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Synthesis and Biological Evaluation of Five-Membered Heterocycles Fused to Cyclopenta[*c*]thiophene as New Antitumor Agents

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Abstract—A series of 10 derivatives **2–6** issued from the fusion of various five-membered heterocycles to cyclopenta[*c*]thiophene were evaluated for potential anticancer activity in the NCI's in vitro human disease-oriented tumor cell line screening panel that consisted of 60 human tumor cell lines arranged in nine subpanels, representing diverse histologies. Among these tested compounds, four were found to be cytotoxic allowing us to point out some structure–activity relationships. The oxazolidinone derivatives **2a–c** displayed further in vivo antitumor activity in the hollow fiber assay and standard xenograft testing developed at the NCI.

Introduction

We recently reported[1] the synthesis and the cytotoxicity of several new cyclopenta[*c*]thiophene related compounds **1** among which some derivatives exerted an in vivo antitumor activity assessed in the NCI's hollow fiber assay (Fig. 1).[2] In order to complete our knowledge of the structure–activity relationships in this original chemical series, for which we have already shown the importance in the cytotoxic activity of the cyclopentane carbonyl group and the thiophene halogen atoms,[1,3] we undertook the synthesis and the biological evaluation of new derivatives **2–6** issued from the fusion of various five-membered heterocycles to cyclopenta[*c*]thiophene.

Chemistry

The oxazolidinones **2a–d** were diastereoselectively prepared in a *cis* form starting from 6-trifluoroacetylamino-5,6-dihydro-4*H*-cyclopenta[*c*]thiophen-4-ones **7a–d**. The latter were first involved in a sequence we previously described,[4] leading to the *cis* 5-hydroxy-4-ammonium chlorides **8a–d** via the *trans* 5-bromo derivatives **9a–d** and the *cis* trifluoromethyloxazoles **10a–d** (Scheme 1). Treatment of **8a–d** by phosgene in refluxing toluene afforded finally **2a–d** in 61–83% yield. Assignment of the relative *cis* configuration of **2a–d** was made on the basis of NOE experiments. Specifically, irradiation of H-7a in **2a** led to an NOE with H-3a (23.9%) but not with NH. This observation was consistent with a *cis* stereochemical relationship between H-7a and H-3a and a *trans* one between H-7a and NH.

The dibromo derivative **2a** was chosen to set out a pharmacomodulation study. The latter concerned firstly the replacement of the thiophene bromine atom in position 6 which we showed previously that it could be

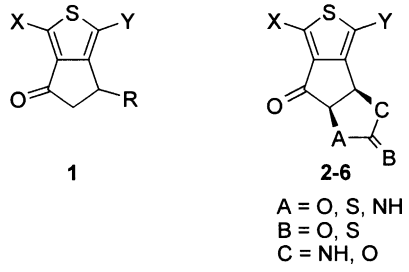

Figure 1. Structures of the 6-substituted cyclopenta[*c*] thiophenones **1** and five-membered heterocycles fused to cyclopenta[*c*]thiophene **2**.

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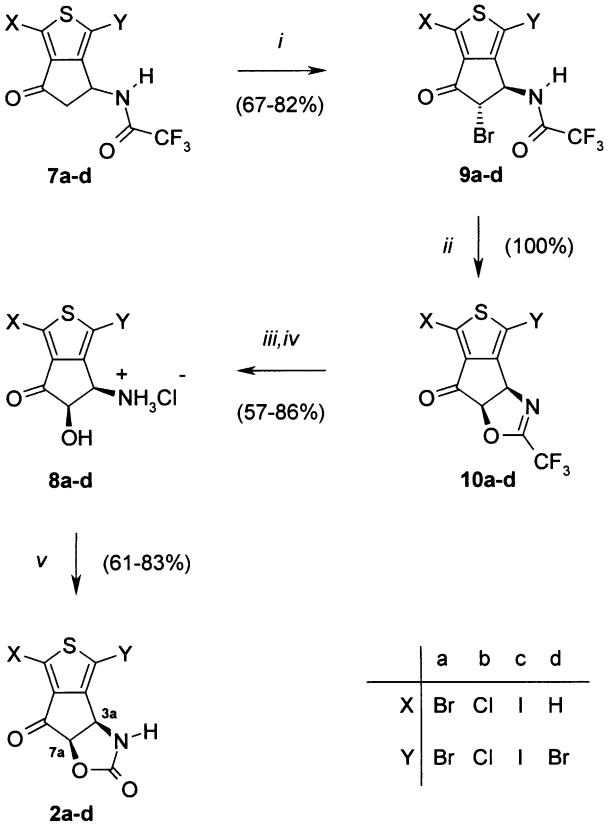
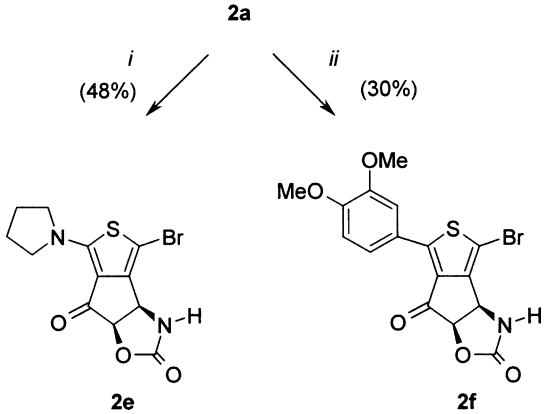
more easily substituted than those in position 4.[5] Thus, the nucleophilic attack of pyrrolidine on **2a** at room temperature led selectively to the 6-pyrrolidinyl derivative **2e** in 48% yield (Scheme 2). On the other hand, cross coupling of **2a** with dimethoxyphenylboronic acid, according to Suzuki's conditions,[6] afforded also selectively the corresponding derivative **2f** in 30% yield.

