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Synthesis and antitumor properties of 2,5-bis(3′-indolyl)thiophenes: Analogues of marine alkaloid nortopsentin

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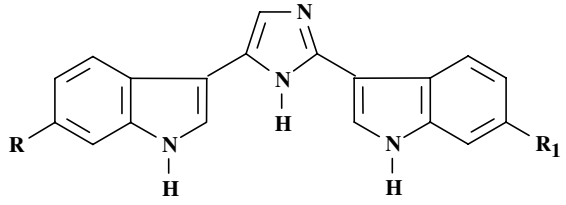
Abstract—A series of 11 bis-indolylthiophenes of type **8–10** were obtained by cyclization of diketones **4** and **7** using Lawesson's reagent. Derivatives **8c**, **9c**, **9d**, and **10c** were selected to be evaluated in the full panel of about 60 human tumor cell lines derived from nine human cancer cell types and showed antiproliferative activity generally in the micromolar range. The most sensitive cell lines were: CCRF-CEM, MOLT-4, HL60 (TB), and RPMI-8226 of the leukemia subpanel, HT29 and HCC-2998 cell lines of the colon sub-panel, NCI-H522 of the non-small cell lung cancer sub-panel, LOX IMVI of the melanoma sub-panel, and UO-31 of the renal cancer sub-panel.

Bis(indolyl)alkaloids are recognized as one of the rapidly growing groups of sponge metabolites because of their broad spectrum of biological properties including antimicrobial, antiviral, and antitumor activities. Nortopsentins A–C, having a characteristic 2,4-bis(3′-indolyl)imidazole skeleton, exhibited in vitro cytotoxicity against P388 cells (IC50 1.7, 7.6, and 7.8 μg/mL, respectively). Their *N*-indolyl methylated derivatives showed significant improvement in P388 activity compared with that of the parent compounds (IC50 0.34–0.90 μg/mL).[1–3]



Nortopsentin A R = R_1 = Br
Nortopsentin B R = Br, R_1 = H
Nortopsentin C R = H, R_1 = Br

A great limitation in the use of the reservoir of marine organisms for therapy is that only very small amounts of the biologically active substances are isolated from the natural material. Due to the interesting biological activities, different analogues of the marine nortopsentins have been synthesized.

Thus, many bis(indolyl)alkaloids in which the imidazole moiety of nortopsentins was replaced by thiazole, pyrimidine, pyrazine, and pyrazinone rings were reported.[4–8]

2,4-Bis(3′-indolyl)thiazole analogues exhibited cytotoxic activities against a wide range of human tumor cell lines at micromolar concentration.[4,5] Also 2,5-bis(3′-indolyl)pyrazines and 3,6-(3′-indolyl)2-(1*H*)pyrazinone showed inhibitory activity against a variety of human tumor cell lines with GI50 values that reached submicromolar level. In these series the pyrazinone derivative was less active than the pyrazine one, whereas the *N*-indolyl methylated compound was the most active showing GI50 values between 0.058 and 7.19 μM.[5,6]

In our effort to search for novel antitumor compounds, we designed new analogues with further modification of indole alkaloids to get more potent and selective agents. Thus we thought to synthesize several different series of bis-indolyl-5-membered heterocycles in order to verify

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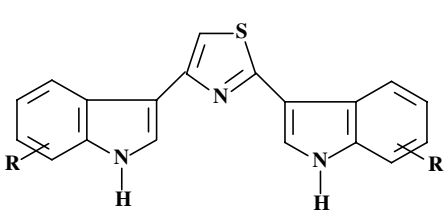
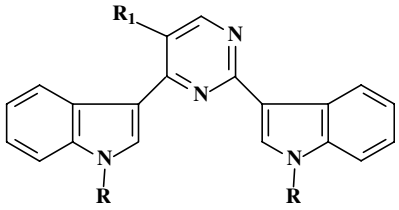
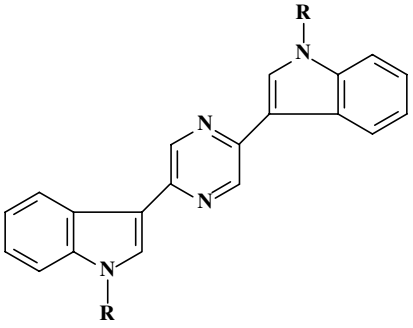


