Synthesis and Cytotoxic Activity against L1210 Leukemia of New Aminocyclopenta[*c*]thiophenones

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Synthesis of some new hydroxyaminocyclopenta[*c*]thiophenones was achieved *via* halogenation reaction, then formation and finally cleavage of an aziridino ring. The *in vitro* cytotoxic activity of these compounds was evaluated against L1210 leukemia. The importance of the ketohydroxyethylamino sequence for their activities is discussed.

Keywords cyclopenta[*c*]thiophene; L1210 leukemia; cytotoxic effect; ketohydroxyammonium sequence

As part of our work on the synthesis of new heterocyclic systems with potential therapeutic interest, we recently described the preparation[1] and the *in vitro* anti-human immunodeficiency virus properties[2] of some cyclopenta[*b*]thiophene derivatives. The activity was often accompanied with high cytotoxicity, especially for compounds having a *cis* ketohydroxyammonium sequence such as **1** (Chart 1), which exhibited a weak antitumor activity against P388 leukemia (T/C = 153% at 60 mg/kg). In order to examine the importance of this structure for the activity, we undertook its introduction into the cyclopenta[*c*]thiophene ring system that we had previously described[3] and we evaluated the *in vitro* cytotoxicity of **1** and its cyclopenta[*c*]thiophene homologues. Substitutions of the amino and hydroxyl groups allowed us to investigate the structure–activity relationships of this system. The results of this study are reported in the present paper.

Chemistry

The cyclopenta[*c*]thiophene ring system was generated starting from thiophene-3-carboxaldehyde **2**.[3] The synthesis proceeded *via* the trifluoroacetamides **3a—d** and the corresponding ammonium chlorides **4a—d**.

Halogenation of the dibromotrifluoroacetamide **3a** with bromine in acetic acid gave selectively the *trans* monobromo derivative **5a**, which allowed the formation of a third fused ring (Chart 2). Treatment of **5a** with sodium carbonate in refluxing acetone[4] afforded quantitatively the aziridino compound **6a**, while the ammonium chloride **7a**, obtained by acidic hydrolysis of **5a**, afforded the oxazolidinone **8a** on treatment at room temperature with sodium carbonate in water.[5]

The aziridino ring of **6a** was cleaved in an ethereal solution with a hydrochloric acid gas flow, leading to the unstable trifluoroacetoxyammonium chloride **9a**, which precipitated in the reaction mixture (Chart 3). Compound **9a**, on exposure to air, was quickly hydrolyzed to give the *cis* hydroxyammonium chloride **10a**. Treatment of **10a** in a mixture of trifluoroacetic acid and trifluoroacetic anhydride afforded the *cis* hydroxytrifluoroacetamide **11a**.[6]

On the other hand the pyrrolidinyl derivative **6e** was prepared from **6a** by treatment with refluxing pyrrolidine. Acidic cleavage of **6e** in a similar manner as described for **6a** gave the *cis* hydroxyammonium chloride **10e**.

The dichloro **3b**, diiodo **3c** and dehalo **3d** homologues of **3a** were treated similarly to give the *cis* hydroxyammonium chlorides **10b—d** *via* the monobromo **5b—d** and aziridino **6b—d** intermediates (Chart 4).