The oxazolidinethione **3** was also obtained starting from **8a** by replacing phosgene in the previous reaction by thiophosgene in refluxing toluene (Scheme 3), while diazotization of **8a**, using sodium nitrite in trifluoroacetic acid, yielded the 5,6-diol **11** in a mixture of



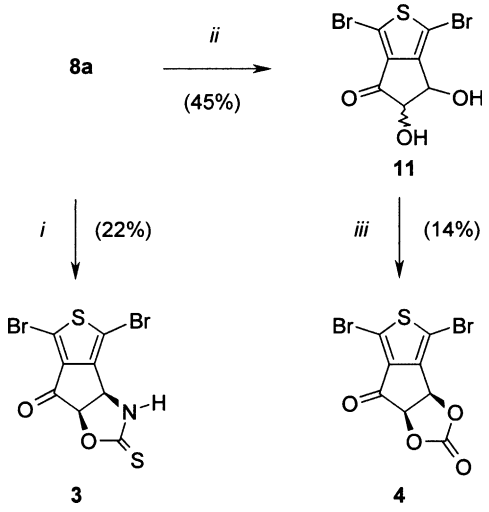
Scheme 1. Reagents: (i) Br_2, AcOH (0 °C/1 h); (ii) Na_2CO_3, Me_2CO (rflx/2 h); (iii) HCl gas, Et_2O (rt/2 min); (iv) ambient air (rt/24 h); (v) $COCl_2$, toluene (rflx/3 h).



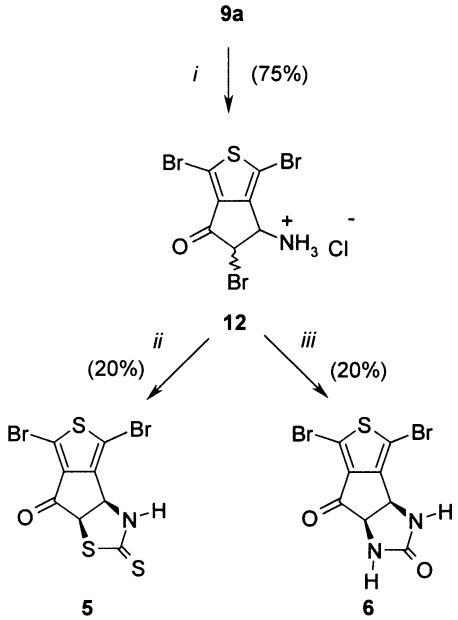
Scheme 2. Reagents: (i) pyrrolidine, dioxane (rt/4 h); (ii) (3,4-diOCH$_3$)–C$_6$H$_3$–B(OH)$_2$, PdCl$_2$ (dppf), TEA, DMF (65 °C/12 h).

cis and *trans* forms. The latter was selectively cyclised by treatment with phosgene in the *cis* dioxolanone **4** in a poor yield (14%) due to the involvement of the sole *cis* isomer of **11** in the reaction. The *trans* isomer was recovered unreacted in the reaction mixture. The configurations of **11** and **4** were assessed on the basis of the chemical shifts and coupling constants measured on their ^{1}H NMR spectra.[7]

The 5-bromo-6-ammonium chloride **12**, prepared in a mixture of its *cis* and *trans* isomers by the acidic hydrolysis of the trifluoroacetamide **9a**, allowed the diastereoselective access to the thiazolidinethione **5** and the imidazolidinone **6** in their *cis* configuration (Scheme 4). The synthesis of **5** was performed by the treatment of **12**, first displaced from its hydrochloric salt by triethylamine, with carbon disulfide, while **6** was obtained by



Scheme 3. Reagents: (i) $CSCl_2$, toluene (rflx/3 h); (ii) $NaNO_2$, 20% TFA (rt/4 h); (iii) $COCl_2$, toluene (rflx/3 h).



