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Synthesis and antitumor activity of 2,5-bis(3′-indolyl)-furans and 3,5-bis(3′-indolyl)-isoxazoles, nortopsentin analogues

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ABSTRACT

A series of novel 2,5-bis(3′-indolyl)furans and 3,5-bis(3′-indolyl)isoxazoles were synthesized as antitumor agents. The antiproliferative activity was evaluated in vitro toward diverse human tumor cell lines. Initially 5 isoxazoles and 3 furan derivatives were tested against a panel of 10 human tumor cell lines and the most active derivatives **3c** and **4a** were selected to be evaluated in an extended panel of 29 cell lines. By exhibiting mean IC_{50} values of 17.4 μg/mL (**3a**) and 20.5 μg/mL (**4c**), in particular **4c** showed a high level of tumor selectivity toward the 29 cell lines.

1. Introduction

Bis-indole alkaloids represent a class of deep-sea sponge metabolites that have received much attention due to their potent biological activities such as antiviral, antimicrobial, anti-inflammatory, and antitumor.[1–4] Nortopsentins A–C, having a 2,4-bis(3′-indolyl)imidazole skeleton (Fig. 1), exhibited in vitro cytotoxicity against P388 cells (IC_{50}, 4.5–20.7 μM). Their N-methylated derivatives showed significant improvement in P388 activity if compared to that of the parent compounds (IC_{50} 0.8–2.1 μM).[5–7]

Due to their interesting biological activities, there has been a rapid growth of interest in the synthesis of this class of compounds and their analogs. Thus, various bis(indolyl)derivatives in which the imidazole moiety of nortopsentin was replaced by thiazole, pyrimidine, pyrazine, and pyrazinone rings have been synthesized (Fig. 2). These analogues showed strong inhibitory activity against a wide range of human tumor cell lines (GI_{50}, molar concentration of the compound that inhibits 50% net cell growth, <0.01–89.4 μM).[8–12]

We have recently reported the synthesis and the antitumor activity of two new series of bis-indolyl-5-membered heterocycles in which the imidazole moiety of nortopsentin was replaced by thiophene **1** and pyrazole **2** rings (Fig. 3). They acted in vitro as inhibitors of growth for many types of human cells generally in the micro- and submicromolar range.[13,14]

These results prompted us to synthesize new analogues of the indole alkaloids introducing other five-membered heterocycles different from the imidazole moiety to verify the influence of the central ring on the antiproliferative activity.

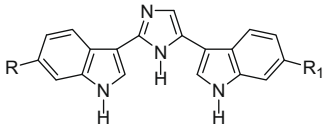
Considering that several isoxazole and furan derivatives have shown antineoplastic activity,[15–20] we planned to synthesize 3,5-bis(3′-indolyl)-isoxazoles **3** and 2,5-bis(3′-indolyl)-furans **4** (Fig. 3).

Thus, 1,3- and 1,4-bis-indolyl-diketones appeared valuable and versatile intermediates for the syntheses of bis(indolyl) heterocycles of type **3** and **4**, respectively.

2. Results and discussion

2.1. Chemistry

Synthesis of the key intermediate 1,3-diketones **7a–e** and 1,4-diketones **8a–e** is outlined in Scheme 1.



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

Figure 1.

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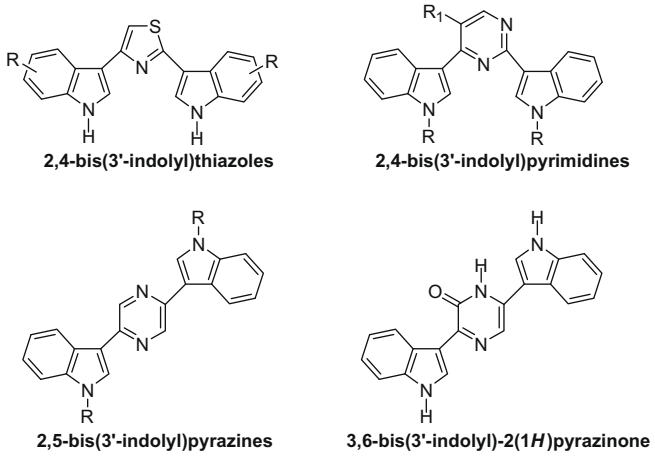


Figure 2.

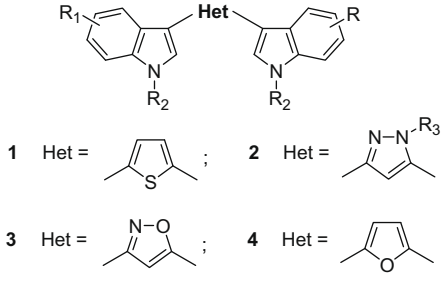


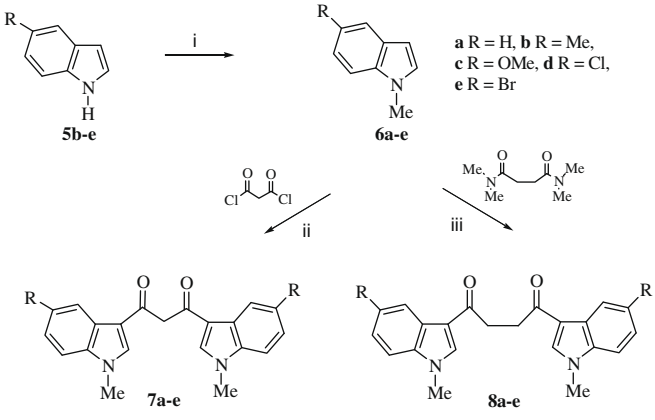
Figure 3.