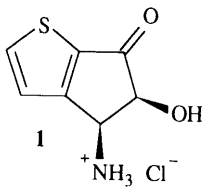

Chart 1

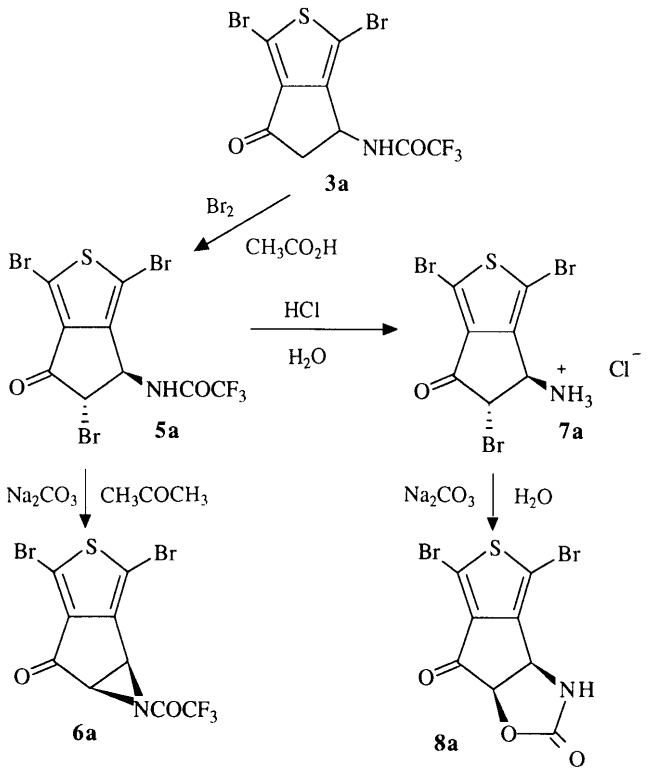

Chart 2

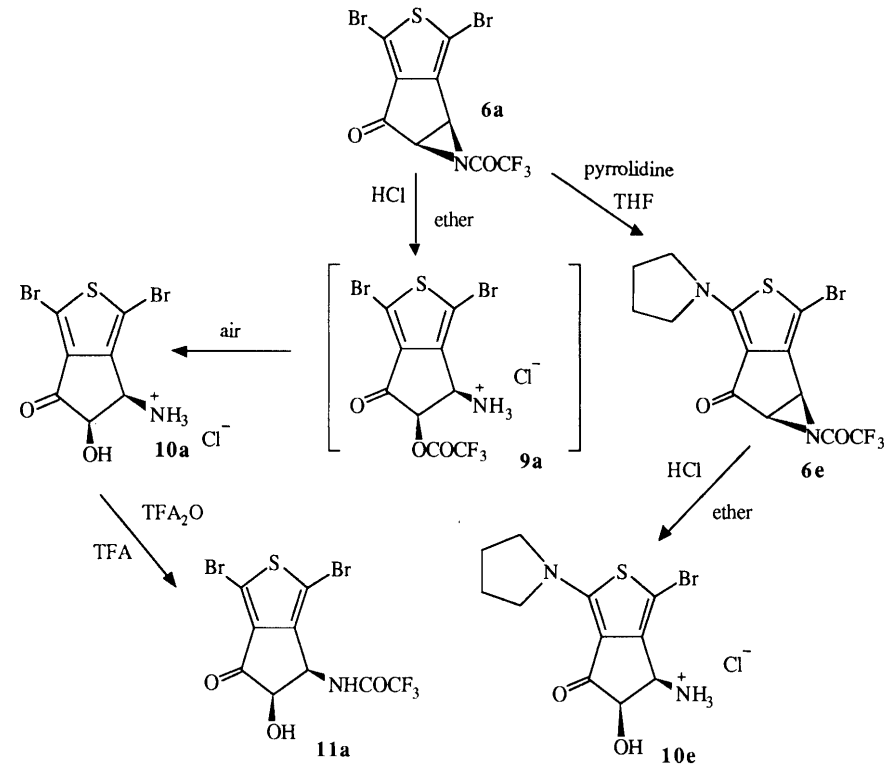

Chart 3

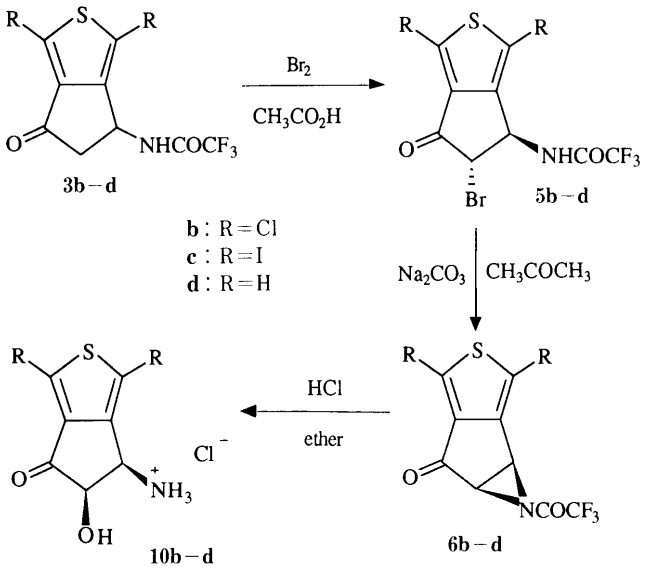

Chart 4

TABLE I. Activity of Cyclopentathiophenones against Murine L1210 Leukemia *in Vitro*

Compound No.	IC_{50} for inhibition of L1210 cell proliferation	
	μg/ml	μM
1	>10	>50
4a	0.3	0.9
5a	1	2
7a	0.3	0.7
8a	0.3	0.9
10a	1.5	4
11a	2	5
10b	2.5	9
10c	2	4
10d	1.5	7
10e	>40	>100

Biological Activity and Discussion

The cytotoxic effects of **1** and of some of the cyclopentane derivatives synthesized above were examined using murine L1210 leukemia cells *in vitro*. The data are shown in Table I.

Compound **1** was not active against L1210 leukemia, but all the cyclopenta[*c*]thiophene derivatives except **10e** were found to inhibit the proliferation of L1210 cells. Compounds **4a**, **7a** and **8a** were the most potent ($IC_{50} < 1$ μM), and the IC_{50} of the other active compounds ranged from 2 to 9 μM. Among the hydroxyammonium chlorides, the dibromo and diiodo derivatives **10a** and **10c** were about two-fold more active than the unsubstituted and dichloro analogs **10d** and **10b**. Replacing one of the bromine atoms of **10a** by a pyrrolidine group led to a loss of the activity (**10e**). Among the 1,4-dibromothiophene derivatives, compounds **4a** (which lacks a substituent on C-5) and **7a** (bearing a bromine atom on this position) were respectively 4 and 6 times more active than their hydroxy analog **10a**. Replacement of the ammonium chloride group in **10a** and **7a** by a trifluoroacetamido group (**11a**, **5a**) did not improve the activity. On the other hand, formation of an oxazolidine ring (**8a**) between the amino and hydroxyl groups of **10a** was rather favorable for the activity. The fact that **8a** could be formed in the culture medium by carbonation of the bromoammonium chloride **7a**, as suggested by its synthetic pathway (Chart 2), could explain the similar activities obtained for both compounds.