Scheme 4. Reagents: (i) HCl, EtOH (rflx, 2 h); (ii) TEA, CS_2, Et_2O (rt/1 h); (iii) KOCN, H_2O (rt/1 h).

Table 1. Cytotoxicity of cyclopenta[*c*]thiophene related compounds

Compd	Log molar drug concentration required for 50% growth inhibition (log GI_{50})									
	Leukemia RPMI 8226	Lung NCI-H522	Colon SW-620	CNS SF-539	Melanoma UACC-257	Ovarian ovcar-4	Renal CAKI-1	Prostate PC-3	Breast MDA-231	MGM[a]
2a	−5.37	−6.61	−5.68	−5.11	−5.74	−5.78	−6.47	−5.02	−5.74	*−5.53*
2b	−4.68	−5.77	−5.67	−5.25	−4.77	−4.66	−4.83	−4.75	−4.73	*−4.94*
2c	−5.25	−5.81	−5.43	−5.58	−5.67	−4.96	−5.66	−5.02	NT[b]	*−5.34*
2d	−4.57	−4.52	−4.69	−4.47	−4.65	NT	−4.68	−4.38	−4.36	*−4.47*
2f	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	*−4.01*
3	−5.41	−5.71	−5.47	−4.74	−4.76	−4.76	−4.73	−4.85	−4.74	*−4.89*
6	−5.37	−5.72	−5.73	−5.73	−5.2	−5.4	−5.11	−5.36	−4.91	*−5.32*

2e, **4**, **5** were found to be inactive on the basis of their evaluation in the three-cell line one-dose primary anticancer assay using NCI-H460 (lung), MCF7 (breast) and SF-268 (CNS).
[a]Mean graph midpoint for all human cancer cell lines tested.
[b]NT, not tested.

reaction of **12** in an aqueous solution with potassium cyanate. In both cases, the yields did not exceed 20% after separation of **5** and **6** from various *cis* derivatives of the starting material, since only the *trans* isomer of **12** was involved in the reactions.

Results and Discussion

Evaluation of the cytotoxicity of the synthesized compounds

Ten five-membered heterocycles fused to cyclopenta[*c*]thiophene were evaluated in the in vitro human disease-oriented tumor cell line screening panel developed at the NCI.[8] The log GI_{50} values (GI_{50} being the molar drug concentration required for half growth inhibition) obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table 1. The MGM is based on a calculation of the average log GI_{50} for all of the cell lines tested (approximately 60) in which GI_{50} values below and above the test range (10^{-4}–10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test. These results indicated a weak cytotoxicity for five derivatives (**2a–c**, **3**, **6**) with a MGM log GI_{50} close or below to −5, corresponding to MGM GI_{50} values ranging from 3.0 to 12.9 μM (Table 2).

The analysis of the structure–activity relationships indicates first that the cytotoxicity seems to be linked to the presence of the oxazolidinone moiety fused to the cyclopenta[*c*] thiophene, since among the most active compounds, three belong to this series (**2a–c**). The in vitro activity of **2a** (MGM log GI_{50} value = −5.53) is conserved for its imidazolidinone derivative **6** (−5.32) but is decreased for its oxazolidinethione one **3** (−4.89).

Table 2. Cytotoxicity of cyclopenta[*c*]thiophene related compounds

MGM (GI_{50} in μM)				
2a	**2c**	**6**	**2b**	**3**
3.0	4.6	4.8	11.5	12.9

On the other hand, the fusion of a dioxolanone (**4**) or thiazolidinethione rings (**5**) to the cyclopenta[*c*]thiophene moiety totally suppresses the cytotoxicity.

Among the oxazolidinone series, the requirement for the cytotoxicity of an halogen atom on the 6-position of the thiophene ring is moreover assessed by these results since the replacement of the 6-bromine atom of **2a** by a proton (**2d**) dramatically decreased the activity (MGM log GI_{50} value = −4.47), while the presence in C-6 of various bulky cycles (**2e**, **2f**) completely abolishes it. Finally, the nature of the halogen atoms seems to play a role with similar MGM log GI_{50} values for the dibromo **2a** and diiodo **2c** derivatives (−5.53 and −5.34 respectively) and a weaker one (−4.94) for the dichloro compound **2b**.

