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Novel antiproliferative analogs of the *Taq* DNA polymerase inhibitor catalpol

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Abstract—The naturally occurring iridoid catalpol (**1**) is a *Taq* DNA polymerase inhibitor. However, its poor lipophilicity might account for the lack of biological activity against human solid tumor cell lines. The traditional prodrug approach by means of peracetylation of the free hydroxyl groups led to a compound, which showed a marginal growth inhibition against the most sensitive cell line A2780 (ovarian cancer). However, the formation of analogs bearing one to three silyl ether groups led to antiproliferative compounds against a panel of six human solid tumor cell lines, with GI_{50} values in the range 1.8–4.8 μM. Cell cycle studies revealed arrest in G_0/G_1 phase that is consistent with DNA polymerase inhibition.

Chemotherapy plays a key role in the treatment of many tumors. In this particular context, DNA polymerases represent important cellular targets in the development of anticancer and antiviral agents. The deoxycytidine analogs ara-C (1-β-D-arabinofuranosyl-cytosine) and gemcitabine (2',2'-difluorodeoxycytidine, dFdC) are DNA antimetabolites used in cancer therapy.[1] These drugs are activated inside the cells to their triphosphate form by deoxycytidine kinase.[2] In the short term, they are potent inhibitors of DNA synthesis by the competitive reversible inhibition of DNA polymerase. After long incubation they can be incorporated into DNA causing irreversible damage.[3] Ara-C is the preferential drug for the treatment of acute myelogenous leukemia (AML), whilst it is not that effective against solid tumors.[4] dFdC is active against several solid tumors, including non-small cell lung cancer (NSCLC) and pancreatic carcinoma.[5]

The iridoid catalpol (**1**) has shown significant inhibition of *Taq* DNA polymerase ($IC_{50} = 48\,\mu M$).[6] In vitro experiments and theoretical calculations suggest that the mechanism of *Taq* DNA polymerase inhibition may occur in a competitive way with deoxynucleoside triphosphates (dNTPs) at the binding site of the enzyme. In fact, iridoids show a certain resemblance with a

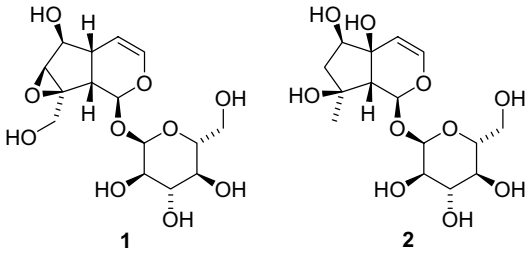

Figure 1. Structure of naturally occurring iridoids catalpol (**1**) and harpagide (**2**).

Keywords: Iridoids; Silyl ethers; Anticancer drugs; Solid tumors; DNA polymerase inhibitors.
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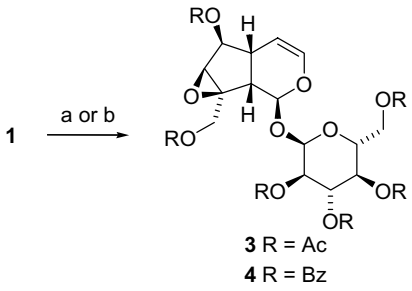
nucleoside framework (Fig. 1). The bicyclic aglycone could mimic the purine scaffold present in nucleosides. In contrast to catalpol (**1**), the natural iridoid harpagide (**2**) is a weak *Taq* DNA polymerase inhibitor (IC_{50} = 417 μM). Considering that the sugar moiety is identical for catalpol (**1**) and harpagide (**2**), the aglycone fragment seems to play a role in *Taq* DNA inhibition.[6b]

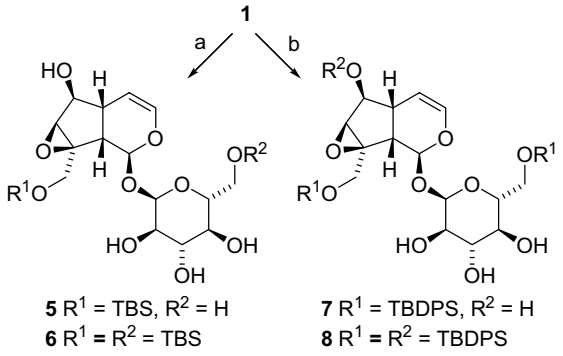
Herein we report on the synthesis and antiproliferative activity of novel lipophilic analogs of catalpol (**1**) against the representative panel of human solid tumor cell lines A2780 (ovarian), SW1573 (non-small cell lung), WiDr (colon), T-47D (breast), HBL-100 (breast), and HeLa (cervix). The in vitro antiproliferative activity was evaluated using the National Cancer Institute (NCI) protocol[7] after 48 h of drug exposure using the sulforhodamine B (SRB) assay.[8] In addition to the antitumor activity, the lipophilicity (C log *P*) of the compounds was evaluated by in silico calculation based on their chemical structure.[9] C log *P* values were calculated to correlate lipophilicity with antitumor activity. Since DNA polymerase inhibitors are known to interfere with the cell cycle and cause arrest in G_0/G_1 phase,[10] we performed cell cycle studies to assess the effect of the new drugs in HBL-100 and SW1573 cells.

