Arsenic in medicine: past, present and future

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Abstract Arsenicals are one of the oldest treatments for a variety of human disorders. Although infamous for its toxicity, arsenic is paradoxically a therapeutic agent that has been used since ancient times for the treatment of multiple diseases. The use of most arsenic-based drugs was abandoned with the discovery of antibiotics in the 1940s, but a few remained in use such as those for the treatment of trypanosomiasis. In the 1970s, arsenic trioxide, the active ingredient in a traditional Chinese medicine, was shown to produce dramatic remission of acute promyelocytic leukemia similar to the effect of all-*trans* retinoic acid. Since then, there has been a renewed interest in the clinical use of arsenicals. Here the ancient and modern medicinal uses of inorganic and organic arsenicals are reviewed. Included are antimicrobial, antiviral, antiparasitic and anticancer applications. In the face of increasing antibiotic resistance and the emergence of deadly pathogens such as the severe acute respiratory syndrome coronavirus 2, we propose revisiting arsenicals with proven efficacy to combat emerging pathogens. Current advances in science and technology can be employed to design newer arsenical drugs with high therapeutic index. These novel arsenicals can be used in combination with existing drugs or serve as valuable alternatives in the fight against cancer and emerging pathogens. The discovery of the pentavalent arsenic-containing antibiotic arsinothricin, which is effective against multidrug-resistant pathogens, illustrates the future potential of this new class of organoarsenical antibiotics.

Keywords Arsenic · Metalloids · Metallodrugs · Anticancer drugs · Antivirals · Antimicrobials

Abbreviations

AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
AQP	Aquaporin/aquaglyceroporin
ArsM	As(III) *S*-adenosylmethionine methyltransferase
ART	Antiretroviral therapy
As(III)	Arsenite
As(V)	Arsenate
AST	Arsinothricin
AST-OH	Hydroxyarsinothricin
ATL	Adult T-cell leukemia/lymphoma
ATO	Arsenic trioxide
ATRA	All-trans retinoic acid
EBV	Epstein–Barr virus
EGCG	(-)-Epigallocatechin-3-gallate
FDA	Food and Drug Administration
GOE	Great Oxidation Event
GS	Glutamine synthetase
HAT	Human African trypanosomiasis
HCV	Hepatitis C virus

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HIV-1	Human immunodeficiency virus-1
HPV	Human papillomavirus
MAs(III)	Methylarsenite
MAs(V)	Methylarsenate
MSMA	Monosodium methylarsenate
MurA	UDP-N-acetylglucosamine enolpyruvyl transferase
Nit(V)	Nitarsone
p-ASA	p-Arsanilic acid
Pin1	Peptidyl-prolyl cis–trans isomerase NIMA (never in mitosis A)-interacting 1
PML	Promyelocytic leukemia
PM-RARα	RARα retinoic acid receptor α
PT	L-phosphinothricin
ROS	Reactive oxygen species
Rox(V)	Roxarsone
SAM	S-Adenosylmethionine
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIV	Simian immunodeficiency virus
UDP	Uridine diphosphate
WHO	World Health Organization

Introduction

History of arsenic in medicine

In this article we review the history and present use of arsenicals in medicine. The origin of the name "arsenic" traces back to the Greek word "*arsenikon*" meaning "*potent*" (Jolliffe 1993; Hoonjan et al. 2018). Arsenic was known empirically as a potent medicinal agent as early as 2000 BC (Fig. 1), when arsenic trioxide (ATO, As_2O_3, also known as white arsenic) (Fig. 2A) obtained from copper smelting was used as both a drug and a poison (Jolliffe 1993). Orpiment (As_2S_3, yellow arsenic) and realgar, (As_4S_4, red arsenic) (Fig. 2B), described as early as the 4th Century BC by the Greek philosopher Aristotle (384–322 BC), were the earliest arsenic minerals in recorded history (Fig. 1) (Gorby 1988; Bentley and Chasteen 2002). Although arsenic-containing minerals were known in antiquity, it was not until 1250 that elemental arsenic was conclusively identified by the German alchemist Albertus Magnus (1193–1280) (https://pubchem.ncbi.nlm.nih.gov/element/Arsenic).