Hollow fiber assay for preliminary in vivo testing

On the basis of these results, three derivatives were selected for a preliminary in vivo testing (**2a–c**). The hollow fiber assay, developed at the NCI, is a screening tool for assessing the potential anticancer activity of compounds against human tumor cells cultivated in hollow fibers and implanted intra-peritoneally and subcutaneously in mice.[2] After treatment by ip route, fiber cultures were collected and the viable cell mass was determined. A scoring system was developed to simplify evaluation of the results (Table 3). For this, a value of 2 was assigned for each compound dose which results in a 50% or greater reduction ($\%T/C \leq 50$) in viable cell mass. The intraperitoneal and subcutaneous samples were scored separately. Compounds with a combined IP + SC score ≥20, a sc score ≥8 or a net cell kill of one or more cell lines are considered as active in this testing.

Table 3. Results of the hollow fiber assay for preliminary in vivo testing

Compd	ip score	sc score	ip + sc score	Cell kill	MTD[a] (mg/kg)
2a	10	6	16	Yes	400
2b	10	4	14	Yes	200
2c	0	14	14	Yes	100

[a]MTD, maximum tolerated dose.

Table 4. Response of early stage subcutaneous tumor model to cyclopenta[*c*]thiophene related compounds

Compd	Cell line	Dose (mg/kg)	Route	% *T*/*C* (day)	Growth delay % *T*−*C*/*C*	Net log cell kill
2a	Lung NCI-H23	200	ip	86 (37)	−1	−0.60
		134	ip	108 (30)	−2	−0.60
		90	ip	132 (27)	−7	−0.70
	Lung NCI-H522	120	ip	106 (27)	−2	−0.40
		80	ip	146 (22)	−8	−0.50
		54	ip	177 (27)	−13	−0.60
	Melanoma LOX IMVI	120	ip	88 (15)	0	−0.70
		80	ip	103 (15)	−1	−0.70
		54	ip	111 (19)	−3	−0.80
	CNS U251	200	ip	100 (28)	−9	−0.40
		134	ip	89 (25)	−1	−0.30
		90	ip	128 (25)	−12	−0.40
2b	Lung NCI-H522	60	iv	51 (25)	28	−0.30
		40	iv	51 (18)	25	−0.30
		27	iv	30 (35)	1	−0.60
	Breast MDA-MB-231	100	iv	Toxic	—	—
		67	iv	Toxic	—	—
		45	iv	Toxic	>56	0.30
		22.25	iv	39 (36)	44.3	0.00
2c	CNS SF-295	30	ip	136 (9)	−5	−0.90
		20	ip	95 (16)	4	−0.80
		13.40	ip	173 (13)	−14	−1.10
	Breast MDA-MB-231	50	ip	64 (37)	−15	−0.70
		33.50	ip	90 (34)	−4	−0.60
		22.40	ip	107 (30)	−14	−0.70

All the tested derivatives met these criteria on account of a net cell kill and moreover for **2c** on the basis of a sc score = 14. The later result, for which the activity of **2c** is greater at the more remote implant site (sc) relative to ip implantation (ip score = 0), is characteristic of a compound being converted into a more potent product after administration in vivo.[9]

Subcutaneous human tumor xenograft assay

Accordingly, **2a–c** have been selected for further in vivo testing in standard subcutaneous xenograft model. This NCI's testing assesses the activity of the test compounds against a human tumor fragment which was implanted subcutaneously into the axillary region of pathogen-free immunodeficient mice. The results are expressed (Table 4) in terms of reduction percentage of the tumor weight in treated mice relative to a control group (%*T*/*C*), of growth delay percentage by which the treated group median tumor weight is delayed in achieving the specified tumor size compared to the control group [%(*T*−*C*)/*C*) and also in term of number of cells killed by the test agent (Net Log Cell Kill).

Among the three test compounds, **2a** and **2c** did not exhibit significant antitumor activity on the treatment schedule. However, criteria for the latter appear to have been met for **2b** (*T*/*C* <40%). Concerning the activity against lung NCI-H522, the result must be considered warily due to the fact that the implanted tumor failed to grow adequately in a significant number of control and treated mice. Against breast MDA-MB-231, a sufficient number of mice died acutely at doses of 100, 67 and 45 mg/kg to eliminate the opportunity for evaluating antitumor potential. However at dose of 22.25 mg/kg, **2b** showed a moderate activity (*T*/*C* = 39% on day 36, growth delay = 44.3%).

