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Short Communication

Synthesis and cytotoxicity evaluation of thiophene analogues of 1-methyl-2,3-bis(hydroxymethyl)benzo[*g*]indole bis[*N*-(2-propyl)carbamate]

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Abstract

The cytotoxicity of the bis[*N*-(2-propyl)carbamates] **2** and **3** which are linked to thieno[*i*,*j*-*g*]indole scaffolds through methylene bridges were studied as thiophene analogues of prototype **1**. Compounds **2** and **3** were evaluated in vitro against 60 human–tumor cell lines derived from nine cancer-cell types and demonstrated, for compound **3** not only strong growth-inhibitory activities against leukemia cancer cells, but also fairly good activities against the growth of certain renal and ovarian cancer cell lines. Compound **2**, the thieno[2,3-*g*]indole bis-carbamate, possessed only significant (MG-MID $\log_{10} GI_{50} = -4.89$) and selective cytoxicity against NCI–HOP92 (non-small cell lung), MALME 3M (melanoma) and IGROV 1 (ovarian) cancer cell with $\log_{10} GI_{50}$ values of -5.66, -5.48 and -5.47, respectively.

Keywords: Biscarbamates; Thieno[*i*,*j*-*g*]indoles; Cytotoxicity

1. Introduction

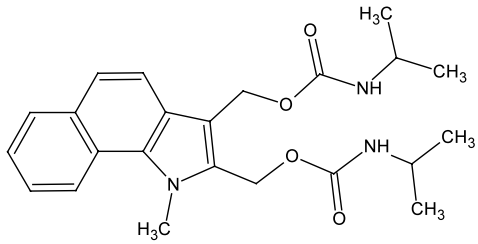
Cancer remains a major threat to the public health. In the challenge to improve modern cancer chemotherapy, the search for new drugs with both higher therapeutic index and lower capacity to induce resistance is, therefore, an active field of investigation in medicinal chemistry [1]. Some time ago, we reported that 1-methyl-2,3-bis(hydroxymethyl)benzo[*g*]indole bis[*N*-(2-propyl)carbamate] (**1**) has strong anticellular activities against cancer cells in in vitro tests [2] (Plate 1).

As a continuation of our work on prototype **1**, thiophene analogues **2** and **3** were synthesized and evaluated for cytotoxicity in the NCI's in vitro disease-oriented antitumor screen (Plate 2).

2. Chemistry

The two thieno[*i*,*j*-*g*]indole bis carbamates **2** and **3** were prepared as outlined in Scheme 1.

Methylation of 4,5-dihydrothieno[*i*,*j*-*g*]indoles **4**,**5** [3] with methyl iodide and sodium hydroxide in dry dimethyl sulfoxide (DMSO) gave **6**, **7** in 64 and 49% yields, respectively. Dehydrogenation of the 4,5-dihydrothienoindoles with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in CH_2Cl_2 provided tricyclic diesters



1

$\log_{10} GI_{50} = -5.85$

Plate 1.

