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Anticancer Activity of Molecular Compounds of Arsenic, Antimony and Bismuth

Edward R.T. Tiekink

Department of Chemistry, University of Malaya, Kuala Lumpur 50603, Malaysia

12.1 Introduction

Metal based drugs continue to play a vital role in contemporary medicine. Accordingly, molecular complexes of platinum(II) for example, cisplatin (I) and carboplatin (II), find wide use as chemotherapeutic agents for the treatment of various forms of cancer; see Figure 12.1 for chemical structures of drugs discussed in this section. Gold(I) compounds, for example, monomeric auranofin (III) and polymeric myochrisine (IV), are still used in the amelioration of the inflammation and pain associated with the debilitating disease, rheumatoid arthritis. The silver(I) complex of sulfadiazine, (V), is used as an antimicrobial and antifungal agent. Age related macular degeneration disease may be remedied by the tin (IV) compound ethyl etiopurpurin, (VI), by exploiting the principles of photodynamic therapy. Owing to its ability to release NO to promote vascular muscle relaxation, sodium nitroprusside, containing iron(II), that is, Na₂[Fe(CN)₅(NO)]·2H₂O (VII), is used clinically as a hypotensive agent. While not a coordination complex, lithium(I) carbonate, Li₂CO₃ (VIII), was introduced in the 1950s for the treatment of bipolar disorders and it still employed for that purpose. Another carbonate but associated with lanthanum(III), i.e. La₂(CO₃)₃ (IX), may be prescribed for chronic renal failure. It is evident from the
foregoing that there is a wide range of metal based chemotherapeutic agents: the important contribution metal complexes play in diagnostic medicine should also be acknowledged. In recognition of the special roles played by metal complexes in modern medicine, it is not surprising that there are books (e.g., [1–7]) and many reviews (e.g., [8–18]) addressing the general concept of metals in medicine.

While no mention has been yet made of the elements that form the focus of this Chapter, namely arsenic, antimony and bismuth, these too form an important part of modern medicine, as may be seen in other chapters collected in this book. Herein, the anticancer activity/potential of arsenic, antimony and bismuth compounds is summarized after a brief introduction of the history of each element in human medicine.

Figure 12.1  Chemical structures of molecules relevant in metal based drugs: cisplatin (I), carboplatin (II), auranofin (III), and polymeric sodium aurothiomate (IV), sulfadiazine, (V), tin ethyl etiopurpurin, (VI) and sodium nitroprusside (VII)
12.2 Arsenic Compounds

Arsenic holds a special place in medicinal inorganic chemistry. Paul Ehrlich’s role in contemporary medicine partially revolved around the development of arsenic based therapeutic compounds for the treatment of syphilis and sleeping sickness, African trypanosomiasis. Indeed, it is the emergence of the arsenic containing compound Salvarsan, developed as a ‘magic bullet’ that probably marks the beginning of modern chemotherapy [19]. However, the use of arsenic in medicine predates Ehrlich by well over 2000 years [20–22]. Hippocrates recorded the use of the arsenic sulfur derivatives, realgar (red arsenic, As₂S₂) and orpiment (yellow arsenic, As₂S₃), for the treatment of ulcers. Arsenic trioxide (white arsenic, As₂O₃; X) was mentioned as a cure for tuberculosis. Arsenic as a medicine has figured prominently in Western society, being used as an important agent for the treatment of leukaemia from the 1700s, for about two centuries. A familiar reagent, Fowlers solution (arsenic trioxide in a potassium bicarbonate solution), was used for the treatment of a variety of human ailments, including leukaemia [23]. Inorganic arsenic compounds also feature in Traditional Chinese Medicine (TCM) and it is probably true to state that the resurgence of interest into their therapeutic use arose in that country. This came about after extensive clinical observations in the 1970s suggesting the efficacy of arsenic trioxide (contained within a TCM used for the treatment of leukaemia, incidentally also containing mercury) in the treatment of Acute Promyelocytic Leukaemia [24]. This is a cancer of the blood and bone marrow, and is a subtype of acute myeloid leukaemia. Subsequently, significant attention has been devoted to the development of arsenic trioxide (Trisenox) as a drug, determining its mechanisms of action and new modes of delivery.

A precise mechanism of action for Trisenox remains unknown and studies investigating the interaction of arsenic with biomolecules continue [25–28]. Patients suffering from acute promyelocytic leukaemia (AML) are characterized by an accumulation of immature granulocytes (i.e., promyelocytes: a category of white blood cells with granules in their cytoplasm) in their bone marrow. Further, patients test positive for a chromosome abnormality, that is, a chromosomal translocation involving the retinoic acid receptor a (RARα or RARA) gene, which usually generates a leukemogenic fusion gene. This latter feature of AML makes this leukaemia susceptible to tretinoin therapy (tretinoin, the acid form of vitamin A, is often referred to as all-trans retinoic acid) [29, 30]. At high concentrations, the dominant effect of Trisenox is cell death by apoptosis while at low concentration, the effect appears due to its ability to disturb cell differentiation [23, 29]. A molecular mechanism for Trisenox is the degradation of the retinoic acid receptor of promyelocyte [30, 31].

