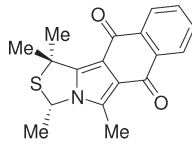


Figure 3. Values of cell viability of tested compounds against WiDr colon adenocarcinoma cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 1

IC₅₀ values of the tested compounds.

Compound	IC ₅₀ (μM) ^a	
	WiDr	A375
 5	8.8 ± 1.14	1.23 ± 0.22
 6a + 6b	>100	>100
 4b	86.0 ± 6.0	46.2 ± 2.8
 4c	>100	36.2 ± 1.8
 4d	>100	65.7 ± 4.6
 4e	>100	47.8 ± 3.8
 10	>100	46.0 ± 6.4
 8	>100	>100

^a Concentration needed to inhibit cell growth by 50% as determined from dose-response curves by exponential decay fitting ($r^2 > 0.9$).

essentially planar, only the substituent at C-3 deviates significantly from the molecular plane.

Anticancer activity of the synthesized compounds against WiDr colorectal adenocarcinoma and A375 melanoma cancer cells lines was determined. These heterocyclic compounds bearing a range of different functionalities showed low anticancer activity.

EXPERIMENTAL

Reagents were commercial grade and were used as supplied. Chromatographic separations were performed using 70–230 mesh silica gel. Juglone (**5**) was prepared by a known procedure [33]. ¹H NMR spectra were recorded on an instrument operating at 300 MHz or at 400 MHz. ¹³C NMR spectra were recorded on an instrument operating at 75.5 MHz or at 100 MHz. The solvent is deuteriochloroform except where indicated otherwise; chemical shifts are expressed in parts per million related to internal TMS, and coupling constants (J) are in hertz. Microanalyses were performed using an EA 1108-HNS-O Fisons instrument. Mass spectra were recorded under electron impact (EI) at 70 eV. HRMS spectra were obtained on a VG Autospect M spectrometer (TOF MS EI⁺).

General procedure for the synthesis of benzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-diones **4, **6**, and **8**.** The appropriate 1,3-thiazolidine-4-carboxylic acid (5 mmol), 1,4-naphthoquinone or juglone (7.5 mmol), and acetic anhydride (20 mL) were heated at 110–120°C for 2 h. The crude product was purified by flash chromatography [hexane/ethyl acetate].

5-Methylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (4a**).** Yellow solid; mp > 250°C; IR (KBr): 721, 1255, 1553, 1650, 1659 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz): δ 2.60 (s, 3H), 4.35 (s, 2H), 5.19 (s, 2H), 7.79–7.82 (m, 2H, ArH), 8.07–8.12 (m, 2H, ArH); ¹³C NMR (DMSO-d₆, 100MHz): δ 11.8, 25.3, 58.4, 117.4, 118.8, 122.0, 123.2, 123.5, 132.1, 135.3, 135.5, 138.7, 185.5, 186.0; HRMS (EI) Calcd. for (M⁺)C₁₅H₁₁NO₂S 269.0511. Found: 269.0519.

(R)-3,5-Dimethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (4b**).** Yellow solid; mp 227–229°C (ethyl acetate/hexane); IR (KBr): 721, 1257, 1546, 1650, 1659 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 1.81 (d, J = 6.3 Hz, 3H), 2.69 (s, 3H), 4.35 (d, J = 15.7 Hz, 1H), 4.49 (dd, J_1 = 1.6 Hz and J_2 = 15.6 Hz, 1H), 5.47 (m, 1H), 7.67–7.70 (m, 2H, ArH), 8.18–8.25 (m, 2H, ArH); ¹³C NMR (CDCl₃, 100MHz): δ 11.8, 25.3, 28.4, 58.2, 113.9, 122.1, 126.5, 126.8, 131.4, 132.8, 132.9, 135.2, 136.1, 138.2, 180.1, 181.2; MS (EI) m/z 283 (M⁺, 100%), 268 (82), 250 (21), 224 (74), 196 (9), 126 (10); HRMS (EI) Calcd. for (M⁺)C₁₆H₁₃NO₂S 283.0667. Found: 283.0671; $[\alpha]_{20}^D$ = + 140 (c 0.5, CH₂Cl₂).