Indole derivatives **5b–e** were converted into the corresponding *N*-methyl derivatives **6b–e** (96–98%) using potassium *t*-butoxide, tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) as a catalyst, and methyl iodide in dry benzene.

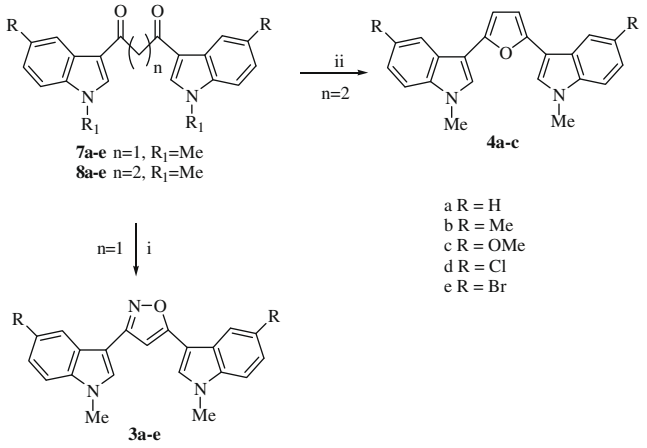
A Friedel–Crafts reaction of the *N*-methylindoles **6a–e** with malonyl-dichloride in dichloromethane yielded the desired symmetrical 1,3-diketones of type **7** (45–70%) (Scheme 1).

Reaction of indoles **6a–e** with succinyl-dichloride to afford the corresponding 1,4-diketones was unsuccessful.

Instead a Vilsmeier–Haack reaction of the same *N*-methylindoles **6a–e** with phosphorus oxychloride and tetramethylsuccinamide produced 1,4-diketones **8a–e** in good yields (56–70%) (Scheme 1).



Scheme 1. Reagent and conditions: (i) *t*-BuOK, TDA-1, benzene, 1–24 h, rt, then, MeI, 0.5–1 h, rt; (ii) malonyl-dichloride, DCM, 2 h, rt; (iii) $POCl_3$, *N,N,N,N*-tetramethylsuccinamide, then 8 h, 55–60 °C (derivative **8a**) or 20 h, rt (derivatives **8b–e**).



Scheme 2. Reagent and conditions: (i) $NH_2OH{\cdot}HCl$, TEA/THF, reflux, 72 h; (ii) PPA, 55–60 °C, 5 h.

All the 1,3- and 1,4-diketones were purified by flash chromatography. It was impossible to purify the halo-diketones **8d,e** since they decomposed in silica gel during the chromatography. Thus these latter were used, for the next step, as crude products.

Symmetrical 1,3-diketones of type **7** were converted into the corresponding 3,5-bis(3′-indolyl)-isoxazoles (**3**) (40–42% yields) using hydroxylamine hydrochloride in refluxing TEA/THF (Scheme 2).

Instead from reaction of 1,4-diketones **8a–e** with PPA at 55–60 °C was possible to isolate only the 2,5-bis(3′-indolyl)-furans **4a–c** (45–60% yields) confirming the instability of **8d,e** in acid medium.

The structure of compounds **3** and **4** was confirmed by spectroscopic (^1H and ^{13}C NMR) as well as analytical data.

2.2. Biology

By using a monolayer cell survival and proliferation assay,[21] the five bis-indolyl-isoxazole **3a–e** and the three bis-indolyl-furan **4a–c** derivatives were screened for in vitro antitumor activity in a panel of 10 human tumor cell lines. Proprietary tumor cell lines from the Oncotest Xenograft collection[22] as well as commercially available cell lines were used.

All compounds showed cytotoxic activity in at least the highest test concentration of 100 μg/mL, exhibiting mean IC_{50} values in the range from 9.6 μg/mL to 44.5 μg/mL (Table 1). Adriamycin tested in parallel was used as cytotoxic positive control and showed concentration-dependent anti-cancer activity towards all cell lines.

The most active candidates **3a** and **4c** were further profiled in an extended panel of 29 cell lines, displaying mean IC_{50} values of 17.4 μg/mL (53.2 μM) (**3a**) and 20.5 μg/mL (53.1 μM) (**4c**), respectively (Table 2).

In particular **4c** showed a high level of tumor selectivity, displaying pronounced activity toward cell lines derived lung cancer (LXFA 526L A549, LXFA 629L) and melanoma (MEXF 276L, MEXF 462NL MEXF 520L). Furthermore, the cell lines GXF 251L (stomach), MAXF 401NL (breast), OVCAR3 (ovary) PANC1 (pancreas), and PC3M (prostate) were shown to be particular sensitive (individual IC_{50} values <1/3 mean IC_{50} value). For compound **3a** selective activity was detected toward A549 and LXFA 629L (lung), as well as UXF 1138L (uterus body).

Moreover compound **4c** was further tested by the National Cancer Institute (Bethesda MD) in a panel of approximately 60 tumor cell lines that have grouped in disease sub-panels including leukemia, non-small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast tumors cell lines.