The above notwithstanding, arsenic is widely, and correctly, regarded as a toxin and is implicated as a carcinogen, that is, it is a paradoxical species in human physiology [22, 32]. The emergence of Trisenox has prompted investigations into alternative and more effective means of delivery, for example exploiting principles of nanotechnology [33] and lipid encapsulation [34]. In addition, the quest for molecular forms of ‘arsenic’ as chemotherapeutic agents is ongoing.

It has already been mentioned that Salvarsan was the first compound to be developed specifically as a chemotherapeutic agent. Having spirochaeticidal activity, this and related compounds were used in the forefront of the eradication of Treponema pallidum, the bacterium implicated in syphilis; their use continued until the advent of penicillin [35].
was not until only recently that the chemical composition of Salvarsan was determined. It turns out that Salvarsan in fact comprises cyclic species, for example, tricyclic (XI) and pentacyclic (XII) [36], see Figure 12.2. Regardless of the true structure of Salvarsan, it functions as a prodrug, providing a slow release source of the therapeutically active species (3-amino-4-hydroxyphenyl)As(OH)$_2$.

Compared with the interest in molecular antimony and bismuth compounds as potential anticancer agents, investigations of molecular arsenic compounds are lagging, probably owing to perceived problems with toxicity. Amongst the first arsenic containing compounds examined for their potential as anticancer agents were those already used clinically. For example, the typanocide, melarsoprol (XIII), Figure 12.2, which is still used in the treatment of African typanosomiasis [37, 38], is effective against leukaemia cell lines, more so than arsenic trioxide and causes apoptosis but not cell differentiation [39]. In vivo studies in immune suppressed mice showed no advantage in terms of inhibiting myeloma growth [40]. More positive were investigations on human breast and prostate cancer cells inoculated in mice which indicated melarsoprol reduced the anticipated growth of the tumours [41]. While the previous study did not present any obvious side effects associated with administration of melarsoprol, further development as an anticancer agent is unlikely owing to (i) severe toxicity to the central nervous system resulting in encephalopathy, and (ii) lack of a clinical response [42–44]. The amoebicidal and bactericidal arsthinol (XIV),

![Molecule Structures]  

*Figure 12.2* Chemical structures of molecules relevant to arsenic pharmacology: tricyclic component of Salvarsan (XI), pentacyclic component of Salvarsan (XII), melarsoprol (XIII), and arsthinol (XIV)
also known as Balarsen, displays significant cytotoxicity against the K562 erythroleukaemia and U937 myelomonocytic leukaemia cell lines [44]. Interestingly, the therapeutic index, calculated as LD$_{50}$ in mice/IC$_{50}$ in cell line, was considerably better than either of arsenic trioxide and melarsoprol. With this background, synthetic arsenic analogues of arsenic based drugs have been investigated.

The simplest organoarsenic compound investigated for putative anticancer activity is dimethylarsenic acid (XV), Figure 12.3. An important, if not the, detoxification mechanism of ‘inorganic’ compounds is biomethylation. Here, methyl-metal bonds are formed to generate organometallic species that are more readily excreted from the body, for example, via respiration. Arsenic species are detoxified this way and dimethylarsenic acid is one of the metabolites. Compounds XV and As$_2$O$_3$ (X) were evaluated against a panel of eight leukaemia and multiple myeloma cell lines [45]. Concentrations of compound XV, on average, 1000 times more were required to achieve the same cytotoxic responses compared with (X). Compound XV induced apoptosis in the cancer cells but not in progenitor cells, and has little effect on the maturation of leukaemic cells [45].

The compound where organoarsenic is complexed to glutathione, 4-((N-(S-glutathionylacetyl)amino)phenylarsinous acid (XVI), inhibits the growth of a variety of cancer cells, including pancreatic, lung and prostate cancers, by a mechanism whereby the blood supply to the tumour is restricted, that is, halting angiogenesis by targeting mitochondria in endothelial cells [46, 47]. Compound XVI exhibits no obvious side effects and Phase I/IIA trials of the compound are ongoing [23]. A closely related compound XVII, a result of the

\[
\text{As} \quad \text{N} \quad \text{As} \\
\text{S} \quad \text{Me} \quad \text{S} \\
\text{OH} \quad \text{OH} \quad \text{OH} \\
\text{Me} \quad \text{Me} \quad \text{Me}
\]

(XVI)

\[
\text{R} = \text{Me, Et, n-Pr and i-Pr}
\]

(XVII)