(R)-5-Methyl-3-phenylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (4c**).** Yellow solid; mp 196–198°C (ethyl acetate/hexane); IR (KBr): 721, 1254, 1546, 1660 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 2.31 (s, 3H), 4.48 (d, J = 15.6 Hz, 1H), 4.64 (dd, J_1 = 1.7 Hz and J_2 = 15.7 Hz, 1H), 6.37 (d, J = 1.7 Hz, 1H), 7.12–7.16 (m, 2H, ArH), 7.35–7.40 (m, 3H, ArH), 7.70–7.73 (m, 2H, ArH), 8.22–8.26 (m, 2H, m, ArH); ¹³C NMR (CDCl₃, 100MHz): δ 11.8, 29.4, 64.5, 113.9, 122.4, 125.8, 126.6, 126.9, 129.3, 129.4, 132.6, 132.9, 133.1, 135.6, 136.2, 139.1, 139.3, 180.3, 181.3; MS (EI) m/z 334 ([M-Me]⁺, 80%), 207 (12), 187 (100), 118

(32); HRMS (EI) Calcd. for $(M^+)C_{16}H_{13}NO_2S$ 283.0667. Found: 283.0671. HRMS (EI) Calcd. for $(M^+)C_{21}H_{15}NO_2S$ 345.0824. Found: 345.0838; $[\alpha]_D^{20} = +230$ (c 0.5, CH_2Cl_2).

(R)-3-(4-Fluorophenyl)-5-methylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (4d). Yellow solid; mp 221–224°C (ethyl acetate/hexane); IR (KBr): 722, 1253, 1547, 1579, 1659 cm^{-1} ; 1H NMR ($CDCl_3$, 300 MHz): δ 2.30 (s, 3H), 4.46 (d, $J = 15.7$ Hz, 1H), 4.62 (dd, $J_1 = 1.6$ Hz and $J_2 = 15.6$ Hz, 1H), 6.36 (s, 1H), 7.05–7.18 (m, 4H, ArH), 7.69–7.72 (m, 2H, ArH), 8.22–8.25 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 100MHz): δ 11.8, 29.4, 63.9, 114.0, 116.4, 116.6, 122.6, 126.6, 126.9, 127.8, 127.9, 132.3, 132.9, 133.1, 135.2, 136.2, 138.9, 163.0 (d, $J = 248$ Hz), 180.3, 181.2; MS (EI) m/z 345 $([M-F]^+, 100\%)$, 312 (22), 224 (21), 121 (54); Anal. Calcd for $C_{21}H_{14}FNO_2S$: C, 69.41; H, 3.88; N, 3.85. Found: C, 69.33; H, 3.82; N, 3.65; $[\alpha]_D^{20} = +210$ (c 0.5, CH_2Cl_2).

(R)-3-(4-Chlorophenyl)-5-methylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (4e). Yellow solid; mp 213–215°C (ethyl acetate/hexane); IR (KBr): 720, 1255, 1547, 1573, 1647, 1659 cm^{-1} ; 1H NMR ($CDCl_3$, 300 MHz): δ 2.32 (s, 3H), 4.47 (d, $J = 15.6$ Hz, 1H), 4.62 (dd, $J_1 = 1.7$ Hz and $J_2 = 15.7$ Hz, 1H), 6.36 (d, $J = 1.7$ Hz, 1H), 7.07–7.10 (m, 2H, ArH), 7.34–7.38 (m, 2H, ArH), 7.69–7.73 (m, 2H, ArH), 8.22–8.25 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 100MHz): δ 11.8, 29.4, 63.9, 114.1, 122.6, 126.6, 126.9, 127.9, 129.7, 132.3, 132.9, 133.3, 135.2, 135.3, 136.2, 137.9, 138.9, 180.3, 181.2; MS (EI) m/z 363 $([M-Me]^+, 100\%)$, 330 (16), 224 (31), 139 (61); Anal. Calcd for $C_{21}H_{14}ClNO_2S$: C, 66.40; H, 3.71; N, 3.69. Found: C, 66.19; H, 3.71; N, 3.42; $[\alpha]_D^{20} = +280$ (c 0.5, CH_2Cl_2).