Table 1
In vitro activity of derivatives **3a–e** and **4a–c** towards 10 human tumor cell lines

Compd	IC_{50} (μg/mL)	Active/total[a]			Tumor selectivity[b,c]	
		1 (μg/mL)	10 (μg/mL)	100 (μg/mL)	A	B
3a	9.6	0/10 (0%)	2/10 (20%)	10/10 (100%)	1/10	+
3b	44.5	0/10 (0%)	0/10 (0%)	7/10 (70%)	0/10	−
3c	17.3	0/10 (0%)	1/10 (10%)	7/10 (70%)	2/10	++
3d	24.5	0/10 (0%)	0/10 (0%)	7/10 (67%)	1/10	+
3e	43.2	0/10 (0%)	0/10 (0%)	3/10 (30%)	1/10	+
4a	27.1	0/10 (0%)	0/10 (0%)	7/10 (70%)	0/10	−
4b	21.1	0/10 (0%)	1/10 (10%)	8/10 (80%)	1/10	+
4c	17.1	0/10 (0%)	2/10 (20%)	7/10 (70%)	2/10	++
Adr[d]	0.007	4/10 (40%)	8/10 (80%)	10/10 (100%)	4/10	+++

[a] Responsive (T/C <30%)/total cell lines.
[b] A = selective (individual IC_{70} <1/3 mean IC_{70})/total cell lines.
[c] B = rating, − (0/10 selective), + (1/10 selective), ++ (2/10 selective), +++ (⩾3/10 selective).
[d] Adr = Adriamycin, active/total is given at 0.03, 0.3, and 3 μg/mL.

The antitumor activity of compound **4c** was given by three parameters for each cell line; pGI_{50} value (GI_{50} is the molar concentration of the compound that inhibits 50% net cell growth), pTGI value (TGI is the molar concentration of the compound leading to total inhibition of net cell growth), and pLC_{50} value (LC_{50} is the molar concentration of the compound that induces 50% net cell death).

An evaluation of the data reported in the Tables 3 and 4 revealed that compound **4c** was cytotoxic showing GI_{50} values against the total number of cell lines investigated at micromolar concentration.

Moreover positive TGI and LC_{50} values were observed with respect to a good number of cell lines (53% and 23%, respectively).

Table 3
Overview of the results of the in vitro antitumor screening of NCI for compound **4c**[a]

	No.[e]	*N*[f]	Range	MG_MID[g]
pGI_{50}[b]	57	50	5.79–4.66	4.99
pTGI[c]	57	30	5.39–4.11	4.39
pLC_{50}[d]	57	13	5.09–4.02	4.1

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen.
[b] pGI_{50} 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] pLC_{50} is the −log of the 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 pGI_{50}, pTGI, and pLC_{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.

Table 2
In vitro activity (IC_{50} values) of **3a** and **4c** towards 29 human tumor cell lines

Cell line	**3a** (μg/mL)	**4c** (μg/mL)
Bladder		
BXF 1218L	20.0	15.7
T24	40.6	>100
CNS		
SF268	24.6	35.1
Colon		
HCT116	29.5	50.4
HT29	32.7	>100
Gastric		
GXF 251L	7.2	3.8
Lung		
LXFL 1121L	21.0	43.4
LXFA 289L	18.7	>100
LXFA 526L	11.5	5.8
A549	5.1	9.6
LXFA 629L	4.2	4.1
H460	18.4	22.2
Mammary		
MAXF 401NL	28.8	8.1
MCF7	21.5	6.2
Melanoma		
MEXF 276L	13.3	3.8
MEXF 462NL	37.8	12.5
MEXF 520L	24.1	6.4
Ovarian		
OVXF 899L	14.9	>100
OVCAR3	11.1	4.2
Pancreatic		
PAXF 1657L	29.9	>100
PANC1	8.8	6.4
Prostate		
22RV1	14.1	33.7
DU145	18.3	>100
LNCAP	11.5	28.2
PC3 M	28.1	4.1
Pleuramesothelioma		
PXF 1752L	24.2	77.4
Renal		
RXF 1781L	38.7	22.0
RXF 486L	29.3	>100
Uterus		
UXF 1138L	4.8	13.7
Mean	17.4	20.5

Table 4
Inhibition of in vitro cancer cell lines by NCI of compound **4c**[a]

Cell lines	GI_{50}[b] (μM)	Cell lines	GI_{50} (μM)
Leukemia			
CCRF-CEM	6.46	SK-MEL-28	14.0
HL-60 (TB)	1.98	SK-MEL-5	10.4
K-562	2.86	UACC-257	27.0
RPMI-8226	2.25	UACC-62	3.81
MOLT-4	>4.00		
SR	1.63		
Non-small cell lung cancer		*Ovarian cancer*	
A549/ATCC	18.4	IGROV1	29.6
EKVX	5.48	OVCAR-3	8.51
HOP-62	>4.00	OVCAR-4	2.06
HOP-92	1.03	OVCAR-5	2.79
NCI-H226	23.6	OVCAR-8	8.02
NCI-H23	17.3	SK-OV-3	>4.00
NCI-H322 M	>4.00	NCI/ADR-RES	3.86
NCI-H460	9.89		
NCI-H522	3.42	*Renal cancer*	
		786-0	>4.00
Colon cancer		A498	>4.00
COLO-205	5.13	ACHN	3.74
HCT-116	8.80	CAKI-1	17.1
HCT-15	4.22	RXF 393	10.4
HT29	22.0	SN12C	21.6
KM12	2.73	TK-10	11.0
SW-620	4.55	UO-31	3.60
CNS cancer			
SF-268	9.25	*Prostate cancer*	
SF-295	13.4	DU-145	>4.00
SF-539	2.47		
SNB-19	29.8		
SNB-75	13.6	*Breast cancer*	
U251	3.66	MCF7	17.3
		NCI/ADR-RES	2.37
Melanoma		MDA-MB-231/ATCC	4.20
LOX IMVI	10.4	HS 578T	5.11
MALME-3 M	52.8	T-47D	2.72
M14	32.9		
SK-MEL-2	2.13		

[a] Data obtained from NCI's in vitro disease-oriented tumor cells screen.
[b] The cytotoxicity GI_{50} value are the concentrations corresponding to 50% growth inhibition of tumor cells.

