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A First Cyclopenta[*c*]thiophene Dimer as a New Bivalent Potent Cytotoxic Derivative

LAN PHAM KHANH, PATRICK DALLEMAGNE*, HENRIETTE LANDELLE and SYLVAIN RAULT

Centre d'Etudes et de Recherche sur le Médicament de Normandie, UFR des Sciences Pharmaceutiques, Université de Caen Basse-Normandie, 1, rue Vaubénard, 14032 Caen cedex, France

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Application of the bivalent approach to the cyclopenta[*c*]thiophene series led to a potent cytotoxic dimer.

Keywords: Cyclopenta[*c*]thiophene; Dimer; Bivalent; Cytotoxicity; Leukemia

INTRODUCTION

During the course of our work aimed at researching new compounds derived from thiophene and showing a biological activity we reported a few years ago the synthesis and the biological evaluation of new cyclopenta[*c*]thiophene derivatives[1] which were characterized as potent cytotoxic derivatives.[2] Among these compounds, the hydrochloric salt of the aminodibromocyclopenta[*c*]thiophenone **1** was recently shown to be particularly active *in vitro* towards leukemia cell lines.[3] In order to increase activity of the latter, we have improved its pharmacomodulation in particular with respect to the replacement of its amino group by various nucleophilic agents. Within this framework, we wish to report here the initial results concerning the synthesis and the cytotoxicity evaluation of new cyclopenta[*c*]thiophene related compounds.

MATERIAL AND METHODS

Chemistry

Procedure for Preparation of 6

To a stirred solution of 0.4 g (0.0014 mol) of **3** in ethanol (20 mL), was added 0.034 g (0.0014 mol) of sodium hydride. The reaction mixture was refluxed for 12 h, then evaporated to dryness. The residue was dissolved in ether (30 mL) and the solution was washed with water, separated, dried over magnesium sulfate and concentrated. The crude product was purified by column chromatography (3.5 × 30 cm, silica gel 60, 0.063–0.200 mm) with petroleum ether / ethyl acetate (95:5) as the eluent to give **6** as a beige solid (26%): mp 150°C; IR 1715 (CO), 3427, 1473, 1102, 801 cm^{-1}; ^1H NMR ($CDCl_3$): 4.76 (dd, J = 7.2 Hz, 1 H, H-6), 3.58 (m, 2 H, CH_2), 3.18 (dd, J = 19.7 Hz, 1 H, H-5a), 2.88 (dd, J = 19.2 Hz, 1 H, H-5b), 1.19 (t, J = 7 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): 192.81 (C-4), 152.61 (C-3a), 141.80 (C-6a), 111.46 (C-3), 107.70 (C-1), 70.27 (C-5), 65.43 (CH_2), 51.23 (C-6), 15.29 (CH_3); MS (EI): m/z = 340 (M^+, 43.9), 296 (41.2), 215 (98.2), 187 (26.9), 83 (100). Anal. Calcd. for $C_9H_8O_2Br_2S$: C, 31.79; H, 2.37. Found: C, 31.69; H, 1.99%.

Procedure for Preparation of 7

To a stirred solution of 1 g (0.0034 mol) of **3** in chloroform (20 mL), was added 0.25 g (0.004 mol) of nitromethane and 0.1 g (0.004 mol) of sodium hydride. The reaction mixture was refluxed for 3 h, then evaporated to dryness. The residue was dissolved in ether (30 mL) and the solution was washed with water, separated, dried over magnesium sulfate and concentrated. The crude product was purified by column chromatography (3.5 × 30 cm, silica gel 60, 0.063–0.200 mm) with petroleum ether / ethyl acetate (95:5) as the eluent to give **7** as a brown oil (30%): IR 1723 (CO), 2974, 1548, 1473, 1087 cm^{-1}; ^1H NMR ($CDCl_3$): 5.03 (dd, J = 14.4 Hz, 1 H, H-1′a), 4.54 (dd, J = 14.10 Hz, 1 H, H-1′b), 3.96 (m, 1 H, H-6),

*Corresponding author. Fax: +33-2-3193-1188. E-mail: dallemagne@pharmacie.unicaen.fr

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3.34 (dd, J = 19.8 Hz, 1 H, H-5a), 2.96 (dd, J = 19.4 Hz, 1 H, H-5b); ^{13}C NMR ($CDCl_3$): 191.92 (C-4), 112.58 (C-3a), 105.57 (C-6a), 100.31 (C-3), 83.65 (C-1), 75.71 (CH_2), 47.98 (C-5), 32.73 (C-6); MS (EI): m/z = 355 (M^+, 35.0), 310 (64.5), 308 (100), 306 (58.9), 230 (20.2), 121 (56.3), 84 (22.3).