Conclusion

The fusion of various five-membered heterocycles to cyclopenta[*c*]thiophene afforded 10 new derivatives whose cytotoxicity was evaluated in vitro in a screening panel consisting of 60 human tumor cell lines. The results of this assay indicated an activity in the micromolar range linked particularly to the oxazolidinone series and three derivatives bearing two thiophene halogen atoms (**2a–c**) were selected for the NCI's hollow fiber assay. The results showed an in vivo activity which led us to involve **2a–c** in the NCI's subcutaneous human tumor xenograft assay. The dichloro derivative **2b** displayed an equivocal result likely to lead us to improve its activity through the pharmacomodulation of this series. Particularly, we have to elucidate whether **2c** acted as a prodrug on account of its high SC score in the hollow fiber assay and to identify its active species. The latter could help us to elucidate moreover the mechanism of action of these compounds that we could not determine through the COMPARE algorithm,[10] since no significant correlations with standard antitumor agents were underlined. This fact is liable to reinforce the potential interest of this original series as new antitumor agents.

Experimental

General experimental procedures

Melting points were determined on a Kofler block and are uncorrected. ^{1}H and ^{13}C NMR spectra were measured on a Jeol JNM-LA 400 spectrometer. NOE experiments were recorded on a Bruker AC 400 spectrometer and the samples were degassed by bubbling nitrogen through the solution. Chemical shifts are

reported in δ (ppm). Column chromatography was performed on Merck silica gel 60, 0.063–0.200 mm, 70–230 mesh. Precoated silica gel plates (Polygram SIL G/UV254, 0.25 mm) were used for TLC analysis. All products and reagents were purchased from Acros, Belgium.

(*cis*)-4,6-Dibromo-3a,7a-dihydro-2*H*-thieno[3′,4′:4,5]cyclopenta[1,2-*d*][1,3]oxazole-2,7(3*H*)-dione (2a). This was prepared by adding 2.5 g (0.007 mol) of **8a** to 80 mL of a 20% solution of phosgene in toluene. The reaction mixture was refluxed for 3 h and then evaporated. The resulting solid was washed with water, filtered, dried and recrystallized from isopropanol: beige crystals (83%), mp 250 °C; ^{1}H NMR (DMSO-d_6) δ 9.0 (bs, 1H, NH), 5.34 (d, J = 7.5 Hz, 1H, H-3a), 5.00 (d, J = 7.5 Hz, 1H, H-7a); ^{13}C NMR (DMSO-d_6) δ 190.5 (C-7), 157.0 (C-2), 152.0 (C-6a), 139.5 (C-3b), 114.7 (C-6), 107.5 (C-4), 83.7 (C-7a), 49.4 (C-3a). Anal. calcd for $C_8H_3NO_3Br_2S$: C, 27.22; H, 0.86; N, 3.97. Found: C, 27.15; H, 0.78; N, 3.90.

(*cis*)-4,6-Dichloro-3a,7a-dihydro-2*H*-thieno[3′,4′:4,5]cyclopenta[1,2-*d*][1,3]oxazole-2,7(3*H*)-dione (2b). This was prepared from **8b**, using the same method as for the synthesis of **2a**. Work up and purification were performed identically, resulting in beige crystals (74%), mp 260 °C; ^{1}H NMR (DMSO-d_6) δ 9.0 (bs, 1H, NH), 5.23 (d, J = 7.3 Hz, 1H, H-3a), 5.08 (d, J = 7.3 Hz, 1H, H-7a); ^{13}C NMR (DMSO-d_6) δ 188.2 (C-7), 157.0 (C-2), 147.8 (C-6a), 136.3 (C-3b), 128.4 (C-6), 121.2 (C-4), 83.5 (C-7a), 49.1 (C-3a). Anal. calcd for $C_8H_3NO_3Cl_2S$: C, 36.39; H, 1.14; N, 5.30. Found: C, 36.33; H, 1.24; N, 5.33.

(*cis*)-4,6-Diiodo-3a,7a-dihydro-2*H*-thieno[3′,4′:4,5] cyclopenta[1,2-*d*][1,3]oxazole-2,7(3*H*)-dione (2c). This was prepared from **8c**, using the same method as for the synthesis of **2a**. Work up and purification were performed identically, resulting in orange crystals (61%), mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 8.9 (bs, 1H, NH), 5.31 (d, J = 7.5 Hz, 1H, H-3a), 4.81 (d, J = 7.5 Hz, 1H, H-7a). Anal. calcd for $C_8H_3NO_3I_2S$: C, 21.50; H, 0.68; N, 3.13. Found: C, 21.75; H, 0.42; N, 2.99.