Derivative **4c** was particularly efficacious against the leukemia sub-panel having GI_{50} in the range 1.63–6.46 μM. The most sensitive leukemia cell lines are SR (GI_{50} 1.63 μM), HL-60 (TB) (GI_{50} 1.98 μM), RPMI-8226 (GI_{50} 2.25 μM), and K-562 (GI_{50} 2.86 μM).

Compound **4c** showed good selectivity with respect to the SK-MEL-2 (GI_{50} 2.13 μM) of the melanoma sub-panel, OVCAR-4 (GI_{50} 2.06 μM), and OVCAR-5 (GI_{50} 2.79 μM) cell lines of the ovarian sub-panel. It also showed selectivity with respect to NCI/ADR-RES (GI_{50} 2.37 μM) and T-47D (GI_{50} 2.72 μM) of the breast cancer sub-panel, SF-539 (GI_{50} 2.47 μM) of the CNS sub-panel, KM12 (GI_{50} 2.73 μM) of the colon sub-panel.

At TGI and LC_{50} level, the best responses were observed in the case of the RPMI-8226 (TGI_{50} 5.07 μM and LC_{50} 20.4 μM) melanoma cell lines and KM12 (TGI_{50} 7.08 μM and LC_{50} 33.4 μM) colon cancer cell line.

3. Conclusion

In conclusion, a series of bis-indolyl-isoxazoles and furans derivatives were synthesized in acceptable overall yields. In vitro profiling in panels of human tumor cell lines for antitumor activity revealed **4c** as the most selective compound, exhibiting pronounced activity toward a wide range of human tumor cell lines.

4. Experimental

4.1. Chemistry

All melting points were taken on a Büchi–Tottoli capillary apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^1H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz, respectively in DMSO-d_6 or $CDCl_3$ solution, using a Bruker Avance II series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230–400 mesh ASTM or with Büchi Sepacore chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ±0.4% of the theoretical values.

4.1.1. General procedure for the preparation of 5-substituted 1-methyl-1*H*-indole (6b–e)

To a cold solution of indoles **5b–e** (5 mmol) in absolute benzene (50 mL), potassium *t*-butoxide (0.76 g, 6.8 mmol), and TDA-1 (1 or 2 drops) were added. The reaction mixture was stirred at rt (1–24 h) and then methyl iodide (0.31 mL, 5 mmol) was added. TLC analysis (DCM/petrolium ether 9/1) revealed that methylation was complete after 0.5–1 h. The solvent was evaporated under reduced pressure. The residue, treated with water, was filtered off and air dried or extracted with DCM. The organic layer was dried over sodium sulfate, and evaporated under vacuum to afford the pure methyl derivatives **6b–e**.

4.1.1.1. 1,5-Dimethyl-1*H*-indole (6b). Conditions: rt, 2 h, then rt, 1 h. Work-up: extraction. Oil; yield: 98%; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.44 (3H, s, CH_3), 3.72 (3H, s, CH_3), 6.38 (1H, d, J = 2.9 Hz, H-3), 6.97 (1H, d, J = 2.9 Hz, H-2), 7.03 (1H, d, J = 8.4 Hz, H-6), 7.19 (1H, d, J = 8.4 Hz, H-7), 7.40 (1H, s, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.4 (CH_3), 32.7 (CH_3), 100.3 (CH), 108.8 (CH), 118.6 (C), 120.5 (CH), 123.1 (CH), 126.6 (C), 128.8 (CH), 135.1 (C). Anal. Calcd for $C_{10}H_{11}N$: C, 82.72; H, 7.64; N, 9.65. Found: C, 82.58; H, 7.54; N, 9.79.

4.1.1.2. 5-Methoxy-1-methyl-1*H*-indole (6c). Conditions: rt, 1 h, then rt, 0.5 h. Work-up: filtration. White solid; yield: 97%; mp: 84 °C; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.67 (3H, s, CH_3), 3.81 (3H, s, CH_3), 6.38 (1H, d, J = 2.9 Hz, H-3), 6.87 (1H, dd, J = 2.9, 8.8 Hz, H-6), 6.96 (1H, d, J = 2.9 Hz, H-4), 7.07 (1H, d, J = 2.9 Hz, H-2), 7.17 (1H, d, J = 8.8 Hz, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 32.8 (CH_3), 55.8 (CH_3), 100.3 (CH), 102.4 (CH), 109.8 (CH), 111.8 (CH), 128.7 (C), 129.2 (CH), 132.1 (C), 153.9 (C). Anal. Calcd for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.76; H, 6.84; N, 8.43.