(XVIII)

Figure 12.3 Chemical structures of synthetic arsenic containing molecules investigated for anticancer potential: dimethylarsinic acid (XV), 4-((N-(S-glutathionylacetyl)amino)phenylarsinous acid (XVI), S-dimethylarsino glutathione (XVII; Darinaparsin) and generic structure of S-dialkylarsino-3-mercaptop-1,2-propanediol (XVIII)
combination of the essential components found in compounds XV and XVI, S-dimethylarsino glutathione (Darinaparsin), also displays exciting potential as a metal based anticancer drug, having a similar profile of activity to XVI. For example, it has been reported that XVII was well tolerated by patients having advanced haematological malignancies in a Phase I trial [23]. In a complementary study based on the National Cancer Institute’s panel of 60 cancer cell lines, that is in vitro, a series of compounds with the general formula shown in (XVIII) were evaluated [48]. Of the R = Me, Et, n-Pr and i-Pr compounds tested, the most cytotoxic was the R = n-Pr derivative clearly indicating that systematic studies of different organoarsenicals is warranted.

As a footnote to this section, it is of some interest that the principal author of the previous study [48] abandoned pioneering studies in this area in the mid 1970s, presumably owing to anxieties over toxicity issues, lack of funding and so on [49]. The foregoing summary of arsenic compounds and their cytotoxicity/antitumour activity quite plainly demonstrates that this is a field of endeavour well deserving of continued attention.

12.3 Antimony Compounds

Various antimony compounds find continued use in the treatment of tropical diseases, most notably leishmaniasis caused by Leishmania species, which are human protozoan parasites of the trypanosomatidae family [50–54]. The two most prominent antimony compounds used in the treatment of cutaneous and visceral leishmaniasis are sodium stibogluconate (XIX, Pentostam) and meglumine antimonate (XX, Glucantime): their molecular compositions let alone precise molecular structures remain uncertain [55]. While each of these drugs features an antimony(V) centre, it is likely that these are reduced to antimony(III) in vivo [51]. Besides these leishmaniacides, the other antimony compound relevant to contemporary medicine is potassium antimony tartrate (XXI, Tartar Emetic) which may also be used against leishmaniasis. This species has antimony present in the +III oxidation state and is also known to be highly toxic [51]. The biological targets of antimony containing therapeutics remain under investigation [50–54] and some progress has been made towards understanding their molecular mechanism (Chapters 3 and 8): this knowledge may have implications for the design of anticancer active antimony compounds. As indicated earlier, the antimony(V) drugs are prodrugs, being reduced in vivo, either enzymatically (e.g. by a thiol dependent reductase (designated TDR1) or the pentavalent antimony reductase, Leishmania major, LmACR2) or by some other bioreduction mechanism, yielding antimony(III). A key feature of the Trypanosomatidae family is their specific redox metabolism which gives a clue as to the mode of action of therapeutic antimonials. While other eukaryotes rely on glutathione/glutathione reductase system, trypanosomatidae utilize a trypanoathione/trypanoathione reductase system. Antimony(III) can inhibit trypanoathione reductase and this disruption of the oxidative-stress defence mechanism is the likely cause of leishmanicidal activity exhibited by antimonials [51]. In support of this postulate, a recent crystal structure determination of the reduced form of trypanoathione reductase, NADPH and antimony(III) shows coordination of antimony(III) by cysteine (x 2), threonine and histidine residues (Chapter 3, Figure 3.14) [52]. The aforementioned results suggest that enzymes or proteins may be biological targets for antimony anticancer agents as opposed to cellular DNA for cisplatin (I) and other transition metal complexes.
Some of the antimony compounds used for the treatment of leishmaniasis have also been investigated for potential antitumour activity [56–59]. Sodium stibogluconate has been evaluated for antileukaemic activity against the myeloid leukaemia cell lines (NB4, HL-60 and U937) and shown to induce differentiation of acute myeloid leukaemia. Further, the results suggest that sodium stibogluconate is functioning as a PTPase inhibitor in the leukaemia cells [57]. It appears that most attention in developing new antimony based anticancer agents has been directed to newly synthesized molecules [58, 59]. The following is an overview of recent studies in this area.

