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Phosphorohydrazines of 4-oxo-4*H*-1-benzopyran and 1-benzopyran-2,4-dione exhibit antitumor activity against L1210 leukemia

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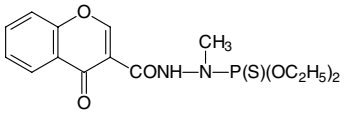
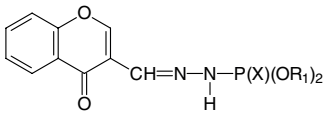
Phosphorohydrazines and phosphorohydrazones of benzopyran-2,4-dione as well as the phosphorohydrazone of 4-hydroxycoumarine were tested for antitumor activity in lymphatic leukemia L1210 bearing mice.

Chromone (4-oxo-4*H*-1-benzopyrane) and coumarin (2-oxo-2*H*-1-benzopyrane) are present in both natural and synthetic compounds of various pharmacological activity (Jimenez-Orozco 2001; Valenti 1996; Kawase 2001; Kayser and Kolodziej 1999; Mao 2002) e.g. antitumor (Jimenez-Orozco 2001; Valenti 1996), antibacterial (Kawase 2001; Kayser and Kolodziej 1999), against HIV (Mao 2002). Recent work from our laboratory has demonstrated that phosphonic derivatives of chromone (Kostka 1994) showed antitumor activity. Phosphoric derivatives of hydrazine showed activity against E0771 tumor in mice (Cates and Lemke 1974).
The mentioned observation initiated the synthesis (Nawrot-Modranka and Kostka 1995; Rybarczyk-Pirek 2003, 2002 b) of chromone phosphohydrazones (**1**–**6**) and phosphorohydrazines of benzopyran-2,4-dione (**7**, **8**) as well as the phosphorohydrazone of 4-hydroxycoumarine (**9**) (Rybarczyk 1999; Rybarczyk-Pirek 2002 a). To allow meaningful pharmacological studies the X-ray structures of **2**, **3**, **4**, **7**, **9** were recently determined (Rybarczyk 1999; Rybarczyk-Pirek 2002 a, 2003, 2002 b). We report herein the preliminary results of the evaluation of *in vivo* antitumor activity of compounds **1**–**9**, performed in lymphatic leukemia L1210 bearing mice.
Out of the 9 compounds administered peritoneally to mice, six were not absorbed from the peritoneal cavity. After administration of the compounds **3**–**6**, **8**, **9** (2 g/kg) no changes were observed. Therefore these compounds have not been further examined in this study. The antitumor activity of the compounds **1**, **2**, **7** have been evaluated.
Compound **7** was given to mice in three doses 0.9; 0.45 and 0.09 g/kg. In the first two doses leukemia development was inhibited. The T/C% value for each of these doses amounted to 169%. However in a dose of 0.09 g/kg this compound did not show any activity, its T/C% value amounted to 108%. Compound **1** also administered in three different doses (1.3; 0.6; 0.13 g/kg) inhibited leukemia development (T/C% was 130%). Compound **2** did not inhibit the neoplastic cells growth in any of the administered doses, T/C% value amounted to 108% (Table).
It has been demonstrated that in the case of compounds **3**–**6** and **8**, **9** due to being unabsorbable from the peritoneal cavity, a different way of administration is required in order to establish their antineoplastic activity. In conclusion, we report of a novel class of phosphorohydrazines of chromone and 1-benzopyran-2,4-dione (tautomeric forms of coumarin) that are endowed with inhibitory against L1210 bearing mice. From our studies, it could be concluded that the 4-oxo-4H-1-benzopyran must bear a phosphorohydrazine group concomitantly on C-3, to display antileukemic activity. The most active compounds of the phosphorohydrazines of chromone and 1-benzopyran-2,4-dione or coumarin series are compounds **1**, **2**, **7**. The antitumor activity was lower if the chain

