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Spirobipyridopyrans, spirobinaphthopyrans, indolinospiropyridopyrans, indolinospironaphthopyrans and indolinospironaphtho-1,4-oxazines: synthesis, study of X-ray crystal structure, antitumoral and antiviral evaluation

Silvana Raić-Malić,[a] Linda Tomašković,[a,†] Draginja Mrvoš-Sermek,[b] Biserka Prugovečki,[b] Mario Cetina,[c] Mira Grdiša,[d] Krešimir Pavelić,[d] Albrecht Mannschreck,[e] Jan Balzarini,[f] Erik De Clercq[f] and Mladen Mintas[a,*]

[a]*Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, PO Box 177, HR-10000 Zagreb, Croatia*
[b]*Laboratory of General and Inorganic Chemistry, Faculty of Science, University of Zagreb, Zvonimirova 8, HR-10000 Zagreb, Croatia*
[c]*Faculty of Textile Technology, University of Zagreb, Pierottijeva 6, HR-10000 Zagreb, Croatia*
[d]*Division of Molecular Medicine, Ruder Bošković Institute, Bijenička 54, PO Box 1016, HR-10001 Zagreb, Croatia*
[e]*Institute of Organic Chemistry, University of Regensburg, Universitätsstr. 31, D-93040, Regensburg, Germany*
[f]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Belgium*

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Abstract—The novel racemic indolinospirobenzopyrans (**5**–**7**), indolinospironaphthopyrans (**11**–**14**) and indolinospironaphtho-1,4-oxazine (**17**) were synthesized by an aldol type of condensation of 1′,3′,3′-trimethyl-2 ′-methyleneindoline and its 5-substituted derivatives with an appropriately substituted hydroxybenzaldehyde, hydroxynaphthaldehyde or nitrosonaphthol. An unequivocal proof of the stereostructures of **9** and **17** was obtained by the single-crystal X-ray diffraction method. A substituted indoline ring and the benzopyran ring in **9** and the naphtho-1,4-oxazine moiety in **17** are interconnected via the common chiral atom and positioned almost perpendicularly to each other. The five-membered 2,3-dihydropyrrolo moiety of the indoline ring adopts an envelope conformation in both structures. Of all the compounds of this series, spirobipyridopyran (**1**) inhibited specifically the growth of human melanoma (HBL) (IC_{50}: 0.9 μM) cells but not the growth of normal fibroblasts (WI38). Indolinospirobenzopyrans (**8**–**10**) showed significant cytostatic activities against all tumor cell lines. However, these compounds also exhibited a cytotoxic effect on normal human fibroblasts. The indolinospirobenzopyrans **4**, **6**–**8**, **10** and the indolinospironaphtho-1,4-oxazine **16** showed, albeit modest, selectivity as antiviral agents against varicella-zoster virus (VZV) and/or cytomegalovirus (CMV) (EC_{50} within the concentration range of 1.0–12.6 μM).

1. Introduction

Chiral spiropyrans have been extensively investigated[1–3] largely because of their thermo- and photochromic properties and application in display and storage of information. In contrast, there is almost no information on biological and pharmacological properties of this class of compounds. The present study deals with various, structurally closely related types of the *spiro*-compounds: spirobipyridopyran (**1**), spirobinaphthopyrans (**2** and **3**) (Fig. 1), indolinospirobenzopyrans (**4**–**10**), indolinospironaphthopyrans (**11**–**14**), indolinospiropyridopyran (**15**) and indolinospironaphtho-1,4-oxazines (**16**–**18**) (Fig. 2) and their cytostatic and antiviral activities. The principal aim of this study was to evaluate the *spiro*-compounds (**1**–**18**) for their cytostatic and antiviral activities.

Keywords: Spiropyrans; ^{1}H and ^{13}C NMR spectra; Single crystal X-ray analysis; Cytostatic activities; Antiviral activities.
* Corresponding author. Tel.: +385-1-4597-214; fax: +385-1-4597-250; e-mail: mladen.mintas@fkit.hr
† Present address: Pliva Research Institute, Prilaz baruna Filipovića 25, HR-1000 Zagreb.

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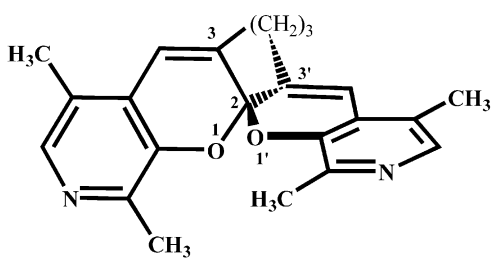
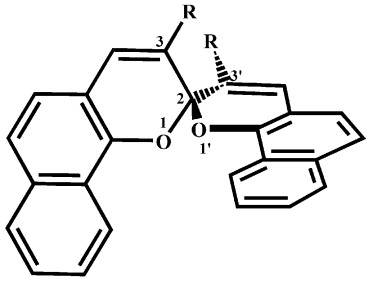
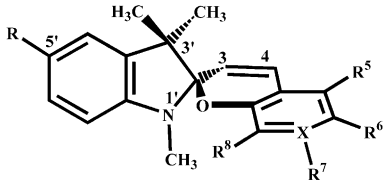


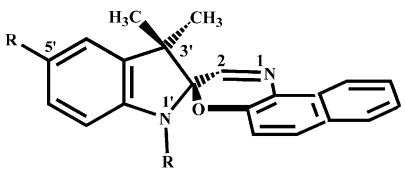
1[1]



	R	*R*
2[2]	CH_2 CH_2	CH_2
3[2]	C_6H_5	C_6H_5

Figure 1. Spirobipyridopyrans **1**[1] and spirobinaphthopyrans **2**[2] and **3**[2].