In probably the most sustained systematic study of antiproliferative activities of antimony compounds, their complexation with various heterocyclic thioamides was researched, with the latter being present in the neutral and/or deprotonated forms [60–63]. As a consequence, a full range of coordination geometries and aggregation patterns have been determined for these derivatives. Compounds formed with antimony trichloride and the heterocyclic thiones 2-mercapto-benzimidazole (MBZIM), 5-ethoxy-2-mercapto-benzimidazole (EtMBZIM) and 2-mercapto-thiazolidine (MTZD) thioamides are uniformly monomeric based on a distorted octahedron or a trigonal bipyramid whereby in each case the thioamide coordinates in the neutral mode via the sulfur atom only and a stereochemically active lone pair of electrons occupies a coordination site [62]. Representative compounds prepared in this study are Sb(MBZIM)$_4$Cl$_2$ (XXII) and Sb(MTZD)$_2$Cl$_3$ (XXIII), Figure 12.4. The antimony compounds were examined for cytotoxicity against the following cancer cell lines: L1210 (Murine Leukaemia Cells), FM3A (Murine Mammary Carcinoma Cells), Molt4/C8, CEM (Human T-lymphocyte Cells), and HeLa (Human Cervix Carcinoma Cells). The key result from this study was that the antimony compounds displayed selective antiproliferative activity against the HeLa cells, with up to and greater than 10-fold more potency against the other cell lines investigated. For example, compound XXII inhibited growth of the HeLa cells at 6.4 ± 1.6 μM compared with 12 ± 7, 36 ± 6, 24 ± 16, 90 ± 19 μM.

![Chemical structures of synthetic antimony containing molecules investigated for anticancer potential: Sb(MBZIM)$_4$Cl$_2$ (XXII), Sb(MTZD)$_2$Cl$_3$ (XXIII), Sb(pmt)$_3$ (XXIV) and [Sb(NNS)Cl$_2$] (XXV)](image-url)
for the L1210, FM3A, Molt4/C8, and CEM cell lines, respectively. It was also reported that the antimony compounds were more potent than the standard drugs cisplatin (I) and carboplatin (II). In another study, comparable reactions with antimony bromides were conducted whereby distinctive structures as well as those resembling XXII and XXIII were isolated [60]. Dimerization and even polymerization was achieved in some of the antimony bromide derivatives owing to the presence of bridging bromide atoms. The antimony bromides were found to be less potent than the corresponding antimony chlorides [60], with only modest cytotoxicity found against almost the same panel of cancer cell lines. Interestingly, evidence was found for an enhanced antiproliferative activity against the HeLa cells.

The binary compound Sb(pmt)$_3$ (XXIV), where pmtH is the heterocyclic thioamide 2-mercapto-pyrimidine, having a coordination geometry based on a pentagonal pyramid, proved ineffective in low doses against leiomyosarcoma cells. However, it inhibited cancer cell induced platelet aggregation [63].

The coordination of a Schiff base to antimony(III), leading to [Sb(NNS)Cl$_2$] (XXV), results in a compound that displays moderate activity against the CEM-SS (T-lymphoblastic leukaemia) human cell line [64].

The foregoing studies allow conclusions based on in vitro assays only, with no definitive indication of antitumour activity. However, antitumour activity is indicated by animal studies on antimony(III) compounds with aminopolycarboxylic acid ligands, that is in the sodium and ammonium salts of [Sb(nta)(Hnta)]$^{2+}$ (Na$^+$ = XXV$_{Na}$, K$^+$ = XXV$_{K}$), where H$_3$nta is nitritolriacetatic acid, N(CH$_2$COOH)$_3$ [65]. The toxicities of XXV$_{Na}$ and XXV$_{K}$ were investigated in allogenic mice and found to be approximately 150 mg/kg (= LD$_{50}$). Next, mice were inoculated with Ehrlich Adenocarcinoma in the ascitic (fluid) form and survival rates were monitored. With doses of 25–50 mg/kg, survival rates increased significantly, up to 90%. The remaining studies to be described involve organoantimony species, with antimony in either the +III or +V oxidation states.

The majority of cytotoxicity trials with organoantimony have the antimony present as antimony(V), with only one study involving antimony(III). A series of diorganoantimony (III)-phthalimide and -succinimide derivatives, see representative structures, XXV, XXVI (and other organoantimony compounds) in Figure 12.5, were synthesized and their cytotoxicity against MCF-7 (breast adenocarcinoma) and EVSA-7 (mammary) cancer cell lines recorded [66]. Compounds XXV and XXVI and their derivatives with partially or fully fluorinated aryl groups displayed moderate to good activity with respect to the standard compound, β-oestradiol. It is also noted that these compounds also displayed antibacterial and antifungal activity [66].