(R)-7-Hydroxy-3,5-dimethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (6a) and (R)-10-hydroxy-3,5-dimethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (6b). (R)-7-Hydroxy-3,5-dimethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (6a) and (R)-10-hydroxy-3,5-dimethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (6b) was obtained as a mixture of regioisomers with a 56:44 distribution; mp 196.2–198.9°C (ethyl acetate/hexane); IR (KBr): 1159, 1249, 1551, 1575, 1624, 1654 cm^{-1} .

Major component. 1H NMR ($CDCl_3$, 300 MHz): δ 1.82 (d, $J = 6.3$ Hz, 3H), 2.69 (s, 3H), 4.34 (d, $J = 15.8$ Hz, 1H), 4.45–4.51 (m, 1H), 5.45–5.51 (m, 1H), 7.16–7.20 (m, 1H, ArH), 7.55–7.59 (m, 1H, ArH), 7.70–7.75 (m, 1H, ArH), 12.94 (s, 1H, OH).

Minor Component. 1H NMR ($CDCl_3$, 300 MHz): δ 1.82 (d, $J = 6.3$ Hz, 3H), 2.69 (s, 3H), 4.34 (d, $J = 15.8$ Hz, 1H), 4.45–4.51 (m, 1H), 5.45–5.51 (m, 1H), 7.16–7.20 (m, 1H, ArH), 7.55–7.59 (m, 1H, ArH), 7.70–7.75 (m, 1H, ArH), 13.18 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100MHz): δ 11.8, 25.3, 28.4, 58.3, 58.4, 117.4, 118.6, 118.8, 122.0, 123.2, 123.6, 132.1, 135.3, 135.5, 136.4, 138.7, 162.6, 162.8, 186.0; MS (EI) m/z 299 (M^+ , 100%), 284 (79), 266 (24), 240 (61), 212 (12); Anal. Calcd for $C_{16}H_{13}NO_3S$: C, 64.20; H, 4.38; N, 4.68. Found: C, 64.04; H, 4.25; N, 4.64; $[\alpha]_D^{20} = +130$ (c 0.5, CH_2Cl_2).

(S)-1,1,3,5-Tetramethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (8). Yellow solid; mp 185.4–188.2°C (ethyl acetate/hexane); IR (KBr): 732, 1262, 1418, 1541, 1589, 1655 cm^{-1} ; 1H NMR ($CDCl_3$, 300 MHz): δ 1.85 (d, $J = 6.3$ Hz, 3H), 1.98 (s, 3H), 2.03 (s, 3H), 2.69 (s, 3H), 5.55 (q, $J = 6.3$ Hz, 1H), 7.67–7.72 (m, 2H, ArH), 8.19–8.24 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.8, 26.6, 28.8, 31.7,

52.2, 58.7, 112.3, 122.7, 126.6, 126.7, 130.6, 132.8, 132.9, 135.5, 135.6, 147.4, 179.7, 181.6; MS (EI) m/z 311 (M^+ , 45%), 296 (100), 281 (7), 250 (14), 236 (30); Anal. Calcd for $C_{18}H_{17}NO_2S$: C, 69.43; H, 5.50; N, 4.50. Found: C, 69.65; H, 5.30; N, 4.52; $[\alpha]_D^{20} = +40$ (c 0.5, CH_2Cl_2).

