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Note

(2-Phenyl-[1,3,2]dithiarsolan-4-yl)-methanol derivatives show in vitro antileukemic activity

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Abstract

The antileukemic activity of a series of (2-Phenyl-[1,3,2]dithiarsolan-4-yl)-methanol derivatives was tested on K562 and U937 human leukemia cell lines. Their systemic toxicity was estimated by the corresponding LD_{50} on mice. The cytotoxic activity of each derivative was significantly better than that of arsenic trioxide and the therapeutic index (T.I. = LD_{50}/IC_{50}) was improved. No correlation between log *P* and the activity or the toxicity was found.

Keywords: Arsenic; Melarsoprol; Arsthinol; Leukemia; Toxicity

1. Introduction

During the past few years, researchers renewed their interest in trivalent organoarsenic compounds. In particular, arsenic trioxide (As_2O_3), which was proved to be an effective drug in the treatment of acute promyelocytic leukemia (AML3) [1,2] was recently marketed in the United States and in France (Trisenox®) for the treatment of refractory AML3. The efficiency of this compound was also shown on the chronic myeloid leukemia [3,4], the lymphoid leukemia [5,6] and the multiple myeloma [7]. Further clinical potencies are still under investigation. Melarsoprol, an organoarsenic compound derived from *p*-arsanilic acid and including a dithiarsolane ring, which remains the only arsenic-containing drug used for the treatment of African trypanosomiasis, exhibited also a broad antileukemic activity against both chronic and acute myeloid and lymphoid leukemia cell lines [3,5]. However, clinical trials using this antiparasitic drug for leukemic diseases were not satisfactory, mainly for major toxic effects [8].

Therefore, and in order to design a new class of (As^{III}) organoarsenic compounds with improved antileukemic properties and better tolerance profile, we have synthesized a series of dithiarsolane derivatives of phenylarsonic acid (**1**) substituted at various ring positions.

2. Experimental

2.1. Synthesis

The starting materials (Scheme 1 and Table 1), 4-aminophenylarsonic acid (arsanilic acid, **2**), 4-hydroxy-3-nitrophenylarsonic acid (**3**) and 3-acetylamino-4-hydroxyphenylarsonic acid (**4**) were commercially available. 2-Iodo 4-aminophenylarsonic acid (**5**) was prepared by iodination of **2** by an iodate/iodide mixture. The 4-amido (**6**, **7**) and 4-sulfamido (**8**) derivatives were prepared by classical acylation. The 4-amino substituted phenylarsonic derivatives (**9**–**12**) were obtained by refluxing the corresponding chloride with **2** in 0.25 N HCl for 1 h [9]. The formation of the dithiarsolane ring with concomitant reduction of As^V to As^{III} was performed by stirring 1 equiv. of the arsonic acid derivative with 6 equiv. of aqueous 5.5 M ammonium thioglycolate during 30 min at 50 °C [10]. Subsequent dropwise addition of 2,3-dimercaptopropanol (BAL, British Anti Lewisite; 1.1 equiv.) led to the expected compounds **13**–**24** [11].

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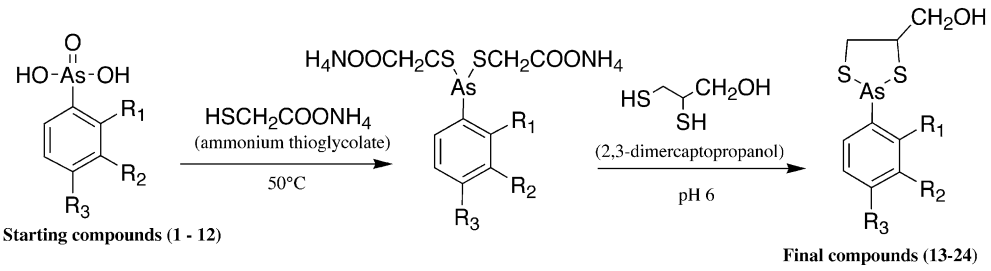
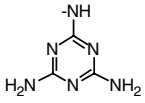
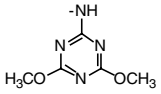
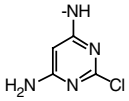


Scheme 1.