4.1.1.3. 5-Chloro-1-methyl-1*H*-indole (6d). Conditions: rt, 24 h, then rt, 1 h. Work-up: extraction. Oil; yield: 98%; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.52 (3H, s, CH_3), 6.32 (1H, d, J = 3.2 Hz, H-3), 6.89 (1H, d, J = 3.2 Hz, H-2), 7.05 (1H, d, J = 8.4 Hz, H-7), 7.51 (1H, d, J = 8.4 Hz, H-6), 7.65 (1H, s, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 32.2 (CH_3), 100.2 (CH), 110.0 (CH), 119.7 (CH), 121.3 (CH), 124.6 (C), 129.2 (C), 129.9 (CH), 134.8 (C). Anal. Calcd for C_9H_8ClN: C, 65.27; H, 4.87; N, 8.46. Found: C, 65.14; H, 5.03; N, 8.29.

4.1.1.4. 5-Bromo-1-methyl-1*H*-indole (6e). Conditions: rt, 24 h, then rt, 1 h. Work-up: extraction. Oil; yield: 96%; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.47 (3H s, CH_3), 6.29 (1H, d, J = 3.1 Hz, H-3), 6.84 (1H, d, J = 3.1 Hz, H-2), 6.94 (1H, d, J = 8.5 Hz, H-7), 7.17 (1H, dd, J = 1.7 , 8.5 Hz, H-6), 7.65 (1H, d, J = 1.7 Hz, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 32.6 (CH_3), 100.2 (CH), 110.5 (CH), 121.2 (C), 122.9 (CH), 123.9 (CH), 127.6 (C), 129.8 (CH), 135.1 (C). Anal. Calcd for C_9H_8BrN: C, 51.46; H, 3.84; N, 6.67. Found: C, 51.62; H, 3.78; N, 6.89.

4.1.2. General procedure for the preparation of 1,3-bis(indol-3-yl)propane-1,3-diones (7a–e)

A solution of malonyl-dichloride (1 mL, 10 mmol) in DCM (10 mL) was added dropwise to a cold solution of *N*-methylindoles 6a–**e** (20 mmol) in DCM (20 mL). The reaction was stirred for 2 h at rt. The mixture was added to 5% aqueous sodium carbonate, shaken for 2 min and extracted with DCM, dried over sodium sulfate and evaporated. The residue was purified by chromatography eluting with DCM/ethyl acetate 9/1 to obtained derivatives **7a–e**.

4.1.2.1. 1,3-Bis(1-methyl-1*H*-indol-3-yl)propane-1,3-dione (7a). Yellow solid; yield: 70%; mp: 220–221 °C; IR 1624 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.78 (3H, s, CH_3), 4.30 (2H, s, CH_2), 7.19–7.32 (3H, m, H-5, H-6 and H-7), 8.14 (1H, s, H-2), 8.33–8.37 (1H, m, H-4), ^{13}C NMR (50 MHz, $CDCl_3$) δ: 33.6 (CH_3), 56.3 (CH_2), 109.6 (CH), 116.0 (C), 122.4 (CH), 122.7 (CH), 123.4 (CH), 126.5 (C), 137.4 (C), 138.4 (CH), 188.2 (C). Anal. Calcd for $C_{21}H_{18}N_2O_2$: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.20; H, 5.63; N, 8.29.

4.1.2.2. 1,3-Bis(1,5-methyl-1*H*-indol-3-yl)propane-1,3-dione (7b). Yellow solid; yield: 66%; mp: 214–217 °C; IR 1610 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.44 (3H, s, CH_3), 3.75 (3H, s, CH_3), 4.28 (2H, s, CH_2), 7.07 (1H, d, J = 8.4 Hz, H-7), 7.09 (1H, d, J = 8.4 Hz, H-6), 8.11 (1H, s, H-2), 8.16 (1H, s H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.4 (CH_3), 33.6 (CH_3), 56.4 (CH_2), 109.2 (CH), 115.5 (C), 122.1 (CH), 124.9 (CH), 126.7 (C), 132.4 (C), 135.8 (C), 138.4 (CH), 188.3 (C). Anal. Calcd for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82. Found: C, 77.29; H, 6.26; N, 8.04.

4.1.2.3. 1,3-Bis(5-methoxy-1*H*-indol-3-yl)propane-1,3-dione (7c). Yellow solid; yield: 68%; mp: 185–187 °C; IR 1620 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.77 (3H, s, CH_3), 3.86 (3H, s, CH_3), 4.26 (2H, s, CH_2), 6.89 (1H, d, J = 8.9 Hz, H-6), 7.15 (1H, d, J = 8.9 Hz, H-7), 7.86 (1H, s, H-4), 8.07 (1H, s H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 33.7 (CH_3), 55.7 (CH_3), 55.9 (CH_2), 103.8 (CH), 110.4 (CH), 113.8 (CH), 115.7 (C), 127.4 (C), 132.4 (C), 138.2 (CH), 156.6 (C), 188.2 (C). Anal. Calcd for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.58; H, 5.49; N, 7.31.

4.1.2.4. 1,3-Bis(5-chloro-1*H*-indol-3-yl)propane-1,3-dione (7d). Yellow solid; yield: 45%; mp: 220–221 °C; IR 1616 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.81 (3H, s, CH_3), 4.26 (2H, s, CH_2), 7.13–7.26 (2H, m, H-6, H-7), 8.13 (1H, s, H-4), 8.33 (1H, s, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 33.9 (CH_3), 56.2 (CH_2), 110.7 (CH), 115.4 (C), 122.0 (CH), 123.2 (C), 123.9 (CH), 128.9 (C), 135.8 (C), 139.1 (CH), 187.8 (C). Anal. Calcd for $C_{21}H_{16}Cl_2N_2O_2$: C, 63.17; H, 4.04; N, 7.02. Found: C, 63.25; H, 3.88; N, 7.12.