Procedure for Preparation of 8

To a stirred solution of 1 g (0.0034 mol) of **3** in chloroform (20 mL), was added 0.45 g (0.0038 mol) of dimethyl malonate and 0.08 g (0.003 mol) of sodium hydride. The reaction mixture was refluxed for 3 h, then evaporated to dryness. The residue was dissolved in ether (30 mL) and the solution was washed with water, separated, dried over magnesium sulfate and concentrated. The crude product was purified by column chromatography (3.5 × 30 cm, silica gel 60, 0.063–0.200 mm) with petroleum ether / ethyl acetate (95:5) as the eluent to give **8** as a beige solid (23%): mp > 260°C; IR 1750 (CO), 1718 (CO), 3420, 1553, 1260, 1023, 801 cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.26 (d, J = 4 Hz, 1 H, H-6), 3.74 (s, 3 H, CH_3), 3.71 (d, J = 4 Hz, 1 H, CH), 3.55 (s, 3 H, CH_3), 3.18 (dd, J = 12.4 Hz, 1 H, H-5a), 3.07 (dd, J = 12.8 Hz, 1 H, H-5b); ^{13}C NMR ($CDCl_3$): 193.50 (C-4), 168.11 (CO_2CH_3), 167.37 (CO_2CH_3), 151.70 (C-3a), 142.48 (C-6a), 111.26 (C-3), 104.64 (C-1), 53.04 (CH_3), 52.65 (CH_3), 51.32 (CH), 47.04 (C-5), 33.78 (C-6); MS (EI): m/z = 426 (M^+, 18.4), 347 (41.9), 313 (100), 287 (71.6), 231 (33.6). Anal. Calcd. for $C_{12}H_{10}O_5Br_2S$: C, 33.83; H, 2.37. Found: C,33.54; H, 2.51%.

Procedure for Preparation of 9

To a stirred solution of 0.5 g (0.0017 mol) of **3** in chloroform (20 mL), was added 0.15 mL (0.0017 mol) of 1,3-diaminopropane. The reaction mixture was refluxed for 1 h, then evaporated to dryness. The residue was dissolved in ether (30 mL) and the solution was washed with water, separated, dried over magnesium sulfate and concentrated. The crude oil was purified by column chromatography (3.5 × 30 cm, silica gel 60, 0.063–0.200 mm) with chloroform-methanol (90:10) as the eluent to give **9** as a colorless oil (9%): IR 3379 (NH), 1720 (CO), 2924, 1536, 1094 cm^{-1}; ^{1}H NMR ($CDCl_3$): 5.21 (dd, J = 7.2 Hz, 2 H, 2 H-6), 3.5 (bs, 2 H, 2 NH), 3.30 (dd, J = 19.7 Hz, 2 H, 2 H-5a), 2.94 (dd, J = 19.2 Hz, 2 H, 2 H-5b), 2.76 (t, J = 7 Hz, 4 H, 2 CH2), 1.60 (t, J = 7 Hz, 2 H, CH2). Anal. Calcd. for $C_{17}H_{14}N_2O_2Br_4S_2$: C, 30.84; H, 2.13; N, 4.23. Found: C, 30.62; H, 2.46; N, 4.68%.

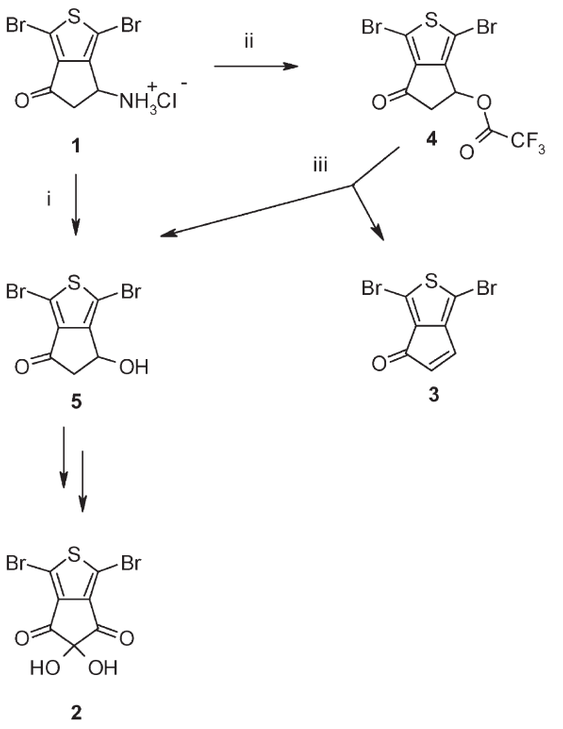
Biology

The cytotoxic activity of the test compounds was evaluated in the NCI's (National Cancer Institute, Bethesda, USA) *in vitro* human disease-oriented antitumor screen.[5] This screening panel consists of 60 human tumor cell lines. Nine subpanels represent diverse histologies, *i.e.* 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 compound are expressed as the molar drug concentration required for 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% cell kill (LC_{50}).