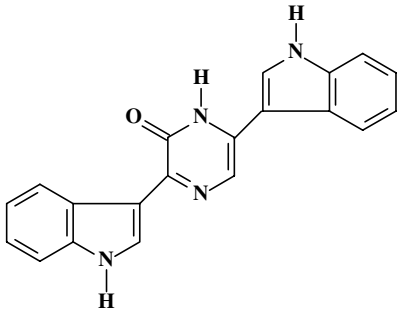
2,4-bis(3'-indolyl)thiazoles



2,4-bis(3'-indolyl)pyrimidines



2,5-bis(3'-indolyl)pyrazines



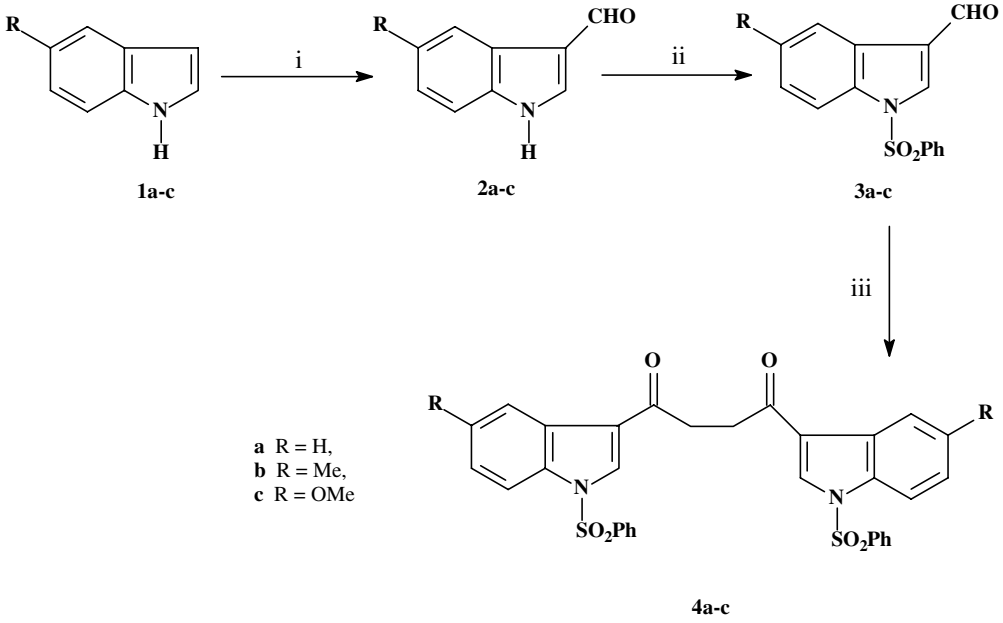
3,6-bis(3'-indolyl)-2(1*H*)pyrazinone

the influence of the central heterocyclic ring on the antineoplastic activity.

In this paper, we report the synthesis of bisindolylthiophene derivatives of type **8**–**10**, in which the imidazole moiety of the nortopsentin was replaced by a thiophene ring, and the NCI's in vitro disease-oriented antitumor screen of the most potent in this series.

1,4-Bis-indolyl-diketones appeared valuable and versatile intermediates for the synthesis of bis(indolyl)thiophenes. Indole derivatives of type **1** were converted into indole-3-carboxaldehydes **2** by a Vilsmeier–Haack reaction with phosphorus oxychloride and dimethylformamide (85–99%). These latter compounds were protected with the benzensulfonyl group at the NH to give *N*-benzensulfonyl-indole-3-carboxaldehydes **3** (95–99%). The next step involves a Stetter reaction between the electron-deficient indole aldehyde and divinyl sulfone using sodium acetate and thiazolium chloride as catalyst in refluxing ethanol to afford 1,4-diketones **4** (55–66%)[9] (Scheme 1).

Alternatively, the treatment of the indole derivatives **1** with potassium *tert*-butoxide, TDA-1 as catalyst, and methyl iodide in benzene yielded the corresponding