Conclusion

The cyclopenta[*c*]thiophene ring system is likely to

afford new compounds with potential cytotoxic effect; 3 of 10 new aminocyclopenta[*c*]thiophenones tested exhibited significant activity. We intend to synthesize nitrosochlorethylureido derivatives for evaluation with a larger panel of cell lines including human tumor cells.

Experimental

Chemistry The term "concentrated" refers to the removal of volatile solvents under reduced pressure at or below 40 °C with the aid of a rotary evaporator. All products were dried to constant weight. All melting points were determined on a Köfler apparatus without correction. Infrared spectra were recorded on a Philips PU 9716 apparatus, and only noteworthy absorptions (reciprocal centimeters) are listed. Proton magnetic resonance spectra were recorded on a JEOL FX 200 in 10% dimethyl sulfoxide-d_6 (DMSO-d_6) solution using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm downfield (δ) from TMS. Analyses were within $\pm 0.4\%$ of the theoretical values.

Synthesis of Compounds 5a—d A solution of 0.005 mol of bromine in 10 ml of acetic acid was added dropwise to a stirred solution of 0.005 mol of **3a**, **3b**, **3c** or **3d** in 150 ml of acetic acid at 0 °C. The mixture was stirred at room temperature for 12 h and the solution obtained was poured into 300 ml of cold water. The precipitate formed was collected by filtration, washed with 2 × 50 ml of water and dried. Recrystallization from ether/petroleum ether gave **5a**, **5b**, **5c** or **5d** as a white solid.

