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3,5-Bis(3′-indolyl)pyrazoles, analogues of marine alkaloid nortopsentin: Synthesis and antitumor properties

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Abstract—A series of 10 bis-indolylpyrazoles of type **9**, **10** were obtained by cyclization of diketones **8** using hydrazine monohydrate or methylhydrazine in refluxing acetic acid/THF. Derivatives **9a**,**c**,**d** were selected, by the National Cancer Institute (NCI, Bethesda, USA), to be evaluated against the full panel of about 60 human tumor cell lines derived from nine human cancer cell types and showed antiproliferative activity in the micromolar range. In particular, **9d**, the most active compound was effective against all the tested cell lines with a GI_{50} mean value of 3.23 μM; TGI and LC_{50} values were 14.5 and 58.9 μM having positive response on 91% and 41% of the tested cell lines, respectively.

Marine sponge of the genera *Spongosorites* has been reported to yield bisindole alkaloids. The interest in this class of compounds has been stimulated by both their unique chemical structure and the wide range of biological properties including antiviral, antimicrobial, and antitumor activity.[1] *Nortopsentins A–C*, with its 2,4-bis(3′-indolyl)imidazole skeleton, exhibited in vitro cytotoxicity against P388 cells (IC_{50} 4.5–20.7 μM) and antifungal activity against *Candida albicans* (Fig. 1). Their *N*-indolyl methylated derivatives showed significant improvement in P388 activity if compared to the activity of parent compounds (IC_{50} 0.8–2.1 μM).[2–4] Due to their interesting biological activities, nortopsentins have been considered important lead compounds for the discovery of new biological active derivatives.

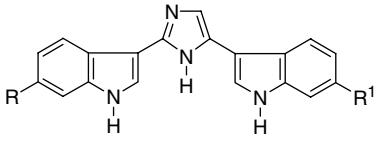

Figure 1.

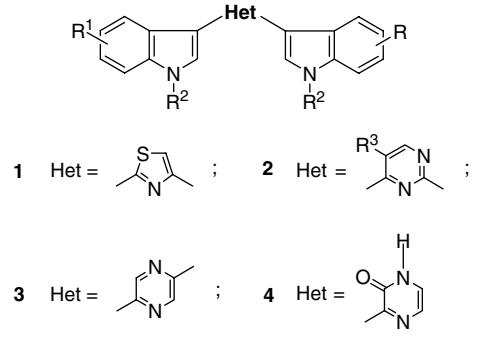

Figure 2.

Various analogues of the marine nortopsentins have been reported. Many bis(indolyl)alkaloids where the imidazole moiety of nortopsentin was replaced by thiazole (**1**), pyrimidine (**2**), pyrazine (**3**), and pyrazinone (**4**) rings were designed and synthesized (Fig. 2). These analogues presented strong inhibitory activity against a wide range of human tumor cell lines (GI_{50} <0.01–89.4 μM).[5–9]

Keywords: Nortopsentins; 3,5-Bis(3′-indolyl)pyrazoles; Antitumor activity; Topoisomerase II.
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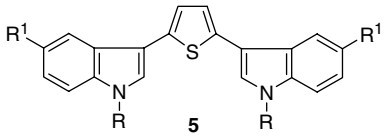


Figure 3.

We have recently reported the synthesis and the antitumor activity of 2,5-bis(3′-indolyl)thiophenes of type **5** where the imidazole moiety of the nortopsentin was replaced by a thiophene ring (Fig. 3). Such derivatives showed antiproliferative activity generally in the micromolar range, and proved to be particularly effective against the leukemia sub-panel (GI_{50} 0.34–3.54 μM).[10]

