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Carbohydrate-conjugated 4-(1,3,2-dithiarsolan-2-yl)aniline as a cytotoxic agent against colorectal cancer

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Arsenic trioxide (As₂O₃) has been approved for the treatment of acute promyelocytic leukemia (APL); however, its use in the treatment of solid tumors is limited due to its pharmacokinetic properties. Organic arsenic compounds provide better options for pharmaceutical optimization. *p*-Aminophenyl arsenoxide (*p*-APAO), an organic arsenic compound, was found to interact with the promyelocytic leukemia-retinoic acid receptor alpha (PML-RAR α) fusion protein in a similar manner to arsenic trioxide. Analogs of *p*-APAO such as 4-(1,3,2-dithiarsolan-2-yl)aniline (*p*-APDTAs) were recently found to show improved cytotoxicity toward several solid tumor cell lines with lower toxicity to normal cells. Here, we synthesized a carbohydrate-conjugated 4-(1,3,2-dithiarsolan-2-yl)aniline (*p*-APDTAs) and showed that it exhibited reduced cytotoxicity to normal cells, suggesting a feasible approach to improve the therapeutic index of arsenic-containing compounds as chemotherapeutic agents.

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Arsenic trioxide (As₂O₃) has been used for various purposes in ancient China and Greece for more than 2400 years.^{1,2} Use of arsenic-containing compounds to treat hematologic disorders such as leukemia, Hodgkin's disease, and pernicious anemia has been reported since the 1700s.³ In the 1970s, a group of physicians in China reported that a formulation of arsenic trioxide induced complete remission in a small group of patients with acute promyelocytic leukemia (APL).¹ Later on, the follow-up clinical trials by American investigators confirmed the clinical efficacy of arsenic trioxide in the management of APL. Subsequently, arsenic trioxide (TrisenoxTM) was approved by the U.S. Food and Drug Administration (FDA) in 2000 for the treatment of relapsed or refractory APL.⁴

Investigations to decipher the mechanism of action of arsenic trioxide revealed that the drug is able to promote the catabolic degradation of an oncogenic fusion protein that derives the proliferation of APL cells and has been found in over 98% of cases of human APL.⁵ The oncogenic fusion protein is the result of chromosomal rearrangements that juxtapose the

promyelocytic leukemia (PML) gene and the retinoic acid receptor alpha (*RAR α*) gene.⁶ Arsenic trioxide is capable of covalently modifying a cysteine-rich region of the PML moiety of the PML-RAR α fusion protein, leading to proteasome-dependent degradation of the oncogenic PML-RAR α fusion protein.^{7,8} Further studies have shown that arsenic trioxide can induce apoptosis of hematologic cancer cells and solid tumor cells *via* a myriad of mechanisms such as disruption of mitochondrial functions and cellular redox processes, activation of different caspases, and downregulation of Bcl-2 expression.^{9,10}

Despite the remarkable success of arsenic trioxide in the treatment of APL, the inorganic arsenic compound has certain limitations as a chemotherapeutic such as the systemic toxicity associated with a high amount of arsenic compounds in the blood, and poor pharmacokinetic properties.^{2,11} Although arsenic trioxide has shown efficacy for solid tumors in many preclinical studies, the therapeutic effects of low-dose arsenic oxide for solid tumors have not been clinically proven yet. This may be attributed to the rapid renal clearance of arsenic trioxide metabolites. This likely results in an insufficient amount of arsenic oxide at the tumor sites.²

Along with the inorganic arsenic trioxide, organic arsenic compounds such as *p*-aminophenylarsine oxide (*p*-APAO)^{5,12,13} and others^{3,14-16} were investigated either as preclinical and clinical experimental drugs or as molecular probes in cancer cells. Arsenic sulfide and its derivatives were reported with potent antitumor activities to solid tumor cell lines such as HCT 116.^{17,18} In comparison with inorganic arsenic compounds, organic arsenic compounds offer certain advantages. They are