***trans*-1,3,5-Tribromo-6-trifluoroacetylamino-5,6-dihydro-4*H*-cyclopenta[*c*]thiophen-4-one (5a)** Yield: 80%; mp 184 °C. IR (KBr): 3260 (NH), 1715 (C=O), 1690 (C=O), 1540, 1210, 1170 cm^{-1}. ^{1}H-NMR δ: 10.23 (d, J=8.3 Hz, 1H, NH), 5.40 (dd, J=8.3, 4.4 Hz, 1H, H-6), 5.23 (d, J=4.4 Hz, 1H, H-5). *Anal.* Calcd for $C_9H_3Br_3F_3NO_2S$: C, 22.25; H, 0.62; N, 2.88. Found: C, 22.31; H, 0.50; N, 2.74.

***trans*-5-Bromo-1,3-dichloro-6-trifluoroacetylamino-5,6-dihydro-4*H*-cyclopenta[*c*]thiophen-4-one (5b)** Yield: 78%; mp 184 °C. IR (KBr): 3300 (NH), 1730 (C=O), 1700 (C=O), 1540, 1480, 1200, 1170 cm^{-1}. ^{1}H-NMR δ: 10.23 (d, J=8.3 Hz, 1H, NH), 5.40 (dd, J=8.3, 4.4 Hz, 1H, H-6), 5.23 (d, J=4.4 Hz, 1H, H-5). *Anal.* Calcd for $C_9H_3BrCl_2F_3NO_2S$: C, 27.23; H, 0.76; N, 3.53. Found: C, 27.31; H, 0.90; N, 3.74.

***trans*-5-Bromo-1,3-diiodo-6-trifluoroacetylamino-5,6-dihydro-4*H*-cyclopenta[*c*]thiophen-4-one (5c)** Yield: 75%; mp 250 °C. IR (KBr): 3300 (NH), 1720 (C=O), 1560, 1460, 1230, 1190, 960, 770 cm^{-1}. ^{1}H-NMR δ: 10.10 (d, J=8.3 Hz, 1H, NH), 5.22 (dd, J=8.3, 4.4 Hz, 1H, H-6), 5.05 (d, J=4.4 Hz, 1H, H-5). *Anal.* Calcd for $C_9H_3BrF_3I_2NO_2S$: C, 18.64; H, 0.52; N, 2.42. Found: C, 18.27; H, 0.48; N, 2.53.

***trans*-5-Bromo-6-trifluoroacetylamino-5,6-dihydro-4*H*-cyclopenta[*c*]-thiophen-4-one (5d)** Yield: 90%; mp 186 °C. IR (KBr): 3250 (NH), 1720 (C=O), 1680 (C=O), 1530, 1200, 1160 cm^{-1}. ^{1}H-NMR δ: 10.14 (d, J=8.3 Hz, 1H, NH), 8.44 (d, J=2.5 Hz, 1H, H-3), 7.71 (d, J=2.5 Hz, 1H, H-1), 5.43 (dd, J=8.3, 4.4 Hz, 1H, H-6), 5.17 (d, J=4.4 Hz, 1H, H-5). *Anal.* Calcd for $C_9H_5BrF_3NO_2S$: C, 32.95; H, 1.54; N, 4.27. Found: C, 32.61; H, 1.50; N, 4.03.

Synthesis of Compounds 6a—d Sodium carbonate (7.4 g, 0.07 mol) was added to a solution of 0.038 mol of **5a**, **5b**, **5c** or **5d** in 50 ml of acetone. The reaction mixture was refluxed for 1 h and concentrated. The residue was taken up in 200 ml of ether and the suspension was filtered. The filtrate was evaporated to dryness to give **6a**, **6b**, **6c** or **6d**. Recrystallization of the crude product from ether gave a white solid except for **6d**, which was an unstable oil that was used immediately for the following reaction.

