Anti-Proliferative Effects of Novel Glyco-Lipid-Arsenicals (III) on MCF-7 Human Breast Cancer Cells

Norbert Wimmer[1], Jodie A. Robinson[2], Nagaraj Gopisetty-Venkata[2], Sarah J. Roberts-Thomson[2], Gregory R. Monteith[2] and Istvan Toth[1,*]

[1]*School of Molecular and Microbial Sciences, and* [2]*School of Pharmacy, The University of Queensland, Brisbane, Queensland 4072, Australia*

Abstract: Arsenic trioxide appears to be effective in the treatment of pro-myelocytic leukaemia. The substituted phenylarsen(III)oxides are highly polar, they have a high tendency to undergo oxidation to As (V) and to form oligomers, to prevent this we protected the As-$(OH)_2$ group as cyclic dithiaarsanes. To increase the compound's biological stability and passive diffusion we conjugated the compound of interest with lipoamino acids (Laas). Alternatively, we further conjugated the dithiaarsane derivative with a carbohydrate to utilize active transport systems and to target compound. We investigated two novel glyco-lipid arsenicals (III) (compounds **9** and **11**) for their ability to initiate MCF-7 breast cancer cell death and characterized the mechanism by which death was initiated. A significant decrease in MCF-7 cell proliferation was observed using 1 μM and 10 μM compound (**11**) and 10 μM of compound (**9**). Treatment with compound (**11**) triggered apoptosis of MFC-7 cells while compound (**9**) induced inhibition of cellular proliferation was not *via* rapid induction of apoptosis and more likely reflected necrosis and/or alterations in the cell cycle. Differences in the anti-proliferative potency of the two compounds indicate that structural modifications influence effectiveness.

Key Words: Arsenicals, trivalent, trivalent organoarsenicals, Lipoaminoacid (Laa), chemotherapeutics.

INTRODUCTION

Almost a century has passed since Paul Ehrlich's work [1] on the anti-parasitic and anti-tumour activity of trivalent arsenicals. Although the toxicity of arsenicals is limited due to their fast elimination and the lack of accumulation in deep organs [2-3], over the years they have fallen out of favour as therapeutic agents. Today there is a renewed interest by researchers in studying arsenicals as drugs for the future, particularly because these drugs are fairly cheap and are therefore suitable as therapeutics for developing countries.

Arsenic trioxide appears to be effective in the treatment of pro-myelocytic leukaemia [4]. While the mechanism of action is based on the induction of apoptosis and cell cycle arrest in lymphoid neoplasms [5-7], the underlying molecular pathways are still far from clear. It is assumed that the electronically soft species RAs^{2+} and R_2As^{+} (R = alkyl, aryl, substituted aryl) block biological sulphhydryl groups, however, the inhibition of thiol-enzymes by As^{III}, although widely quoted, is poorly understood [8]). Closely spaced sulphhydryl groups occur in several intracellular proteins [9] such as disulfide isomerase (PDI) [10], lipoic acid in the pyruvate dehydrogenase complex [11] and other proteins important in cell functions. A recent study shows that Arsenic trioxide at submicromolar concentrations is capable of inhibiting Hepatitis C virus (HCV) replication (50) without causing cellular toxicity.

The redox activity of these enzymes is based on the sulphhydryl-disulfide interchange [12]. Trivalent arsenicals (e.g. Phenylarsen(III)oxides [13-22] react readily with vicinal dithiols or closely spaced dithiols forming high affinity chelating ring structures, whereas monothiols do not give stable complexes. The driving force of the reaction is the entropic gain due to the formation of a cyclic compound. The stability of these cyclic complexes drops with the ring-size, whereby a 5-membered ring in an envelope configuration displays the highest stability. Following the literature [23-26] the lone pair favours the structures where the substituent on the As-atom is in the axial position, similar to the anomeric effect in cyclic conformations of sugars.

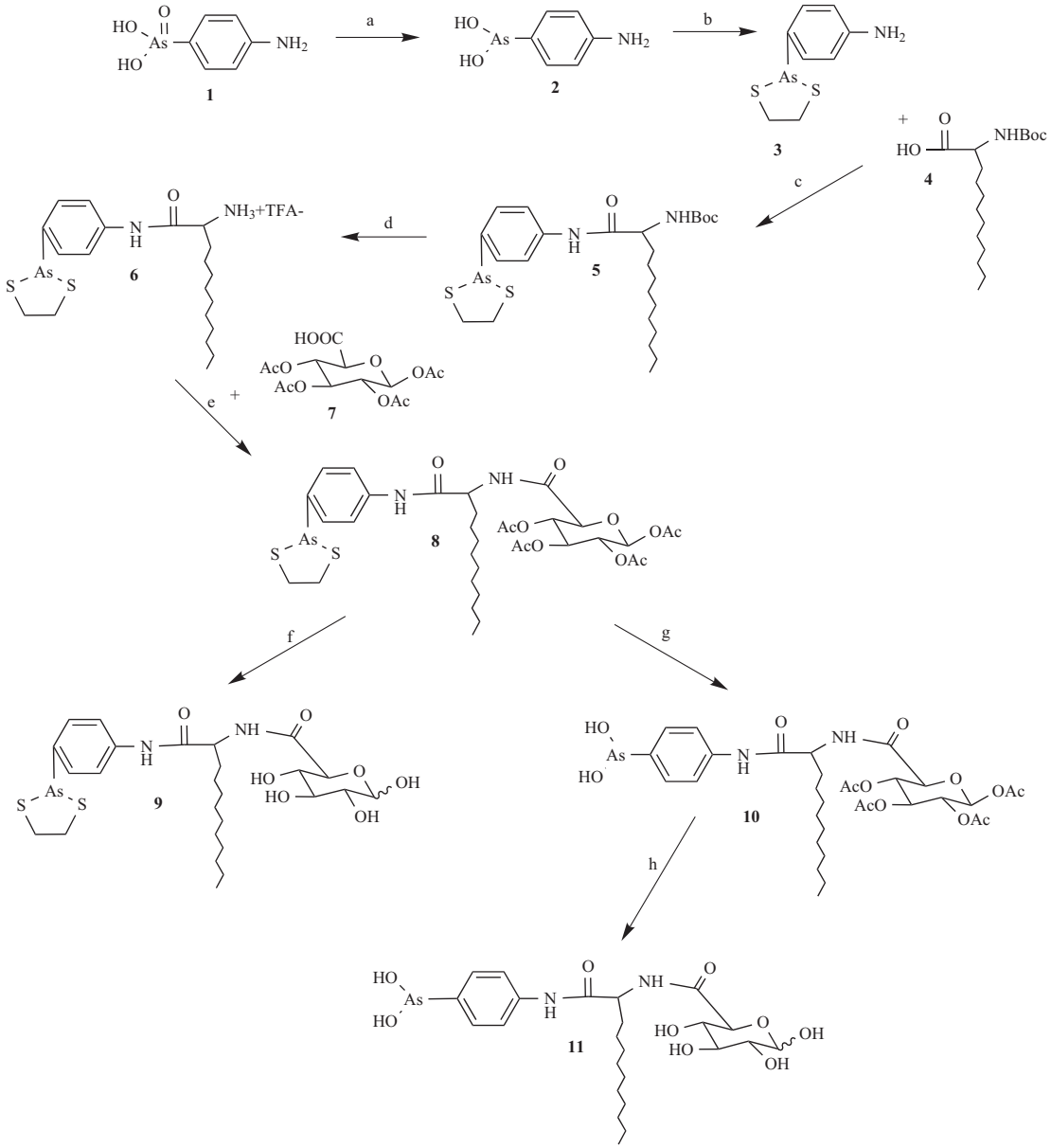
Since the substituted phenylarsen(III)oxides are highly polar, they have a high tendency to undergo oxidation to As (V) and to form oligomers, to prevent this we protected the As-$(OH)_2$ group as cyclic dithiaarsanes. Dithiaarsanes comprise relatively stable compounds that are easy to purify and can be oxidatively converted to the active phenylarsen-oxide derivative. Alternatively, the dithiaarsane derivative (**9**) was considered as a target compound due to the possibility of enzymatic *in-vivo* cleavage of the thioacetal ring or sulphhydryl-disulfide interchange with proteins forming the arsenoxide derivative (**11**).