4-15

	*R*5′	*R*5	*R*6	*R*7	*R*8	*X*
4[4]	H	H	NO_2	H	H	C
5	NO_2	H	H	H	H	C
6	NO_2	H	NO_2	H	H	C
7	Cl	H	NO_2	H	H	C
8	H	H	H	OCH_3	H	C
9	H	H	OCH_3	H	H	C
10	Cl	H	H	OCH_3	H	C
11	H	H	H	benzo		C
12	Cl	H	H	benzo		C
13	NO_2	H	H	benzo		C
14	Cl	benzo		H	H	C
15[1]	NO_2	H	H	-	H	N

16–18

	*R*5′	*R*
16[5]	H	CH_3
17	Cl	CH_3
18[6]	H	C_6H_5

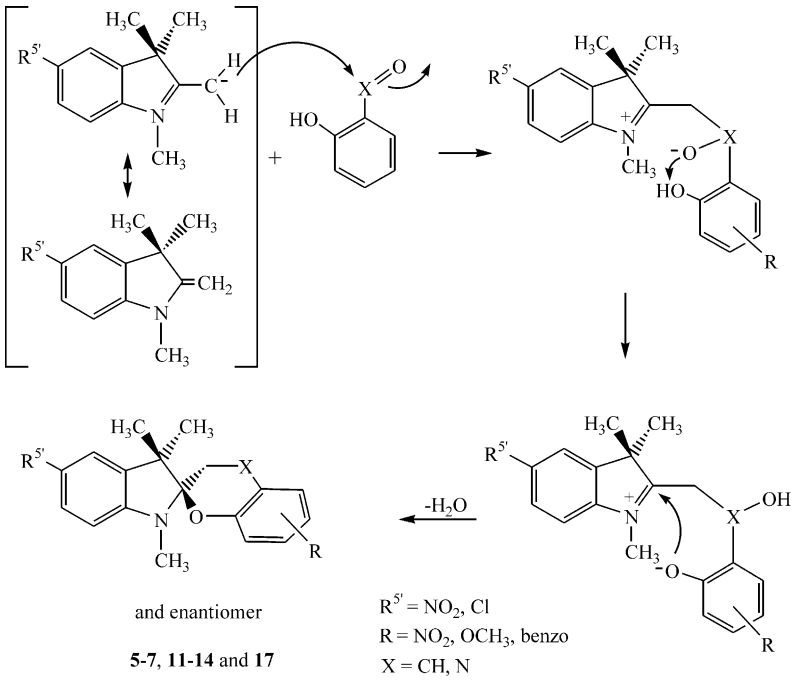
Figure 2. Indolinospirobenzopyrans **4–10**, indolinospironaphthopyrans **11–14**, indolinospiropyridopyran **15** and indolinospironaphtho-1,4-oxazines **16–18**.

2. Results and discussion

2.1. Chemistry

The novel indolinospirobenzopyrans **5–7**, indolinospironaphthopyrans **11–14** and indolinospironaphtho-1,4-oxazine **17** (Fig. 2) were synthesized by an aldol type of condensation of equimolar amounts of 1′,3′,3′-trimethyl-2′-methyleneindoline and its 5′-substituted derivatives with 2-hydroxybenzaldehyde substituted with a nitro or methoxy group at position 6 or 7, 1-hydroxy-2-naphthaldehyde, 2-hydroxy-1-naphthaldehyde or 1-nitroso-2-naphthol (Scheme 1). This reaction involves nucleophilic attack of the enamine to the carbonyl or nitroso group and subsequent ring closure by intramolecular addition of the phenolic oxygen to the immonium group which gave under elimination of water the *spiro*-compounds (**5–7**, **11–14** and **17**).[6]

The structures of the novel compounds **5–7**, **11–14** and **17** were elucidated by analysis of their one- and two-dimensional ^1H and ^{13}C NMR spectra, mass spectra, and elemental analyses (see Experimental). The ^1H NMR spectroscopic data of **5–7**, **11–14** and **17** are displayed in Table 1. The chemical shifts and H–H coupling constants are consistent with the proposed structures. The chemical shift assignments are in agreement with the corresponding ones of the related *spiro*-systems.[1–3]



Scheme 1. Synthesis of indolinospirobenzopyrans **5–7**, indolinospironaphtopyrans **11–14** and indolinospironaphtho-1,4-oxazine **17** (*c.f.* Figs 1 and 2).

2.2. X-Ray crystal structure analysis

In order to determine the exact structures of the indolinospirobenzopyran **9** and indolinospironaphthoxazine **17**, their X-ray crystal structure analyses were performed. The molecular structures of the racemic compounds **9** and **17** with the atom numbering Schemes are shown in Figures 3 and 4.