3,5-Dibromo-1-trifluoroacetyl-1a,2,5b-trihydro-1*H*-cyclopenta[*c*]-thieno[4,5-*b*]azirin-2-one (6a) Yield: 80%; mp 130 °C. IR (KBr): 1710 (C=O), 1470, 1200, 1180, 1110 cm^{-1}. ^{1}H-NMR δ: 5.69 (d, J=7.3 Hz, 1H, H-1a), 5.59 (d, J=7.3 Hz, 1H, H-5b). *Anal.* Calcd for $C_9H_2Br_2F_3NO_2S$: C, 26.69; H, 0.50; N, 3.46. Found: C, 26.19; H, 0.61; N, 3.27.

3,5-Dichloro-1-trifluoroacetyl-1a,2,5b-trihydro-1*H*-cyclopenta[*c*]-thieno[4,5-*b*]azirin-2-one (6b) Yield: 82%; mp 135 °C. IR (KBr): 1730 (C=O), 1480, 1190, 1160, 1110 cm^{-1}. ^{1}H-NMR δ: 5.69 (s, 2H, H-1a and H-5b). *Anal.* Calcd for $C_9H_2Cl_2F_3NO_2S$: C, 34.20; H, 0.64; N, 4.43. Found: C, 34.20; H, 0.63; N, 4.57.

3,5-Diiodo-1-trifluoroacetyl-1a,2,5b-trihydro-1*H*-cyclopenta[*c*]thieno-[4,5-*b*]azirin-2-one (6c) Yield: 80%; mp 236 °C. IR (KBr): 1710 (C=O), 1670, 1540, 1460, 1380, 1205, 1180, 1110 cm^{-1}. ^{1}H-NMR δ: 5.68 (d, J=7.3 Hz, 1H, H-1a), 5.45 (d, J=7.3 Hz, 1H, H-5b). *Anal.* Calcd for $C_9H_2I_2F_3NO_2S$: C, 21.66; H, 0.40; N, 2.81. Found: C, 21.69; H, 0.43; N, 2.82.

5-Bromo-3-pyrrolidinyl-1-trifluoroacetyl-1a,2,5b-trihydro-1*H*-cyclopenta[*c*]thieno[4,5-*b*]azirin-2-one (6e) Pyrrolidine (1.65 ml, 0.020 mol) was added to an ice-cooled solution of 2 g (0.005 mol) of **6a** in tetrahydrofuran (50 ml). The reaction mixture was stirred at room temperature for 1 h and then evaporated to dryness under reduced pressure. The residue was taken up in methanol (5 ml) and the insoluble solid was filtered, dried and recrystallized from ether to give **6e** as white crystals. Yield: 70%; mp 216 °C. IR (KBr): 1670 (C=O), 1590, 1535, 1460, 1400, 1340, 1200, 1170, 1130, 930 cm^{-1}. ^{1}H-NMR δ: 5.51 (d, J=7.3 Hz, 1H, H-1a), 5.37 (d, J=7.3 Hz, 1H, H-5b), 3.54 (m, 4H, 2CH$_2$), 2.00 (m, 4H, 2CH$_2$). *Anal.* Calcd for $C_{13}H_{10}BrF_3N_2O_2S$: C, 39.51: H, 2.55; N, 7.09. Found: C, 39.58; H, 2.55; N, 7.00.

Synthesis of Compounds 10a—e A stirred solution of 0.01 mol of **6a**, **6b**, **6c**, **6d** or **6e** in 100 ml of ether was bubbled for 1 min at room temperature with gaseous hydrochloric acid. The precipitate formed was collected by filtration, washed with ether and exposed to air for 12 h. Recrystallization from 2-propanol gave **10a**, **10b**, **10c**, **10d** or **10e** as a white solid.