We can increase a compound's biological stability and passive diffusion by increasing its lipophilicity [27] by conjugation of the compound of interest with lipoamino acids (Laas). Laas are α-amino acids with alkyl side-chains that can be varied in length, substitution and degree of unsaturation. Additionally Laa modification on the p-amino function of compound (**3**) may have a favourable effect on toxicity. We attached Laa (**4**) on the 4-amino group of the dithiaarsane (**3**) [13] using straight forward peptide coupling-procedures. Due to the fact that Laas often cause solubility

*Address correspondence to this author at the School of Molecular and Microbial Sciences, School of Pharmacy, The University of Queensland, Brisbane Qld 4072 Australia; Tel: (07) 3346 9892, Int: +61 7 3346 9892; Fax: (07) 3365 4273; E-mail: i.toth@uq.edu.au

1573-4064/06 $50.00+.00

problems the combination of lipid and sugar conjugation was favoured [28-29]. The polyhydroxylated nature of sugars provides an efficient and biocompatible way of altering the physicochemical properties of a drug, particularly increasing water solubility. Herein we attached a peracetylated β-D-glucuronic acid (**7**) unit onto the *N*-terminus of the lipid arsenical **6** to give compound **8.** Zemplén-deacetylation and oxidative cleavage of the thioacetal ring using mercury(II) perchlorate gave rise to target compounds (**9**) and (**11**), respectively. Having synthesized compounds (**9**) and (**11**)



Reagents and conditions: (a) phenylhydrazine, methanol, reflux, 1 h; (b) ethanedithiol, ethanol, reflux, 10 min;(c) HBTU, DIEA, DMF/THF, o/n;(d) TFA/ DCM (30 %),45 min; (e) HBTU, DIEA, DMF/THF, 16 h; (f) NaOMe/MeOH (0.1 M), 3 h, (2) Dowex 50-AG WX8 (H^+); (g) $Hg(ClO_4)_2$ in methanol, K_2CO_3 , 10 min; (h) NaOMe/MeOH (0.1 M), 4 h, (2) Dowex 50-AG WX8 (H^+)

Fig. (1). Synthesis of sugar-liposaccharide linked arsenicals (III) **9** and **11**.

our goal was to characterise both compounds for their ability to initiate cell death in MCF-7 cells, and additionally to determine the mode of cell death. MCF-7 cells are a commonly used model of breast cancer. In addition both compounds were also tested regard their effect on the proliferation of HT-29 colon cancer cells, since the literature concerning arsenicals indicates high cell selectivity of arsenicals. (48, 49) The apoptosis pathway is often targeted for therapeutic design because of the controlled mechanism of removal of cellular components and a lack of release of intracellular contents, which could affect surrounding normal cells.

EXPERIMENTAL PART

^{1}H NMR spectra were recorded at 297 K with a Bruker 400 MHz and 500 MHz instrument operating at 500 MHz for ^{1}H using $CDCl_3$, CD_3OD or Aceton-d_6 as solvent and tetramethylsilane as internal standard, unless stated otherwise. Coupling constants are given in Hz (first or values). ^{13}C spectra were measured at 100.62 MHz and 125.77 MHz and referenced to $CDCl_3$ (77.0 Hz), CD_3OD (49.0 Hz) or Aceton-d_6 (30.5 Hz). Homo- and heteronuclear 2D NMR spectroscopy was performed with Bruker standard software.

TLC was performed on Merck pre-coated aluminium sheet (Silica Gel 60F254); spots were detected by spraying with H_2SO_4. Amine derivatives were detected by ninhydrin spray [30], followed by heating the sheets. Column chromatography was performed on silica gel columns (size A, 28 × 2; B, 30 × 2.5; and c, 43 × 4 cm; silica gel 0.040-0.063 mm.

Analytical RP-HPLC was performed on a Shimadzu instrument (LC-10AT liquid chromatograph, SC_L-10A system controller, SPD-6A UV detector, a SIL-6B auto injector with a SCL-6B system controller, and a C18- HPLC-column using a acetonitrile / water gradient as well as a isopropanol / water gradient. HPLC purification was done on a Waters HPLC system (Model 600 controller, 490E UV detector, F pump, and 0.46 x 15 cm Vydac RP-C18 column with 0.005 mm particle size) using a acetonitrile / water gradient. He-gas was applied for degassing of HPLC-solvents.

Mass spectra were obtained on a quatropol-electrospray-MS (Percin Elmer API 3000 instrument) in the positive ion mode using CH_3CN / water mixtures. Concentration of solutions was performed at reduced pressure at temperatures <40 °C.

Synthesis of 2-*N*-D, L-(Tert-Butoxycarbonyl)Tetradecanoic Acid (C14Laa) (4)

This synthesis has been frequently described in the literature [31].

Synthesis of 4-Aminophenylarsenoxide (2)

p-Arsanilic acid (**1)** (21.7 g, 100 mmol) was dissolved in methanol (115 mL) under reflux conditions and phenylhydrazine (20.6 mL, 200 mmol) was added within 10 minutes. When N_2-production was becoming less refluxing was continued for 1 h. The mixture was concentrated at 100 °C, treated with water (170 mL) an aqu. NaOH-solution (0.1 M, 120 mL) and washed with Ether (300 mL). The aqueous solution was treated with aqueous NH_4Cl-solution (5M80 mL) kept 1h at 0 °C. Precipitate was then filtered through a Buechner-funnel, washed with ice-water (100 mL) and dried over KOH, which gave (**2)** as white powder (9.94 g, 49 % yield) [1].

NMR data were identical to literature values [32].

Synthesis of 2-*p*-Aminophenyl-1, 3, 2-Dithiarsenolane (3)

A solution of *p*-Aminophenylarsenoxide (**2)** (3.04 g, 15.2 mmol), ethanedithiol (1.67 mL, 20 mmol) in dry ethanol (10 mL) was stirred for 10 min under reflux. The solution was chilled in dry ice / acetone, co-distilled with toluene (250 mL) and concentrated. A sample was recrystallized from ethanol; mp 69.5-71 °C (Lit: 69-70.5 °C); 3.33 g (84% yield).

ES-MS: ($C_8H_{10}NAsS_2$) 259.2 m/z (%): $(M+H)^+$: 259.01 (40%), $(M\text{-}SCH_2As+2H_2O)^+$: 202.21 (20%), $(M\text{-}SCH_2As+H_2O)^+$ 184.02 (100%).

NMR data were identical to literature values [33].

^{1}H NMR (Aceton-d_6): δ 7.35-7.32 (m, 2 H, arom. H), 6.66-6.68 (m, 2 H, arom. H), 3.37-3.23 (m, 4 H, CH_2), 2.77 (s, 2 H, NH_2).

^{1}H-^{13}C HMQC Correlation (d_6-Aceton): Crosspeaks: 7.35-7.32 and 122.2 (Aromat.H(A) and aromat. C (A), 2C), 6.66-6.68 and 103.5 (, Aromat. H (B), 2H, and aromat. C (B), 2C)), and between 3.37-3.23 (m, 4 H, CH_2) and 31.8 (CH_2, 2 C). ^{13}C NMR data were identical to literature values [36].