The analysis of the X-ray data shows that the skeleton of the compound **9** consists of a substituted indoline ring connected to a methoxy-substituted benzopyran ring via the *spiro*-C2′ atom. Similarly, in the compound **17**, a substituted indoline ring is linked *via* the *spiro*-C2′ atom to a naphtho-1,4-oxazine moiety. The dihedral angles between the planes defined by the atoms N1′/C2′/C3′ and O1/C2′/C3 amount to 87.4(2)° in **9** and 86.1(3)° in **17**. This means that the indoline ring and the benzopyran moiety in **9**, and the naphtho-1,4-oxazine moiety in **17** are almost perpendicular to each other. The C_{spiro}-N bond lengths are equal in both *spiro*-compounds [1.447(3) Å]; the C_{spiro}–O bond lengths are also equal within standard uncertainties amounting to 1.469(3) Å in **9** and 1.464(3) Å in **17**. These both lengths are in agreement with those found in the structurally related 1′,3′,3′ - trimethyl - 5′ - nitro(indoline - 2,2′ - (2H) - spirobenzopyran),[7] 1′,3′,3′-trimethyl-7-methoxy(indoline-2,2′-(2H - 1)spirobenzopyran),[8] 1,3,3-trimethyl(indoline-2,3′-(3H)-naphtho(2,1-b)(1,4)spirooxazine),[9] and (1,3,3-trimethyl-6-nitroindoline-2,3′-3H-naphtho(2,1-b)(1,4)spirooxazine).[10] The spiro carbon atom C2′ in the five-membered moiety of the indoline ring is out of the plane defined by the atoms N1′, C7A′, C3A′ and C3′. It means that the 2,3-dihydropyrrole ring in both structures adopts an envelope conformation. This is shown by the values of the dihedral angles between the mean plane defined by the atoms N1′/C7A′/C3A′/C3′, and the plane defined by the atoms N1′/C2′/C3′ amounting to 32.7(2)° in **9** and 24.2(2)° in **17**. This means that the deviation from the planarity of the five-membered ring is more evident in **9** than in **17**. The atoms N1′/C7A′/C3A′/C3′ of the five-membered ring and the fused benzene ring via the common carbon atoms C3A′ and C7A′ are coplanar. The dihedral angle between these mean planes is equal in both structures [2.5(2)°]. The consequence of the five-membered ring folding is an increase in pyramidality of the indoline nitrogen atom N1′, the sum of the angles about this atom amounting to 347.4 and 349.7° in **9** and **17**, respectively.

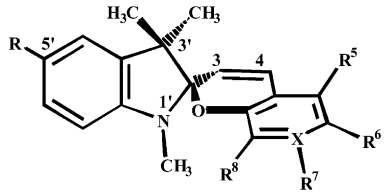
3. Biological results

3.1. Cytostatic activities

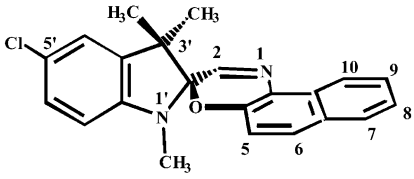
The compounds **1–18** were evaluated for their activities against malignant tumor cell lines: murine leukemia (L1210) and human T-lymphocytes (Molt4/C8 and CEM), melanoma (HBL), cervical carcinoma (HeLa), breast carcinoma (MCF7), colon carcinoma (HT29 and SW620), laryngeal carcinoma (Hep2), pancreatic carcinoma (MiaPaCa2) cell lines as well as normal human fibroblasts (WI38) (Table 2). Of all compounds in the series, spyrobipyridopyran **1** had the most pronounced inhibitory activity against human melanoma (HBL) cells (IC_{50}: 0.9 μM). This compound also exhibited the highest cytostatic selectivity: this compound inhibited specifically the growth of human melanoma (HBL) cells but not that of normal fibroblasts (WI38).

Among other classes, indolinospirobenzopyrans (**8–10**) showed significant cytostatic activities in all tumor cell

Table 1. ^{1}H NMR chemical shifts (δ/ppm)[a] and coupling constants (*J*/Hz)[b] of compounds **5–7**, **11–14** and **17**



5-7, **11-13** and **14**



17

Compd		**5**	**6**	**7**	**11**	**12**	**13**	**14**	**17**[c]
C-(CH$_3$)$_2$	δ	1.20(s, 3H) 1.35(s,3H)	1.22(s, 3H) 1.34(s,3H)	1.18(s, 3H) 1.25(s,3H)	1.23(s, 3H) 1.35(s,3H)	1.25(s, 3H) 1.35(s,3H)	1.26(s, 3H) 1.40(s,3H)	1.20(s, 3H) 1.30(s,3H)	1.33(s, 3H) 1.36(s,3H)
N-CH$_3$/	δ	2.83(s,3H)	2.87(s,3H)	2.70(s,3H)	2.73(s,3H)	2.72(s,3H)	2.85(s,3H)	2.70(s,3H)	2.74(s,3H)
H-4′	δ *J*	6.1–8.4(1H)	7.96(1H)	6.3–7.3(1H)	7.12(1H) 6.4(d)	7.10(s, 1H)	7.99(1H) 2.1(d)	6.2–8.2(1H)	7.03(1H)
H-5′	δ *J*	—	—	—	6.88(1H) 6.8(t)	—	—	—	—
H-6′	δ *J*	6.1–8.4(1H)	8.20(1H) 2.3; 8.7(dd)	6.3–7.3(1H)	7.18(1H) m	7.18(1H) 8.7(d)	8.22(1H) 2.3;8.7(dd)	6.2–8.2(1H)	7.16(1H) 8.3(d)
H-7′	δ *J*	6.1–8.4(1H)	6.80(1H)	6.3–7.3(1H)	6.54(1H) 7.6(d)	6.47(1H) 8.2(d)	6.51(1H) 8.7(d)	6.2–8.2(1H)	6.47(1H)
H-3	δ *J*	5.64(1H) 10(d)	5.85(1H) 10.4(d)	5.85(1H) 10(d)	5.71(1H) 10.2(d)	5.71(1H) 10.2(d)	5.69(1H) 10.1(d)	5.75(1H) 10.6(d)	—
H-4	δ *J*	6.1–8.4(1H)	7.0(1H)	6.3–7.3(1H)	6.97(1H) 10.1(d)	6.70(1H) 10.0(d)	7.04(1H) 10.2(d)	6.2–8.2(1H)	—
H-5	δ *J*	6.1–8.4(1H)	8.03(1H)	6.3–7.3(1H)	7.36(1H) 8.1(d)	7.35(1H) 8.5(d)	7.36(m,1H)	6.2–8.2(1H)	7.0(1H) 8.9(d)
H-6	δ *J*	6.1–8.4(1H)	—	—	7.39(1H) 8.1(d)	7.42(1H) 8.3(t)	7.42(m,1H)	6.2–8.2(1H)	7.68(1H) 8.9(d)
H-7	δ *J*	6.1–8.4(1H)	8.06(1H) 8.9(d)	6.3–7.3(1H)	7.70(1H) 8.1(d)	7.74(1H) 8.0(d)	7.73(1H) 8.2(d)	6.2–8.2(1H)	7.75(1H) 8.1(d)
H-8	δ	6.1–8.4(1H)	6.54(1H)	6.3–7.3(1H)	7.30(m,1H)	7.33(m,1H)	7.32(m,1H)	6.2–8.2(1H)	7.40(1H)
H-9	δ *J*	—	—	—	7.9(m,1H)	7.21(1H) 8.5(d)	7.21(1H) 8.3(d)	6.2–8.2(1H)	7.58(1H) 8.5(d)
H-10	δ *J*	—	—	—	7.88(1H) 8.3(d)	7.91(1H) 8.2(d)	7.86(1H) 8.4(d)	6.2–8.2(1H)	8.55(1H)