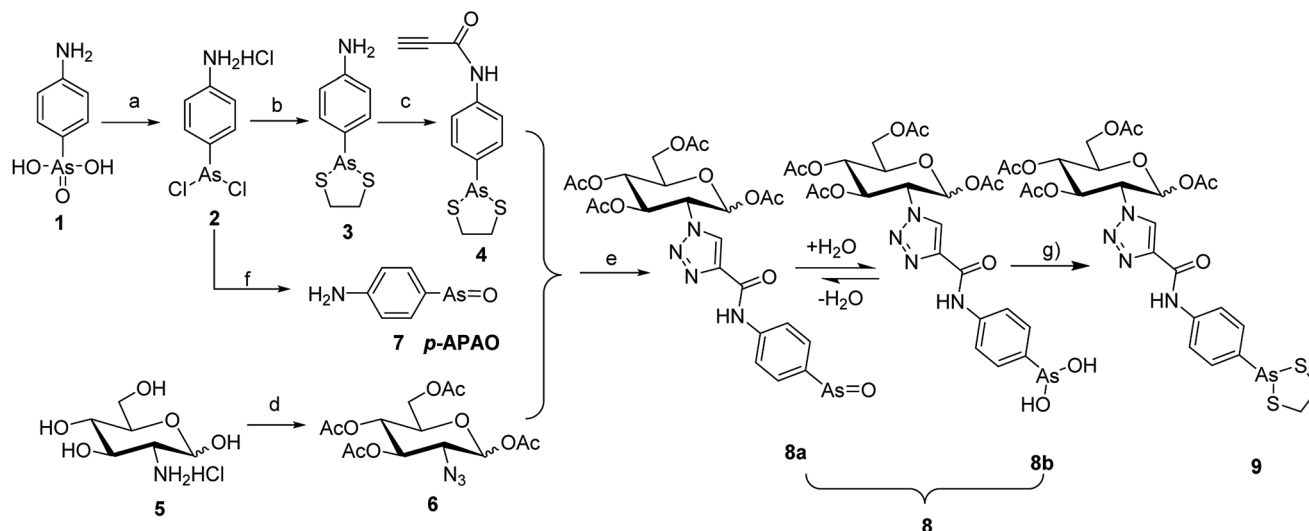
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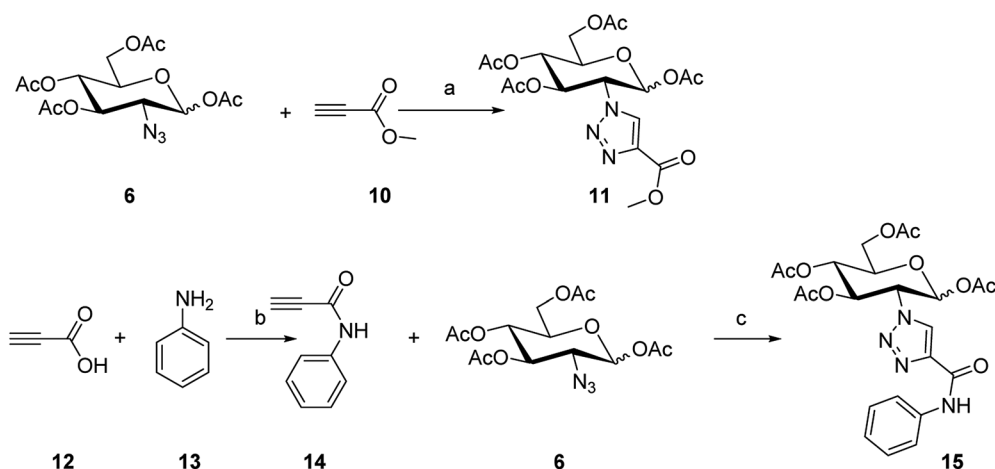


Scheme 1 Synthesis routes of compounds **7** and **9**. Reaction conditions: (a) SO_2 , KI, HCl, MeOH; (b) ethane-1,2-dithiol, NaHCO_3 , MeOH, 82%; (c) propiolic acid, dicyclohexyl carbodiimide, CH_2Cl_2 , r.t., 64%; (d) (i) TfN_3 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, CH_3CN ; (ii) Ac_2O , pyridine; (e) sodium L-ascorbate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, microwave, 100°C , 38.3%; (f) $\text{NH}_3 \cdot \text{H}_2\text{O}$, N_2 , 53.9%; (g) ethane-1,2-dithiol, MeOH, 69%.

and the dose dependent inhibition of proliferation by compound **9** and **5-FU** are illustrated in Fig. 2.

Similar to **5-FU**, arsenic containing compounds **3**, **4**, **8**, **9** and **p-APAO** all exhibited anti-proliferation activity in the three colorectal cancer cell lines. Particularly, arsenic compound **4** showed significantly enhanced activity cross the three colorectal cancer cell lines in comparison with **5-FU**. However, these compounds showed higher cytotoxicity in normal cells except the *p*-APDTAs analogue (**9**), which exhibited potent activity against HCT116 cells with IC_{50} value of $1.29 \pm 0.21 \mu\text{M}$. It also showed strong activities against DLD1 and RKO cell lines with IC_{50} values of $13.96 \pm 2.05 \mu\text{M}$ and $12.55 \pm 1.90 \mu\text{M}$, respectively. Compound **9** showed stronger cytotoxicity than **5-FU** in HCT-116 and DLD1 cells, but not in RKO cells. Compare to **5-FU**, compound **9** displayed similar cytotoxicity towards normal colorectal endothelial cells NCM460, but with over 1.8 fold increase of cytotoxicity to colorectal cancer HCT-116 cells.

