# Synthesis and Biological Evaluation of New Naphthoquinone-Containing Pyrrolo-Thiazoles as Anticancer Agents

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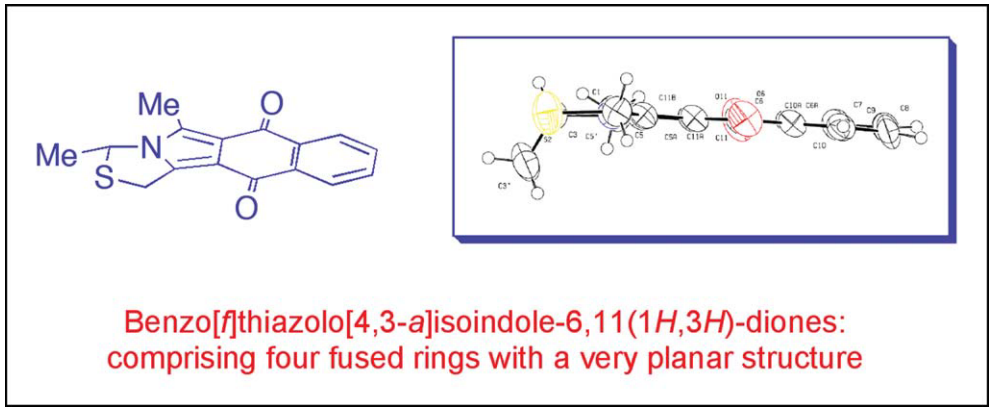


Naphthoquinones undergo 1,3-dipolar cycloaddition with bicyclic münchnones generated from thiazolidines affording new pyrrolo-thiazoles with a fused quinone nucleus. The products were obtained as single enantiomers in good yields. These benzo[*f*]thiazolo[4,3-*a*]isoindole-6,11(1*H*,3*H*)-diones are comprised of four fused rings and present a very planar structure. The evaluation of their anticancer activity against melanoma A375 and colorectal adenocarcinoma WiDr human cell lines showed only moderate activity but gave insight into the modeling of new structures. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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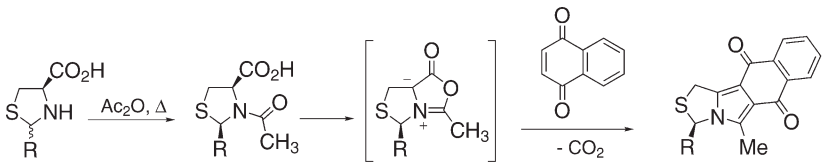
## INTRODUCTION

Quinone-containing drugs such as adriamycin, daunorubicin, and mitoxantrone have been established as one of the most effective classes of antitumor agents in clinical use. However, the drawbacks are the risk of dose-related cardiotoxicity and the development of resistance toward these compounds. To overcome these problems, there is a demand for the search of new lead compounds retaining the ''core quinone'' chromophore [1–4]. Hence, there is particular interest in combining the nucleus of a quinone with heterocyclic rings to achieve molecules with anticancer activity [5,6]. On the other hand, the thiazolidine ring is known to be involved in biologically active compounds with anti-inflammatory [7], anti-HIV [8], antimicrobial [9,10], or anticancer properties [11,12]. Particularly relevant is the anticancer activity of 2-arylthiazolidine carboxylic acid derivatives that are effective against the melanoma [13,14].

Our goal was to prepare structures combining the ''core quinone'' chromophore with a thiazolidine ring via the construction of the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles ring system. One important mechanism of action of quinone-containing drugs is thought to be related to intercalation processes with DNA in which planarity of the active nucleus is important.[6] Thus, a naphthoquinone ring system fused to a pyrrolo[1,2-*c*]thiazole should allow the system to attain the required planarity. On the other hand, pyrrolo[1,2-*c*]thiazoles are a class of compounds some of which showing biological activity namely antitumoral activity [15,16].