4.1.2.5. 1,3-Bis(5-bromo-1*H*-indol-3-yl)propane-1,3-dione (7e). Yellow solid; yield: 48%; mp: 238–240 °C; IR 1626 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.84 (3H, s, CH_3), 4.28 (2H, s, CH_2), 7.15 (1H, d, J = 8.7 Hz, H-6), 7.37 (1H, d, J = 8.7 Hz, H-7), 8.14 (1H, s, H-2), 8.51 (1H, s, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 33.9 (CH_3), 56.6 (CH_2), 111.1 (CH), 116.7 (C), 124.5 (C), 125.1 (CH), 126.6 (CH), 128.1 (C), 133.4 (C), 139.0 (CH), 187.8 (C). Anal. Calcd for $C_{21}H_{16}Br_2N_2O_2$: C, 51.67; H, 3.30; N, 5.74. Found: C, 51.88; H, 3.56; N, 5.67.

4.1.3. Synthesis of *N,N,N′,N′*-tetramethylsuccinamide

5.3 mL (0.05 mol) of succinyl chloride at 0 °C was added dropwise to a solution of dimethylamine (40% in water, 2 mmol). The mixture was stirred for 30 min and then extracted with DCM, dried and evaporated to afford the pure *N,N,N′,N′*-tetramethylsuccinamide: white solid; yield: 100%; mp: 68–69 °C; IR 1633 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.63 (2H, s, CH_2), 2.91 (3H, s, CH_3), 3.04 (3H, s, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 28.0 (CH_2), 35.5 (CH_3), 37.2 (CH_3), 172.3 (C). Anal. Calcd for $C_8H_{16}N_2O_2$: C, 55.79; H, 9.36; N, 16.27. Found: C, 55.62; H, 9.50; N, 16.01.

4.1.4. General procedure for the preparation of 1,4-bis(indol-3-yl)butane-1,4-diones (8a–e)

Phosphorus oxychloride (5.3 mL, 57 mmol) was slowly added to *N,N,N′,N′*-tetramethylsuccinamide (2.58 g, 15 mmol) at 10–20 °C and the mixture was stirred for 24 h. Then *N*-methylindoles **6a–e** (30 mmol) were slowly added keeping the temperature below 45 °C. After the addition was complete the mixture was heated for 8 h to 55–60 °C (for derivative **8a**) or stirred at rt for 20 h (for derivatives **8b–e**). The solution was poured onto crushed ice, made basic with sodium hydroxide 10 M and filtered. The solid was washed with water, dried and purified by chromatography using (DCM/ethyl acetate 9/1) as eluent to afford the pure derivatives **8a–c**; whereas derivatives **8d,e** were used for next step without purification.

4.1.4.1. 1,4-Bis(1-methyl-1*H*-indol-3-yl)butane-1,4-dione (8a). Yellow solid; yield: 80%; mp: 224–226 °C; IR 1631 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.35 (2H, s, CH_2), 3.78 (3H, s, CH_3), 7.25–7.29 (3H, m, H-5, H-6, H-7), 7.83 (1H, s, H-2), 8.37 (1H, d, J = 1.6, 7.3 Hz, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 33.4 (CH_3), 34.0 (CH_2), 109.5 (CH), 116.2 (C), 122.4 (CH), 122.5 (CH), 123.2 (CH), 126.3 (C), 135.7 (CH), 137.4 (C), 194.3 (C). Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 76.80; H, 6.02; N, 8.30.

4.1.4.2. 1,4-Bis(1,5-methyl-1*H*-indol-3-yl)butane-1,4-dione (8b). Yellow solid; yield: 56%; mp: 300–302 °C; IR 1620 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.40 (3H, s, CH_3), 3.21 (2H, s, CH_2), 3.85 (3H, s, CH_3), 7.10 (1H, d, J = 8.7 Hz, H-6), 7.42 (1H, d , J = 8.7 Hz, H-7), 7.98 (1H, s, H-4), 8.36 (1H, s, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.5 (CH_3), 33.5 (CH_2), 34.1 (CH_3), 109.2 (CH), 114.8 (C), 122.3 (CH), 124.8 (CH), 125.5 (C), 132.2 (C), 135.7 (CH), 136.5 (C), 194.7 (C). Anal. Calcd for $C_{24}H_{24}N_2O_2$: C, 77.39; H, 6.49; N, 7.52. Found: C, 77.62; H, 6.35; N, 7.76.

4.1.4.3. 1,4-Bis(5-methoxy-1*H*-indol-3-yl)butane-1,4-dione (8c). White solid; yield: 67%; mp: 213–214 °C; IR 1631 (CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.21 (2H, s, CH_2), 3.75 (, 3H, s, CH_3), 3.85 (3H, s, CH_3), 6.90 (1H, d, J = 8.8 Hz, H-6), 7.43 (1H, d, J = 8.8 Hz, H-7), 7.70 (1H, s, H-4), 8.35 (1H, s, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 33.7 (CH_2), 33.7 (CH_3), 55.7 (CH_3), 103.7 (CH), 110.4 (CH), 113.8 (CH), 115.8 (C), 127.2 (C), 132.4 (C), 135.7 (CH), 156.4 (C) , 194.3 (C). Anal. Calcd for $C_{24}H_{24}N_2O_4$: C, 71.27; H, 5.98; N, 6.93. Found: C, 71.05; H, 5.88; N, 6.76.