***cis*-1,3-Dibromo-5-hydroxy-4-oxo-5,6-dihydro-4*H*-cyclopenta[*c*]thien-6-ylammonium Chloride (10a)** Yield: 70%; mp >260 °C. IR (KBr): 3420 (OH), 2940, 2760, 1700 (C=O), 1550, 1475, 1055 cm^{-1}. ^{1}H-NMR δ: 8.5 (br, 3H, NH$_3$), 7.3 (br, 1H, OH), 4.87 (d, J=6.8 Hz, 1H, H-5), 4.71 (d, J=6.8 Hz, 1H, H-6). *Anal.* Calcd for $C_7H_6Br_2ClNO_2S$: C, 23.13; H, 1.66; N, 3.85. Found: C, 23.33; H, 1.73; N, 3.91.

***cis*-1,3-Dichloro-5-hydroxy-4-oxo-5,6-dihydro-4*H*-cyclopenta[*c*]thien-6-ylammonium Chloride (10b)** Yield: 81%; mp 240 °C. IR (KBr): 3460 (OH), 2980, 2800, 1720 (C=O), 1565, 1500, 1070 cm^{-1}. ^{1}H-NMR: δ 8.5 (br, 3H, NH$_3$), 7.2 (br, 1H, OH), 4.83 (d, J=6.8 Hz, 1H, H-5), 4.74 (d, J=6.8 Hz, 1H, H-6). *Anal.* Calcd for $C_7H_6Cl_3NO_2S$: C, 30.62; H, 2.20; N, 5.10. Found: C, 30.44; H, 2.06; N, 5.21.

***cis*-5-Hydroxy-1,3-diiodo-4-oxo-5,6-dihydro-4*H*-cyclopenta[*c*]thien-6-ylammonium Chloride (10c)** Yield: 65%; mp 250 °C. IR (KBr): 3440 (OH), 2980, 2600, 1710 (C=O), 1540, 1460, 1290, 1260, 1050, 980, 770 cm^{-1}. ^{1}H-NMR: δ 8.3 (br, 3H, NH$_3$), 4.85 (d, J=6.8 Hz, 1H, H-5), 4.48 (d, J=6.8 Hz, 1H, H-6), 3.6 (br, 1H, OH). *Anal.* Calcd for $C_7H_6ClI_2NO_2S$: C, 18.38; H, 1.32; N, 3.06. Found: C, 18.28; H, 1.06; N, 3.04.

***cis*-5-Hydroxy-4-oxo-5,6-dihydro-4*H*-cyclopenta[*c*]thien-6-ylammonium Chloride (10d)** Yield: 95%; mp 210 °C. IR (KBr): 3250 (OH), 3150, 3040, 1700 (C=O), 1570, 1455, 1015 cm^{-1}. ^{1}H-NMR δ: 8.5 (br, 3H, NH$_3$), 8.33 (d, J=2.0 Hz, 1H, H-3), 7.92 (d, J=2.0 Hz, 1H, H-1), 4.82 (s, 2H, H-5 and H-6), 2.5 (br, 1H, OH). *Anal.* Calcd for $C_7H_8ClNO_2S$: C, 40.88; H, 3.92; N, 6.81. Found: C, 40.84; H, 3.71; N, 6.87.

***cis*-1-Bromo-5-hydroxy-4-oxo-3-pyrrolidinyl-5,6-dihydro-4*H*-cyclopenta[*c*]thien-6-ylammonium Chloride (10e)** Yield: 80%; mp >260 °C. IR (KBr): 3420—2600, 1650 (C=O), 1580, 1520, 1320, 805, 770 cm^{-1}. ^{1}H-NMR δ: 8.3 (br, 3H, NH$_3$), 6.7 (br, 1H, OH), 4.62 (d, J=6.8 Hz, 1H, H-5), 4.40 (d, J=6.8 Hz, 1H, H-6), 3.57 (m, 4H, 2CH$_2$), 2.09 (m, 4H, 2CH$_2$). *Anal.* Calcd for $C_{11}H_{14}BrClN_2O_2S$: C, 37.36; H, 3.99; N, 7.92. Found: C, 37.40; H, 4.02; N, 7.87.