(*cis*)-4-Bromo-3a,7a-dihydro-2*H*-thieno[3′,4′:4,5]cyclopenta[1,2-*d*][1,3]oxazole-2,7(3*H*)-dione (2d). This was prepared from **8d**, using the same method as for the synthesis of **2a**. Work up and purification were performed identically, resulting in beige crystals (36%), mp 180 °C; ^{1}H NMR (DMSO-d_6) δ 9.0 (bs, 1H, NH), 8.45 (s, 1H, H-6), 5.33 (d, J = 6.4 Hz, 1H, H-3a), 5.05 (d, J = 6.4 Hz, 1H, H-7a). Anal. calcd for $C_8H_4NO_3BrS$: C, 35.06; H, 1.47; N, 5.11. Found: C, 35.26; H, 1.24; N, 5.07.

(*cis*)-4-Bromo-6-pyrrolidin-1-yl-3a,7a-dihydro-2*H*-thieno[3′,4′:4,5]cyclopenta[1,2-*d*][1,3]oxazole-2,7(3*H*)-dione (2e). This was prepared by adding 1.4 g (0.02 mol) of pyrrolidine to a solution of 1 g (0.003 mol) of **2a** in 20 mL of dioxane. The reaction mixture was stirred at room temperature for 4 h and then evaporated to dryness. The residue was dissolved in 50 mL of $CHCl_3$ and the solution was washed with water, separated and dried over $CaCl_2$. The solvent was removed to afford crystals which were recrystallized from Et_2O: Beige crystals (48%), mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 8.7 (bs, 1H, NH), 5.16 (d, J = 7.5 Hz, 1H, H-3a), 4.79 (d, J = 7.5 Hz, 1H, H-7a), 3.33 (m, 4H, pyrrolidine), 1.98 (m, 4H, pyrrolidine). Anal. calcd for $C_{12}H_{11}N_2O_3BrS$: C, 42.00; H, 3.23; N, 8.16. Found: C, 41.78; H, 3.15; N, 8.12.

(*cis*)-4-Bromo-6-(3,4-dimethoxyphenyl)-3a,7a-dihydro-2*H*-thieno[3′,4′:4,5]cyclopenta[1,2-*d*][1,3]oxazole-2,7 (3*H*)-dione (2f). This was prepared by adding under argon 116 mg (0.64 mmol) of 3,4-dimethoxyphenylboronic acid, 62.2 mg (0.08 mmol) of dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium and 0.6 mL (4.2 mmol) of TEA to a solution of 150 mg (0.42 mmol) of **2a** in 5 mL of DMF. The reaction mixture was heated at 65 °C for 12 h, poured into 50 mL of water and extracted with 100 mL of ethyl acetate. The organic layer was washed with water, separated, dried over $MgSO_4$ and evaporated to dryness to afford a red residue which was purified by column chromatography with CH_2Cl_2–MeOH (99.5:0.5) as the eluent: Orange crystals (30%), mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 8.93 (bs, 1H, NH), 7.72 (s, 1H, H-2′), 7.48 (d, J = 10 Hz, 1H, H-6′), 7.07 (d, J = 10 Hz, 1H, H-5′), 5.39 (d, J = 7.5 Hz, 1H, H-3a), 5.03 (d, J = 7.5 Hz, 1H, H-7a), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3); MS (EI): m/z 411 (M^{+1}, 100), 409 (M^{-1}, 88), 84 (24), 66 (37). Anal. calcd for $C_{16}H_{12}NO_5BrS$: C, 46.84; H, 2.94; N, 3.41. Found: C, 46.33; H, 2.58; N, 3.23.

(*cis*)-4,6-Dibromo-2-thioxo-2,3,3a,7a-tetrahydro-7*H*-thieno[3′,4′:4,5]cyclopenta[1,2-*d*][1,3]oxazol-7-one (3). This was prepared by refluxing for 3 h a mixture of 2.3 g (0.006 mol) of **8a** and 6 mL of thiophosgene in 15 mL of toluene. After evaporation of the reaction mixture, the resulting solid was washed with water, filtered, dried and recrystallized from isopropanol: beige crystals (22%), mp 120 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (bs, 1H, NH), 5.38 (d, J = 7.5 Hz, 1H, H-3a), 4.86 (d, J = 7.5 Hz, 1H, H-7a); ^{13}C NMR (DMSO-d_6) δ 188.9 (C-7), 157.2 (C-2), 152.1 (C-6a), 139.6 (C-3b), 114.9 (C-6), 107.6 (C-4), 83.7 (C-7a), 49.5 (C-3a). Anal. calcd for $C_8H_3NO_2Br_2S_2$: C, 26.04; H, 0.82; N, 3.80. Found: C, 26.18; H, 1.03; N, 3.87.