In a preliminary in vitro antiproliferative screening, catalpol (**1**) and harpagide (**2**) did not reach growth inhibition at the maximum concentration tested, which is 100 μM (Table 1). We speculated that the poor lipophilicity of the compounds might account for the lack of biological activity. This major drawback also occurs to the antimetabolites used in current therapy ara-C and dFdC. Antimetabolites cannot enter cells easily by passive diffusion due to their low lipophilicity. Instead they enter the cells using specialized transport systems.[11]

In an effort to increase drug accumulation and prolong drug retention inside cancer cells, a first series of peracetylated catalpol analogs **3**[12] and **4** were synthesized by standard methods (Scheme 1).[13] The rationale behind this prodrug approach is that the ester groups would be hydrolyzed in vivo by the action of esterases,[14] which are prevalent inside the cells. The results on antiproliferative activity showed a marginal antiproliferative activity for the peracetylated analog **3** against the ovarian cancer cell line (GI_{50} = 60 μM). A2780 is the most sensitive cell line of the aforementioned panel to anticancer drugs. It is noteworthy that compound **3** was not able to inhibit *Taq* DNA polymerase at 500 μM.[6b]



Scheme 1. Reagents and conditions: (a) Ac_2O, py, rt, 80%; (b) BzCl, Et_3N, CH_2Cl_2, 68%.



Scheme 2. Reagents and conditions: (a) TBSCl, imidazole, DMF, 72% for **5**, 79% for **6**; (b) TBDPSCl, imidazole, CH_2Cl_2, 86% for **7**, 67% for **8**.

Within our program directed at the synthesis of novel antitumor compounds, we have reported recently that silyl ethers represent a plausible strategy to introduce lipophilicity in drugs.[15] Therefore, our novel approach was to synthesize *tert*-butyldimethylsilyl (TBS) or *tert*-butyldiphenylsilyl (TBDPS) ethers of catalpol (**1**). To the best of our knowledge, there are neither enzymes known to cleave silyl ether bonds nor reports in the literature related to such process. We speculate that the silyl ether bond is stable enough to the metabolic machinery of the cells.

Table 1. In vitro antiproliferative activity of catalpol (**1**) and its analogs against a representative panel of human solid tumor cell lines[a]

Compound	Cell line						
	C log *P*[b]	A2780 (ovarian)	SW1573 (NSCLC)	WiDr (colon)	T-47D (breast)	HBL-100 (breast)	HeLa (cervix)
1	−3.30	>100	>100	>100	>100	—	—
2	−3.77	>100	>100	>100	>100	—	—
3	0.57	60[c]	>100	>100	>100	—	—
4	11.6	>100	>100	>100	>100	>100	>100
5	−0.58	36 (±0.3)	88 (±21)	81 (±33)	87 (±22)	52 (±4.4)	77 (±21)
6	1.10	2.6 (±0.9)	4.8 (±2.4)	2.7 (±0.5)	3.6 (±0.6)	2.8 (±1.4)	3.6 (±1.0)
7	7.86	2.4 (±0.5)	2.7 (±1.0)	3.4 (±0.3)	4.1 (±0.1)	2.7 (±1.4)	4.4 (±2.7)
8	13.9	2.4 (±1.1)	1.8 (±0.1)	2.0 (±0.3)	2.7 (±1.0)	2.4 (±0.5)	2.1 (±0.3)

[a] Values expressed as GI_{50} (50% growth inhibition) are given in micromolar and are means of two to four experiments, standard deviation is given in parentheses.
[b] Ref. 9.
[c] One experiment was performed.

Table 2. Flow cytometric analysis of HBL-100 and SW1573 cells treated with the novel lipophilic catalpol analogs **6**–**8**

Cell line	Drug	Dose (μM)	Cell cycle population (%)		
			G_0/G_1	S	G_2/M
HBL-100	Control		44	40	16
	6	5	54	35	11
	7	5	52	33	15
	8	5	56	32	12
	6	10	51	30	19
	7	10	59	28	13
	8	10	59	30	11
SW1573	Control		47	38	15
	6	5	49	37	14
	7	5	54	32	14
	8	5	58	29	13
	6	10	63	20	17
	7	10	61	28	11
	8	10	59	37	4

Thus, functional groups containing silicon might just provide lipophilicity to the drug, allowing it to pass the cell membrane by passive diffusion. This strategy has been successfully applied in the antitumor drug analogs silatecans[16] (silicon-containing camptothecins) and silaplatins[17] (cisplatin analogs), and the HIV-1 reverse transcriptase inhibitor TSAO-T.[18]

The silylation of hydroxyl groups with TBS and TBDPS can be carried out in a regioselective manner. Thus, primary hydroxyl groups are preferentially silylated to secondary hydroxyl groups when the reaction is carried out at 0 °C. Furthermore, the primary hydroxyl group of the bicyclic aglycone is more reactive than that of the glucose moiety. If one equivalent of TBSCl is reacted at 0 °C with catalpol (**1**), the monoprotected analog **5** is obtained as the sole product. The addition of an extra equivalent of TBSCl allows obtaining the diprotected derivative **6**. Diprotected compound **7** is prepared in a similar manner using two equivalents of TBDPSCl as silylating agent. Finally, the trisilyl ether **8** is obtained when catalpol (**1**) reacts with three equivalents of TBDPSCl. Likewise primary alcohols, the secondary hydroxyl group at the aglycone scaffold is more reactive than those located at the glucose fragment.[19]