[a] $CDCl_3$, chemical shifts referred to TMS. Multiplicity of coupling and number of protons are given in parentheses.
[b] Digital resolution ± 0,29 Hz.
[c] δ = 7.72 ppm for H-2.

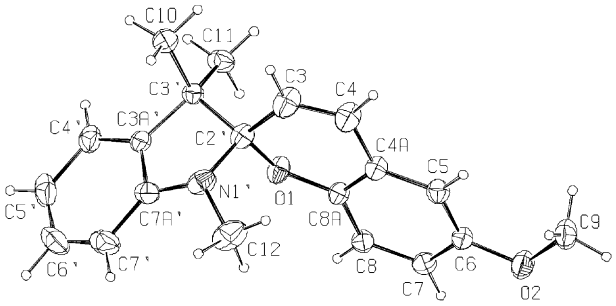


Figure 3. The molecular structure and atom labelling of the indolinospirobenzopyran **9**. Displacement ellipsoid are drawn at the 30% probability level.

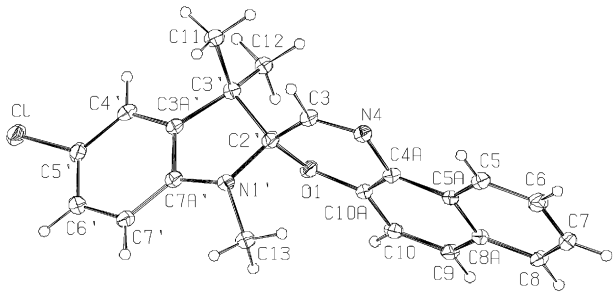


Figure 4. The molecular structure and atom labelling of the indolinospironaphtho-1,4-oxazine **17**. Displacement ellipsoid are drawn at the 30% probability level.

Table 2. Inhibitory effects of spirobipyridopyran **1**, spirobinaphthopyrans **2** and **3** (*c.f.* Fig. 1), indolinospirobenzopyrans **4–10**, indolinospironaphthopyrans **11–14**, indolinospiropyridopyran **15** and indolinospironaphtho-1,4-oxazines **16–18** (*c.f.* Fig. 2) on the growth of malignant tumor cells lines in comparison with their effects on the growth of normal human fibroblasts (WI38)

Comp	Tumor cell growth IC_{50}[a] (μM)										
	L1210	Molt4/C8	CEM	HBL	SW620	HeLa	MCF7	HT29	Hep2	MiaPaCa2	WI38
1	144.5	150.3	14.5	0.9	48	600	>100	35	700	300	>1000
2	>500	>500	>500	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
3	>500	>500	>500	40	54	>1000	>1000	>1000	>1000	>1000	>1000
4	31.1	40.4	24.9	>1000	>1000	105	166	178	178	199	126
5	348.3	317.2	283	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
6	285.6	>500	>500	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
7	26.6	11.5	10.6	420	380	>1000	23.9	>1000	>1000	50.1	72.4
8	32.6	17.6	11.1	14.3	13.2	10	34.7	25	15.8	5.5	10
9	4.9	2.3	2.2	3.5	4.5	2.0	3.1	3.8	2.5	3.2	5.6
10	49.6	35	19	16.2	15.8	15.8	44.7	41.6	41.6	40.7	29.5
11	>500	156.1	244.8	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
12	>500	>500	>500	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
13	>500	>500	>500	>1000	>1000	>1000	>1000	400	>100	600	>1000
14	281.5	93.8	80	>1000	>1000	890	427	>1000	>1000	>1000	417
15	120.9	114.7	49.6	30	35	10	10	10	10	90	600
16	103.7	54.9	57.9	420	295	>1000	10.9	>1000	>1000	100	14.8
17	332.8	167.8	170.5	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
18	>500	>500	>500	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000

[a] 50% Inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%.

lines. However, these compounds also exhibited a cytotoxic effect on normal human fibroblasts. The compound **9** of this class containing a methoxy group at the position 6 of the benzopyran ring exhibited pronounced inhibitory effects on the growth of all tumor cell lines (IC_{50} within the concentration range of 2.0–4.9 μM) (Fig. 5). Indolinospirobenzopyran **8** with the methoxy group at position 7 was approximately 2–10 fold less active against all examined cell lines than **9**, while indolinospirobenzopyrans **4** and **7** with a nitro group at the position 6 of the benzopyran ring exhibited more selective cytostatic activity. These compounds showed better inhibition of the growth of murine L1210 (IC_{50}: 31.1 μM for **4** and 26.6 μM for **7**) and human Molt4/C8 (IC_{50}: 40.4 μM for **4** and 11.5 μM for **7**) as well as CEM (IC_{50}: 24.9 μM for **4** and 10.6 μM for **7**) cell lines than other cells. Furthermore, compound **14** as representative of the indolinospironaphthopyran class showed only slight activity against human T-lymphocytes, while indolinospiropyridopyran **15** containing a nitro group at position 5′, was rather active against all examined tumor cell lines, particularly against HeLa, MCF7, HT29 and Hep2 cells (IC_{50}: 10 μM). Among the indolinospironaphtho-1,4-oxazines, compound **16** showed a moderate cytostatic activity, particularly against human breast carcinoma (MCF7) cells (IC_{50}: 10.9 μM).