The results from compounds **11** and **15** indicated that the *p*-APDTAs moiety is critical for the anticancer effects of compound **9**. The absence of the arsolane group significantly reduced antitumor activity as observed from compound **15**. Similarly, the triazole-containing compound **11**, which is derived from 1,3,4,6-tetra-*O*-acetyl- β -D-glucosamine (OADG), was not responsible for the anticancer effects of compound **9**. The compound **8** is the OADG-conjugated derivative of *p*-APAO and the compound **9** is the OADG-conjugated derivative of *p*-APDTAs. In comparison with their non-conjugated count part (*i.e.* **8** vs. *p*-APAO, and **9** vs. **3**), the OADG-conjugated organic arsenic compounds **8** and **9** showed significantly reduced toxicity towards normal colorectal cells while their anti-proliferation activities were generally maintained. We also found that deacetylation of compound **9** with sodium methoxide and then neutralization with acid resin couldn't give deacetylation compound. Actually dithiol protective group on



Scheme 2 Synthesis routes of compounds **11** and **15**. Reaction conditions: (a) sodium L-ascorbate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, microwave, 100°C , 61.1%; (b) propiolic acid, dicyclohexyl carbodiimide, CH_2Cl_2 , r.t., 70%; (c) sodium L-ascorbate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, microwave, 100°C , 32.5%.



Table 1 Cytotoxicity of carbohydrate-conjugated and non-conjugated dithiarsolanes in colorectal cancer cells and normal cells

IC ₅₀ (μM) ± SD (μM)									
Compounds	5-FU	<i>p</i> -APAO	3	4	8	9	11	15	
NCM460 ^a	44.57 ± 4.69	3.67 ± 0.10	11.11 ± 0.82	3.20 ± 0.78	16.15 ± 2.21	50.75 ± 0.97	ND ^b	ND ^b	
HCT-116	3.62 ± 1.22	5.03 ± 0.66	1.86 ± 0.34	0.53 ± 0.20	17.69 ± 1.44	1.29 ± 0.21	>50	43.39 ± 4.17	
DLD1	>50	2.17 ± 0.21	6.90 ± 1.81	1.41 ± 0.59	14.96 ± 2.72	13.96 ± 2.05	>50	20.28 ± 2.30	
RKO	2.09 ± 0.87	2.99 ± 0.04	3.74 ± 0.88	0.79 ± 0.05	4.57 ± 0.17	12.55 ± 1.90	20.03 ± 3.38	>50	
Selectivity index ^c	12.31	0.73	5.97	6.04	0.91	39.34	—	—	

^a NCM460 cell is used as the model of normal cell. ^b ND = not determined. ^c Selectivity index: the IC₅₀ against HCT116 over the IC₅₀ against NCM460.

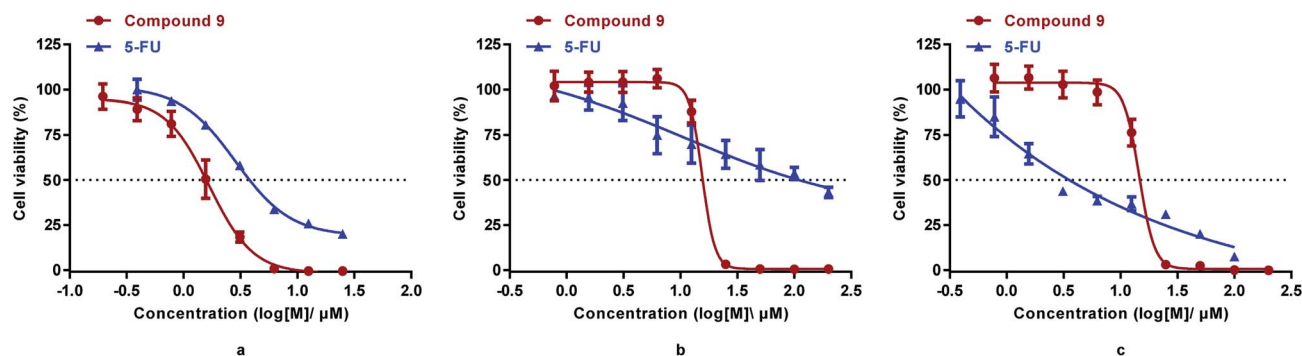


Fig. 2 Cytotoxicity of compound 9 and 5-FU in HCT-116 (a), DLD1(b), and RKO (c) cells measured by the MTS assay (mean ± SD).

the arsenic compound 9 was also deprotected under acid resin.^{36,37} When we used dithiol to protect arsenic acid of deacetylation carbohydrate-conjugated arsenic compound again, all the naked hydroxyl groups could be protected. So it is very difficult to get the deacetyl compound from 9. The usefulness of 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine (OADG) in anticancer molecules were also demonstrated by others. The acetyl protective groups were deemed beneficial in comparison with the unprotected β-D-glucosamine bearing analogues.²²

Conclusions

In this investigation, it was found that conjugation of cytotoxic arsenic-containing compound *p*-APDTAs with β-D-glucose 9 derived 1, 3, 4, 6-*O*-acetyl-β-D-glucoamine (OADG) can significantly reduce the toxicity to normal colorectal endothelial cells while the cytotoxicity towards colorectal cancer cells can be maintained. The conjugation linker introduced by click chemistry did not abolish the anticancer activity of the resultant compound, which showed anticancer activity comparable to the standard chemotherapeutic 5-fluorouracil (5-FU) in colorectal cancer models. These results suggested a new approach for the discovery and development of arsenic-containing compounds as novel chemotherapeutics for the treatment of solid tumors.

Conflicts of interest

There are no conflicts to declare.

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