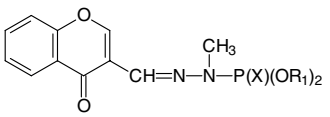


1



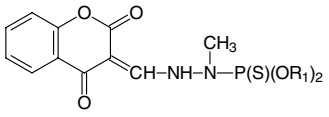
2,3

2: X = O, $R_1 = C_6H_5$
3: X = S, $R_1 = C_2H_5$



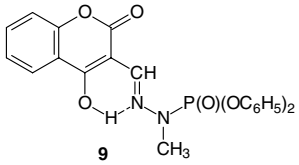
4-6

4: X = O, $R_1 = C_6H_5$
5: X = S, $R_1 = CH_3$
6: X = S, $R_1 = C_2H_5$



7,8

7: $R_1 = CH_3$
8: $R_1 = C_2H_5$



9

Table: Activity of compounds 1, 2, 7 in L1210 leukemia

Compd.	ALD^{*}_{50} (g/kg) i.p.	Dose i.p. (ratio of ALD^{*}_{50})	Dose (g/kg) of body weight	T/C (%)	Antineoplastic effect according NCl-USA**
3-{[2-Diethoxythiophosphoryl)-2-methylhydrazino]-carbonyl}-3,4-dihydro-4*H*-1-benzopyran-4-one (**1**)	1.3	1.0	1,30	130	+[a]
		0.5	0.65	130	+[a]
		0.1	0.13	130	+[a]
3-{[2-Diphenoxyphosphoryl)-hydrazono]methyl}-4*H*-1-benzopyran-4-one (**2**)	0.57	1.0	0.57	108	−[b]
		0.5	0.285	108	−[b]
		0.1	0.057	108	−[b]
(*E*)-3-{[Dimethoxythiophosphoryl)-2-methylhydrazino]methylidene} 3,4-dihydro-2*H*-1-benzopyran-2,4-dione (**7**)	0.9	1.0	0.90	169	+[a]
		0.5	0.45	169	+[a]
		0.1	0.09	108	−[b]

* The approximate LD_{50} (ALD_{50}) was calculated according to Deichmann and Le Blance (1943)
** [a] $T/C(\%) > 125$ (+); [b] $100 < T/C(\%) < 125$ (−)

phosphorohydrazine was replaced by the phosphorohydrazone chain.

These presented results suggest that the new compounds (**1**, **2**, **7**) can be considered as potentially antineoplastic agents and encourage further studies of antibacterial properties of these ligands and their Pd(II) complexes.

Experimental

1. Animals

For the experiments hybrid male, F_1 (BALB/c × DBA/2) mice, weighing 23–29 g, 8–10 weeks old, purchased from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wrocław) were used. They received standard laboratory food and water *ad libitum*.

2. Leukemia

Leukemia L1210 was purchased from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wrocław) and was maintained by serial passages *in vivo*. Leukemia cells from the fluid were resuspended in 0.9% sodium chloride, so that 3×10^5 L1210 cells could be injected intraperitoneally (i.p.) into mice.

3. Therapeutics

The compounds were administrated in a volume of 0.01 ml/g mouse weight in 1% methylcelulose solution. Control mice received equivalent volumes of 1% methylcelulose solution.

4. Toxicity determination

The approximate lethal dose was determined by the method described by Deichmann and Le Blance (1943).

5. Antileukemic assay

On day $0{,}3 \times 10^5$ of L1210 leukemic cells were implanted i.p. into F1 mice. Five mice were used per groups. Beginning on day 1, the mice received solution of investigated compounds ($1 \times$ i.p.) after leukemia implanted. The mice of the control group received 1% methylcelulose solution on treatment day. The mice were observed daily for survival. The median survival time (MST) according to Geran's method (Geran 1972) is: $MST = (x + y)/2$, where × denotes the earliest day when the number of dead animals is $\geq$ N/2 y denotes the earliest day when the number of dead animals is $\geq (N/2 + 1)$; N denotes the number of animals in the group. The antileukemic effect of the drugs was assessed as percentage ratio of MST of the treated group (T) to that of the control group (C):
$T/C(\%) = (MST_T/MST_C) \times 100\%$

6. Statistical analysis

The results were evaluated using student's test for differences between means. Differences were considered significant when $p \leq 0.05$.

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