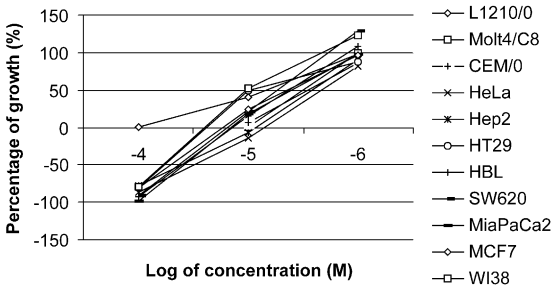


Figure 5. Cytostatic effect of compound **9** on different human cell lines. The cells were treated with compound **9** at different concentration and percentage of growth was calculated. Each point represents a mean value of three parallel samples in three individual experiments.

3.2. Antiviral activities

Compounds **4–10**, **14** and **16–18** were evaluated against varicella-zoster virus (VZV) and cytomegalovirus (CMV) in human embryonic lung (HEL) cells and their activities were compared with those of acyclovir (**ACV**), 5-(2-bromovinyl)-2′-deoxyuridine (**BVDU**), ganciclovir (**GCV**) and (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]cytosine (**HPMPC, cidofovir, CDV,** Table 3). Compounds **6** and **7** showed best and selective activity against VZV, whereas compounds **4**, **8**, **10** and **16** had some lower activity against VZV. Thus, **6** showed significant activity against thymidine kinase-positive (TK^+) strains (IC_{50}: 1.5 μM against the YS strain and 1.0 μM against the OKA strain) and thymidine kinase-deficient (TK^-) (IC_{50}: 1.1 μM) 07/1 strain of VZV. Indolinospirobenzopyran **10** with the methoxy group at position 7 of the benzopyran ring and chloro at position 5′ of the indolino moiety exhibited selective activity against CMV (AD-169 strain: IC_{50}: 12.6 μM and Davis strain: IC_{50}: 7.0 μM). Comparison of antiviral activity of **10** to that of **GCV** ascertained that activity against CMV of compound **10** was close to that of GCV. However, compound **10** exhibited also a more pronounced cytotoxic effect (Table 3).

Compounds **4–10**, **14** and **16–18** were also evaluated for their activity against human immunodeficiency virus type 1 and 2 (HIV-1, HIV-2), vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus. No specific antiviral effects

Table 3. Activity of **4–10**, **14** and **16–18** against varicella-zoster virus (VZV) and cytomegalovirus (CMV) in human embryonic lung (HEL) cells

Comp	Antiviral activity EC_{50} (μM)[a]					Cytotoxicity (μM)	
	TK^+ VZV		TK^- VZV	CMV		Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
	YS strain	OKA strain	07/1 strain	AD-169 strain	Davis strain		
4	5.7	3.6	3.1	>12.4	7.5	62.2	16.2
5	ND	>9.9	>9.9	>49.8	>49.8	248.8	141.5
6	1.5	1.0	1.1	>8.7	>8.7	43.5	18.5
7	2.7	1.9	1.7	>1.8	>1.8	8.9	8.9
8	9.2	6.0	5.5	>10.4	>10.4	52.2	18.9
9	ND	>2.1	>2.1	>10.4	>10.4	52.2	<6.5
10	4.4	3.7	2.5	12.6	7.0	54.5	32.1
14	ND	>44.2	>8.8	>44.2	>44.2	220.8	119.2
16	4.7	4.5	5.1	>9.8	>9.8	48.8	20.1
17	ND	>8.8	>8.8	>44	>44	220	107.5
18	ND	>8.2	>8.2	>204.8	>40.9	204.8	96.3
ACV	1.1	0.8	53.3	ND	ND	>222	888
BVDU	0.0033	0.0048	>150	ND	ND	ND	489
GCV	ND	ND	ND	7.9	9.1	>196	674.2
CDV	ND	ND	ND	2.7	2.1	>179	236.3

ND, not determined.
[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

were noted for any of the compounds against any of those viruses (data not shown).

4. Conclusions

The principal aim of the presented work was to evaluate various but structurally closely related classes of the chiral *spiro*-compounds for their cytostatic and antiviral activities. For this reason the new indolinospirobenzopyrans **5–7**, indolinospironaphtopyrans **11–14** and indolinospironaphtho-1,4-oxazine **17** were synthesized. The exact stereostructures of the representatives of two classes, namely indolinospirobenzopyran **9** and indolinospironaphtho-1,4-oxazine **17**, were determined by X-ray structural analysis.

Among the evaluated compounds, spyrobipyridopyran **1** showed the most pronounced inhibitory activity against human melanoma (HBL) cells (IC_{50}: 0.9 μM). This compound also exhibited the highest cytostatic selectivity. Compound **9**, containing a methoxy group at the position 6 of the benzopyran ring, exhibited significant cytostatic activities against all examined cell lines (IC_{50} within the concentration range of 2.0–4.9 μM). However, this compound also exhibited a cytotoxic effect on normal human fibroblasts. Compounds **4**, **6–8, 10** and **16** showed a selective activity against VZV, whereas compound **10** also showed selectivity against CMV (EC_{50}: within the concentration range of 1.0-12.6 μM).