Tert-Butyl-1-(4-(1,3,2-Dithiarsolan-2-yl)Phenylamino)-1-Oxotetradecan-2-yl-Carbamate (5)

A solution of (**3)** (738 mg, 2.84 mmol) in dry THF (8 mL), then molecular sieve (4Å, 500 mg), DIEA (1.5 mL, 8.6 mmol), a solution of R/S-Boc C14-LAA (1952 mg, 5.68 mmol) in dry DMF (3 mL) and finally HBTU (2153 mg, 5.68 mmol) was added. pH 10 was adjusted by adding DIEA (0.2 mL, 1.15 mmol) and stirred overnight. After co-evaporation with toluene (3x, each 50 mL), the residue was dissolved in DCM (100 mL), washed with water (150 mL), ice-cold aqueous HCl-solution (5%), satd. $NaHCO_3$-solution (3x, each 100 mL), brine (3x, each 100 mL), and dried ($MgSO_4$). The organic layer was taken to dryness and the residue was purified on a column of silica gel (C, 4:1 hexane- EtOAc; solvents containing 0.5% triethylamine) to afford **5** as a pale yellow syrup (953 mg, 57 % yield).

ES-MS: ($C_{27}H_{45}O_3N_2S_2As$) 584.7 m/z (%): $(M\text{-}Boc+H)^+$ 485.4 (25)

$(M+H)^+$: 585.6 (40), $(M\text{-}t\text{-}Butyl)^+$: 529.4 (100), $(M+Na)^+$ 607.6 (25)

^{1}H NMR (d_6-Aceton): δ 8.32 (s, 1 H, arom. NH), 7.57-7.47 (m, 4 H, arom. H), 4.96 (s, 1 H, NH), 4.12 (m, 1 H, CH), 3.33 (m, 2 H, SCH_2), 3.13 (m, 2 H, SCH_2), 1.89-1.55 (m, 4 H, β-CH2-, SCH_2-$\underline{C}H_2$), 1.43 (s, 9 H, t-butyl), 1.40-1.20 (m, 20 H, 10 CH_2), 0.86 (t, 3, CH_3).

^{13}C NMR ($CDCl_3$): δ 169.02, 169.40 (2 C, C=O (A, B)), 139.78, 139.20 (2 C, NH linked quart. aromat. C (A, B),

130.90 (1 C, quart. aromat. C (A*), 132.39, 132.28 (2 C, 4 C, aromat.CH (A,B), 130.25 (1 C, quart. aromat.C (B*), 120.80, 120.71 (2 C, 4 C, aromat.CH (A,B), 57.44 (1 C, -CH (A*)), 54.31 (1 C, -CH (B*)), 42.54 (1 C, SCH_2 (A*), 42.55 (2 C, SCH_2 (A, B), 42.30 (1 C, SCH_2 (B*), 32.14, 31.38, 29.89, 29.87, 29.86, 29.83, 29.61, 29.57, 28.55, 25.78, 22.91 (11 C, CH_2), 20.98, 20.85, 20.73, 20.72 (4 C, CH_3C=O), 14.32 (1 C, CH_3).

_N_-(4-(1, 3, 2-Dithiarsolan-2-yl)Phenyl)-2-Aminotetradecanamide Trifluoroacetat (6)

Compound (**5**) (73 mg, 0.125 mmol) was stirred with a solution (2 mL) of TFA/ DCM (30%) for 45 min. The solution was co-evaporated with toluene (3x, each 100 mL), dried overnight at high-vacuum, which furnished **6** as a slightly yellow syrup (72 mg, 96% yield).

ES-MS: ($C_{22}H_{38}O_3N_2F_3AsS_2$) 598.6 m/z (%): (M-$CF_3COO$+H)+ 486.06 (100)

^{1}H NMR (^{6}d-Aceton): δ 7.79-7.51 (m, 4 H, arom. H), 4.47 (m, 1 H, CH(A)), 4.22 (m, 1 H, CH(B)), 3.80 (m, 2 H, SCH_2), 3.20 (m, 2 H, SCH_2), 2.05-1.98 (m, 2 H, -CH_2 (A), 2.05, 1.87 (m, each 1 H of -CH_2 (B)), 1.61-1.17 (m, 20 H, 10 x CH_2), 0.87 (t, 3 H, CH_3).

^{13}C NMR ($CDCl_3$): δ 169.14, 169.61 (2 C, C=O (A, B)), 140.78, 140.20 (2 C, NH linked quart. aromat. C (A, B), 131.17 (1 C, quart. aromat. C (A*), 132.86, 132.75 (2 C, 4 C, aromat.CH (A,B), 130.47 (1 C, quart. aromat.C (B*), 121.07, 121.01 (2 C, 4 C, aromat.CH (A,B), 58.14 (1 C, -CH (A*)), 55.59 (1 C, -CH (B*)), 43.27 (1 C, SCH_2 (A*), 43.07 (2 C, SCH_2 (A, B), 43.01 (1 C, SCH_2 (B*), 33.28, 31.05, 30.99, 30.95, 30.87, 30.85, 30.75, 30.65, 30.62, 30.60, 30.56, 27.24, 26.81, 23.98 (11 C, CH_2), 15.03 (1 C, CH_3).

1, 2, 3, 4-Tetra-*O*-Acetyl- -Glucopyranuronic Acid (7)

D-Glucuronic acid (4.950 g, 25.5 mmol) was stirred with acetic anhydride (15 mL, 145 mmol), J_2 (400 mg, 1.6 mmol) and 4-*N*, *N*-dimethylaminopyridine (cat. amounts) at 0 °C for 5h. The reaction was quenched by drop-wise adding of dry methanol (30 mL) for 18h. The solution was concentrated washed with DCM (100 mL), 1 M $Na_2S_2O_3$, dried and evaporated under vacuum. The white solid was washed with a mixture of ether, hexane and $CHCl_3$ (1:1:1, 100 mL) and recrystallised (EtOAc-hexane) to afford compound (**7**) as white crystals (7.3 g, 79% yield).

ES-MS: ($C_{14}H_{18}O_{11}$) 362.08 m/z (%): $(M\text{-OAc+H})^+$: 303 (34), $(M\text{-OAc+Na})^+$: 325 (64), $(M+Na)^+$: 385 (86), $(M+2Na\text{-H})^+$: 407 (100).

^{1}H NMR ($CDCl_3$): δ 5.78 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 5.28 (m, 2 H, H-3, H-4), 5.12 (m, 1 H, H-2), 4.24 (m, 1 H, H-4), 2.10, 2.04, 2.02, and 2.01 (4 s, each 3 H, 4 Ac). ^{13}C NMR data were identical to literature values [34].

4-(2-(2,3,4,5-Tetra-*O*-Acetyl-D-Glucopyranosyl-6-Carboxamido)Tetradecanamido)- 1,3,2- Dithiarsolane (8)

To a stirred suspension of (**6**) (301 mg, 0.503 mmol) in dry THF (2 mL), molecular sieve (4Å, 500 mg), DIEA (1 mL, 5.7 mmol), glucuronic acid peracetate **7** (364 mg, 1.01 mmol) and a solution of HBTU (383 mg, 1.01 mmol) in DMF (2 mL) was added. After overnight stirring the suspension was filtered through celite and the clear solution was co-evaporated with toluene (3x, each 70 mL) to dryness. The resulted residue was dissolved in $CHCl_3$ (100 mL) and washed with water (150 mL), ice-cold aqueous HCl-solution (5%, 2x, each 50 mL), satd. $NaHCO_3$-solution (2x, each 50 mL), brine (3x, each 30 mL), and dried ($MgSO_4$). The organic layer was taken to dryness and the residue was purified by flash-chromatography on a column of silica gel (B, 1:1 hexane- EtOAc; solvents containing 0.5% triethylamine) to afford first one diastereomer of (**8a**) (56 mg, 13%). Continued elution gave a diastereomeric mixture (R/S) of (**8**) (262 mg, 63%), both appeared as colourless syrup.