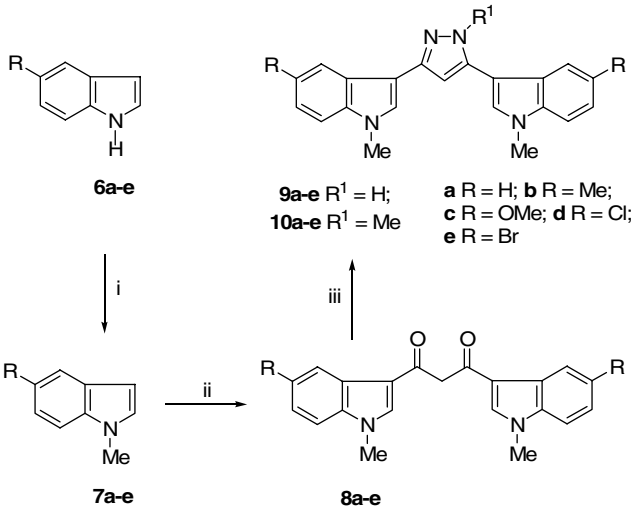
In the attempt of looking for novel antitumor compounds, we designed new analogues with further modification of indole alkaloids in order to get more potent and selective agents. We therefore planned to synthesize a new series of bis-indolyl-5-membered heterocycles so as to verify the influence of the central heterocyclic ring on the antineoplastic activity.

In this paper, we report the synthesis of 3,5-bis(3′-indolyl)pyrazoles **9**, **10**, where a pyrazole central ring substituted the imidazole ring of nortopsentin, and the NCI's in vitro disease-oriented antitumor screen of the most potent compounds of this series.

1,3-Bis-indolyl-diketones **8** appeared as valuable and versatile intermediates for the synthesis of bis(indolyl)pyrazoles. The synthetic approach to the pyrazole compounds involved the indole derivatives of type **6**, which were converted into the corresponding *N*-methyl derivatives **7** using potassium *t*-butoxide, tris(3,6-dioxaheptyl)amine (TDA-1) as a catalyst and methyl iodide in dry benzene (96–98%). Friedel-Craft reaction of the *N*-methylindoles **7** with malonyldichloride in dichloromethane yielded the expected 1,3-diketones of type **8** (45–70%). The resulting symmetrical 1,3-diketones **8** were converted into corresponding 3,5-bis(3′-indolyl)-pyrazoles **9**, **10** using hydrazine monohydrate or methylhydrazine in refluxing acetic acid/THF (54–92%) (Scheme 1).

All the bis(indolyl)pyrazoles **9**, **10**[11] were submitted to the National Cancer Institute (Bethesda, MD). Three of them were selected (**9a**,**c**,**d**) for evaluation against the full panel of about 60 human cancer cell lines derived from nine human cancer cell types, grouped into disease sub-panels including leukemia, non-small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast tumors 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}, 10^{-8} M.[12]

In this series bis(indolyl)pyrazoles **9a**,**d** resulted the most active compounds. The antitumor activity is given by three parameters for each cell line; GI_{50} value, TGI value, and LC_{50} value. Moreover, a mean graph midpoint (MG_MID) was calculated for each of the mentioned



Scheme 1. Reagents and conditions: (i) *t*-BuOK, MeI, TDA-1/benzene rt, 24 h; (ii) malonyldichloride/DCM, rt, 2 h; (iii) $NH_2NH_2{\cdot}H_2O$ or NH_2NHMe, THF/AcOH, reflux, 24 h.

parameters, giving an average activity parameter over all cell lines.

Data evaluation of the data reported in Table 1 pointed out that compounds **9a**,**d** exhibited antineoplastic activity against most of the human cell lines. Compound **9d**, bearing a chloro group, is more active than the unsubstituted derivative **9a** both in terms of GI_{50} (mean value 3.23 and 18.2, respectively) and of percentage of sensitive cell lines out of the total number of cell lines investigated (100% and 90%, respectively). Derivative **9d** was

Table 1. Overview of the results of the in vitro antitumor screening for compounds **9a**,**d**[a]

		Compound	
		9a	**9d**
No[e]		58	58
N[f]		52	58
GI_{50}[b]	Range	1.95 to 93.1	1.55 to 9.60
	MG_MID[g]	18.2	3.23
No		59	58
N		16	53
TGI[c]	Range	2.06 to >100	1.63 to 94.8
	MG_MID	79.4	14.5
No		59	59
N		4	24
LC_{50}[d]	Range	59.7 to 74.3	6.62 to 96.8
	MG_MID	97.7	58.9

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen.
[b] GI_{50} is the μM concentration that inhibits 50% net cell growth.
[c] TGI is the μM molar concentration giving total growth inhibition.
[d] LC_{50} is the μM molar concentration leading to 50% net cell death.
[e] No is the number of the cell lines investigated.
[f] N is the number of cell lines giving positive GI_{50}, TGI and LC_{50}.
[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.