***trans*-1,3,5-Tribromo-4-oxo-5,6-dihydro-4*H*-cyclopenta[*c*]thien-6-ylammonium Chloride (7a)** **5a** (4.2 g, 0.01 mol) was added to a 6 N aqueous solution of hydrochloric acid (50 ml). The reaction mixture was refluxed for 30 min and then evaporated to dryness under reduced pressure. The solid residue was recrystallized from propan-2-ol to give **7a** as white crystals. Yield: 45%; mp >260 °C. IR (KBr): 3500—2620, 1715 (C=O), 1480, 1400, 1260, 1090, 750 cm^{-1}. ^{1}H-NMR δ: 9.6 (br, 3H, NH$_3$), 5.14 (d, J=2.9 Hz, 1H, H-6), 4.80 (d, J=2.9 Hz, 1H, H-5). *Anal.* Calcd for $C_7H_5Br_3ClNOS$: C, 19.72; H, 1.18; N, 3.29. Found: C, 19.85; H, 1.32; N, 2.99.

4,6-Dibromo-3,3a,7,7a-tetrahydro-2*H*-cyclopenta[*c*]thieno[4,5-*d*]-oxazol-2,7-dione (8a) Sodium carbonate (0.25 g, 0.002 mol) was added to a solution of **7a** (0.5 g, 0.001 mol) in water (50 ml). The reaction mixture was stirred at room temprature for 15 min and filtered. The filtrate was left overnight at room temperature and the precipitate which appeared was filtered, dried and recrystallized from ether to give **8a** as white crystals. Yield: 70%: mp 250 °C. IR (KBr): 3290 (NH), 1740 (C=O), 1550, 1480, 1380, 1360, 1220, 800 cm^{-1}. ^{1}H-NMR δ: 8.97 (s, 1H, NH), 5.34 (d, J=7.3 Hz, 1H, H-7a), 5.00 (d, J=7.3 Hz, 1H, H-3a). *Anal.* Calcd for $C_8H_3Br_2NO_3S$: C, 27.22; H, 0.86; N, 3.97. Found: C, 27.15; H, 0.78; N, 3.90.

***cis*-1,3-Dibromo-5-hydroxy-6-trifluoroacetylamino-5,6-dihydro-4*H*-cyclopenta[*c*]thiophen-4-one (11a)** **10a** (2 g, 0.0055 mol) was dissolved in a mixture of trifluoroacetic acid (10 ml) and trifluoroacetic anhydride (10 ml). The reaction mixture was stirred at room temperature for 1 h and then poured into water (50 ml). The precipitate that appeared was collected by filtration, washed with water (20 ml), dried and recrystallized from ether to give **11a** as white crystals. Yield: 85%; mp 220 °C. IR (KBr): 3390 (OH), 3290 (NH), 1710 (C=O), 1690 (C=O), 1540, 1470, 1300, 1200, 1170, 1090, 940 cm^{-1}. ^{1}H-NMR δ: 9.6 (d, J=8.8 Hz, 1H, NH), 6.4 (d, J=6.8 Hz, 1H, OH), 5.24 (dd, J=8.8, 7.8 Hz, 1H, H-6), 4.77 (dd, J=7.8, 6.8 Hz, 1H, H-5). *Anal.* Calcd for $C_9H_4Br_2F_3NO_3S$: C, 25.56; H, 0.95; N, 3.31. Found: C, 25.71; H, 0.92; N, 3.19.

Biology Murine leukemia L1210 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (105 UI/l), streptomycin (0.1 g/l) and fungizon (2.5 mg/l) in humidified air containing 5% CO_2 at 37 °C. The test compound, dissolved in DMSO, was diluted in RPMI (final concentration of DMSO: 0.2%) and added at various concentrations to the cell suspension (0.15×10^6 cells/ml). The cytotoxic activity (IC_{50} value) was defined as the concentration causing a 50% growth inhibition after 48 h, measured by cell counting on a Coulter ZM. It was determined from dose-response curves obtained from at least three independent experiments.

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