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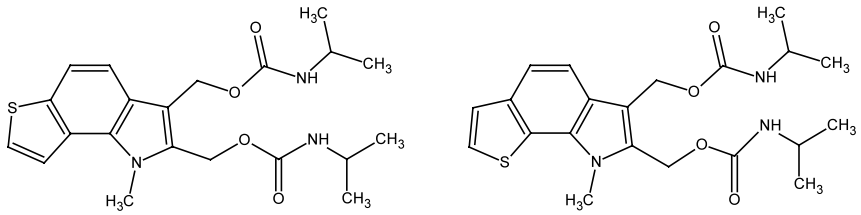
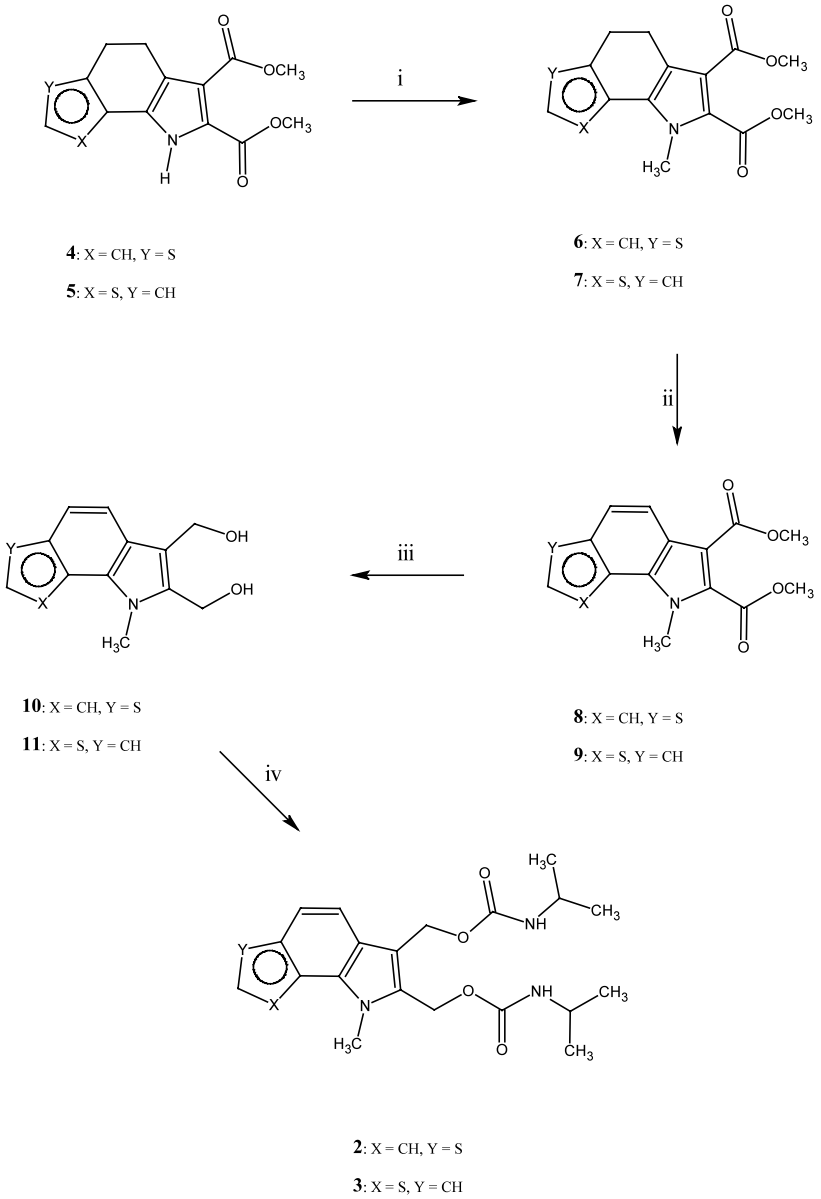


2 **3**

Plate 2.



Scheme 1. Reagents: (i) CH_3I/NaOH, dry DMSO; (ii) DDQ, CH_2Cl_2; (iii) DibAl-H, dry toluene; (iv) $(CH_3)_2CHNCO$, dry dioxane, dibutyltin diacetate.

8 and **9** whose structures were confirmed by ^1H NMR analyses. Reduction of the ester groups of **8** and **9** to **10** and **11** using diisobutylaluminium hydride (DibAl-H) followed by acylation of the carbinol groups with isopropylisocyanate provided the desired compounds **2** and **3** in good yields.

3. Results and discussion

The prepared thieno[*i*,*j*-*g*]indole biscarbamate compounds were evaluated for cytotoxicity in the NCI's in vitro disease-oriented antitumor screen [4] against approximately a panel of 60 human tumor cell lines derived from leukemia, non small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. In general the two compounds were found to be active with MG–MID values of -4.89 and -5.24, respectively. The results report in Table 1, as $\log_{10} GI_{50,}$ are those, in that cell line, lesser than the mean value over all cell lines tested.