The Clog*P* values for analogs **5**–**8** are within a wide range (Table 1). However, the antiproliferative data reveal that all these new compounds are able to induce growth inhibition in the six cell lines. The derivative **5** bearing a single TBS group is the least active compound of the series, with GI_{50} values in the range 36–88 μM. The ovarian cancer cells are the most sensitive to analog **5**. This is consistent with previous results found in conventional anticancer drugs.[20] The remaining derivatives **6**–**8** are the most active of the series and show similar growth inhibition values against all cell lines. The GI_{50} values are in the range 1.8–4.8 μM. Two important consequences can be inferred from these results. First, the introduction of at least one silyl group is enough to induce growth inhibition. However, the antiproliferative effect is enhanced with two or three silyl groups. There is no difference observed on the biological activity between the TBS and the TBDPS analogs (**6** vs **7**). Second, the hydroxyl groups of the aglycone fragment seem not relevant for the activity. (Scheme 2).

Cell cycle control is the major regulatory mechanism of cell growth. Many cytotoxic agents and/or DNA damaging agents arrest the cell cycle at the G_0/G_1, S, or G_2/M phase and then induce apoptotic cell death. We examined cell cycle phase distribution by flow cytometry to determine if cell growth inhibition involves cell cycle changes. Since the GI_{50} values were similar in all cell lines, we selected HBL-100 and SW1573 as representative examples to study the influence on the cell cycle. The cells were exposed for 24 h to a low and a high drug concentration.[21] These doses were chosen based on the GI_{50} values of the drugs, 5 (largest GI_{50} of compounds **6**–**8**) and twice this value (10 μM). The results are given in Table 2.

For HBL-100 cells exposed to 5 μM of the drugs, it is possible to observe a slight increase in the percentage of cells in the G_0/G_1 phase. At the high dose, the arrest in G_0/G_1 phase augments. The rise is concomitant with a decrease in the S phase compartment. Overall, the same

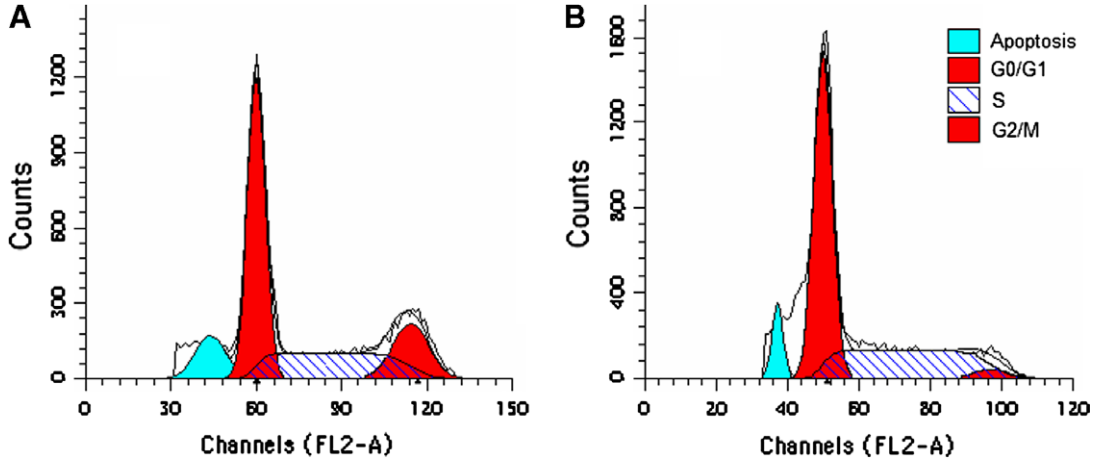


Figure 2. Effects on cell cycle distribution after 24 h exposure to 10 μM of (A) **6** against HBL-100 cells and (B) **8** against SW1573 cells.

effect is obtained for the cell cycle distribution of SW1573 cells exposed to analogs **6**–**8**. The effect in both cell lines is a G_0/G_1 arrest, which is consistent with DNA polymerase inhibition. At the high dose, 11% of apoptosis is observed for HBL-100 cells exposed to **6**, while analog **8** induced 7.6% of apoptosis in SW1573 cells (Fig. 2).

In summary, we have reported a series of novel lipophilic catalpol analogs by the regioselective addition of silyl ether groups. The new compounds inhibit in a dose-dependent manner the proliferation of a panel of diverse human cancer cell lines through G_0/G_1 phase arrest. Although the results are preliminary, we found that they are consistent with the inhibition properties of DNA polymerase exhibited by the parent compound catalpol (**1**). Additional studies on the mechanism by which these catalpol analogs induce apoptosis are currently underway and will be reported elsewhere.

Acknowledgments

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