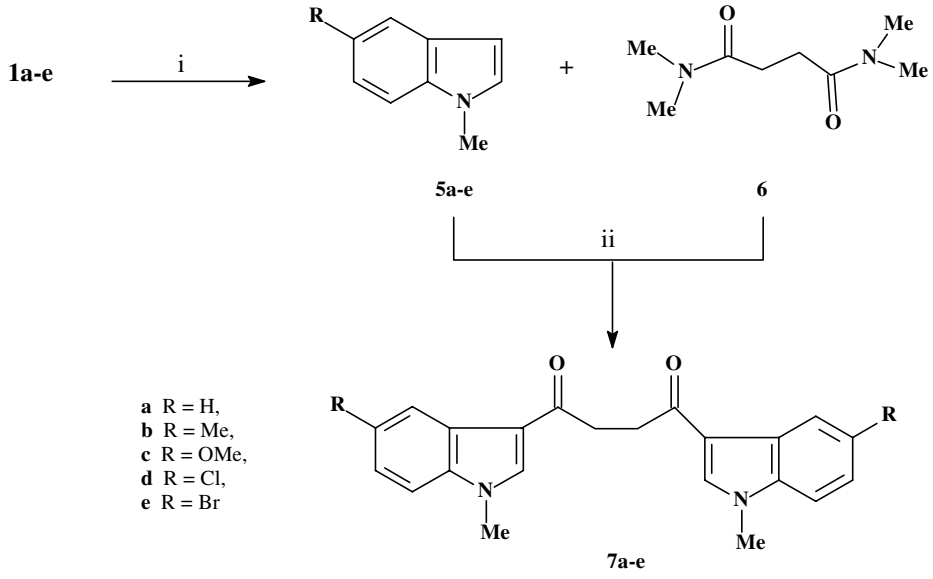
1a-c 2a-c 3a-c 4a-c

Scheme 1. Synthesis of intermediates **4a**–**c**. Reagents and condition: (i) $POCl_3$/DMF, NaOH; (ii) NaH/THF, $ClSO_2Ph$; (iii) thiazolium salt/EtOH, NaOAc/Divinyl sulfone, reflux.

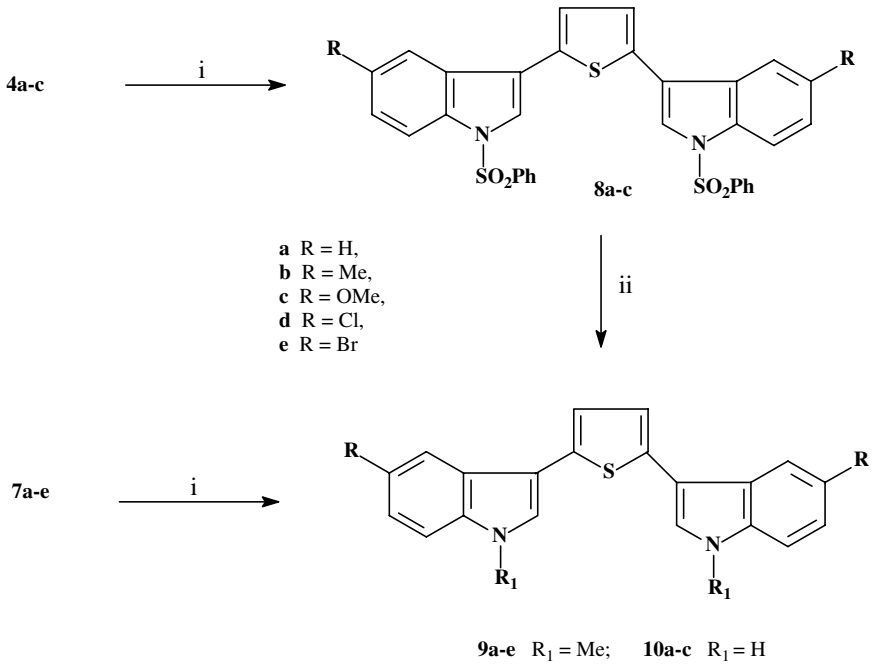
N-methyl derivatives **5** (96–98%). These latter compounds were converted into 1,4-diketones of type **7** by Vilsmeier–Haack reaction using phosphorus oxychloride and tetramethylsuccinamide **6** (56–70%) (Scheme 2).

The resulting diketones **4a**–**c** and **7a**–**e** were converted into the corresponding bis(indolyl)thiophenes **8a**–**c** and **9a**–**e** in excellent yields with Lawesson's reagent in refluxing toluene (88–99%). Hydrolysis of compounds **8a**–**c**, with KOH in refluxing ethanol, gave the corresponding thiophenes of type **10a**–**c** (65–86%) (Scheme 3).

All the bis(indolyl)thiophene compounds **8**–**10**[10] were submitted to the National Cancer Institute (Bethesda, MD); four of them were selected (**8c**, **9c**, **9d**, and **10c**), for evaluation the full panel of about 60 human cancer cell lines derived from nine human cancer cell types, that have grouped in disease sub-panels including leukemia, non-small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast tumor cell lines. The compounds were tested at five concentrations at 10-fold dilution the highest being 10^{-4} M and the others 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M.[11]



Scheme 2. Synthesis of intermediates **7a**–**e**. Reagents: (i) *t*-BuOK/TDA-1, benzene, CH_3I; (ii) $POCl_3$/DMF.