We have been interested in exploring a straightforward approach to new chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives via 1,3-dipolar cycloaddition of bicyclic münchnones [17–19]. Therefore, we used this synthetic strategy to prepare a range of new chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles retaining the ''core quinone''

**Scheme 1**




1 2 3

| Compound | R | Yield (%) |
|---|---|---|
| **4a** | H | 60 |
| **4b** | Me | 71 |
| **4c** | Ph | 76 |
| **4d** | $C_6H_4$-F-*p* | 74 |
| **4e** | $C_6H_4$-Cl-*p* | 73 |

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*,3H-pyrrolo[1,2-*c*]thiazole derivatives **4b** as being *R*. The compound **4b** crystallizes in the chiral space group $P3_2$, 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.

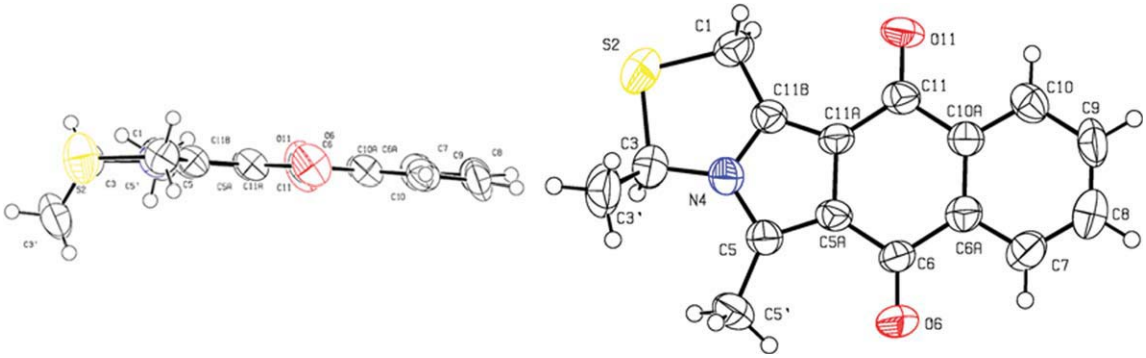
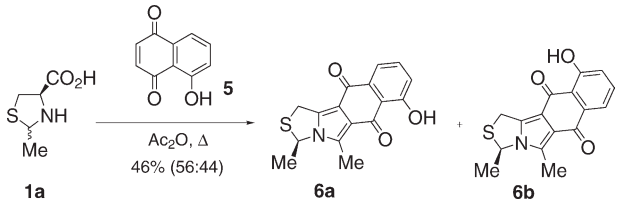



**Figure 1.** Ortep diagrams [21] for (*R*)-3,5-dimethylbenzo[*f*]thiazolo[4,3-*a*]isoindole-6,11(1*H*,3*H*)-dione **4b**. The displacement ellipsoids are drawn at the 50% probability level. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

**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).

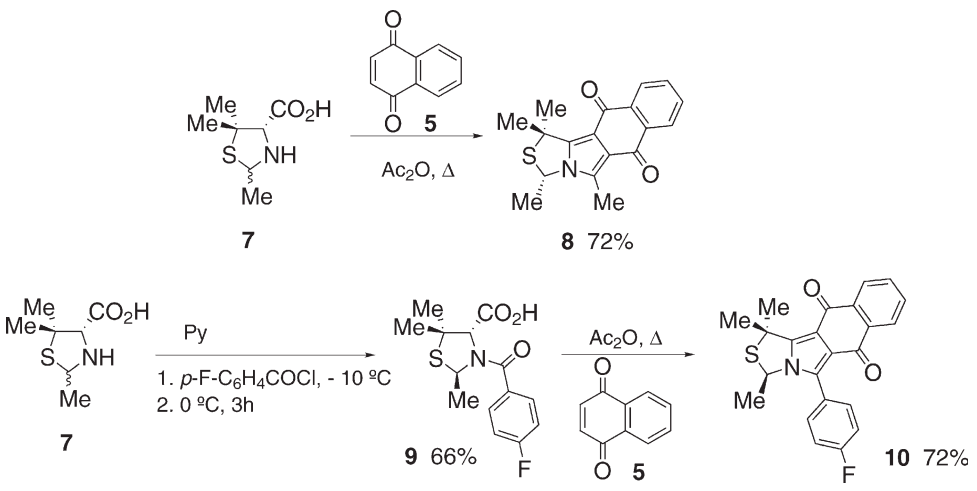
Similar chemistry can be carried to prepare the chiral benzo[*f*]thiazolo[4,3-*a*]isoindole-6,11(1*H*,3*H*)-dione **8**. In this case, D-penicilamine, an α-amino acid with *S* configuration, was condensed with acetaldehyde leading to (*4S*)-2,5,5-trimethyl-1,3-thiazolidine-4-carboxylic acid (**7**) [27]. 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** ($IC_{50} = 86$ μ*M*), using concentrations of up to 100 μ*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 refering to different cell line [12]. The only exception to our results is