(*cis*)-4,6-Dibromo-3a*H*-thieno[3′,4′:3,4]cyclopenta[1,2-*d*][1,3]dioxole-2,7(7a*H*)-dione (4). This was prepared by adding 1 g (0.003 mol) of **11** to 10 mL of a 20% solution of phosgene in toluene. The reaction mixture was refluxed for 3 h and then evaporated. The resulting solid was washed with water, filtered, dried and recrystallized from isopropanol: beige crystals (14%), mp 174 °C; ^{1}H NMR (DMSO-d_6) δ 4.79 (d, J = 5.8 Hz, 1H, H-3a), 4.50 (d, J = 5.8 Hz, 1H, H-7a); ^{13}C NMR (DMSO-d_6) δ 190.4 (C-7), 164.1 (C-2), 151.5 (C-6a), 138.6 (C-3b), 111.2 (C-6), 108.1 (C-4), 81.3 (C-7a), 64.9 (C-3a); MS (EI): m/z 355 (M^{+1}, 1), 353 (M^{-1}, 2), 282 (20), 95 (41), 80 (100). Anal. calcd for $C_8H_2O_4Br_2S$: C, 27.15; H, 0.57. Found: C, 27.43; H, 0.72.

(*cis*)-4,6-Dibromo-2-thioxo-2,3,3a,7a-tetrahydro-7*H*-thieno[3′,4′:4,5]cyclopenta[1,2-*d*][1,3]thiazol-7-one (5). This was prepared by adding 3 mL of triethylamine to a suspension of 3 g (0.007 mol) of **12** in 20 mL of Et_2O. After filtration, 2 mL of carbon disulfide were added to the filtrate and the reaction mixture was stirred at room temperature for 1 h. Crystals (20%) were formed, filtered, washed with water, dried and recrystallized from chloroform: beige crystals, mp 160 °C; ^{1}H NMR (DMSO-d_6) δ 10.0 (bs, 1H, NH), 5.78 (d, J = 7 Hz, 1H, H-3a), 5.28 (d, J = 7 Hz, 1H, H-7a); ^{13}C NMR (DMSO-d_6) δ 189.0 (C-7), 157.2 (C-2), 152.1 (C-6a), 139.5 (C-3b), 115.1 (C-6), 107.8 (C-4), 83.8 (C-7a), 49.5 (C-3a). Anal. calcd for $C_8H_3NOBr_2S_3$: C, 24.95; H, 0.79; N, 3.64. Found: C, 24.87; H, 0.98; N, 3.92.

(*cis*)-4,6-Dibromo-1,3,3a,7a-tetrahydrothieno[3′,4′:3,4]cyclopenta[1,2-*d*]imidazole-2,7-dione (6). This was prepared by adding portionwise 3 g (0.03 mol) of potassium cyanate to a solution of 4 g (0.01 mol) of **12** in 30 mL of water. The reaction mixture was then stirred at room temperature for 1 h. Crystals (20%) were formed, filtered, washed with water, dried and recrystallized from EtOH: yellow crystals, mp 170 °C; ^{1}H NMR (DMSO-d_6) δ 9.5 (bs, 1H, NH), 9.0 (bs, 1H, NH), 5.79 (d, J = 7.4 Hz, 1H, H-3a), 5.35 (d, J = 7.4 Hz, 1H, H-7a); ^{13}C NMR (DMSO-d_6) δ 157.4 (C-4), 150.0 (C-2), 144.9 (C-4a), 135.3 (C-7a), 120.0 (C-5), 118.2 (C-7), 79.0 (C-3a), 51.2 (C-7b). Anal. calcd for $C_8H_4N_2O_2Br_2S$: C, 27.30; H, 1.15; N, 7.96. Found: C, 27.08; H, 1.48; N, 7.72.