cytotoxic against the total number of cell lines investigated at micromolar concentration, and in particular it proved to be selective with respect to the melanoma sub-panel, having all the sub-panel cell lines GI_{50} in the range 1.63–9.64. The most sensitive cell lines were UACC-62, LOX IMVI, and SK-MEL-5 GI_{50} 1.63, 1.70, and 1.79, respectively). It also showed selectivity with respect to MOLT-4, SR, and K-562 (GI_{50} 1.55, 2.36, and 2.78, respectively) of leukemia sub-panel, HCC-2998 and COLO 205 (GI_{50}) 1.71 and 2.22, respectively) of colon cancer, CAKI-1 (GI_{50} 1.70) of renal cancer, BT-549 (GI_{50} 2.03) of breast cancer, and SF-539 (pGI_{50} 1.81) of CNS sub-panel. Derivative **9a** was particularly effective against the colon sub-panel having GI_{50} in the range 4.58–19.0. The most sensitive colon cell lines were KM12 and HCC-2998 (GI_{50}) 4.58 and 4.74, respectively). Compound **9a** showed good selectivity with respect to the HOP-92 (GI_{50} 2.06) and NCI-H460 (GI_{50} 4.48) of the non-small-cell lung cancer sub-panel and MCF7 (GI_{50} 3.95) of the breast cancer sub-panel (Table 2).

Moreover, as to **9d** derivative, positive TGI and LC_{50} responses were observed with respect to a noticeable number of cell lines (91% and 41%, respectively).

The best responses were observed in the case of the SF-539 (TGI 3.47 and LC_{50} 6.62) CNS cancer, HCC-2998 (TGI 3.84 and LC_{50} 8.63) colon cancer, and LOX IMVI (TGI 3.80 and LC_{50} 8.53) melanoma cell lines (data not shown).

In order to discern the mechanism of action of bis(indolyl)pyrazoles, we performed COMPARE computations for derivatives **9a**,**d** against the NCI 'Standard Agents' database.[13] Compound **9a**,**d** had a Pearson Correlation Coefficient (PCC) <0.6 suggesting that the antiproliferative activity of 3,5-bis(3′-indolyl)pyrazoles would be mechanistically unrelated to that of any known drug.

Experiments aiming at the evaluation of **9a**,**d** derivatives' capability of interacting with DNA—a crucial target for most of the known antitumor drugs—revealed that such derivatives were unable to form a molecular complex with the macromolecule. In particular, linear flow dichroism spectra obtained by salmon testes DNA, where different concentrations of **9a**,**d** were present, resulted practically overlapping to those recorded without those test compounds (spectra not shown). Furthermore, the ability of interfering with the activity of the nuclear enzyme topoisomerase II, which catalyzes the interconversion of different topological forms of DNA,[14] was assayed. Figure 4A and B show the relaxation of supercoiled plasmid DNA mediated by the enzyme, when **9a**,**d** were absent (lanes b) and when **9a**,**d** were present in increasing concentrations (lanes c–e). As to **9a**, the results obtained point out that the inhibitory capability becomes detectable at about 50 μM (Fig. 4A, lane d), while in the case of **9d** it happens at 100 μM (Fig. 4B, lane e). Furthermore, both test compounds present a scored inhibition which is significantly weaker than the one of *m*-amsacrine used at 8 μM concentration (lane f).

Table 2. Inhibition of in vitro cancer cell lines by compound **9a**,**d**[a]