Table 1
Inhibition of in vitro tumor cell growth by **2** and **3**

Panel/cell line	Cytotoxicity ($\log_{10} GI_{50}$ in μM)	
	2	**3**
Leukemia		
CCRF-CEM	−5.31	−5.64
HL-60 (TB)		−5.71
K-562	−5.22	
MOLT-4	−5.04	−6.05
RPMI-8226	−5.17	−5.53
SR	−5.37	
Non small cell lung cancer		
EKVX	−5.31	
HOP-92	−5.66	
NCI-H226	−5.34	
NCI-H23	−4.94	−5.51
NCI-H460	−5.17	−5.56
NCI-H522		−5.72
Colon cancer		
HCT-116	−4.92	
HCT-15	−4.92	
SW-620	−4.97	−5.74
CNS cancer		
SF-268	−4.94	−5.75
SF-539	−4.91	
SNB-75		−5.35
U251	−5.16	
Melanoma		
LOX IMVI	−5.19	−5.28
MALME-3M	−5.48	−5.47
UACC-62		−5.37
Ovarian cancer		
IGROV1	−5.47	
OVCAR-4	−4.92	−6.10
Renal cancer		
786-0	−5.02	
A498	−4.95	−5.25
ACHN	−4.91	−5.56
CAKI-1	−5.16	−6.21
RXF393	−4.95	−5.45
SN12C		−5.31
Prostate cancer		
DU-145		−5.47
Breast cance		
MCF7	−4.92	−5.36
NCI/ADR-RES		−5.42
HS 578T	−4.92	−5.60
MDA-MB-435	−4.97	−5.43
BT-549		−5.46

Data obtained from NCI's in vitro disease oriented tumor cell screen; the numerical values listed are $\log_{10} GI_{50}$ values which are the logs of the molar concentration causing 50% cell growth inhibition.

Interestingly, the cytotoxic potency and selectivity depended on the tricyclic scaffold on which the biscarbamate groups were linked. Compound **3** demonstrated potent and selective cytotoxicity against MOLT-4 (leukemia), OVCAR-4 (ovarian) and CAKI-1 (renal) cancer cells with $\log_{10} GI_{50}$ values of -6.05, -6.10 and -6.21, respectively. Cytotoxicities against other cell lines were significant or marginal ($\log_{10} GI_{50}$ values ranging from -5.74 to -4.62). Compound **2** showed significant selective cytotoxicity against NCI-HOP92 (non-small cell lung cancer), MALME-3M (melanoma) and IGROV1 (ovarian) with $\log_{10} GI_{50}$ values of -5.66, -5.48 and -5.47, respectively. It also displayed marginal cytotoxicity toward one-fourth of the tested cell lines ($\log_{10} GI_{50} < -4.89$). In summary, these results suggest that the thieno-[3,2-*g*]indole biscarbamate derivative **3** has potential as cytotoxic agent with cell line selectivity.

4. Experimental

IR spectra were recorded on a Perkin–Elmer 781 infrared spectrophotometer. ^{1}H NMR spectra (reference: TMS int.) were taken at 200 MHz on a Varian XL-200 instrument. UV spectra were registered on a Perkin–Elmer Lambda 5 model. Column chromatography was performed on a Baker silica gel F254. Melting points (m.p.) were determined on a Electrothermal 9100 apparatus and are uncorrected. Elemental analysis was performed by the Laboratorio di Microanalisi of Padova and analytical results were within $\pm 0.4\%$ of theoretical values.

4.1. General procedure for preparation of compounds *6*, *7*

To a stirred mixture of sodium hydroxide (13.7 mmol) in dry DMSO (8 ml) the diester (**4**,**5**) (3.4 mmol) was added. After 45 min stirring, the mixture was cooled with an external ice bath, then iodomethane (6.85 mmol) was added and stirring was prolonged for the same time at room temperature (r.t.) The mixture was then diluted with the same volume of water and the solid product collected, air-dried and crystallized from ethanol/water to give the desired product (**6**,**7**).