Compound (**8a**):

ES-MS: ($C_{36}H_{53}O_{11}N_2S_2As$) 828.9 m/z (%): $(M+H)^+$: 829.2 (100%), $(M\text{-Ac+H})^+$: 787.4 (20%), $(M+Na)^+$: 851.4 (30 %).

^{1}H NMR ($CDCl_3$): δ 8.18 (s, 1 H, -C_6H_4-NH), 7.62-7.56 (m, 4 H, aromat. H), 6.66 (d, 1 H, $J_{NH\text{-}CH}$ 8.7 Hz, NH), 5.76 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.33 (t, 1 H, $J_{2,3}$ 9.3 Hz, H-3), 5.13 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 5.09 (dd, 1H, H-2), 4.47 (m, 1 H, -CH-), 4.08 (d, 1 H, H-5), 3.36-3.29 (m, 2 H, -SCH_2-), 3.17-3.10 (m, 2 H, -SCH_2-), 2.10, 2.05, 2.02, and 2.00 (4 s, each 3 H, 4 Ac), 2.05 (m, 2 H, -CH_2-), 1.69-1.58 (m, 2 H, γ-CH_2-), 1.26-1.21 (m, 20 H, 10 CH_2), and 0.86 (t, 3 H, CH_3).

^{13}C NMR ($CDCl_3$): δ 169.88, 169.36, 169.17, 168.84, 168.83, 167.07 (6 C, C=O), 139.09, 131.75 (2 C, quart. aromat. C), 125.72, 120.22 (4 C, aromat. CH), 91.62 (1 C, sugar C-1), 73.46 (1 C, sugar C-5), 71.73 (1 C, sugar C-3), 70.51 (1 C, sugar C-2), 69.60 (1 C, sugar C-4), 53.62 (1 C, -CH), 42.01 (2 C, SCH_2), 32.13, 31.47, 30.56, 29.85, 29.82, 29.73, 29.60, 29.54, 29.47, 25.78, 22.89, (11 C, CH_2), 20.94, 20.89, 20.71, 20.69 (4 C, CH_3C=O), 14.30 (1 C, CH_3).

Compound (**8**) (R/S-mixture):

ES-MS: ($C_{36}H_{53}O_{11}N_2S_2As$) 828.9 m/z (%): $(M+H)^+$: 829.2 (100), $(M\text{-Ac+H})^+$: 787.4 (15), $(M+Na)^+$: 851.4 (25).

^{1}H NMR ($CDCl_3$): δ 8.18 (s, 1 H, -C_6H_4-NH), 7.62-7.56 (m, 4 H, aromat. H (A)), 7.54-7.46 (m, 4 H, aromat. H (B)), 6.75 (d, 1 H, $J_{NH\text{-}CH}$ 8.1 Hz, NH, (B), 6.66 (d, 1 H, $J_{NH\text{-}CH}$ 8.7 Hz, NH, (A)), 5.76 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1, (A)), 5.75 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1, (B)), 5.33 (t, 1 H, $J_{2,3}$ 9.3 Hz, H-3, (A)), 5.29 (t, 1 H, $J_{2,3}$ 9.3 Hz, H-3, (B)), 5.17-5.08 (m, 2 H, H-2 (A,B), H-4 (A,B), 4.47 (m, 1 H, -CH-(A)), 4.36 (dd, 1 H, -CH-(B)), 4.08 (d, 1 H, H-5, (A)), 4.07 (d, 1 H, H-5 (B), 3.36-3.29 (m, 2 H, -SCH_2-(A,B)), 3.17-3.10 (m, 2 H, -SCH_2-, A,B), 2.10, 2.05, 2.02, and 2.00 (4 s, each 3 H, 4 Ac, (A)), 2.11, 2.03, 2.02, and 2.00 (4 s, each 3 H, 4 Ac, (B)), 2.05 (m, 2 H, -CH_2-, (A,B)), 1.69-1.58 (m, 2 H, γ-CH_2-, (A,B)), 1.26-1.21 (m, 20 H, 10 CH_2, (A,B)), and 0.86 (t, 3 H, CH_3, (A,B)).

^{13}C NMR ($CDCl_3$): Significant shift-differences from diastereomer (**8a**):

δ 119.95 (2 C, aromat.CH), 91.49 (1 C, sugar C-1), 73.46 (1 C, sugar C-5), 70.51 (1 C, sugar C-2), 54.23 (1 C, –CH).

4-(2-(D-Glucopyranosyl-6-Carboxamido)Tetradecanamido)-1,3,2- Dithiarsolane (9)

A solution of (**8**) (10 mg, 0.012 mmol) in dry MeOH (1 mL) was stirred with 0.1 M methanolic NaOMe (0.1 mL) for

3 h at room temperature. The solution was set to pH 5 by addition of Dowex 50-AG WX8 (H^+) cation-exchange resin, filtered, and evaporated. The resulted residue was purified on a RP-C4 column (0.46 x 15 cm, acetonitrile / water gradient, and lyophilised to afford compound (**9)** as colourless syrup (7.8 mg, 99% yield).

Analytical HPLC on RP-C4 column (0.46 x 15 cm) using 2 different solvent systems:

Solvent system 1: Solvent A (H_2O, 0.1 % TFA), Solvent B (90 % CH_3CN, 10 % H_2O, 0.1 % TFA)

Retention time: 23.45 min

Solvent system 2: Solvent A (H_2O, 0.1 % TFA), Solvent B (90 % Isopropanol, 10 % H_2O, 0.1 % TFA)

Retention time: 20.60 min

Gradient: 0-100 % B within 30 min

Flowrate: 1mL/min

Wave length: 214 nm

ES-MS: ($C_{28}H_{45}O_7N_2S_2As$) 661.7 m/z (%): (M+H)+ 662

^{1}H NMR (d_6-Aceton): δ d 7.84-7.55 (m, 4 H, aromat. H, (A,B), 6.52 (d, 1 H, aromat.-NH (A*), 6.52 (d, 1 H, aromat.-NH (B*), 5.20 (d, 1 H, $J_{1,2}$ 3.2 Hz, α-H-1), 4.57 (m, 3 H, α*-C<u>H</u>-(A), α*-C<u>H</u>-(B), β-H-1), 4.28-4.18 (m, 2H, α, β-H-5), 3.82 (m, 1 H, α-H-3), 3.50-3.31 (m, 6H, α-H-2, β-H-3, α,β-H-4, -SCH_2-), 3.28-3.17 (m, 3H, β-H-2, -SCH_2-), 1.95-1.68 (m, 2 H, β–CH_2-), 1.65-1.19 (m, 20 H, 10 CH_2), and 0.87 (t, 3 H, CH_3).

^{13}C NMR (d_6-Aceton): δ 172.05, 171.21 (2 C, C=O), 139.01, 132.09, 132.04, 132.01, 131.98, 131.60 (quart. aromat. C, R/S, α,β), 121.55, 121.50, 121.10, 121.05 (aromat.<u>C</u>H, R/S), 98.59, 98.10 (2 C, R/S- sugar C-1β), 94.10, 93.88 (2 C, R/S- sugar C-1α), 77.85, 77.71 (2 C, sugar R/S C-2α), 76.25, 76.10 (2 C, sugar R/S C-2β), 76.20, 75.97 (2 C, sugar R/S-C-5β), 74.67, 74.58 (2 C, sugar C-3 α), 74.30, 74.04, 73.80, 73.60, 73.50, 73.40 (4 C, sugar R/S-C-4α,, β; C-2 α,, C-3β), 72.10, 72.02 (2 C, sugar R/S-C-5α), 55.44, 55.33, 55.30, 55.23 (4 C, R/S, α,β), 42.24 (2 C, SCH_2), 33.18, 30.80, 30.79, 30.75, 30.67, 30.61, 30.57, 30.51, 30.43, 26.70, 23.80 (11 C, CH_2), and 14.51 (1 C, CH_3).