4.1.5. General procedure for the preparation of isoxazoles (3a–e)

Hydroxylamine hydrochloride (57 mmol) was dissolved in THF (10 mL) and added dropwise to a stirred solution of 1,3-diketones **7a–e** (1 mmol) in THF (10 mL) with a stoikiometric amount of TEA (1 mL). The reaction was heated at reflux for 72 h. The mixture was concentrated in vacuo prior to the addition of water (50 mL) and the reaction products were extracted with DCM (3 × 50 mL). The combined organic extracts were dried over sodium sulfate, evaporated and purified by silica gel chromatography using DCM as eluent to give the isoxazoles **3a–e**.

4.1.5.1. 3-3′-Isoxazole-3,5-diylbis(1-methyl-1*H*-indole) (3a). Yellow solid; yield: 42%; mp: 214–215 °C; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.90 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 7.19 (s, 1H, H-4′), 7.22–8.20 (m, 10H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.8 (CH_3), 32.9 (CH_3), 95.2 (CH), 102.7 (C), 104.0 (C), 110.2 (CH), 110.7 (CH), 119.6 (CH), 120.4 (CH), 120.8 (CH), 121.3 (CH), 122.2 (CH), 122.4 (CH), 124.1 (C), 124.9 (C), 129.6 (CH), 131.0 (CH), 136.9 (C), 137.1 (C), 158.3 (C), 164.7 (C). Anal. Calcd for $C_{21}H_{17}N_3O$: C, 77.04; H, 5.23; N, 12.84. Found: C, 77.24; H, 5.13; N, 13.09.

4.1.5.2. 3-3′-Isoxazole-3,5-diylbis(1,5-dimethyl-1*H*-indole) (3b). Yellow solid; yield: 50%; mp: 214–215 °C; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.47 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 3.87 (s, 3H, CH_3) 7.09–8.08 (m, 9H, Ar) ; ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2 (CH_3), 21.3 (CH_3), 32.8 (CH_3), 32.9 (CH_3), 95.0 (CH), 102.2 (C), 103.6 (C), 109.9 (CH), 110.3 (CH), 119.3 (CH), 121.0 (CH), 123.7 (CH), 123.9 (CH), 124.3 (C), 125.1 (C), 129.1 (C), 124.5 (CH), 129.8 (C), 131.0 (CH), 135.4 (C), 135.6 (C), 158.4 (C), 164.8 (C). Anal. Calcd for $C_{23}H_{21}N_3O$: C, 77.72; H, 5.96; N, 11.82. Found: C, 77.57; H, 6.09; N, 12.00.

4.1.5.3. 3-3′-Isoxazole-3,5-diylbis(5-methoxy-1-methyl-1*H*-indole) (3c). Yellow solid; yield: 52%; mp: 201–202 °C; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.84 (s, 3H, CH_3), 3.86 (s, 1H, CH_3), 3.87 (s, 1H, CH_3), 3.90 (s, 1H, CH_3), 6.90–8.07 (m, 9H, Ar) ; ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.8 (CH_3), 33.9 (CH_3), 55.3 (CH_3), 55.6 (CH_3), 94.7 (CH), 101.7 (CH), 102.4 (C), 102.9 (CH), 103.7 (C), 111.1 (CH), 111.5 (CH), 112.2 (CH), 112.3 (CH), 124.6 (C), 125.3 (C), 129.9 (CH), 131.3 (CH), 132.1 (C), 132.3 (C), 154.5 (C), 154.9 (C), 158.4 (C), 164.8 (C). Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.45; H, 5.67; N, 10.64.

4.1.5.4. 3-3′-Isoxazole-3,5-diylbis(5-chloro-1-methyl-1*H*-indole) (3d). Yellow solid; yield: 40%; mp: 246–247 °C; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.90 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 7.28–8.24 (m, 9H, Ar); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.1 (CH_3), 33.2 (CH_3), 95.5 (CH), 102.3 (C), 103.7 (C), 112.1 (CH), 112.5 (CH), 118.7 (CH), 120.3 (CH), 122.2 (CH), 122.4 (CH), 124.9 (C), 125.2 (C), 125.7 (C), 125.9 (C), 131.1 (CH), 132.7 (CH), 135.5 (C), 135.7 (C), 158.1 (C), 164.1 (C). Anal. Calcd for $C_{21}H_{15}Cl_2N_3O$: C, 63.65; H, 3.82; N, 10.60. Found: C, 63.38; H, 4.03; N, 10.81.

4.1.5.5. 3-3′-Isoxazole-3,5-diylbis(5-bromo-1-methyl-1*H*-indole) (3e). Yellow solid; yield: 42%; mp: 246–247 °C; ^1H NMR (200 MHz,

DMSO-d_6) δ: 3.91 (s, 3H, CH_3), 3.92 (s, 3H, CH_3), 7.27–8.28 (m, 9H, Ar); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.0 (CH_3), 33.1 (CH_3), 95.5 (CH), 102.2 (C), 103.5 (C), 112.5 (CH), 112.9 (CH), 121.6 (CH), 123.3 (CH), 124.7 (CH), 125.0 (CH), 125.5 (C), 126.3 (C), 128.6 (C), 131.0 (C), 131.5 (CH), 132.6 (CH), 135.7 (C), 135.9 (C), 158.0 (C), 164.1 (C). Anal. Calcd for $C_{21}H_{15}Br_2N_3O$: C, 51.99; H, 3.12; N, 8.66. Found: C, 52.18; H, 3.35; N, 8.89.