5. Experimental

5.1. General methods

Melting points (uncorrected) were determined with Kofler micro hot-stage (Reichert, Wien) and Büchi 530 (Göppingen). Precoated Merck silica gel 60F-254 plates were used for thin layer chromatography (**TLC**) and the spots were detected under UV light (254 nm). Column chromatography (**CLC**) was performed using silica gel (0.063–0.2 mm), a Kemika; glass column being slurry-packed under gravity. The electron impact mass spectra were recorded with a Varian MAT 311A spectrometer with ionising energy 70 eV. Elemental analyses were performed in the Micro-analytical Laboratory of the University of Regensburg. ^1H and ^{13}C NMR spectra were recorded on Varian Gemini 300, Bruker WM-250 and Bruker ARX-400 spectrometers. The samples were dissolved in $CDCl_3$ and measured in 5 mm NMR tubes. The ^1H and ^{13}C NMR chemical shift values (δ) are expressed in ppm referred to TMS and coupling constants (*J*) in Hz. Two-dimensional COSY (Correlated Spectroscopy), NOESY (Nuclear Overhauser Effect Spectroscopy) and DEPT (Distortionless Enhancement by Polarization Transfer) ^1H and ^{13}C NMR spectra were also used for structure elucidation of the novel compounds. IR spectra were measured on a Beckman Acculab 1 instrument. UV spectra were recorded on a Hitachi U 2000 spectrometer.

5.2. Compounds preparations

The compounds **1**,[1] **2–3**,[2] **4**,[4] **15**,[1] **16**[5] and **18**[6] had been prepared as described previously.

5.2.1. (±)-5′-Nitro-1′,3′,3′-trimethylspiro(2H-1-benzopyran-2,2′-indoline) (5). The mixture of 5-nitro-1,3,3-trimethyl-2-methyleneindoline (1.1 g, 5 mmol) and 2-hydroxybenzaldehyde (0.6 g, 5 mmol) in absolute ethanol (20 mL) was heated under reflux for 2 h and the hot reaction mixture was then filtered. The solidified product was obtained by cooling the reaction mixture to room temperature. Recrystallization from light petroleum (80–110 °C) gave yellow-orange crystals. Yield

30%, mp = 178–182 °C; UV (MeOH): λ_{max} (log ε) = 214 nm (4.55), 255 (4.08), 308 (3.75), 372 (4.20); IR (KBr): ν = 1610 cm^{-1} (C=C), 1500 (C=$C_{aromat.}$, N-O), 1310 (N-O), 1255 (C-O), 970 (N-$C_{sp.}$-O); MS *m/z* 322.4 ($M^{+}\cdot$). Anal. calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.57; H, 5.62; N, 8.71.

5.2.2. (±)-5′-Nitro-6-nitro-1′,3′,3′-trimethylspiro(2H-1-benzopyran-2,2′-indoline) (6). 5-Nitro-1,3,3-trimethyl-2-methyleneindoline (2.1 g, 9.62 mmol) and 2-hydroxy-5-nitrobenzaldehyde (1.67 g, 10 mmol) were dissolved in ethanol (99%, 35 mL) and heated under reflux for 2 h. After cooling the reaction mixture to room temperature yellow-orange crystals were obtained, filtered off, washed and dried *in vaccuo*. The crude solidified product was recrystallized from ethanol. Yield 67%, mp = 201–204 °C; IR (KBr): ν = 3060 cm^{-1} (=C–H), 2980, 2965 (–C–H), 1590, 1495, 1485 (C=C, N–O), 1300 (N–O); MS *m/z* 367.4 ($M^{+}\cdot$). Anal. calcd for $C_{19}H_{17}N_3O_5$: C, 62.12; H, 4.66; N, 11.44. Found: C, 62.18; H, 4.64; N, 11.41.

5.2.3. (±)-5′-Chloro-6-nitro-1′,3′,3′-trimethylspiro(2H-1-benzopyran-2,2′-indoline) (7). Compound **7** was prepared by heating the mixture of 5-chloro-1,3,3-trimethyl-2-methyleneindoline (1.05 g, 5 mmol) and 2-hydroxy-5-nitrobenzaldehyde (835 mg, 5 mmol) in ethanol (25 mL) under reflux for 4 h. The solidified crude product was filtered off and recrystallized from ethanol to give crystals of **7**. Yield 28%, mp = 152–154 °C; UV(MeOH): λ_{max} (log ε) = 227 nm (4.10), 249 (4.31), 312 (3.87), 334 (3.85); MS *m/z* 356.8 ($M^{+\cdot}$). Anal. calcd for $C_{19}H_{17}ClN_2O_3$: C, 63.96; H, 4.80; N, 7.85. Found: C, 63.81; H, 4.82; N, 7.83.

Compounds **8**–**10** were prepared according to S. Kraml, Diploma Thesis, University of Regensburg, Germany, 1994.

5.2.4. (±)-1′,3′,3′-Trimethylspiro[indoline-2,2′-3H-naphtho(1,2-b)pyran] (11). Compound **11** was prepared by reacting of 1-hydroxy-2-naphthaldehyde (1 g, 5.8 mmol) with 1,3,3-trimethyl-2-methyleneindoline (1 mL, 5.8 mmol) in absolute ethanol (15 mL). After refluxing for 2 h, the reaction mixture was cooled to room temperature and a precipitate was formed. After filtration of this precipitate, the crude product was purified by column chromatography using light petroleum (40–60°):chloroform = 1:2 as eluent. Recrystallization of the crude crystals from ethanol gave red crystals. Yield 32%, mp = 208–210°; UV(MeOH): λ_{max} (log ε) = 206nm (4,51); 218 (4,40); 260 (4,33); 268 (4,35); 308 (3,82); 386 (3,59); 554 (4,28); 588 (4,32). IR(KBr): ν = 2926 cm^{-1} (C–H), 1586 (C=C), 1204 (C–O), 932 (N–$C_{sp.}$–O); ^{13}C NMR δ 28.82 (N–CH_3), 105.04 (C-2/2′), 51.34 (C-3′), 20.19 (3′-CH_3), 25.68 (3′-CH_3), 136.80 (C-3′a), 121.72 (C-4′), 118.96 (C-5′), 127.36 (C-6′), 106.72 (C-7′), 147.99 (C-7a), 117.42 (C-3), 129.57 (C-4), 134.49 (C-4a), 118.99 (C-5), 126.28 (C-6), 112.50 (C-6a), 127.36 (C-7), 125.17 (C-8), 124.43 (C-9), 121.42 (C-10), 123.43 (C-10a), 149.34 (C-10b); MS (70 eV) *m/z* = 327 $M^{+\cdot}$. Anal. calcd for $C_{23}H_{21}NO$: C, 84.37; H, 6.46; N 4.28. Found: C, 84.14; H, 6.47; N 4.27.