4-(2-(2,3,4,5-tetra-*O*-acetyl-glucopyranosyl-6-carboxamido) tetradecanamido)phenylarsonous acid (10)

A solution of mercury(II)perchlorate (55 mg, 0.132 mmol) in MeOH (0.6 mL) was drop-wise added to a suspension of dithioacetal (**8a)** (50 mg, 0.060 mmol) and K_2CO_3 (18 mg, 0.13 mg) in $CHCl_3$/MeOH (2.5 mL, 31; v/v) under stirring. After 10 min a white precipitate was filtered from the product-containing solution. The clear filtrate was neutralised to pH 6 by adding a satd. $NaHCO_3$ –solution (3 mL), washed with $CHCl_3$/MeOH (100 mL, 3 1), 10 % aqueous KI-solution (3x, each 25 mL) and dried ($MgSO_4$) and evaporated. Purification of the crude product on a short plug of silicagel (EtOAc) afforded compound (**10)** as yellow syrup (23mg, 88 % yield).

ES-MS: ($C_{34}H_{51}O_{13}N_2As$) 770.7 m/z (%): $(M+Na)^+$: 793.21 (100 %), $(M+H-H_2O)^+$: 753.3 (25 %), $(M-H_2O-Ac+H)^+$: 711.31 (10 %).

^{1}H NMR ($CDCl_3$): δ 8.18 (s, 1 H, -C_6H_4-N<u>H</u>), 7.72-7.53 (m, 4 H, aromat. H), 6.73 (d, 1 H, J_{NH-CH} 8.7 Hz, NH(A)), .6.62 (d, 1 H, J_{NH-CH} 8.7 Hz, NH(B))5.78 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.34 (t, 1 H, $J_{2,3}$ 9.3 Hz, H-3), 5.18-5.06 (m, 2 H, H-2, H-4), 4.49 (m, 1 H, α-C<u>H</u>-), 4.37 (m, 1 H, β-C<u>H</u>-), 4.09 (d, 1 H, $J_{4,5}$ 9.7 Hz, H-5), 2.11, 2.06, 2.02, and 2.00 (4 s, each 3 H, 4 Ac), 2.05 (m, 2 H, β-CH_2-), 1.69-1.58 (m, 2 H, γ-CH_2-), 1.28-1.18 (m, 22 H, 11 CH_2), and 0.86 (t, 3 H, CH_3).

^{13}C NMR ($CDCl_3$): δ 169.77, 169.40, 169.20, 168.90, 168.80, 167.00 (6 C, C=O), 139.01, 131.60 (2 C, quart. aromat.C), 125.35, 120.12 (4 C, aromat.<u>C</u>H), 91.55 (1 C, sugar C-1), 73.44 (1 C, sugar C-5), 71.70 (1 C, sugar C-3), 70.47 (1 C, sugar C-2), 69.55 (1 C, sugar C-4), 53.81 (1 C, α-CH), 32.10, 31.45, 30.54, 29.87, 29.80, 29.70, 29.61, 29.56, 29.48, 25.74, 22.75, (11 C, CH_2), 20.79, 20.80, 20.65, 20.60 (4 C, <u>C</u>H_3C=O), 14.25 (1 C, CH_3).

4-(2-(D-Glucopyranosyl-6-carboxamido)tetradecanamido) phenylarsonous acid (11)

A solution of compound (**10)** (32 mg, 0.041 mmol) in dry MeOH (3 mL) was stirred with 0.1 M methanolic NaOMe (0.5 mL) for 4 h at room temperature. The solution was diluted with CH_3OH (2 mL) and deionized by addition of Dowex 50-AG WX8 (H^+) cation-exchange resin to pH 7, filtered, and evaporated. The resulted residue was purified on a RP-C18 column (0.46 x 15 cm, acetonitrile / water gradient, and lyophilised to afford compound (**11)** as amorphous powder (16 mg, 64 % yield).

Analytical HPLC on RP-C18 column (0.46 x 15 cm) using 2 different solvent systems:

Solvent system 1: Solvent A (H_2O, 0.1 % TFA), Solvent B (90 % CH_3CN, 10 % H_2O, 0.1 % TFA)

Retention time: 25.1 min

Solvent system 2: Solvent A (H_2O, 0.1 % TFA), Solvent B (90 % Isopropanol, 10 % H_2O, 0.1 % TFA)

Retention time: 23.38 min

Gradient: 0-100 % B within 30 min

Flow rate: 1mL/min

Wave length: 214 nm

ES-MS: ($C_{26}H_{43}O_9N_2As$) 602.5 m/z (%): $(M+H)^+$: 603.4 (25 %), $(M+Na)^+$: 625.4 (100 %), $(M+NH_4)^+$: 620.6 (30 %).

^{1}H NMR (MeOD): δ d 7.72-7.57 (m, 4 H, aromat. H, (A)), 7.57-7.33 (m, 4 H, aromat. H, (B)), 5.20 (d, 1 H, $J_{1,2}$ 3.2 Hz, α-H-1), 4.53 (m, 3 H, -C<u>H</u>-(A), α*-C<u>H</u>-(B), β-H-1), 4.33-4.22 (m, 2H, α,β-H-5), 3.72 (m, 1 H, α-H-3), 3.52 (m, 1 H, β-H-4), 3.48 (m, 1 H, α-H-4), 3.45-3.40 (m, 1 H, α-H-2, β-H-3, 3.20 (m, 1 H, β-H-2), 2.11, 2.06, 2.02, and 2.00 (4 s, each 3 H, 4 Ac), 1.92-1.72 (m, 2 H, β-CH_2-), 1.28-1.18 (m, 20 H, 10 CH_2), and 0.86 (t, 3 H, CH_3).

^{13}C NMR (MeOD): δ 173.09, 173.04 (2 C, C=O), 138.13, 134.56, 133.87, 133.55, 130.01 (quart. aromat. C, R/S, α, β), 132.09, 121.66, 121.59, 121.19, 121.13 (aromat.<u>C</u>H, R/S),

98.73, 98.69 (2 C, R/S- sugar C-1), 94.54, 94.47 (2 C, R/S-sugar C-1α), 77.81, 77.77 (2 C, sugar R/S C-2α), 76.39, 76.28 (2 C, sugar R/S C-2), 76.03, 75.97 (2 C, sugar R/S-C-5), 74.65, 74.61 (2 C, sugar C-3 α), 74.42, 74.05, 73.92, 73.57, 73.52, 73.44 (4 C, sugar R/S-C-4α,, ; C-2 α,, C-3), 72.04, 72.00 (2 C, sugar R/S-C-5α), 55.39, 55.34, 55.32, 55.27 (4 C, R/S, α,), 33.20, 30.87, 30.85, 30.78, 30.68, 30.63, 30.59, 30.50, 30.44, 26.83, 23.85 (11 C, CH_2), and 14.55 (1 C, CH_3).