The coupling of N-phenylglycine with triarylantimony(V) results in compounds of the general formula (XXVII), Figure 12.5 [67]. The amino acid derivatives displayed greater potency than Ph$_3$SBr$_2$ in KB (human epidermoid), Bel-7402 (hepatocellular carcinoma) and HCT-8 (colon) cancer cells. Interestingly, the screening of triarylantimony compounds showed evidence of systematic variations in that the triphenyl derivative was markedly most potent against the KB cell line compared with the other compounds tested, similarly the p-tolyl derivative exhibited the same trend against Bel-7402 and the p-chloro derivative against HCT-8, with the latter being the most potent compound of the series investigated [67]. Compound selectivity is of vital importance in developing new drug therapies and the foregoing results might provide pointers in this regard. This being stated,
Figure 12.5  Chemical structures of synthetic organoantimony containing molecules investigated for anticancer potential: Ph$_2$Sb(phthalimide) (XXV), Ph$_2$Sb(succinimide) (XXVI), generic triarylantimony bis(N-phenylglycinate) (XXVII), triphenylantimony(V) bis(phenylhydroxamate) (XXVIII), tetra(p-tolyl)antimony phenylhydroxamate (XXIX), generic structure for phenylantimony (N-hydroxy-demethyldehydrogencantharimide) derivatives (XXX), generic structure for phenylantimony (N-hydroxy-demethyldehydrogencantharimide) derivatives (XXXI), Ph$_{15-n}$Sb[O$_2$CCH($R'$)CH($R''$)GePh$_3$]$_n$; $n = 1$ (XXXII) and $n = 2$ (XXXIII), and Ph$_{15-n}$Sb[O$_2$C(C$_5$H$_4$)FeC$_3$H$_5$]$_n$; $n = 1$ (XXXIV) and $n = 2$ (XXXV)
A comprehensive series of tri- and tetra-arylantimony(V) arylhydroxamates, with examples XXVIII and XXIX shown in Figure 12.5, was synthesized and investigated for their cytotoxicity against the following panel of cancer cell lines: HL-60 (human immature granulocyte leukaemia), BGC-823 (human gastric carcinoma) and MDA-MB-435 (human mammary gland carcinoma) [68]. The neutral tetraorganoantimony(V) compound displayed significantly greater cytotoxicity than the charged (anionic) triorganoantimony(V) compounds. Of the latter series, with the additional substitution of –NH₂ in the 2-position of the arylhydroxamate ligands were more potent than those derivatives without this substitution pattern. The precursor compound was also included in this study, that is, (4-CH₃C₆H₄)₃SbBr₂, which had inhibition ratios compared to the –NH₂ substituted compounds. Against all three cell lines, cisplatin (I) was more potent than all of the triorganoantimony compounds. However, the (4-CH₃C₆H₄)₄Sb[ON(H)(=O)Ph] compound (XXIX) was more effective than cisplatin (I) against the HL-60 and MDA-MB-435 cell lines [68].

The influence of having three or four aryl substituents in organoantimony(V) compounds was further probed for two series of neutral compounds represented by compounds XXX and XXXI in Figure 12.5. Using a wider panel of human cancer cell lines, i.e. HL-60 (immature granulocyte leukaemia), PC-3MIE8 (prostatic carcinoma), BGC-823 (gastric carcinoma), MDAMB-435 (mammary gland carcinoma), Bel-7402 (hepatocellular carcinoma), and HeLa (carcinoma), it was again demonstrated that the tetraorganoantimony(V) compounds were markedly more potent than their triorganoantimony(V) analogues. For the tetraorganoantimony(V) series, the antimony-bound p-tolyl substituent generally gave rise to greater potency. No clear cut trends that could be related to the nature of saturation in the demethylcantharimide based ligands were discerned. Comparing the most active compounds with cisplatin (I) suggests that the organoantimony(V) compounds were more potent by up to a factor of two against the HL-60, MDA-MB-435 and Bel-7402 cell lines [69].

The remaining two studies to be summarized incorporate additional metal centres, that is, germanium and iron, with the aim to improve the efficacy of the organoantimony species. For the series of compounds with the generic formulae R₄Sb[O₂CCH(R')CH(R'')(R''')GePh₃] (XXXII) and R₃Sb[O₂CCH(R')CH(R'')(R''')GePh₃]₂ (XXXIII), it was reported that the antimony compounds had greater potency than that exhibited by the carboxylic acid precursor compound, HO₂CCH₂CH₂GePh₃ [70]. No discernible trends in terms of the utility of tetra- versus tri-arylantimony derivatives were evident although the most potent compound against the cancer cell lines tested, that is HL-60 (leukaemia), EJ (bladder), SKOV-3 (ovarian), HeLa (cervical), BGC-823 (gastric) cancer cells, was the triaryl derivative, (p-ClC₆H₄)₃Sb[O₂CCH₂CH(Me)GePh₃]₂ [70]. In a related study, arylantimony(V) ferrocenecarboxylate derivatives, Arylₖ₋ₙSb[O₂C(CH₃)₄FeC₆H₃]ₙ, where n = 1 and 2 for XXXIV and XXXV respectively, were investigated for their cytotoxicity against the HL-60, Bel-7402, KB and HeLa cancer cell lines [71]. Although, again, details are sparse, it is possible to conclude from the data included in the publication that (i) the presence of antimony increased potency and (ii) no discernible trends were found, viz a viz, whether tetra- versus tri-arylantimony compounds have greater potency. Within the tetraaryl series, the presence of a halide in the 4-position of the antimony bound aryl group clearly increased potency. Within the triaryl series, the p-tolyl derivative proved consistently more potent across the four cancer cell lines tested [71].
12.4 Bismuth Compounds