5.2.5. (±)-5′-Chloro-1′,3′,3′-trimethyspiro[indoline-2,2′-3H-naphtho(1,2-b)pyran] (12). Compound **12** was prepared by reacting equimolar quantities of 1-hydroxy-2-naphthaldehyde and 5-chloro-1,3,3-trimethyl-2-methyleneindoline using the procedure analogous to that for preparing **11**. Recrystallization of the crude product from ethanol gave crystals of **12**. Yield 17%, mp = 200–203 °C; UV(MeOH): λ_{max} (log ε) = 208nm (4,63); 218 (4,57); 260 (4,63); 268 (4,61); 310 (3,93); 354 (3,61); 388 (3,11); 556 (3,90); 592 (3,91). IR(KBr): ν = 2966 cm^{-1} (C–H), 1605 (C=C), 1274 (C–O), 934 (N–$C_{sp.}$–O); ^{13}C NMR δ 28.80 (N–CH_3), 105.39 (C-2/2′), 51.62 (C-3′), 20.09 (3′-CH_3), 25.61 (3′-CH_3), 139.13 (C-3′a), 122.33 (C-4′), 123.90 (C-5′), 127.39 (C-6′), 107.90 (C-7′), 147.03 (C-7′a), 117.12 (C-3), 130.26 (C-4), 134.88 (C-4a), 119.70 (C-5), 126.75 (C-6), 112.70 (C-6a), 127.76 (C-7), 125.65 (C-8), 124.72 (C-9), 121.90 (C-10), 123.68 (C-10a), 149.50 (C-10b); MS(70 eV) *m/z* = 362 $M^{+\cdot}$. Anal. calcd for $C_{23}H_{20}$ ClNO: C, 76.34; H, 5.57; N, 3.87. Found: C, 76.11; H, 5.58; N, 3.86.

5.2.6. (±)-5′-Nitro-1′,3′,3′-trimethylspiro[indoline-2,2′-3H-naphtho(1,2-b)pyran] (13). Reaction of equimolar quantities of 1-hydroxy-2-naphthaldehyde and 5-nitro-1,3,3-trimethyl-2-methyleneindoline by a procedure analogous to that for **11** gave crystals of **13**. Yield 23%, mp = 230–232 °C; UV(MeOH): λ_{max} (log ε) = 206 nm (4,51); 218 (4,59); 260 (4,56); 268 (4,62); 358 (4,25). IR(KBr): ν = 2924 cm^{-1} (C–H), 1608 (C=C), 1271/1239 (C–O), 924 (N–$C_{sp.}$–O); ^{13}C NMR δ 28.58 (N–CH_3), 105 (C-2/2′), 50.82 (C-3′), 19.86 (3′-CH_3), 25.38 (3′-CH_3), 137.56 (C-3′a), 121.40 (C-4′), 153.16 (C-5′), 126.76 (C-6′), 105.26 (C-7′), 140.49 (C-7′a), 115.82 (C-3), 130.61 (C-4), 134.75 (C-4a), 118.35 (C-5), 126.23 (C-6), 112.22 (C-6a), 127.64 (C-7), 125.70 (C-8), 124.40 (C-9), 120.11 (C-10), 123.31 (C-10a), 148.74 (C-10b); MS(70 eV) *m/z* = 372 $M^{+\cdot}$. Anal. calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 74.09; H, 5.40; N, 7.50.

5.2.7. (±)-5′-Chloro-1′,3′,3′-trimethylspiro[indoline-2,3′-3H-naphtho(1,2-b)pyran] (14). Compound **14** was prepared by reaction of 2-hydroxy-1-naphthaldehyde (0.85 g, 5 mmol) with 5-chloro-1,3,3-trimethyl-2-methyleneindoline (1.05g, 5 mmol) in absolute ethanol (25 mL). After refluxing for 4 h, the reaction mixture was cooled. A pink solid was precipitated, filtered and washed with ethanol. The product was purified by recrystallization from ethanol (96%). Yield 20%, mp = 220–224 °C; UV(MeOH): λ_{max} (log ε) = 241 nm (4.70), 300 (4.03), 313 (4.02), 339 (3.70), 360 (2.50)sh; IR (KBr): ν = 1635 cm^{-1} (C=C), 1248 (C–O), 990 (N–$C_{sp.}$–O); MS *m/z* 361.9 ($M^{+\cdot}$). Anal. calcd for $C_{23}H_{20}ClNO$: C, 76.34; H, 5.57; N, 3.87. Found: C, 76.39; H, 5.58; N, 3.86.

5.2.8. (±)-5′-Chloro-1′,3′,3′-trimethylspiro[indoline-2,3′-3H-naphtho(1,2-b)(1,4)oxazine] (17). 1-Nitroso-2-naphthol (3 g, 17.3 mmol) reacted with freshly destilled 5-chloro-1,3,3-trimethyl-2-methyleneindoline (3.59 g, 17.3 mmol) under reflux for 18 h. Purification of crude solid product by column chromatography (toluene and light petroleum:ethyl acetate = 15:1) gave an oily product.