Synthesis of (R)-5-(4-fluorophenyl)-1,1,3-trimethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (10). The (2R,4S)-3-(4-fluorophenylcarbonyl)-2,5,5-trimethylthiazolidine-4-carboxylic acid (**9**) [7] (1.49 g, 5 mmol), 1,4-naphthoquinone (0.79 g, 7.5 mmol), and acetic anhydride (20 mL) were heated at 110–120°C for 2 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [hexane/ethyl acetate]. Compound **10** was obtained as a yellow solid; mp 183.4–185.0°C (ethyl acetate/hexane); IR (KBr): 735, 1268, 1498, 1542, 1594, 1609, 1656 cm^{-1} . 1H NMR ($CDCl_3$, 300 MHz): δ 1.37 (d, $J = 6.3$ Hz, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 5.72 (q, $J = 6.3$ Hz, 1H), 7.19–7.26 (m, 2H, ArH), 7.56–7.71 (m, 4H, ArH), 8.13–8.25 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 100MHz): δ 26.1, 29.0, 31.4, 52.2, 59.6, 112.8, 115.8, 116.1, 123.2, 125.4, 125.43, 126.7, 130.9, 131.6, 131.7, 133.0, 133.1, 135.3, 135.5, 148.6, 163.3 (d, $J = 249$ Hz), 179.8, 180.5; MS (EI) m/z 391 (M^+ , 65%), 376 (100), 331 (50), 316 (52); Anal. Calcd for $C_{23}H_{18}FNO_2S$: C, 70.57; H, 4.63; N, 3.58. Found: C, 70.58; H, 4.58; N, 3.61; $[\alpha]_D^{20} = +50$ (c 0.5, CH_2Cl_2).

Crystal data for (R)-3,5-dimethylbenzo[f]thiazolo[4,3-a]isoindole-6,11(1H,3H)-dione (4b). $C_{16}H_{13}N_1O_2S_1$, $M = 283.33$, hexagonal, $a = 16.1968(3)$ Å, $c = 4.44350(10)$ Å, $V = 1009.52(3)$ Å³, $T = 293(2)$ K, space group $P3_2$, $Z = 3$, $m(MoK\alpha) = 0.240$ mm⁻¹, 3340 reflections measured, of which 1677 unique, used for direct methods structure determination [34] and full matrix least-squares refinement. The H atoms were placed at calculated idealized positions and refined as riding atoms. The final R (F^2) was 0.053 (for $I > 2\sigma(I)$) and $R_w(F^2)$ was 0.150 (for all reflections). The crystal used in data collection was twinned. Twin ratios refined to nearly 0.80:0.20. The Flack parameter refined to 0.0(2) [35].

Measurement of cell viability. The *in vitro* cytotoxic effect of the molecules was evaluated in human colorectal adenocarcinoma (WiDR) and human melanoma (A375) cell lines both purchased from American Type Culture Collection. The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated fetal bovine serum and 100 μM sodium pyruvate at 37°C, in a humidified incubator 95% air and 5% CO_2 . For each experiment, cells were plated in 48-well plates, in a concentration of 40,000 cells/mL and kept overnight in the incubator, allow the attachment of the cells. The molecules tested were reconstituted on dimethylsulfoxide (DMSO) to achieve solutions with a concentration of 4 mg/mL. Several concentrations (1, 5, 10, 20, 50, and 100 μM) of the molecules were tested by addition to the cell media. Final concentration of DMSO varied from 0.17 to 0.99%. For each experiment, two controls were performed: untreated cell cultures and cells treated with 1% DMSO, the vehicle of administration of the molecules. Cell-plates were incubated for 48 h. To analyze the proliferation inhibition, the MTT assay was performed. The ratio of absorbance of treated cultures to that of DMSO control cultures was obtained for all concentrations of every drug. From the dose-response curve obtained, a 50% inhibitory concentration (IC_{50}) was determined. Each experiment was performed in triplicate and repeated in two different sets of tests.

Acknowledgments. The authors thank *Chymiotecnnon* and *FCT* (Project PTDC/QUI/64470/2006) and FEDER for financial support. S.M.M.L. also thanks FCT for the Ph.D. (Grant SFRH/BD/45128/2008). The authors acknowledge the Nuclear Magnetic Resonance Laboratory of the Coimbra Chemical Centre (www.nmrccc.uc.pt), University of Coimbra for obtaining the NMR data.