Table 1
In vitro cytotoxicity, the lethal dose 50 and the log *P* of various (2-phenyl-[1,3,2]dithiarsolan-4-yl)-methanol derivatives

Final compounds	Starting compounds	R_1	R_2	R_3	IC_{50} K562 (μM)[a]	IC_{50} U937 (μM)[a]	log *P*	LD_{50} (μmol/kg)[b]
13 (Melarsoprol)	**9**	H	H		2.81 ± 0.12	2.77 ± 0.11	2.53	112 ± 1
14	**6**	H	H	NH–CO-phenyl	1.79 ± 0.16	1.62 ± 0.17	2.97	ND[c]
15	**10**	H	H		0.65 ± 0.10	0.53 ± 0.04	2.95	11 ± 1
16	**11**	H	H		1.47 ± 0.38	1.51 ± 0.22	2.85	10 ± 1
17	**12**	H	H	NH–CH_2–CO–NH_2	3.98 ± 0.18	4.51 ± 0.25	1.74	258 ± 1
18	**8**	H	H	NH–SO_2-phenyl	5.50 ± 0.85	6.69 ± 0.14	3.10	66 ± 7
19	**7**	H	H	NH–CO–CH_3	2.78 ± 0.26	3.19 ± 0.27	1.57	123 ± 48
20	**2**	H	H	NH_2	1.64 ± 0.19	1.61 ± 0.23	2.18	103 ± 1
21	**5**	I	H	NH_2	0.44 ± 0.02	0.34 ± 0.02	3.04	60 ± 3
22 (Arsthinol)	**4**	H	NH–CO–CH_3	OH	1.44 ± 0.17	2.86 ± 0.23	2.34	402 ± 12
23	**3**	H	NO_2	OH	1.73 ± 0.42	2.07 ± 0.37	1.90	74 ± 15
24	**1**	H	H	H	0.19 ± 0,01	0.08 ± 0,01	2.61	26 ± 7
As_2O_3					24.95 ± 1.76	16.73 ± 1.54	ND[c]	57[d]

The starting compounds (**1**–**12**) were not active ($IC_{50} > 1000$ μM).
[a] IC_{50} after a 48-h incubation period, are the mean ± SD of 10 determinations (MTT test).
[b] LD_{50} were obtained using five mice per dose.
[c] ND, not determined.
[d] Obtained from Kreppel et al. [16].

All of the compounds gave satisfactory NMR spectra (400.133 MHz; 300 K; DMSO-d^6) and FT-IR spectra (KBr, diffuse reflectance). Selected data were listed:

*2.1.1. {2-[4-(4,6-Dimethoxy-[1,3,5]triazin-2-ylamino)-phenyl]-[1,3,2]dithiarsolan-4-yl}-methanol (**15**)*

Yield: 52%. m.p. 149 °C. ^{1}H NMR (DMSO-d^6), major conformer (75%): δ 10.22 (s, 1H, ArN*H*CH), 7.57–7.77 (d, 2H, *aromatics*), 7.59–7.61 (d, 2H, *aromatics*), 5.14 (t, 1H, CH_2O*H*), 3.98 (m, 1H, SC*H*), 3.91 (s, 6H, OCH_3), 3.79–3.82 (dd, 1H, SCH_2), 3.25–3.29 (dd, 2H, CH_2OH), 2.80–2.84 (dd, 1H, SCH_2). ^{1}H NMR (DMSO-d^6), minor conformer (25%): δ 10.22 (s, 1H, ArN*H*CH), 7.57–7.77 (d, 2H, *aromatics*), 7.64–7.66 (d, 2H, *aromatics*), 5.20 (t, 1H, CH_2O*H*), 3.98 (m, 1H, SC*H*), 3.91 (s, 6H, OCH_3), 3.79–3.82 (dd, 1H, SCH_2), 3.25–3.29 (dd, 2H, CH_2OH), 2.80–2.84 (dd, 1H, SCH_2), ^{13}C NMR (DMSO-d^6): δ 171.87 (N*C*(NH)N), 165.90 (N*C*(OCH3)N), 139.84 (*aromatic para*), 136.41 (As-*C*), 131.09 (*aromatics ortho*), 119.88 (*aromatics meta*), 62.93 (*C*H_2OH), 58.12 (S*C*H), 54.41 (O*C*H_3), 42.16 (S*C*H_2). UV (acetonitrile) λ_{max} 200, 285 nm. IR (KBr) υ_{max} 1066, 1250 cm^{-1}. Anal. Calc. for $C_{14}H_{17}AsN_4O_3S_2$: C, 39.25; H, 4.00; As, 17.49; N, 13.08; O, 11.21; S, 14.97. Found: C, 38.63; H, 3.92; As, 18.92; N, 12.92; O, 9.72; S, 15.89%.