Scheme 3. Synthesis of 2,5-bis-indolylthiophenes. Reagents and conditions: (i) Lawesson's reagent/toluene, reflux; (ii) KOH/EtOH, reflux.

The most active compound is derivative **10c**. The antitumor activity is given by three parameters for each cell line; pGI50 value, pTGI value, and pLC50 value. Moreover, a mean graph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving an average activity parameter over all cell lines.

An evaluation of the data reported in Tables 1 and 2 revealed that compound **10c** was cytotoxic showing GI50 values against the total number of cell lines investigated at micromolar concentration. Moreover positive TGI and LC50 values were observed with respect to a congruous number of cell lines (88% and 46%, respectively).

Derivative **10c** was particularly efficacious against the leukemia sub-panel having GI50 in the range 0.34–3.54 μM. The most sensitive leukemia cell lines are CCRF-CEM (GI50 0.34 μM), MOLT-4 (GI50 1.91 μM), HL60 (TB) (GI50 2.27 μM), and RPMI-8226 (GI50 2.83 μM).

Compound **10c** showed good selectivity with respect to the HT29 (GI50 2.79 μM) and HCC-2998 (GI50 2.83 μM) cell lines of the colon sub-panel. It also showed selectivity with respect to NCI-H522 (GI50 1.31 μM) of the non-small cell lung cancer sub-panel, LOX IMVI (GI50 2.55 μM) of the melanoma sub-panel, and UO-31 (GI50 2.66 μM) of the renal cancer sub-panel.

At TGI and LC50 level, the best responses were observed in the case of the HCC-2998 (TGI 6.50 μM and LC50 27.2 μM,) colon cancer cell line and LOX IMVI (TGI 7.08 μM and LC50 38.3 μM), melanoma cell lines.

In order to discern the mechanism of action of derivative **10c** we performed COMPARE computations against the NCI 'Standard Agents' database.[12] Compound **10c** had a Pearson correlation coefficient (PCC) <0.6 suggesting that the antiproliferative activity would be mechanistically unrelated to that of any known drug.

Table 1. Overview of the results of the in vitro antitumor screening for compound **10c**[a]

	No.[e]	No.[f]	Range	MG_MID[g]
pGI50[b]	56	56	6.46–4.66	5.2
pTGI[c]	56	49	5.40–4.18	4.55
pLC50[d]	57	26	4.57–4.01	4.1

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cell screen.
[b] pGI50 is the −log of the molar concentration that inhibits 50% net cell growth.
[c] pTGI is the −log of the molar concentration giving total growth inhibition.
[d] pLC50 is the −log of the molar concentration leading to 50% net cell death.
[e] No. is the number of cell lines investigated.
[f] No. is the number of cell lines giving positive pGI50, pTGI, and pLC50.
[g] MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.

Table 2. Inhibition of in vitro cancer cell lines by compound **10c**[a]

Cell lines	GI50 (μM)[b]
Leukemia	
CCRF-CEM	0.34
HL-60 (TB)	2.27
K-562	3.54
RPMI-8226	2.83
MOLT-4	1.91
Non-small cell lung cancer	
A549/ATCC	3.66
EKVX	13.8
HOP-62	9.88
HOP-92	4.64
NCI-H226	16.2
NCI-H23	11.1
NCI-H322M	21.8
NCI-H460	3.24
NCI-H522	1.31
Colon cancer	
COLO-205	10.6
HCC-2998	2.83
HCT-15	3.17
HT29	2.79
KM12	3.39
SW-620	3.66
CNS cancer	
SF-268	13.0
SF-295	3.03
SF-539	5.81
SNB-19	19.0
SNB-75	Nd[c]
U251	3.91
Melanoma	
LOX IMVI	2.55
MALME-3M	17.3
M14	13.5
SK-MEL-2	16.4
SK-MEL-28	14.5
SK-MEL-5	5.69
UACC-257	18.0
UACC-62	15.8
Ovarian cancer	
IGROV1	10.8
OVCAR-3	10.8
OVCAR-4	9.04
OVCAR-5	12.9
OVCAR-8	6.35
SK-OV-3	19.2
Renal cancer	
786-0	3.29
A498	3.29
ACHN	4.21
CAKI-1	12.0
RXF 393	3.98
SN12C	14.0
TK-10	11.4
UO-31	2.66
Prostate cancer	
PC-3	4.51
DU-145	6.05
Breast cancer	
MCF7	6.71
NCI/ADR-RES	3.76