Along with arsenic and antimony, bismuth has a long history in medicine; its use was again motivated by the need to treat bacterial infections, such as caused by the pathogenic organism *Helicobacter pylori* [72, 73]. Gastric complaints began to be treated with bismuth formulations in the nineteenth century and this use continues today whereby De-Nol (colloidal bismuth subcitrate), Pepto-Bismol (bismuth subsalicylate) and Pylorid (ranitidine bismuth citrate), for example, are readily available, often over the counter [72–74]. Such availability attests to the relative nontoxic character of bismuth to humans. The utility of bismuth formulations has motivated many studies into their possible mechanism of action and to the discovery of their biological targets [51, 75]; as with antimony containing drugs, the precise molecular structures of bismuth drugs remain unknown. Studies show that bismuth can interact with enzymes generated by *Helicobacter pylori* such as the Jack Bean urease (responsible for the catalysis of the process whereby urea is hydrolysed to ammonia and carbon dioxide), ATPase (the class of enzymes that are responsible for catalysing the decomposition of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and free phosphate with the release of energy), and cytosolic alcohol dehydrogenase (ADH; the zinc containing enzyme responsible for the oxidation of alcohol to aldehyde). Bismuth is also known to interact with proteins such as transferrin, the glycoproteins responsible for the transportation of ferric ion [51, 73]. Thus, as for antimony, bismuth appears to target non-DNA sites, a feature which offers new opportunities for novel mechanisms of action in the treatment of cancer. Additional impetus to studying the medicinal chemistry of bismuth compounds was gained recently with the report of the ability of several bismuth compounds to inhibit the SARS coronavirus [76]. The antitumour potential of bismuth compounds has not attracted as much attention as have the lighter elements of this group. A bibliographic review summarising the screening of bismuth compounds appeared in 2002 [58] and herein, recent studies are summarized.

The first studies to be summarized are those on binary bismuth 1,1-dithiolates. Three xanthate structures, Bi(S₂COR)₃ where R = Et (XXXVI), Figure 12.6, i-Pr, and cyclohexyl, displayed similar cytotoxicity against Calu-6 (lung adenocarcinoma), which is highly sensitive to cisplatin (I), indicating little influence exerted by the R group [77]. By contrast, the R = cyclohexyl derivative was significantly less cytotoxic against MCF 7 (mammary carcinoma), which is relatively non responsive to cisplatin (I), than the compounds with smaller R groups. Interestingly, when cytotoxicity was scored at pH = 6.8, mimicking the pH of a solid tumour, the potency was greater for the R = Et and i-Pr compounds [77]. It was determined that the comparable binary platinum(II) xanthates were significantly more cytotoxic than the bismuth compounds which have potency comparable to cisplatin (I). Changing the nature of the 1,1-dithiolate ligand to dithiocarbamate, S₂CNR₂, a species renowned for its use/potential use in medicine [78], a very potent series of compounds is generated.

Compounds of general formula Bi(S₂CNR₂)₃, for example R = Et (XXXVII), Figure 12.6, were screened for potency against a panel of seven human cancer cell lines: A498 (renal), MCF-7 (breast), EVSA-T (breast), H226 (non-small cell lung), IGROV (ovarian), M19 MEL (melanoma), and WIDR (colon) [79]. Several compounds proved quite potent and a qualitative structure activity was established. Thus, for the compounds with straight chain R groups, potency varied in the following order:
Figure 12.6  Chemical structures of synthetic bismuth containing molecules, including organobismuth molecules, with additional metal centres investigated for anticancer potential: Bi(S$_2$COEt)$_3$ (XXXVI), Bi(S$_2$CNEt)$_3$ (XXXVII), the 1,4,7,10-tetraakis(2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane macrocyclic ligand in the structure of (XXXVIII), N-tert-butyl-bi-chlorodibenzo[c,f][1,5]azabismocine (XXXIX), bi-chlorodibenzo [c,f][1,5] thiabismocine (XL), bi-chlorophenothiabismin-S,S-dioxide (XLI), Ph$_3$Bi[O$_2$CCH(Me)CH$_2$GePh$_3$]$_2$ (XLII), (p-tolyl)$_3$Bi (O$_2$CCH$_2$N=C(H)C$_6$H$_4$OH-2)$_2$ (XLIII), generic structure for triarylbismuth N-(p-toluenesulfonyl) aminoacetate (XLIV), tris[2-(N,N-dimethylaminomethyl)phenyl]bismuth (XLV), and generic structure for bismuth tris(8-quinolinethiolate) (XLVI)
Me < Et > n-Pr > n-Bu. The introduction of branching decreased potency, for example n-Pr > i-Pr. For examples where NR₂ was a ring, smaller rings were more potent, such as, n = 4 > n = 6 for N(CH₃)₄. Finally, introducing aromatic rings decreased potency. Of the full series, compound XXXVII was chosen as the lead compound as it displayed uniformly high cytotoxicity and a broad range of potency in the cancer cell lines tested. Gross toxicity trials in a Balb/C murine model gave a maximum tolerated dose (LD₉₀) of 7 mg Bi/kg mouse; LD₅₀ = 3.6 mg Bi/kg mouse. While this value is low, the very low concentrations required to arrest the growth in the in vitro trial, for example IC₅₀ (µM): 0.009 [EVSA-T], 0.015 [H226], <0.005 [IGROV], and 0.006 [MCF-7] cf. <0.004 µM for Taxol in all cell lines, clearly justified in vivo trials for antitumour activity. The tumours inoculated in Nude Balb/C mice were OVCAR-3 (ovarian) and HT-29 (colon). Compound XXXVII returned an average tumour weight inhibition (TWI) score on Day 26 of 54%, indicating significant anti-tumour activity. Against the virulent HT-29 colon cancer, the tumour growth was curtailed compared to untreated animals. The foregoing results suggest that promising in vitro results obtained for the Bi(S₂CNR₂)₃ compounds are translated to antitumour activity and that further studies are clearly justified.