(*cis*/*trans*)-1,3-Dibromo-5,6-dihydroxy-5,6-dihydro-4*H*-cyclopenta[*c*]thiophen-4-one (11). This was prepared by adding portionwise at 0 °C 4.5 g (0.066 mol) of sodium nitrite to a solution of 4 g (0.011 mol) of **8a** in 150 mL of a 20% aqueous solution of TFA. The reaction mixture was stirred at room temperature for 4 h. The precipitate formed was filtered, washed with water, dried and recrystallized from ether: yellow crystals (45%), mp 130 °C; ^{1}H NMR (DMSO-d_6) δ 6.3 (bs, 2H, OH *cis* and *trans*), 4.79 (d, J = 5.8 Hz, 1H, H-6 *cis*), 4.65 (d, J = 4.6 Hz, 1H, H-6 *trans*), 4.50 (d, J = 5.8 Hz, 1H, H-5 *cis*), 4.28 (d, J = 4.6 Hz, 1H, H-5 *trans*); ^{13}C NMR (DMSO-d_6) δ 196.5 (C-4 *cis*), 193.2 (C-4 *trans*), 151.5 (C-3a *cis*), 149.5 (C-3a *trans*), 138.9 (C-4a *trans*), 138.6 (C-4a *cis*), 111.2 (C-3 *cis* and *trans*), 108.2 (C-1 *cis*), 106.6 (C-1 *trans*), 87.2 (C-5 *trans*), 81.3 (C-5 *cis*), 72.0 (C-6 *trans*), 64.9 (C-6 *cis*). Anal. calcd for $C_7H_4O_3Br_2S$: C, 25.64; H, 1.23. Found: C, 25.87; H, 1.35.

(*cis*/*trans*)-1,3,5-Tribromo-4-oxo-5,6-dihydro-4*H*-cyclopenta[*c*]thien-6-ylammonium chloride (12). This was prepared by adding 20 mL of an 6 N aqueous solution of hydrochloric acid to a solution of 2 g (0.004 mol) of **9a** in 50 mL of EtOH. The reaction mixture was refluxed for 2 h and then evaporated to dryness. The residue was washed with acetone, filtered, dried and recrystallized from EtOH: beige crystals (75%) mp 210 °C; ^{1}H NMR (DMSO-d_6) δ 9.6 (bs, 3H, $^+$NH$_3$ *trans*), 9.0 (bs, 3H, $^+$NH$_3$ *cis*), 5.62 (d, J = 6.8 Hz, 1H, H-6 *cis*), 5.14 (d, J = 3 Hz, 1H, H-6 *trans*), 5.02 (d, J = 6.8 Hz, 1H, H-5 *cis*), 4.80 (d, J = 3 Hz, 1H, H-5 *trans*). Anal. calcd for $C_7H_5NOBr_3S$: C, 19.72; H, 1.18; N, 3.29. Found: C, 19.35; H, 1.32; N, 2.97.

Cytotoxic assays

The cytotoxic activity of tested compounds was evaluated in the NCI's in vitro human disease-oriented antitumor screen. This screening panel consists of 60 human tumor cell lines. Nine subpanels represent diverse histologies, that is nonsmall cell lung, renal, breast cancers, central nervous system, colon, melanoma, prostate, ovarian, and leukemia. Compounds were tested at a minimum of five concentrations at 10-fold dilutions. Results are evaluated in terms of specificity and potency. The cytotoxic effects of each of these compounds are expressed as the molar drug concentration required for 50% growth inhibition (GI_{50}).

Hollow fiber assay

Human tumor cells were cultivated in polyvinylidene fluoride hollow fibers, and a sample of each cell line was implanted into each of two physiologic compartments (intraperitoneal and subcutaneous) in mice. After treatment with tested compounds at each of two test doses using a QD × 4 schedule, fiber cultures were collected and the viable cell mass was determined using a formazan dye conversion assay. A scoring system was developed to simplify evaluation of the results. The cell lines used were: MDA-MB-231 and MDA-MB-435 (breast cancer), NCI-H23 and NCI-H522 (lung cancer), OVCAR-3 and OVCAR-5 (ovarian cancer), SF-295 and U-251 (CNS cancer), LOX IMVI and UACC-62 (melanoma), COLO 205 and SW-620 (colon cancer).

Early stage subcutaneous tumor model

Human tumor fragments (30 mg) are implanted subcutaneously into the axillary region of pathogen-free immunodeficient mice, on experimental day 0. Test agent treatment is initiated either on the day which is historically associated with the start of tumor growth, or when the tumor is palpable. Tumor size and body weights are obtained approximately two times per week. Tumor weights are calculated from caliper measurements of tumor dimensions in mm using the formula for a prolate ellipsoid: $(L \times W^2)/2$ where L is the longer of the two measurements.

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