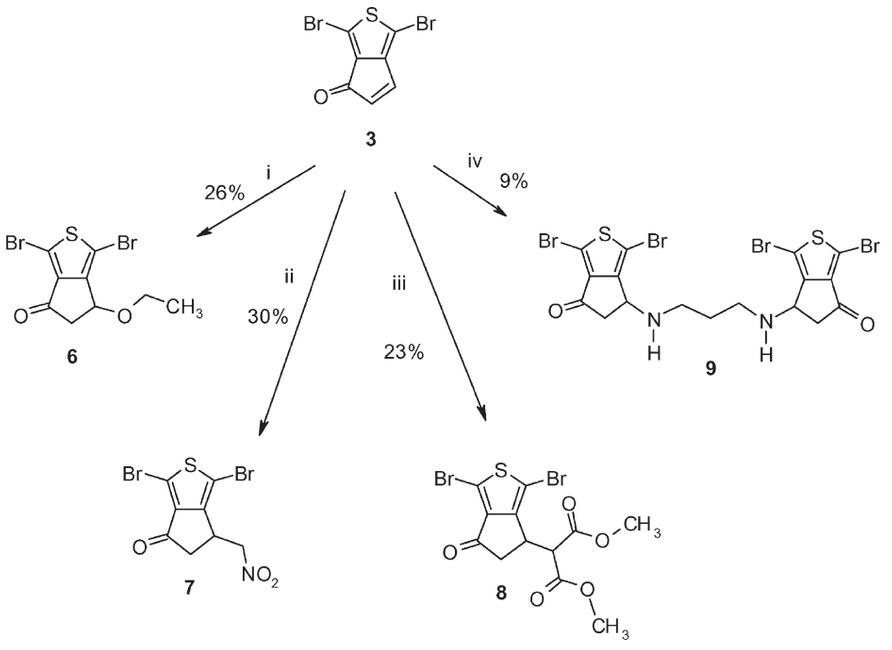
RESULTS AND DISCUSSION

Chemistry

Apart from its interesting biological properties, derivative **1** also constitutes a useful scaffold that we have profited from for access to dibromoisothianinhydrin **2**, a thiophene isostere of ninhydrin with fluorogenic properties useful in forensic science.[4] During the course of this chemical pathway (Scheme 1), involving the diazotisation of the amino group of **1**, we demonstrated the formation, as a by-product, of the cyclopentenone **3**. The latter arose from the elimination, by exposure to ambient air, of the trifluoroacetoxy group of the intermediate **4**, concomitantly with its hydrolysis leading to the desired alcohol **5**.



SCHEME 1 (i): $NaNO_2$, AcOH, H_2O; (ii): $NaNO_2$, TFA, H_2O; (iii): ambient air.



SCHEME 2 (i): EtOH, NaH; (ii): CH_3NO_2, NaH, $CHCl_3$; (iii): $CH_2(CO_2CH_3)_2$, NaH, $CHCl_3$; (iv): $H_2N(CH_2)_3NH_2$, $CHCl_3$.

The chemical behavior of the cyclopentenone **3** is that of a Michaël acceptor and we have capitalized on its reactivity in particular in various nucleophilic substitutions (Scheme 2). Thus, treatment of **3** in refluxing ethanol and in the presence of sodium hydride led to the ethoxy derivative **6** in 26% yield. By using either nitromethane or dimethyl malonate carbanions in refluxing chloroform in the presence of the same base, the reaction afforded the nitromethyl derivative **7** and the cyclopentyl malonate **8**, in 30% and 23% yields, respectively. On the other hand, **3** was sufficiently reactive in refluxing chloroform to add 1,3-diaminopropane in the absence of another base, leading to the dimer **9** although the yield was quite poor (9%).

Biology

The compounds **7**, **8** and **9** were selected and evaluated in the *in vitro* human disease-oriented tumor cell line screening panel developed at the NCI[5] and the results were compared with those obtained with derivative **1**. The log GI_{50} values (GI_{50} being the molar drug concentration required for half growth inhibition) obtained with leukemia cell lines are summarized in Table I.

TABLE I Cytotoxicity of cyclopenta[c]thiophene derivatives towards leukemia cell lines

	log molar drug concentration for 50% growth inhibition (log GI_{50})			
Compd	CCRF-CEM	K-562	MOLT-4	RPMI 8226
1	−5.81	−5.79	−5.73	−6.53
7	−5.14	−4.76	−4.84	−4.75
8	−5.39	−4.68	−5.48	−4.94
9	−6.91	−6.52	−6.57	−7.45

These results showed that while **7** and **8** broadly lose cytotoxicity towards leukemia cell lines compared to structure **1**; **9** appears more active with GI_{50} values in the submicromolar range. This increased potency confirms the involvement of the so-called "bivalent approach",[6,7] since many examples of derivatives including two pharmacophores in a single structure have been found to have enhanced activity over their respective monomer counterparts.[8,9]

This result encourages us to synthesize further dimers of **1** and to vary the length of their spacer chain, with the aim of obtaining higher activities in this series. This objective, as well as the elucidation of the mechanism of action of these novel compounds, will form future studies. The latter, in particular need to attempt to show if **9** possesses inhibiting properties towards cdc2 kinase, as is the case for derivative **1** ($IC_{50} = 18\,\mu M$), and if this activity is connected to its cytotoxic activity.

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