In a rare example of a study linking cytotoxicity assays with possible mechanisms of action, the cytotoxicity and DNA binding ability of a water soluble bismuth macrocyclic complex have been determined [80]. The precise structure of the compound XXXVIII is not known but is likely to be cationic. The structure of the macrocyclic ligand was shown in Figure 12.6. The complex of Bi³⁺ with the ligand (XXXVIII) is approximately 100 times more potent against B16-BL6 (melanoma) cells than cisplatin (I). Under physiologically relevant conditions, the compound was shown to form a non covalent interaction with calf thymus-DNA [80]. While studies on the antitumour potential of bismuth compounds currently used in medicine are lacking, some interesting indicators are forthcoming from studies of their putative mechanism of action. For example, very recent studies indicate that methylbismuth species, a biomethylated metabolite of bismuth drugs, are responsible for cytotoxic effects rather than the originally applied bismuth drugs [81]. Further, another recent report suggests that organobismuth compounds can induce apoptosis [82]. In this study, heterocyclic organobismuth compounds XXXIX – XLI, Figure 12.6, showed good activity against leukaemic cell lines and displayed lower cytotoxicity towards TIG cells (normal human fibroblasts). The IC₅₀ values in the ranges 0.059–4.7, 0.036–4.8 and 0.20–5.1 µM, respectively, indicate compound (XLI), with a six-membered heterocyclic ring, was less cytotoxic compared with the more flexible molecules with eight-membered rings. Studies on the possible mechanism of cell death were conducted and showed that at low concentrations of compound XLI, that is 0.22–0.44 µM, the cell death was by apoptosis but at higher concentrations, that is, > 1.1 µM, the cell death was by acute necrosis [82]. Several more studies on the potential anticancer activity of organobismuth compounds have been reported. A range of triarylbumuth bis(carboxylates) related to the structure of Ph₃Bi[O₂CCH(Me)CH₂GePh₃]₂ (XLI), shown in Figure 12.6 and the antimony derivatives described above [70], have been synthesized and their cytotoxicity against the cancer cell lines KB (carcinoma of the nasopharynx), Bel-7402 (hepatocellular carcinoma) and HCT-8 (intestinal adenocarcinoma) cells explored [83]. As with the study of the antimony derivatives, the influence of varying the aryl group and the substituents in the carboxylate
ligand, \([\text{O}_2\text{CCH(R')CH(R'')}\text{GePh}_3]\), were investigated. First and foremost, many of the triarylbismuth bis(carboxylates) were significantly more potent than either of \(\text{Ph}_3\text{GeCH}_2\text{CH}_2\text{COOH}\) and \(\text{Ph}_3\text{BiCl}_2\). In terms of the bismuth-bound aryl groups, phenyl and \(p\)-tolyl derivatives were more potent than their \(p\)-chlorophenyl and \(p\)-bromophenyl analogues. Interestingly, two of the three \(p\)-fluorophenyl derivatives synthesized in this study, namely \((p\text{-FC}_6\text{H}_4)\text{Bi(O}_2\text{CCH(Me)CH}_2\text{GePh}_3)_2\) (all three cell lines) and \((p\text{-FC}_6\text{H}_4)\text{Bi(O}_2\text{CCH}_2\text{CH(Ph)GePh}_3)_2\) (in the KB and Bel-7402 cell lines) gave indications of significant potency. The \(\text{Ph}_3\text{Bi(O}_2\text{CCH(Me)CH}_2\text{GePh}_3)_2\) compound was selected for a comparative assay with cisplatin (I) and proven to be more substantially more potent in the KB cell line when administered at concentrations of 0.5, 0.05 and 0.005 g ml\(^{-1}\) [83].