REFERENCES AND NOTES

- [1] Hadden, M. K.; Hill, S. A.; Davenport, J.; Matts, R. L.; Blagg, B. S. J. *Bioorg Med Chem* 2009, 17, 634.
- [2] Tandon, V. K.; Maurya, H. K.; Tripathi, A.; ShivaKeshava, G. B.; Shukla, P. K.; Srivastava, P.; Panda, D. *Eur J Med Chem* 2009, 44, 1086.
- [3] Valderrama, J. A.; Leiva, H.; Rodríguez, J. A.; Theoduloz, C.; Schmeda-Hirschmann, G. *Bioorg Med Chem* 2008, 16, 3687.
- [4] Pérez-Sacau, E.; Díaz-Penate, R. G.; Estévez-Braun, A.; Ravelo, A. G.; García-Castellano, J. M.; Pardo, L.; Campillo, M. *J Med Chem* 2007, 50, 696.
- [5] Krapcho, A. P.; Menta, E.; Oliva, A.; Di Domenico, R.; Fiocchi, L.; Maresch, M. E.; Gallagher, C. E.; Hacker, M. P.; Beggiolin, G.; Giuliani, F. C.; Pezón, G.; Spinelli, S. *J Med Chem* 1998, 41, 5429.
- [6] Gomez-Monterrey, I.; Santelli, G.; Campiglia, P.; Califano, D.; Falasconi, F.; Pisano, C.; Vesci, L.; Lama, T.; Grieco, P.; Novellino, E. *J Med Chem* 2005, 48, 1152.
- [7] Zarghi, A.; Najafnia, L.; Daraee, B.; Dadrass, O. G.; Hedayati, M. *Bioorg Med Chem Lett* 2007, 17, 5634.
- [8] Barreca, M. L.; Balzarini, J.; Chimirri, A.; De Clercq, E.; De Luca, L.; Holtje, H. D.; Holtje, M.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Rao, A.; Zappalà, M. *J Med Chem* 2002, 45, 5410.
- [9] Gouveia, F. L.; Oliveira, R. M. B.; Oliveira, T. B.; Silva, I. M.; Nascimento, S. C.; Sena, K. X. F. R.; Albuquerque, J. F. C. *Eur J Med Chem* 2009, 44, 2038.
- [10] Bozdag-Dundar, O.; Ozgen, O.; Mentese, A.; Altanlar, N.; Atli, O.; Kendi, E.; Ertan, R. *Bioorg Med Chem* 2007, 15, 6012.
- [11] Gududuru, V.; Hurh, E.; Dalton, J. T.; Miller, D. D. *J Med Chem* 2005, 48, 2584.
- [12] Ottanà, R.; Carotti, S.; Maccari, R.; Landini, I.; Chiricosta, G.; Caciagli, B.; Vigorita, M. G.; Mini, E. *Bioorg Med Chem Lett* 2005, 15, 3930.
- [13] Li, W.; Lu, Y.; Wang, Z.; Dalton, J. T.; Miller, D. D. *Bioorg Med Chem Lett* 2007, 17, 4113.
- [14] Chen, J.; Wang, Z.; Lu, Y.; Dalton, J. T.; Miller, D. D.; Li, W. *Bioorg Med Chem Lett* 2008, 18, 3183.
- [15] Anderson, W. K.; Mach, R. H. *J Med Chem* 1987, 30, 2109.
- [16] Dureé, D.; Lancelot, J.; Robba, M.; Chenu, E.; Mathé, G. *J Med Chem* 1989, 32, 456.
- [17] Pinho e Melo, T. M. V. D.; Barbosa, D. M.; Ramos, P. J. R. S.; Rocha Gonsalves, A. M. d'A.; Gilchrist, T. L.; Beja, A. M.; Paixão, J. A.; Silva, M. R.; Alte da Veiga, L. *J Chem Soc Perkin Trans I* 1999, 1219.