*4.1.1. Dimethyl 1-methyl-4,5-dihydro-1H-thieno[2,3-g]indol-2,3-dicarboxylate (**6**)*

(64% yield). M.p. 112–113 °C. IR (NUJOL) cm^{-1}: 1730, 1700. UV λ_{max} (EtOH) nm: 219, 301. ^1H NMR ($CDCl_3$) δ: 2.86–3.01 (4H, m), 3.85 (6H, s), 4.05 (3H, s), 7.19 (1H, d, $J = 5.2$ Hz), 7,29 (1H, d, $J = 5.2$ Hz). *Anal.* Calc. for $C_{15}H_{15}NO_4S$: C, 59.00; H, 4.95; N, 4.59; S, 10.50. Found: C, 59.02; H, 4.72; N, 5.12; S, 10.14%.

*4.1.2. Dimethyl 1-methyl-4,5-dihydro-1H-thieno[3,2-g]indole-2,3-dicarboxylate (**7**)*

(49% yield). M.p. 81–82 °C. IR (NUJOL) cm^{-1}: 1730, 1700. UV λ_{max} (EtOH) nm: 218, 252, 332, 349. ^1H NMR ($CDCl_3$) δ: 2.87 (4H, s); 3.85 (6H, s); 4.05 (3H, s); 6.98 (1H, d, $J = 5.0$ Hz); 7.21 (1H, d, $J = 5.0$ Hz). *Anal.* Calc. for $C_{15}H_{15}NO_4S$: C, 59.00; H, 4.95; N, 4.59; S, 10.50. Found: C, 59.13; H, 4.80; N, 5.02; S, 10.38%.

*4.2. General procedure for preparation of compounds **8**, **9***

DDQ was added, under stirring and in small quantities, to a solution of the *N*-methyl diester (**6**,**7**) (3.2 mmol) in dichloromethane (10 ml), until the green color persisted. The mixture was stirred at r.t. for additional 30 min. After removal of the solvent, the residue was flash-chromatographed over a silica gel column, eluting with a mixture of ethyl acetate and light petroleum in the ratio of 2:8 to give the desired product (**8**,**9**) as solid.

*4.2.1. Dimethyl 1-methyl-1H-thieno[2,3-g]indole-2,3-dicarboxylate (**8**)*

(76% yield). M.p. 67–68 °C. IR (NUJOL) cm^{-1}: 1730, 1710, 1610. UV λ_{max} (EtOH) nm: 233, 241, 249, 300. ^1H NMR ($CDCl_3$) δ: 3.95 (3H, s), 4.03 (3H, s), 4.21 (3H, s), 7.58 (1H, d, $J = 6.4$ Hz), 7.76 (1H, d, $J = 8.6$ Hz), 7.85 (1H, d, $J = 6.4$ Hz), 8.10 (1H, d, $J = 8.6$ Hz). *Anal.* Calc. for $C_{15}H_{13}NO_4S$: C, 59.39; H, 4.32; N, 4.62; S, 10.57. Found: C, 59.78; H, 4.37; N, 5.01; S, 10.81%.

*4.2.2. Dimethyl 1-methyl-1H-thieno[3,2-g]indole-2,3-dicarboxylate (**9**)*

(65% yield). M.p. 101–102 °C. IR (NUJOL) cm^{-1}: 1740, 1710, 1610. UV λ_{max} (EtOH) nm: 244, 256, 319. ^1H NMR ($CDCl_3$) δ: 3.95 (3H, s), 4.03 (3H, s), 4.21 (3H, s), 7.34 (1H, d, $J = 4.8$ Hz), 7.50 (1H, d, $J = 4.8$ Hz), 7.71 (1H, d, $J = 8.8$ Hz), 8.06 (1H, d, $J = 8.8$ Hz). *Anal.* Calc. for $C_{15}H_{13}NO_4S$: C, 59.39; H, 4.32; N, 4.62; S, 10.57. Found: C, 59.45; H, 4.41; N, 4.98; S, 10.63%.