4.1.6. General procedure for the preparation of furans (4a–c)

The appropriate 1,4-diketones **8a–c** (8 mmol) and polyphosphoric acid (10.0–12.0 g) were stirred and heated for 5 h at 55–60 °C. The reaction mixture was poured onto crushed ice, neutralized with aqueous sodium carbonate and extracted with DCM. The extracts were dried over sodium sulfate and evaporated to yield the crude products which were purified by chromatography using DCM as eluent to give the furans **4a–c**.

4.1.6.1. 3-3′-Furan-2,5-diylbis(1-methyl-1*H*-indole) (4a). Green solid; yield: 52%; mp: 201–202 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.82 (3H, s, CH_3), 6.61 (1H, s, H-2′), 7.23 (1H, t, J = 7.7 Hz, H-5), 7.29 (1H, t, J = 7.7 Hz, H-6), 7.31 (1H, d, J = 7.7 Hz, H-7), 7.44 (1H, s, H-2), 8.02 (1H, d, J = 7.7 Hz, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 32.9 (CH_3), 104.7 (CH), 108.0 (C), 109.5 (CH), 120.2 (CH), 120.5 (CH), 122.1 (CH), 124.9 (C), 125.3 (CH), 137.2 (C), 148.1 (C). Anal. Calcd for $C_{22}H_{18}N_2O$: C, 80.96; H, 5.56; N, 8.58. Found: C, 80.73; H, 5.67; N, 8.83.

4.1.6.2. 3-3′-Furan-2,5-diylbis(1,5-dimethyl-1*H*-indole) (4b). Green solid; yield 47%; mp: 163–164 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.53 (3H, s, CH_3), 3.79 (3H, s, CH_3), 6.60 (1H, s, H-2′), 7.11 (1H, dd, J = 8.2 Hz, H-6), 7.23 (1H, d, J = 8.2 Hz, H-7), 7.39 (1H, s, H-2), 7.81 (1H, s, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.6 (CH_3), 32.9 (CH_3), 104.5 (CH), 107.5 (C), 109.1 (CH), 117.2 (C), 120.2 (CH), 123.7 (CH), 125.4 (CH), 126.9 (C), 129.3 (C), 135.6 (C). Anal. Calcd for $C_{24}H_{22}N_2O$: C, 81.33; H, 6.26; N, 7.90. Found: C, 81.09; H, 6.37; N, 8.12.

4.1.6.3. 3-3′-Furan-2,5-diylbis(5-methoxy-1-methyl-1*H*-indole) (4c). Green solid; yield: 55%; mp: 167 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.78 (3H, s, CH_3) 3.90 (3H, s, CH_3), 6.56 (1H, s, H-2′), 6.94 (1H, dd, J = 2.3, 8.8 Hz, H-6), 7.22 (1H, d, J = 8.8 Hz, H-7), 7.38 (1H, s, H-2), 7.48 (1H, d, J = 2.3 Hz, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 33.1 (CH_3), 56.0 (CH_3), 102.7 (CH), 104.3 (CH), 107.5 (C), 110.2 (CH), 112.2 (CH), 125.3 (CH), 125.9 (C), 132.5 (C), 148.1 (C), 154.6 (C). Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.77; H, 5.59; N, 7.05.

4.2. Biology

Antitumor activity of the compounds was tested in a monolayer cell survival and proliferation assay using human tumor cell lines. Studies using panels of human tumor cell lines of different origin/histotype allow the analysis of potency and tumor selectivity of test compounds and to identify active compounds that qualify for further preclinical evaluation.

4.2.1. Cell lines

Sixteen out of the 29 cell lines as tested were established at Oncotest from patient-derived human tumor xenografts passaged subcutaneously in nude mice.[22] The origin of the donor xenografts was described.[23,24] The cell lines T24, A549, Panc1, and 22RV1 were obtained from ATCC (Rockville, MD, USA), the cell line LnCAP from DSMZ (Braunschweig, Germany) and the other eight cell lines were kindly provided by the National Cancer Institute (Bethesda, MA, USA). Cells were cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum and 0.1 mg/mL gentamicin under standard conditions (37 °C, 5% CO_2).

4.2.2. Cytotoxicity assay (monolayer assay)

A modified propidium iodide assay was used to assess the compounds' activity toward human tumor cell lines.[21] Briefly, cells were harvested from exponential phase cultures by trypsinization, counted and plated in 96-well flat-bottom microtiter plates at a cell density dependent on the cell line (4.000–20.000 cells/well). After 24 h recovery period to allow the cells to adhere and resume exponential growth, test compounds were added at five concentrations in log increments and left for further 4 days. The inhibition of proliferation was determined by measuring the DNA content using an aqueous propidium iodide solution (7 μg/ml). Fluorescence was measured using the Cytofluor micro-plate reader (excitation λ = 530 nm, emission λ = 620 nm), providing a direct relationship to the total viable cell number. In each experiment, all data points were determined in triplicates. Three independent experiments were performed for compounds **3a** and **4c** as tested in the 29 cell line panel.

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