*2.1.2. [2-(4-Amino-2-iodo-phenyl)-[1,3,2]dithiarsolan-4-yl]-methanol (**21**)*

Yield: 79%, m.p. 158 °C. ^{1}H NMR (DMSO-d^6): δ 7.75 (s, 1H, *aromatics meta*), 7.33 (d, 1H, *aromatics meta*), 6.76 (d, 1H, *aromatics ortho*), 5.49 (s, 2H, NH_2), 5.15 (s, 1H, CH_2O*H*), 3.98 (m, 1H, SC*H*), 3.76–3.80 (dd, 1H, SCH_2),

3.25–3.28 (t, 2H, CH_2OH), 2.83–2.87 (dd, 1H, SCH_2). ^{13}C NMR (DMSO-d^6): δ 150.24 (*aromatic para*), 141.56 (*aromatic ortho*), 132.59 (*aromatics*), 131.09 (*aromatics*), 115.04 (*aromatic meta*), 84.42 (*C*-I *aromatic ortho*), 63.81 (*C*H_2OH), 59.09 (S*C*H), 42.96 (S*C*H_2). UV (acetonitrile) λ_{max} 225, 275 nm. IR (KBr) υ_{max} 385, 1066 cm^{-1}. Anal. Calc. for $C_9H_{11}AsINOS_2$: C, 26.04; H, 2.67; As, 18.05; I, 30.57; N, 3.37; O, 3.85; S, 15.45. Found: C, 25.53; H, 2.65; As, 18.01; I, 30.50; N, 3.20; O, 3.49; S, 14.37%.

2.2. Determination of log *P*

The log *P* values of the compounds were determined by HPLC analysis of the corresponding log k'_w (C_{18} column; 5 μm, 4.6 × 25 cm, Macherey-Nagel, Eckbolsheim, France). The log k'_w of each derivative was determined by measuring its log k' using several mixtures of water/acetonitrile as the mobile phase and extrapolating back to 100% of water. A standard curve log *P* vs. log k'_w was constructed using the log k'_w of the compounds with known log *P*.

2.3. Cytotoxic activity

The cytotoxic activity of each starting compound and each final compound was performed on K562 erythroleukemia and U937 myelomonocytic leukemia cell lines. Briefly, exponential growing cells were seeded into a 96-well plate at a final density of 4.10^4/well using different concentrations of organoarsenic compounds (0.01 μM–1 mM). Cells were incubated for 2 or 3 days at 37 °C in a humidified 5% CO_2 atmosphere. Cell viabilities were determined using the classical MTT test [12].

2.4. Determination of the LD_{50}

To determine the lethal dose (LD_{50}) of each compound, six groups of five mice, weighing 22–24 g, were housed in cages and observed throughout the quarantine-period experiments. Organoarsenic compounds were dissolved and diluted in a mixture of propylene glycol, sodium chloride and DMSO (25:50:25; v/v). The mortality was assessed at the 96th hour after injecting 0.25 mL of the solution.