HAEMOLYSIS ASSAY [35]

Fresh blood (10 ml) was collected from Sprague Dawley (SD) rats and centrifuged at 800 g for 10 min. The red blood cells were thoroughly washed three times with phosphate buffer PBS (10 mM), in order to remove plasma and hemoglobin. The washed cells were resuspended in a volume of PBS approximate to the original volume of blood. In 96 well plate, 0.1 mL of the RBC suspension was aliquoted into each well and allowed to warm to 37 °C in a bench top incubator for 10 min. 0.1 mL of samples of compound (**9**) and the arsenoxide derivative (**11**) respectively (1 mM, 2 mM, 4 mM in 10 mM PBS), control (PBS; 10 mM) and SDS (1 mM, 2 mM, 4 mM in 10 mM PBS) as positive control were added to the wells and incubated for 30 min at 37 °C without exposure of light. After incubation samples 0.02 mL) of the positive control (100% haemolysis) were added to Drabkins reagent (5 mL), all the other samples were first zentrifuged for 2 min at maximum speed and supernatants (0.020 mL) of those samples (0.020 mL) were added to Drabkins reagent (5 mL) under mixing. After 30 min standing without exposure of light the absorption at 540 nm was measured and the equation below was used to calculate the haemolysis of red blood cells. The negative control defines spontaneous haemolysis, the positive control (SDS) displays 100% haemolysis. All samples were done in triplicates. SDS was chosen as a comparison known to be toxic to cells by disintegrating their membranes.

CELL CULTURE

a. MCF7 Cells

The breast cancer cell line MCF7 was purchased from the ATCC and maintained in high glucose Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Mount Waverley, VIC, Australia) supplemented with 10% foetal bovine serum (FBS; JRH Biosciences), 2 mM L-glutamine (Invitrogen), 100 U/ml penicillin G (Invitrogen) and 100 µg/ml streptomycin sulphate (Invitrogen). The cells were cultured at 37 ºC in a humidified 5% CO_2/95% air incubator and passaged prior to confluence.

b. HT-29 Colon Cancer Cells

HT-29 cells were cultured and maintained in RPMI-1640 media, supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 100U/ml penicillin G and 100µg/ml of streptomycin sulphate and maintained at 37 ^{o}C in a humidified 5% CO_2 / 95% air incubator.

PROLIFERATION ASSAYS

a. MCF7 Cells

MCF7 cells were plated into 96-well plates at 800 cells/well in normal growth media and allowed to adhere for 7 hours. The media was removed and replaced with either serum free supplemented DMEM or normal growth media containing compound (**9**) or (**11**) (0.001-10 µM) or vehicle (DMSO). The media was replaced every 2-3 days. Cell density was measured at 2, 6 and 10 days using the CellTitre 96® Aqueous One Solution Proliferation Assay (Promega, Annandale, NSW, Australia). Plates were incubated for 2 hr at 37 ºC in 5% CO_2 and the absorbance read at 490 nm with a Bio-Rad model 550 microplate reader (Hercules, CA).

b. HT-29 Colon Cancer Cells

The HT-29 cells were seeded into 96-well plates at 600 cells/well and allowed to adhere. The media was removed and replaced with either serum free supplemented RPMI-1640 or complete growth RPMI media with compounds 9 or 11 at concentrations 0.001 – 10µM or vehicle (DMSO). The media was replaced every 2 days. Cell density was measured at day 10 using CellTitre 96® Aqueous one solution Proliferation assay (Promega, Annandale, NSW, Australia). The plates were incubated for 2 hr at 37 ^{o}C in 5% CO_2 and the absorbance was read at 490nm as for MCF-7 cells.

In situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) assay

MCF7 cells were plated onto 13-mm glass coverslips in 24-well plates in normal growth media at 2 x 10^5 cells/well. After 24 h, compounds (**9**) or (**11**) or vehicle were added and the cells incubated for a further 24 hours. Apoptotic cell death was confirmed using the Dead End Colorimetric Apoptosis Detection System (Promega). Coverslips were fixed in 4% paraformaldehyde, permeabilized in 0.2 % Triton X-100, and then incubated with biotinylated nucleotides and terminal deoxynucleotidyl transferase for 60 min at 37°C. The reaction was terminated by immersing the coverslips in 2x SSC, and the endogenous peroxidases were blocked with 0.3% hydrogen peroxide. The cells were incubated with streptavidin-horseradish peroxidase (1:500) for 30 min at room temperature prior to staining with diaminobenzidine. Stained cells were viewed using an Olympus microscope.

RESULTS

Generation of Arsenic Derivatives

Due to cheap starting materials such as *p*-arsanilic acid (**1**), Laa (**4**)and D-glucuronic acid the synthesis was straight forward and cost effective. Direct acylation of *p*-amino-phenyl arsenoxide (**2**) resulted in very low yields due to the instability of the arsenoxide functionality under the coupling conditions. Therefore, the As-O group was protected as a thioacetal using ethane dithiole. The protected derivative (**3**) was more nonpolar and easier to monitor on TLC and more facil to purify on silicagel. Subsequent removal of the Boc group with HCl in DCM, oxidative cleavage of thioacetal group with mercury (II) perchlorate and finally zemplén-deacylation afforded the target compound (**11**).

The tendency towards oxidation of As (III) to As (V) was decreased by degassing all solvents with N_2 and storage under Ar at -80 °C. Impurities of oxidized material (As (V-)) could be monitored in the ES-MS (M +16) and in the ^{1}H NMR which displayed the aromatic protons with an additional multiplet at 7.93 ppm instead of two multiplets

between 7.60 ppm and 7.76 ppm in the "intact" As (III) derivative (**11**). In the studies of N. Donoghue at al [36] the As (III) compounds especially in solution were stabilized by the addition of glycine, in which most likely a five-membered cyclic 1,3,2- oxazarsolidin-5-one was formed.

Effects of Compounds (9**) and (**11**) on Hemolysis**

RBC toxicity = % hemolysis = $AvgAbs_{540}$-Avg(-ve)cont Abs_{540} / (Avg(+ve)cont Abs (max) - Avg (-ve)cont Abs_{540} (min)) x 100

Compound (**9**)**:**

0.5 mM SDS = 13.5% hemolysis compared to SDS

1 mM SDS = 26.8%

2 mM SDS = 57.8%

Compound (**11**):

0.5 mM = 5.2% hemolysis compared to SDS

1 mM = 11.1%

2 mM = 54.4%

The toxicity of compound (**9**) and (**11**) at concentrations that were used in *in vitro* assays (10 nM-100 μM) was negligible.

SDS

0.5 mM SDS = 15.4% hemolysis

1 mM SDS = 35.4%

2 mM SDS = 58.6%

Effects of Compounds 9 **and** 11 **on Growth and Apoptosis of MCF-7 Cells**

The effect of the two arsenic derivatives (**9**) and (**11**) on the growth of human cancer cells was determined by adding the two compounds separately to cultures of MCF-7 breast cancer cells (Fig. **2**). At days 2, 6 and 10 the proliferation of the cells was measured. Compound (**11**) at 10 μM was completely cytotoxic after only 48 h in culture. Modest decreases in cell proliferation were also observed using 1 μM of compound (**11**) after 6 and 10 days. In contrast, a significant decrease in cell proliferation was observed when MCF-7 cells were treated with 10 μM of compound (**9**) for 6 and 10 days.

The mechanism of action of arsenicals is thought to involve the induction of apoptosis and cell cycle arrest [5-7]. To determine whether compound (**9**) and (**11**) were triggering apoptosis, MCF-7 cells treated with these two compounds were analysed using a TUNEL assay (Fig. **3**). Compound (**11**) was apoptotic at 10 μM and 50 μM as evidenced by the presence of dark brown nuclei. In contrast to compound (**11**), compound (**9**) did not induce apoptosis at any concentration tested.

Effects of Compounds 9 **and** 11 **on Growth and Apoptosis of HT-29 Colon Cancer Cells**

There were no effects of compounds **9** and **11** on the proliferation of HT-29 colon cancer cells indicating the high cell selectivity of arsenicals (48, 49).