**Scheme 3**



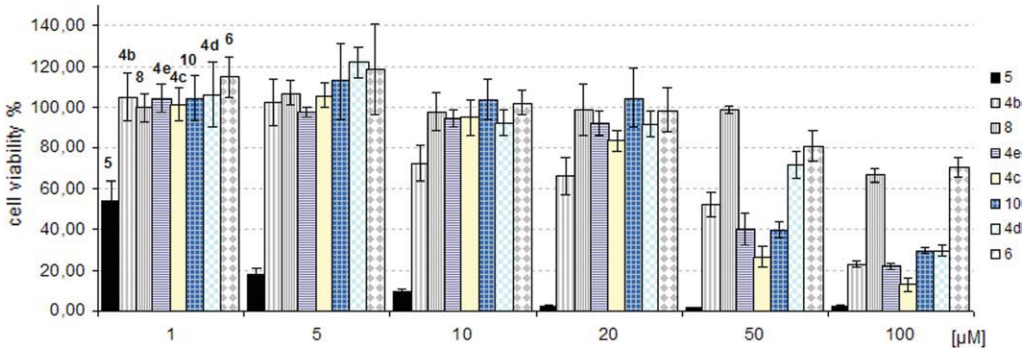



**Figure 2.** Values of cell viability of tested compounds against A375 melanoma cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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 μ*M* for A375 cells and 8.8 μ*M* 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 cytotoxycity of the pyrrolo-thiazole compounds was somewhat unexpected considering the geometry of the molecule (see Fig. 1). Molecular shape of thiazolidinones, characterized by the preferencial ''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*]isoindole-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*]isoindole-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 heterocylic systems are comprised of four fused rings that are

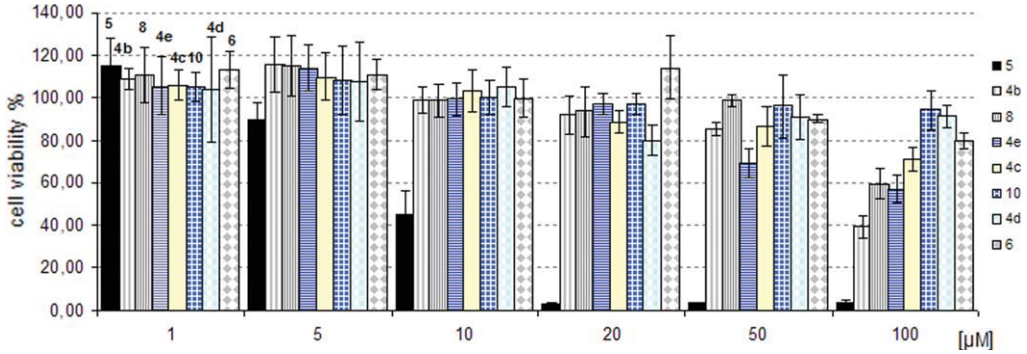
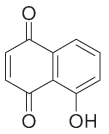
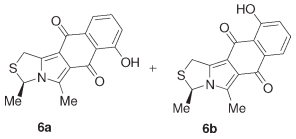
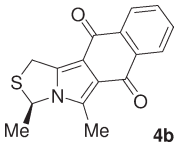
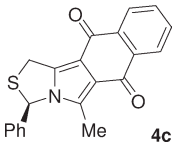
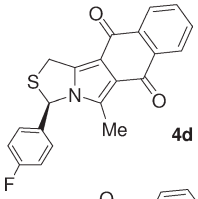
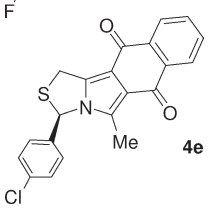
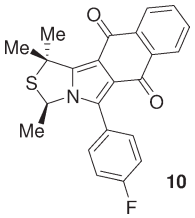
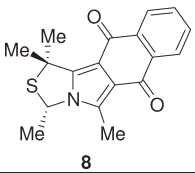