*4.3. General procedure for preparation of compounds **10**, **11***

To a solution of thieno[*i*,*j*-*g*]indole (**8**,**9**) (3.2 mmol) in dry toluene (28 ml) under argon atmosphere was added dropwise a solution of 20% of DibAl-H in toluene (6.4 mmol) kept at 0 °C. The mixture was stirred at r.t. for 1 h, then cooled with an external ice bath, added of a aqueous solution of 10% sodium hydroxide and stirred overnight at r.t. The desired product (**10**, **11**) was collected by filtration.

*4.3.1. (1-Methyl-1H-thieno[2,3-g]indol-2,3-yl)-bis-hydroxymetyle (**10**)*

(93% yield). M.p. 124 °C. IR (NUJOL) cm^{-1}: 3415, 1605. UV λ_{max} (EtOH) nm: 238, 241, 296, 310, 323. ^1H NMR ($CDCl_3$) δ: 4.13 (2H, br s, exc. with D_2O), 4.17 (3H, s), 4.84 (4H, dd, $J = 6.2$ and 5.6 Hz), 7.50 (1H, d, $J = 5.6$ Hz), 7.59 (1H, d, $J = 8.6$ Hz), 7.68 (1H, d, $J = 8.6$ Hz), 7.86 (1H, d, $J = 5.6$ Hz). *Anal.* Calc. for $C_{13}H_{13}NO_2S$: C, 63.13; H, 5.30; N, 5.66; S, 12.97. Found: C, 62,98; H, 5.43; N, 5.77; S, 12.57%.

*4.3.2. (1-Methyl-1H-thieno[3,2-g]indol-2,3-yl)-bis-hydroxymetyle (**11**)*

(68% yield). M.p. 161–162 °C. IR (NUJOL) cm^{-1}: 3320, 3200, 1610. UV λ_{max} (EtOH) nm: 234, 240, 250, 255, 319. ^1H NMR ($CDCl_3$) δ: 4.13 (2H, br s, exc. with D_2O), 4.17 (3H, s), 4.85 (4H, dd, $J = 6.6$ and 6.2 Hz), 7.36 (1H, d, $J = 5.6$ Hz), 7.47 (1H, d, $J = 5.6$ Hz), 7.55 (1H, d, $J = 8.4$ Hz), 7.69 (1H, d, $J = 8.4$ Hz). *Anal.* Calc. for $C_{13}H_{13}NO_2S$: C, 63.13; H, 5.30; N, 5.66; S, 12.97. Found: C, 63.25; H, 5.12; N, 5.53; S, 12.68%.

*4.4. General procedure for preparation of compounds **2**, **3***

A mixture of diol (**10**, **11**) (3.85 mmol) in an excess of isopropylisocyanate (12.36 mmol) and in the presence of few drops of dibutyltin diacetate was heated at 50 °C for 30 min under argon atmosphere. Then dry dioxane (21 ml) was added and the resulting solution was heated at 70–75 °C for 1 h. After concentration under reduced pressure, the crude residue was purified by crystallization from a mixture of ethyl acetate and hexane to give the target compound (**2**, **3**).