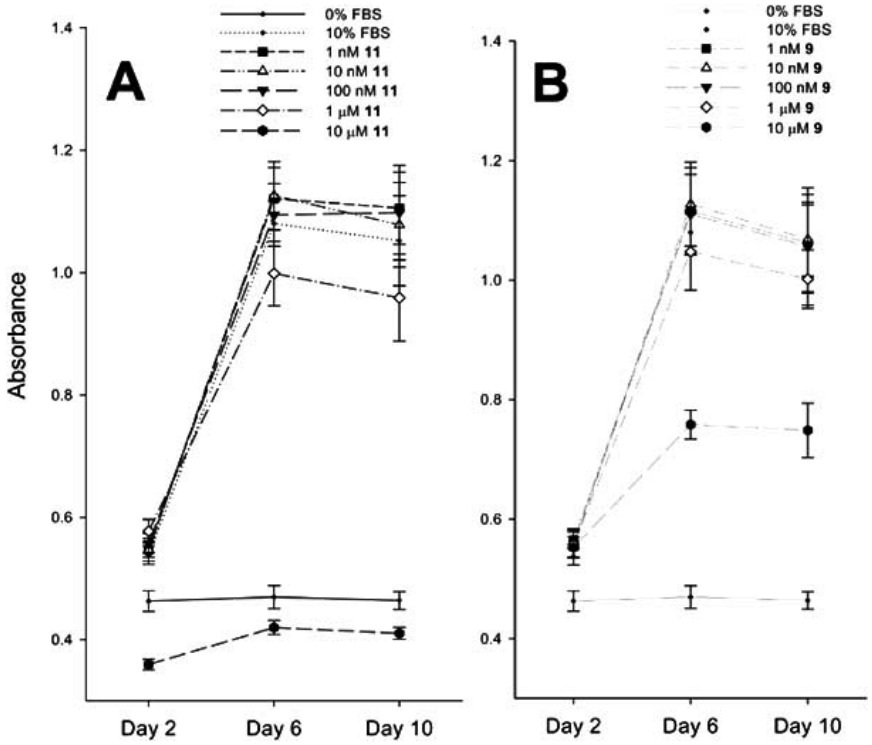


Fig. (2). The effect of compounds **9** and **11** on the proliferation of MCF-7 cells. MCF-7 cells were grown in the presence of 1 nM to 10 μM of compound **11** (A) or **9** (B) for 10 days. Cell numbers were determined indirectly by MTT absorbance assays at days 2, 6 and 10. Values are the mean absorbance ± SD (n=8).

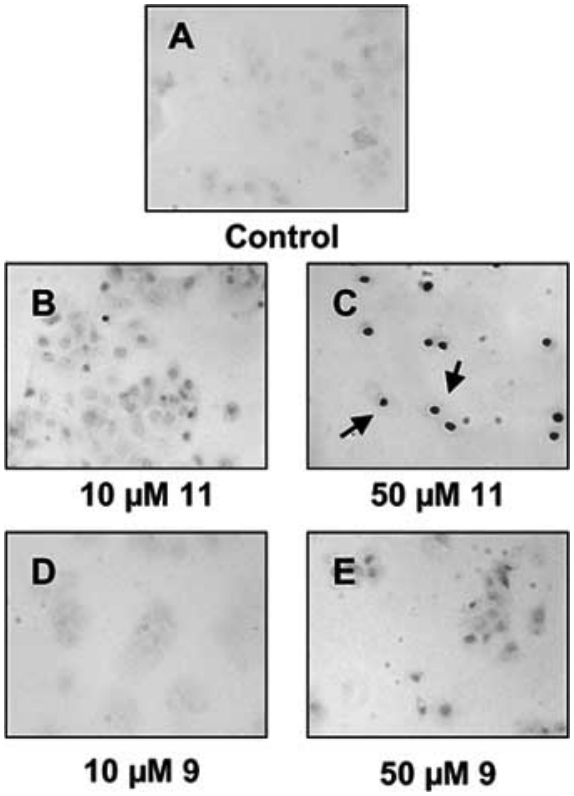


Fig. (3). Representative photomicrographs of *in situ* detection of apoptotic MCF-7 cells by TUNEL staining in coverslip cultures treated with compounds **9** and **11** for 24 h. TUNEL-positive nuclei were stained dark brown. (A) MCF-7 cells treated with vehicle; (B) MCF-7 cells treated with compound **11** (10 µM); (C) MCF-7 cells treated with **11** (50 µM); (D) MCF-7 cells treated with compound **9** (10 µM); (E) MCF-7 cells treated with compound **9** (50 µM).

DISCUSSION

The arsenicals are a class of therapeutics, which are receiving increasing attention as possible cost-effective anticancer agents. Structural modifications of arsenicals may confer improved bioavailability, selectivity and potency. In this study we investigated two novel glyco-lipid arsenicals (III) for their ability to initiate MCF-7 breast cancer cell death and characterized the mechanism by which death was initiated.

A significant decrease in MCF-7 cell proliferation was observed using 1 µM and 10 µM of compound (**11**) and 10 µM of compound (**9**). Treatment with compound (**11**) triggered apoptosis of MFC-7 cells while compound (**9**) induced inhibition of cellular proliferation was not *via* rapid induction of apoptosis and more likely reflected necrosis and/or alterations in the cell cycle. Differences in the anti-proliferative potency of the two compounds indicate that structural modifications influence effectiveness. An increase in lipophilicity did not correlate with increased anti-proliferative effects. It is possible that the reduced potency observed for compound (**9**) is related to the requirement for cleavage of the five-membered dithiarsolane ring to the arsenoxide group.

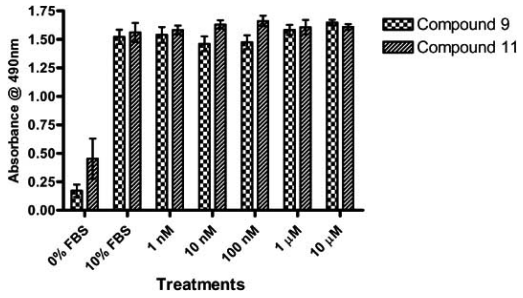


Fig. (4). The effect of compounds **9** and **11** on the proliferation of HT-29 cells on day 10. HT-29 cells were grown in the presence of 1nM to10µM of compound **9** and **11** for 10 days in serum supplemented RPMI-1640 media. Cell numbers were determined by MTT absorbance assay at 490nm. The values represented are the mean absorbance ± SD (n=8).

Our observation that inhibition of proliferation was mediated *via* apoptosis only for compound (**11**) demonstrates that structural alterations to the arsenoxide group may yield compounds which inhibit proliferation *via* mechanisms other than non-rapid (< 24 hr) apoptosis, thus reducing their favourable properties as anti-cancer agents. The altered mechanism initiated by compound (**9**) may be related to its enhanced lipophilicity allowing greater access to more intracellular targets, the consequence of which is uncontrolled cell death and/or alterations in the cell cycle [37].

The high micromolar concentrations required to significantly inhibit MCF-7 proliferation suggests that these compounds are not potential therapies for the treatment of breast cancer. However, the ability of these novel glyco-lipid arsenicals (III) to induce cell death may indicate that further studies of other novel arsenical derivatives are warranted. Future work will be based on modifying the lipid-chain, the sugar entity and more importantly using less stable As-protective groups in order to liberate the arsenoxide functionality *in-vivo* more effectively.