**Figure 3.** Values of cell viability of tested compounds against WiDr colon adenocarcinoma cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

**Table 1**

$IC_{50}$ values of the tested compounds.

| Compound | $IC_{50}$ ($\mu M$)[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]. $^1$H NMR spectra were recorded on an instrument operating at 300 MHz or at 400 MHz. $^{13}$C 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(1*H*,3*H*)-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$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz): δ 2.60 (s, 3H), 4.35 (s, 2H), 5.19 (s, 2H), 7.79–7.82 (m, 2H, Ar*H*), 8.07–8.12 (m, 2H, Ar*H*); $^{13}$C NMR (DMSO-d$_6$, 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$^+$)$C_{15}H_{11}NO_2S$ 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$^{-1}$; $^1$H NMR ($CDCl_3$, 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, Ar*H*), 8.18–8.25 (m, 2H, Ar*H*); $^{13}$C NMR ($CDCl_3$,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$^+$)$C_{16}H_{13}NO_2S$ 283.0667. Found: 283.0671: $[\alpha]_{20}^{D} = +140$ ($c$ 0.5, $CH_2Cl_2$).

***(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$^{-1}$; $^1$H NMR ($CDCl_3$, 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, Ar*H*), 7.35–7.40 (m, 3H, Ar*H*), 7.70–7.73 (m, 2H, Ar*H*), 8.22–8.26 (m, 2H, m, Ar*H*); $^{13}$C NMR ($CDCl_3$, 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; $[\alpha]_{20}^{D} = +230$ (*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, Ar*H*), 7.69–7.72 (m, 2H, Ar*H*), 8.22–8.25 (m, 2H, Ar*H*); $^{13}C$ 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; $[\alpha]_{20}^{D} = +210$ (*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, Ar*H*), 7.34–7.38 (m, 2H, Ar*H*), 7.69–7.73 (m, 2H, Ar*H*), 8.22–8.25 (m, 2H, Ar*H*); $^{13}C$ 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; $[\alpha]_{20}^{D} = +280$ (*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, Ar*H*), 7.55–7.59 (m, 1H, Ar*H*), 7.70–7.75 (m, 1H, Ar*H*), 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, Ar*H*), 7.55–7.59 (m, 1H, Ar*H*), 7.70–7.75 (m, 1H, Ar*H*), 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; $[\alpha]_{20}^{D} = +130$ (*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, Ar*H*), 8.19–8.24 (m, 2H, Ar*H*); $^{13}C$ 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; $[\alpha]_{20}^{D} = +40$ (*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 (2*R*,4*S*)-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, Ar*H*), 7.56–7.71 (m, 4H, Ar*H*), 8.13–8.25 (m, 2H, Ar*H*); $^{13}C$ 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; $[\alpha]_{20}^{D} = +50$ (*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) Å$^3$, *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 μ*M* sodium piruvate 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 μ*M*) 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.

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## 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-Hirshmann, 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-Peredy, 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.