*4.4.1. (1-Methyl-1H-thieno[2,3-g]indol-2,3-yl)bismethyl N-isopropylcarbamate (**2**)*

(63% yield). M.p. 184–185 °C. IR (NUJOL) cm^{-1}: 3320, 1680. UV λ_{max} (EtOH) nm: 235, 309, 322. ^1H NMR ($CDCl_3$) δ: 1.13 (12H, dd, $J = 6.2$ and 6.6), 3.82 (2H, m), 4.15 (3H, s), 4.79 (2H, br s, exc. with D_2O), 5.40 (4H, s), 7.51 (1H, d, $J = 8.2$ Hz), 7.67 (1H, d, $J = 8.2$ Hz), 7.72 (1H, d, $J = 5.4$ Hz), 7.84 (1H, d, $J = 5.4$ Hz). *Anal.* Calc. for $C_{21}H_{27}N_3O_4S$: C, 60.41; H, 6.52; N, 10.06; S, 7.68. Found: C, 59.99; H, 6.52; N, 10.01; S, 7.82%.

*4.4.2. (1-Methyl-1H-thieno[3,2-g]indol-2,3-yl)bismethyl N-isopropylcarbamate (**3**)*

(57% yield). M.p. 185–187 °C. IR (NUJOL) cm^{-1}: 3290, 1670. UV λ_{max} (EtOH) nm: 204, 242, 293, 318. ^1H NMR ($CDCl_3$) δ: 1.14 (12H, dd, $J = 5.8$ and 6.4), 3.83 (2H, m), 4.13 (3H, s), 4.81 (2H, br s, exc. with D_2O), 5.44 (4H, s), 7.51 (1H, d, $J = 8.2$ Hz), 7.67 (1H, d, $J = 8.2$ Hz), 7.72 (1H, d, $J = 5.4$ Hz), 7.84 (1H, d, $J = 5.4$ Hz). *Anal*. Calc. for $C_{21}H_{27}N_3O_4S$: C, 60.41; H, 6.52; N, 10.06; S, 7.68. Found: C, 60.03; H, 6.48; N, 10.22; S, 7.73%.

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References

[1] Wilson, Gisvold, in: J.N. Delgado, W.A: Remers (Eds.), Textbook of Organic Medicinal and Phamaceutical Chemistry, Lippincott-Raven publishers, Philadelphia, NY, 1998, pp. 343–401.

[2] G.A. Pinna, M.A. Pirisi, M. Sechi, G. Paglietti, Synthesis and in vitro anticancer activity evaluation of biscarbamic esters of 2,3-(hydroxymethyl)-1-methyl-7- and 7,8- substituted-benzo[*g*]indoles, Farmaco 53 (1998) 161–168.

[3] (a) G.A. Pinna, M.A. Pirisi, G. Paglietti, Addition reactions of acetylenic esters upon 1-(2-thienyl)- and 1-(3-thienyl)-ethanone oximes and upon 6,7-dihydro-benzo[*b*]thiophen-4(5*H*)-one and 5,6-dihydrobenzo[*b*]thiophen-7(4*H*)-one oximes. Formation of 2-(thienyl)-pyrroles and 4,5-dihydro-1*H*-thieno[*g*]indoles, J. Chem Research (S) (1993) 210–211;
(b) G.A. Pinna, M.A. Pirisi, G. Paglietti, Addition reactions of acetylenic esters upon 1-(2-thienyl)- and 1-(3-thienyl)-ethanone oximes and upon 6,7-dihydro-benzo[*b*]thiophen-4(5*H*)-one and 5,6-dihydrobenzo[*b*]thiophen-7(4*H*)-one oximes. Formation of 2-(thienyl)-pyrroles and 4,5-dihydro-1*H*-thieno[*g*]indoles, J. Chem Research (M) (1993) 1279–1296.

[4] (a) A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A.Waigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines, J. Natl. Cancer Inst. 83 (1991) 757–766;
(b) K.D. Paull, R.H. Shoemaker, L. Hodes, A. Monks, D.A. Scudiero, L. Rubinstein, J. Plowman, M.R. Boyd, Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm, J. Natl. Cancer Inst. 81 (1989) 1088–1092;
(c) M.R. Boyd, K.D. Paull, L.R. Rubinstein, In: F.A. Valeriote, T. Corbett, L. Baker, (eds.), Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery, Kluwer Academic Publishers, Amsterdam (1992) 11–34.