REFERENCES

[1] Ehrlich, P.; Bertheim, A. *Chem. Ber*., **1910**, *43*, 917.
[2] Dreyfuss, G.; Pénicaut, B.; Wolf, J.G.; Dubost, G.; Nicilas, J.A. *Bull. Soc. Pathol. Exot*., **1990,** *83*, 360.
[3] Rekik, L.; Coulais, M.A.; Rochas, M.A.; Campistron, G.; Wolf, J.G.; Houin, G. *J. Pharm. Sci*., **1989**, *78*, 203.
[4] Shen, Z.X; Chen, G.Q.; Ni, J.H.; Li, X.S.; Xiong, S.M.; Qiu, Q.Y.; Zhu, J.; Tang, W.; Sun, G.L.; Yang, K.Q.; Chen, Y.; Zhou, L.; Fang, Z.W.; Wang, Y.T.; Ma, J.; Zhang, P.; Zhang, T.D.; Chen, S.J.; Chen, Z.; Wang, Z.Y. *Blood*, **1997,** *89*, 3354.
[5] Zhang, W.; Ohnishi, K.; Shigeno, K.; Fujisawa, S.; Naito, K.; Nakamura, S.; Takeshita, K.; Takeshita, A.; Ohno, R. *Leukemia*, **1998,** *12*, 1383.
[6] Wang, Z.G.; Rivi, R.; Delva, L.; König, A.; Scheinberg, D.A.; Gambacorti-Passerini, C.; Gabrilove, J.L.; Warrell, R.P.; Pandolfi, P.P. *Blood*, **1998,** *92*, 1497.
[7] Look, A.T. *J. Natl. Cancer Inst*., **1998,** *90*, 86.
[8] Ni Dhubhghaill, O.M.; and Sadler, P.J. *Struct. Bonding* (Berlin), **1991,** *78*, 129.
[9] Don, A.; Kisker, O., Dilda, P.; Donoghue, N.; Zhao, X.; Decollogne, S.; Creighton, B.; Flynn, E.; Folkman, J.; and Hogg, P.J. *Cancer Cell*, **2003,** *3 (5)*, 497.
[10] Gilbert, H.F. *J. Biol.Chem*., **1997,** *272*, 29399.
[11] Stevenson, K.J.; Hole, G.; Perham, R.N. *Biochemistry*, **1978,** *17*, 2189

[12] Singh, R.; Whitesides, G.M. *J. Am. Chem. Soc*., **1990,** *112*, 6304.
[13] Bardos, T.J.; Datta-Gupta, N.; Hebborn, P.; Triggle, D.J. *J. Med. Chem*., **1965**, *EN, 8*, 167.
[14] Banks, C.K.; Controulis, J.; Walker, D.F.; Sultzaberger, J.A. *J. Amer. Chem. Soc*., **1947**, *69*, 5.
[15] Ainley, A.D.; Davey, D.G. *Br. J. Pharmacol. Chemother*., **1958,** *13*, 244.
[16] Banks, C.K.; Controulis J. *J. Amer. Chem. Soc*., **1946,** *68*, 944.
[17] Doak, G.O.; Eagle, H.; Steinman, H.G. *J. Amer. Chem. Soc*., **1940,** *62*, 168.
[18] Erlenmeyer, W. *Helv. Chim. Acta*, **1935,** *18*, 733.
[19] Banks, C.K. *J. Amer. Chem. Soc*., **1944,** *66*, 1771, 1774.
[20] Friedheim, E.A. *J. Amer. Chem. Soc*., **1944,** *66*, 1775, 1778.
[21] Bertheim, A. *Chem. Ber*., **1911.** *44*, 1073.
[22] Steinman, H.G.; Doak, G.O.; Eagle, H. *J. Amer. Chem. Soc*., **1944,** *66*, 192.
[23] Aksnes, D.W.; Vikane, O. *Acta Chem. Scand*., **1971,** *29*, 672.
[24] Adams, E.; Jeter, D.; Cordes, A.W.; Kolis, J.W. *Inorg. Chem*., **1990,** *29*, 1500.
[25] Dill, K.; Adams, E.R.; O'Connor, R.J. *Magn. Reson. Chem*., **1987,** *26*, 1074.
[26] Dill, K.; Adams, E.R.; O'Connor, R.J. McGown, E.L. *Chem. Res. Toxicol*., **1989,** *2*, 181.
[27] Toth, I.; Flinn, N.; Hillery, A.M.; Gibbons, W.A.; Artursson, P. *Int. J. Pharm.,* **1994,** *105*, 241.
[28] Wong, A.; Toth, I. *Curr. Med. Chem*., **2001,** *8,* 1123.
[29] Blanchfield, J.T.; Toth, I. *Curr. Med. Chem*., **2004**, *11*, 2375.
[30] Fahmy, R.A.; Niederwieser, A.; Pataki, G.; Brenner M. *Helv. Chim. Acta*, **1961,** *44*, 2022.
[31] Gibbons, W. A.; Hughes, R. A.; Chralambous, M.; Szeto, A.; Aulabaugh, A. E.; Mascagni, P.; Toth, I. *Liebigs Ann. Chem.,* **1990**, 1175.
[32] Adams, E.; Jeter, D.; Cordes, A.W.; Kolis, J.W. *Inoorg. Chem*., **1990**, *29*, 1550.
[33] Bardos, T.J.; Datta-Gupta, N.; Hebborn, P. *J. Med. Chem*., **1966**, *EN, 9*, 221.
[34] Malkinson, J.P.; Falconer, R.A.; Toth, I. *J. Org. Chem*., **2000,** *65(17)*, 5249.
[35] Hatlelid, K.M.; Brailsford, C.; Carter, D.E. *Fundamen. App. Toxicol.,* **1995,** *25*, 302.
[36] Donoghue, N.; Yam, P.T.W.; Hogg, P.J. *Protein Sci.*, **2000,** *9*, 2436.
[37] Okada, H.; Mak, T.W. *Nat. Cancer* **2004,** *4*, 592.
[38] Doak, G.O.; Steinman, H.G.; Eagle, H. *J. Amer. Chem. Soc*., **1944,** 66, 197, 199.
[39] Von Doellen, A.; Strasdeit, H. *Eur. J. Inorg. Chem*., **1998,** *1*, 61.
[40] Petit, L. D.; Turner, D. *Spectrochim. Acta,* **1968,** *B24A*, 999.
[41] Davidson, G.; Kenneth, P. E. *Spectrochim. Acta,* **1983,** *39A*, 419.
[42] Rank, A.; Allen, L.C.; Mislow, K. *Angew. Chem. Int. Ed*., **1970,** *9*, 400.
[43] Loiseau, PM.; Lubert, P.; Wolf, JG. *Arzneim.-Forsch./Drug Res*., **1999,** *49 (II)*, 944.
[44] Loiseau, PM.; Lubert, P.; Wolf, JG. *Antimicrob. Agents Chemother*., **2000,** *44(11)*, 2954.
[45] Bi, X.; Gu, J.; Chen,; Z.; Min, Q. *Zhonghua Pifuke Zazhi*, **2002,** *35 (6)*, 457.
[46] Addullaev, F.I.; Rivera-Luna, R.; Garcia-Carranca, A.; Ayala-Fierro, F.; Espinosa-Aguirre, J.J. *Mutat. Res.*, **2001,** *493 (1-2)*, 31.
[47] Li, Y.M.; Bromme, J.D.; John, D. *Cancer Res.*, **1999,** *59 (4)*, 776.
[48] Akay, C.; Gazitt, Y. *Cell Cycle*, **2003**, 2 (4), 358.
[49] Hogg, P.J. *PCT Int. Appl*., **2003,** WO 2003039564.
[50] Hwang, D.R.; Tsai, Y.C.; Lee, J.C.; Huang, K.K.; Lin, R.K.; Ho, C.H.; Chiou, J.M.; Lin, Y.T.; Hsu, J.T.; Yeh, C.T. *Antimicrob. Agents Chemother.*, **2004,** *48(8)*, 2876.

Received: 28 June, 2005 **Revised: 03 October, 2005** **Accepted: 04 October, 2005**