(*continued on next page*)

Table 2 (*continued*)

Cell lines	GI50 (μM)[b]
MDA-MB-231/ATCC	4.20
HS 578T	11.7
MDA-MB-435	13.7
BT-549	11.0
T-47D	11.1

[a] Data obtained from NCI's in vitro disease-oriented tumor cell screen.
[b] The cytotoxicity GI50 values are the concentrations corresponding to 50% growth inhibition of tumor cells.
[c] Not determined.

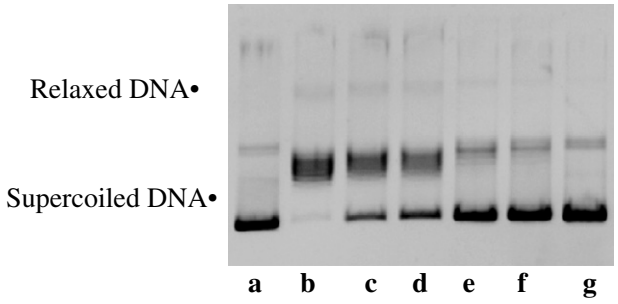


Figure 1. Effect of derivative **10c** on the relaxation of supercoiled plasmid DNA by human recombinant Topoisomerase II.[14]

Moreover, we performed experiments on the ability of derivative **10c** to interfere with the catalytic activity of DNA Topoisomerase II.

Indeed, it was widely demonstrated that a number of anticancer drugs exert their antiproliferative effect by inhibiting this nuclear enzyme.[13]

Figure 1 shows the relaxation of supercoiled plasmid DNA mediated by Topoisomerase II in the absence (lane b) and in the presence of increasing concentrations of **10c** (lanes c–f). The results obtained indicate a somewhat weaker inhibitory capacity at the lower concentrations taken into consideration (1 and 10 μM, lanes c and d, respectively). The inhibitory effect increases in a dose-dependent manner and at 200 μM concentration is comparable with that induced by the well-known Topoisomerase II inhibitor *m*-amsacrine, used at 8 μM concentration (compare lanes f and g). Considering that the antiproliferative effect exerted by **10c** for a number of cell lines is in the 0.34–21.8 μM range (Tables 1 and 2), the inhibition of Topoisomerase II does not seem the main cause of cell death, even though an undoubtable effect on the enzyme occurs. Topoisomerase II modifies the topological states of DNA and a number of antiproliferative drugs, which affect its catalytic activity, are known to be DNA binders.[13] Therefore, it appeared of interest to investigate on the ability of **10c** to form a molecular intercalative complex by means of flow linear dichroism experiments, performed with salmon testes DNA. The spectra of DNA alone and in presence of **10c** do not evidence any detectable difference suggesting the incapacity of the test derivative to intercalate inside the macromolecule (data not shown). In conclusion, it is conceivable that more than one cellular molecular target contributes to the antiproliferative ability of **10c**.

Acknowledgments

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10. H and ^{13}C NMR, IR, elemental analysis (C, H, and N), and melting point determination. For example, for compound **10c**: mp 201–202 °C; IR 3402 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.84 (s, 3H, OCH_3), 6.85 (dd, $J = 2.0$, 8.8 Hz, 1H, H-6), 7.31 (s, 1H, H-3′), 7.37 (d, $J = 8.8$ Hz, 1H, H-7) 7.39 (s, 1H, H-2), 7.68 (d, $J = 2.0$ Hz, 1H, H-4), 11.3 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 55.3 (q), 100.9 (d), 109.8 (s), 111.9 (d), 112.7 (d), 121.9 (d), 123.5 (d), 125.0 (s), 131.7 (s), 134.2 (s), 154.0 (s).
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14. Supercoiled pBR322 DNA (0.25 μg, lane a) was incubated for 60 min at 37 °C with Topoisomerase II (1 U) in the absence (lane b) or presence of **10c** at 1, 10, 100, and 200 μM (lanes c–f, respectively). Addition of *m*-amsacrine 8 μM (lane g) was used as reference. DNA samples were separated by electrophoresis on a 1% agarose gel. The gel was stained with ethidium bromide 1 μg/mL in TAE buffer, transilluminated by UV light, and fluorescence emission visualized using a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.