3. Results and discussion

Since previous results showed that chelating the —As=O group by B.A.L. in phenyl arsenoso derivatives induced a very significant increase of their therapeutic index by reducing their systemic toxicity [13], all tested compounds included an identical dithiarsolane ring and thus, were derivatives of (2-phenyl-[1,3,2]dithiarsolan-4-yl)-methanol. Actually, the compound **20** (LD_{50} = 103 μmol/kg) was clearly less toxic than the corresponding As=O derivative, 4-arsenoso-phenylamine (**25**; LD_{50} = 33 μmol/kg). This dithiarsolane ring permitted to stabilize the molecule since the As^{III} atom in the As=O group exhibits a pronounced tendency to polymerization and oxidation [14].

As tested in vitro on human leukemic cell lines, the cytotoxic activity of each derivative was quite similar for both cell lines but was significantly better (IC_{50} ranging from 0.08 ± 0.01 to 6.69 ± 0.14 μM) than that of arsenic trioxide (IC_{50} = 24.95 ± 1.76 μM for K562 and 16.73 ± 1.54 for U937; $p < 0.001$ vs. all derivatives). Despite the fact that no strong structure–activity relationship was noticed, some significant informations were obtained in this series of compounds. The substitution of one hydrogen atom of the NH_2 group of *para*-arsanilic acid by various residues (**13**–**19**) did not add any significant advantages compared with the compound with a primary amine (**20**), except for the compound **15** (substituted by 2,4-dimethoxy 1,3,5-triazine ring). This compound (**15**) was also more cytotoxic than melarsoprol (**13**) and (2-chloro-4-amino pyrimidine ring) **16**, who both carried the carbon–nitrogen NH_2—CH=N— arrangement, which was related to the trypanocidal activity of melarsoprol [15], thus indicating that this arrangement is not critical for antileukemic properties. However, an additional substitution of the arsanilic acid by an iodine atom (**21**) at the 2-position led to a significant improvement of the antileukemic activity of **20** (IC_{50} on U937 cells = 0.34 ± 0.02 vs. 1.61 ± 0.21 μM, respectively; $p < 0.001$), with a limited increase of the systemic toxicity (60 vs. 103 μmole/kg). Interestingly enough, a nitrogen or an oxygen atom at the *para*-position does not seem to be required for the cytotoxicity since the un-substituted compound **24** showed high antileukemic activity. Moreover, no correlation between the log *P* and the activity or the toxicity was found.

Since all derivatives were more cytotoxic than As_2O_3, the toxicity/activity ratio (therapeutic index T.I., LD_{50} in mice/IC_{50} on cell lines) was used to select the lead compounds. This ratio was significantly improved for all derivatives when compared to As_2O_3 [16]. Indeed, T.I. ranged from 6.80 ± 2.44 (**16**) to 279.17 ± 41.32 (**22**) vs. 2.88 ± 0.16 (As_2O_3) for K562 cells and 6.62 ± 1.63 (**16**) to 325.00 ± 20.2 (**24**) vs. 3.41 ± 0.32 (As_2O_3). Thus, the compounds **21**, **22** and **24** which exhibited a strong improvement of T.I., were considered as starter structures for further studies.

The mechanism of the antileukemic properties of As^{III} derivatives (As_2O_3 and melarsoprol) was partially elucidated during the last few years and seems quite similar for both compounds. It was attributed to the linkage between these compounds and the cystine moieties of numerous proteins, especially apoptosis proteins [17]. Nevertheless, some differences were shown on multiple myeloma cells [7]. In contrast to As_2O_3, melarsoprol only slightly reduces the plasma cell differentiation of normal B cell induced by pokeweed mitogen [7]. Both pokeweed mitogen-induced normal plasma cells and malignant plasma cells showed a normal nuclear distribution of PML protein, which was disrupted by As_2O_3 but not by melarsoprol, suggesting that these As^{III} organic derivatives could act by different mechanisms. As expected, the starting compounds (As^V) were not active (IC_{50} > 1000 μM).

However, some pentavalent organoarsenics have demonstrated an activity against two human lymphoblastic cell lines (NALM-6, MOLT-3), but no data concerning their cytotoxicity against another leukemic cell line were available [18]. Nevertheless, the interesting results obtained with As^{III} derivatives of phenyl arsonic acid encouraged us to pursue further developments of the lead compounds, which could provide the basis for more effective treatments.

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