Formation of the green crystals was effected by adding a small amount of acetone. That product was additionaly washed with ice cold petrolether and dried in vacuo. Yield 15%, mp = 176–179 °C; UV(MeOH): λ_{max} (log ε) = 231 nm (4.71), 313 (3.87), 347 (3.58)sh; IR (KBr): ν = 3040 cm^{-1} (=C–H), 2840, 2870, 2905, 2970 (–C–H), 1470, 1573, 1590, 1600 (C=C, C=N). MS *m/z* 362.9 ($M^{+\cdot}$). Anal. calcd for $C_{22}H_{19}ClN_2O$: C, 72.82; H, 5.28; N, 7.72. Found: C, 72.63; H, 5.29; N, 7.73.

5.3. X-Ray determination

The single crystals of **9** and **17** suitable for X-ray structure analysis were obtained by growth at room temperature of a very dilute ethanol solution. For compound **9**, the intensities were collected at room temperature on a Philips PW1100 diffractometer updated by Stoe and Cie[11,12] using Mo-K_α radiation (λ = 0.71073 Å) with the ω-scan mode. For compound **17**, the intensities were collected at 100 K on a Nonius KappaCCD diffractometer[13,14] using Cu-K_α radiation (λ = 1.54184 Å) with the ω-scan mode. The intensities were corrected for Lorentz and polarization effects for both compounds. The crystal structures were solved by direct methods using *SHELXS97*[15] program. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares calculations based on F^2 using *SHELXL97*[16] program. The hydrogen atoms were treated using appropriate riding models and their coordinates were included in structure factor calculations. The final difference map contained no significant features ($\Delta\rho_{max.}/\Delta\rho_{min.}$ = 0.209/−0.146 eÅ^{-3} for **9** and 0.257/−0.235 eÅ^{-3} for **17**). The molecular drawings were prepared by *PLATON*[17] program. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-213813 and CCDC-213814. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data for **9**: $C_{20}H_{21}NO_2$, M_r = 307.38, orthorhombic space group *P*bca; *a* = 38.699(5), *b* = 11.972(2), *c* = 7.215(1) Å, *V* = 3342.7(8) Å^3; *Z* = 8; *F*(000) = 1312; d_x = 1.222 g cm^{-3}; μ(MoK_α) = 0.078 mm^{-1}; *S* = 0.986; *R*/*wR* = 0.0594/0.1340 for 210 parameters and 1578 reflections with $I \geq 2\sigma(I)$, *R*/*wR* = 0.1769/0.1791 for all 3990 independent reflections measured in the range 5.42°−2θ−55.96°.

Crystal data for **17**: $C_{22}H_{19}ClN_2O$, M_r = 362.84, monoclinic space group $P2_1/a$; *a* = 8.3312(1), *b* = 16.2963(1), *c* = 12.9924(1) Å, β = 95.223(6)°; *V* = 1756.63(1) Å^3; *Z* = 4; *F*(000) = 760; d_x = 1.372 g cm^{-3}; μ(CuK_α) = 2.022 mm^{-1}; *S* = 1.047; *R*/*wR* = 0.0474/0.1240 for 238 parameters and 1827 reflections with $I \geq 2\sigma(I)$, *R*/*wR* = 0.0507/0.1266 for all 1993 independent reflections measured in the range 8.72°−2θ−108.48°.

5.3.1. Biological tests antitumor activity assays. Biological evaluation of the compounds **1**–**18** was performed for potential antitumor activity. The effects of different concentrations of each compound on proliferation of tumor and normal cell lines was examined. The cells (melanoma, HBL; cervical carcinoma, HeLa; breast carcinoma, MCF7; colon carcinoma, HT29 and SW620; laryngeal carcinoma, Hep2; pancreatic carcinoma, MiaPaCa2 and human normal fibroblasts, WI38) were seeded in 96-well plates at a concentration of 3×10^4 / mL in D-MEM, supplemented with 10% FBS and glutamine (2 mM) and grown in humidified atmosphere with 5% CO_2. At 24 h later, the test compounds were added at a final concentration of 10^{-6}, 10^{-5} and 10^{-4} M. The number of cells was determined imediatelly before (day 0) and 72 h after addition of compounds, using the MTT test.[18] A method is based on a reduction of MTT (yellow) with a mitochondrial dehydrogenase from alive cells to yield a formazan product (red). The number of living cells is linearly proportional to the amount of reduced MTT. The medium was discarded and MTT (20 μg/40 μL) was added to each well. Formed precipitates were disolved in 160 μL DMSO and the absorbance was measured on an ELISA reader at 570 nm. The compounds were dissolved in DMSO as a 10^{-1} M solution and diluted with a medium to the appropriate concentration. The final concentration of DMSO was less than 0.1%, and at that concentration it did not influence cell growth. Control cells were grown in D-MEM without any addition. The antitumor activity results were expressed as IC_{50}, that is the concentration required to afford 50% inhibition of cell growth. Each result was a mean value from three parallel samples in three individual experiments.

Antitumor activity against L1210 (murine leukemia), FM3A (murine mammary carcinoma), Molt4/C8 and CEM (human T-lymphocytes) cell lines were measured essentially as originally described for the mouse leukemia (L1210) cell line.[19]

5.3.2. Antiviral activity assays. Antiviral activity against VZV, CMV, HIV-1, HIV-2, vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus was determined essentially as described previously.[20,21] The antiviral activity results were expressed as EC_{50}, that is the effective concentration required to afford 50% protection against viral cytopathogenicity or viral plaque formation.

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