Cell line	GI_{50}[b]	
	9a	**9d**
Leukaemia		
CCRF-CEM	14.4	6.40
HL-60 (TB)	15.4	4.45
K-562	42.1	2.78
RPMI-8226	37.8	6.79
MOLT-4	4.75	1.55
SR	3.46	2.36
Non-small cell lung cancer		
A549/ATCC	16.3	3.24
EKVX	27.2	3.15
HOP-62	17.3	7.98
HOP-92	2.06	1.86
NCI-H226	19.8	5.29
NCI-H23	22.0	2.49
NCI-H322M	>100	5.73
NCI-H460	4.48	2.35
NCI-H522	22.1	1.75
Colon cancer		
COLO-205	7.98	2.22
HCC-2998	4.74	1.71
HCT-116	19.0	3.82
HCT-15	8.68	3.01
HT29	5.35	3.57
KM12	4.58	3.45
SW-620	>100	3.52
CNS Cancer		
SF-268	16.0	5.29
SF-295	17.1	2.52
SF-539	16.2	1.81
SNB-19	27.0	4.09
SNB-75	18.4	4.25
U251	12.9	3.37
Melanoma		
LOX IMVI	5.38	1.70
MALME-3M	93.1	9.64
M14	>100	2.15
SK-MEL-2	57.5	2.34
SK-MEL-28	>100	4.80
SK-MEL-5	13.1	1.79
UACC-257	80.6	4.27
UACC-62	16.4	1.63
Ovarian cancer		
IGROV1	8.90	2.55
OVCAR-3	28.4	4.41
OVCAR-4	34.9	5.82
OVCAR-5	>100	3.11
OVCAR-8	28.8	4.03
SK-OV-3	31.6	5.81
Renal cancer		
786-0	14.1	3.41
A498	19.6	2.44
ACHN	7.67	3.23
CAKI-1	>100	1.70
RXF 393	14.7	3.03
SN12C	1.95	Nd[c]
TK-10	37.4	5.16
UO-31	37.6	3.01
Prostate cancer		
PC-3	8.11	3.05
DU-145	15.6	9.60

Table 2 (*continued*)

Cell line	GI_{50}[b]	
	9a	**9d**
Breast cancer		
MCF7	3.95	2.64
NCI/ADR-RES	27.6	2.25
MDA-MB-231/ATCC	8.06	2.95
HS 578T	18.5	3.27
MDA-MB-435	Nd	2.99
BT-549	15.9	2.03
T-47D	79.7	4.06

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen.
[b] GI_{50} is the μM concentration that inhibits 50% net cell growth.
[c] Not determined.

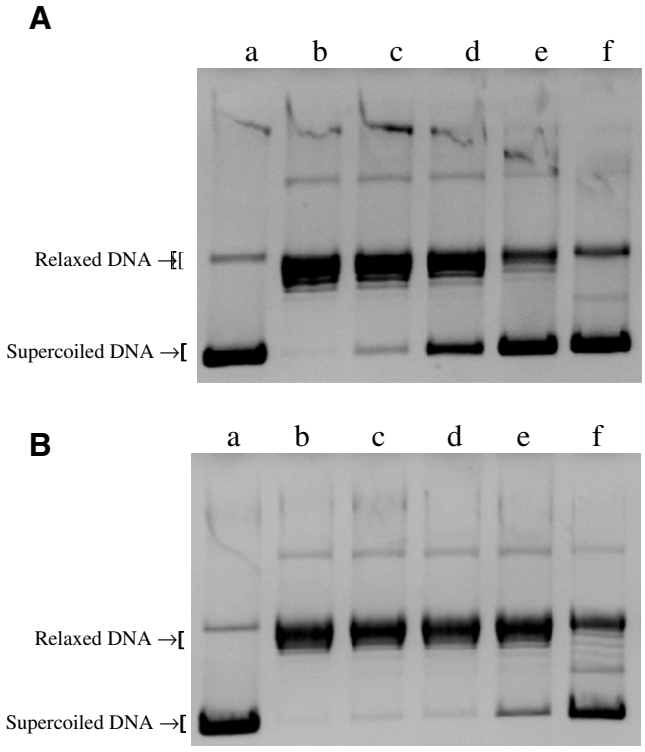


Figure 4. (A and B) Effect of derivatives **9a** (**4A**) and **9d** (**4B**) on the relaxation of supercoiled plasmid DNA by human recombinant topoisomerase II. Supercoiled pBR322 DNA (0.25 μg, lanes a) was incubated for 60 min at 37 °C with topoisomerase II (1 U) in the absence (lanes b) or presence of test compounds at 10, 50, 100 μM (lanes c–e, respectively). Addition of *m*-amsacrine 8 μM (lane f) 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.

These results point out that DNA cannot be considered the main cause of cell death, suggesting that other cellular molecular targets participate in the antiproliferative effect of **9a**,**d**.

In conclusion, the substitution of the imidazole core of nortopsentin with a pyrazole ring displayed a similar potency to the natural product but a wider spectrum of activity as testified by the GI_{50} at μM level observed for the 58 tested cell lines.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.042.

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