A related study of several carboxylate derivatives where the carboxylate is derived from \(N\)-salicylidene amino acids, e.g. (LXIII), again indicated significant cytotoxicity for the compounds investigated [84]. In this study, the advantage of combining bismuth with carboxylate was demonstrated as the bismuth compounds had significant better cytotoxicity profiles compared with the precursor compounds and cisplatin (I). The most potent compound in the trial was (LXIII), against the MDA-MB-435 (mammary carcinoma) but across the three human cell lines investigated, that is, MDA-MB-435, HL-60 (immature granulocyte leukaemia), and BGC-823 (gastric carcinoma), the triphenyl derivative with \(R = \text{CH}_2\text{C(H)(CH}_3)_2\) at the \(\alpha\)-position of the carboxylic acid was the most potent [84]. Continuing the carboxylic acid theme, a series of triarylbismuth \(N\)-(p-toluenesulfonyl) aminoacetates were synthesized, see generic structure (LXIV) [85]. Similar observations were observed, that is, the compounds were more active than their precursor carboxylic acid and cisplatin (I) and the triphenylbismuth derivative was the most potent compared with the \(p\)-methyl, \(p\)-chloro and \(p\)-bromo derivatives. Particularly noteworthy was the potency of the triphenyl derivative against the PC-3MIE8 (prostatic carcinoma) cancer cells [85].

An obvious difficulty in gauging the potential of one series of compounds against another is that experimental protocols, let alone cell lines, vary form study to study. There are relatively few trials where both antimony and bismuth compounds have been studied concurrently. In a rare example, a study designed to discover a bismuth compound selective for vascular endothelial cells, a comprehensive series of organobismuth compounds and their antimony analogues were investigated [86]. The study revealed that a single derivative, namely tris[2-\((N,N\text{-dimethylaminomethyl})\text{phenyl}\)]bismuth (LXV), was cytotoxic to bovine aortic endothelial cells but not to bovine aortic smooth muscle cells and porcine kidney epithelial LLC-PK\(_1\) cells. The antimony derivative did not exhibit cytotoxicity under the same conditions [86].

In a systematic study of compounds related to bismuth tris(8-quinolinethiolate) (LXVI), Lukevics et al. included both arsenic and antimony derivatives (along with many other metals). In a cytotoxicity trial of the selenolate derivatives, with methyl substituents in the ring with the heteroatom, against the HT-1080 (fibrosarcoma), MG-22A (mouse hepatoma), B16 (mouse melanoma), and Neuro 2A (mouse neuroblastoma) cell lines, arsenic compounds were generally more potent than the bismuth compounds which were in turn more potent than the antimony analogues [87]. It is noted that the tested compounds were also cytotoxic to non cancerous cells. No significant differences between bismuth (LXVI) and antimony 8-quinolinethiolates [88] and their methyl- and methoxy-substituted 8-quinolinethiolates [89] were noted in subsequent studies: again the compounds were highly toxic to non cancerous cells.
12.5 Conclusions

The foregoing summary of the development of arsenic, antimony and bismuth compounds as anticancer drugs clearly indicates the potential of these elements to augment their therapeutic roles. Perceived toxicity issues associated with these and other metal based drugs are clearly laid to rest by the arsenic containing drug used for the treatment of Acute Promyelocytic Leukaemia, i.e. Trisenox. Evidently, there are therapeutic windows in which these metal based drugs can provide significant clinical benefit. Systematic studies of arsenic, antimony and bismuth compounds in the context of anticancer potential are, regrettably, sparse. One can only wonder whether an easily synthesized, stable and effective molecule is awaiting discovery by the diligent chemist working in tandem with biomedical colleagues. The low toxicity and potent cytotoxicity exhibited by some of the reported compounds surely demand more attention. Fundamental questions such as (i) the relative efficacy of arsenic versus antimony versus bismuth compounds, (ii) ‘inorganic’ versus ‘organometallic’ species, and so on remain unanswered. The reliance on in vitro studies, the importance of which cannot be denied as an early indicator of potential antitumour activity, is also regrettable. For serious advances to be made, in vivo studies are essential. Further impetus to continued studies of arsenic, antimony and bismuth compounds as anticancer agents is warranted when it is considered that non DNA sites are the likely biological targets for these compounds offering differing mechanisms of action compared to the currently used anticancer platinum drugs. The prevalence of cancer diseases and the human suffering they cause is surely sufficient motivation for the necessary investment of resources, in all their guises, to investigate the potential utility of arsenic, antimony and bismuth compounds as anticancer agents.

References