- [18] Pinho e Melo, T. M. V. D.; Soares, M. I. L.; Barbosa, Dália M.; Rocha Gonsalves, A. M. d'A.; Paixão, J. A.; Beja, A. M.; Ramos Silva, M.; Alte da Veiga, L. *Tetrahedron* 2000, 56, 3419.
- [19] Pinho e Melo, T. M. V. D.; Soares, M. I. L.; Barbosa, D. M.; Rocha Gonsalves, A. M. d'A.; Paixão, J. A.; Beja, A. M.; Ramos Silva, M.; Alte da Veiga, L.; Costa Pessoa, J. *J Org Chem* 2002, 67, 4045.
- [20] Gilchrist, T. L.; Rocha Gonsalves, A. M. d'A.; Pinho e Melo, T. M. V. D. *Tetrahedron* 1994, 50, 13709.
- [21] Johnson, C. K. ORTEPII. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA, 1976.
- [22] Szilágyi, L.; Györgydeák, Z. *J Am Chem Soc* 1979, 101, 427.
- [23] Györgydeák, Z.; Kajtár-Perey, M.; Kajtár, J.; Kajtár, M. *Liebigs Ann Chem* 1987, 927.
- [24] Benedini, F.; Ferrario, F.; Sala, A.; Sala, L.; Soresinetti, P. A. *J Heterocycl Chem* 1994, 31, 1343.
- [25] Lázár, L.; Fülöp, F. *Eur J Org Chem* 2003, 3025.
- [26] Pinho e Melo, T. M. V. D.; Soares, M. I. L.; Barbosa, D. M.; Rocha Gonsalves, A. M. d'A.; Paixão, J. A.; Beja, A. M.; Ramos Silva, M.; Alte da Veiga, L. *Tetrahedron* 2000, 56, 3419.
- [27] Pinho e Melo, T. M. V. D.; Soares, M. I. L.; Nunes, C. M. *Tetrahedron* 2007, 63, 1833.
- [28] Pinho e Melo, T. M. V. D.; Gomes, C. S. B.; Rocha Gonsalves, A. M. d'A.; Paixão, J. A.; Beja, A. M.; Ramos Silva, M.; Alte da Veiga, L. *Tetrahedron* 2002, 58, 5093.
- [29] Soares, M. I. L.; Lopes, S. M. M.; Cruz, P. F.; Brito, R. M. M.; Pinho e Melo, T. M. V. D. *Tetrahedron* 2008, 64, 9745.
- [30] Hodnett, E. M.; Wongwiechintana, C.; Dunn, W. J., III; Marrs, P. *J Med Chem* 1983, 26, 570.
- [31] (a) Hennig, L.; Christner, C.; Kipping, M.; Schelbert, B.; Rücknagel, K. P.; Grabley, S.; Küllertz, G.; Fischer, G. *Biochemistry* 1998, 37, 5953; (b) Fila, C.; Metz, C.; Van der Sluijs, P. *J Biol Chem* 2008, 283, 21714.
- [32] Krapcho, A. P.; Petry, M. E.; Getahun, Z.; Landi, J. J.; Stallman, J.; Polsenberg, J. F.; Gallagher, C. E.; Maresch, M. J.; Hacker, M. P.; Giuliani, F. C.; Beggiolin, G.; Pezzoni, G.; Menta, E.; Manzotti, C.; Oliva, A.; Spinelli, S.; Tognella, S. *J Med Chem* 1994, 37, 828.
- [33] Ribeiro, S. M.; Serra, A. C.; Rocha Gonsalves, A. M. d'A. *Tetrahedron* 2007, 63, 7885.
- [34] Sheldrick, G. M. SHELXS97 & SHELXL97; University of Göttingen: Germany, 1997.
- [35] Flack, H. D. *Acta Cryst* 1983, A39, 876.