History is rife with stories of arsenic used as a poison for both royalty and commoners. Odorless and tasteless ATO has been used as a poison for millennia due to its availability and low cost (Jolliffe 1993; Hoonjan et al. 2018; Gorby 1988; Hughes et al. 2011). One of the earliest recorded cases of arsenic poisoning was in the year 55 AD, when the fifth Roman emperor Nero ordered the poisoning of his 13-year-old stepbrother Britannicus to secure his Roman throne (Jolliffe 1993; Gorby 1988; Bentley and Chasteen 2002; Doyle 2009). Pope Alexander VI (1431–1503), a member of the Borgia family, one of the most eminent dynasties of the Italian Renaissance, used the infamous powder called *cantarella*, which is widely believed to have consisted mainly of arsenic, to murder cardinals for their property and
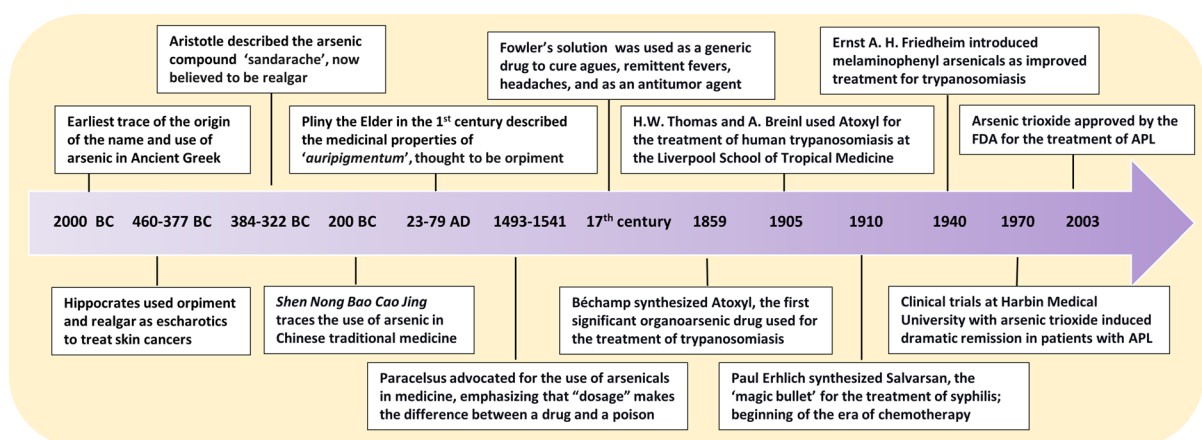

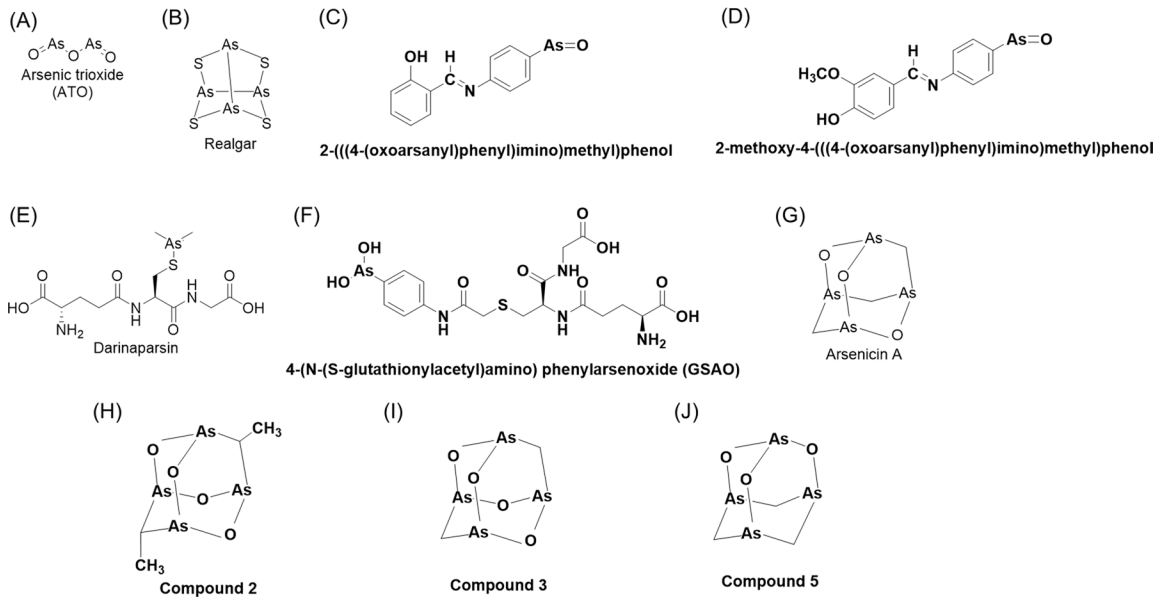**Fig. 1** Milestones of the use and development of arsenicals in medicine

wealth (Gorby 1988). A well-known example of arsenic poisoning is "*The Affair of the Poisons*" in the French court of Louis XIV, where Catherine Deshayes provided the arsenic-based poison *La Poudre de Succession* or "*inheritance powder*" to women to help them rid themselves of their husbands (Gorby 1988; Bentley and Chasteen 2002). The inheritance powder continued to be popular in France until the nineteenth century, when it became the most favorite poison, as recorded by early forensic toxicologists (Gorby 1988). The incidence of arsenic poisoning dramatically waned after the advent of the Marsh test, a sensitive forensic test for arsenic developed in 1836 by the English chemist James Marsh (Gorby 1988; Hughes et al. 2011).

Behind its inglorious history as a poison, however, arsenic has an even more prestigious history as a pharmaceutical agent. Arsenic has been in use as therapeutics since ancient times in the Greek and Roman civilizations, as well as in Chinese and Indian traditional medicine (Doyle 2009). Hippocrates (460–377 BC), the Greek physician, often referred to as the Father of Medicine, is thought to have administered the arsenic minerals orpiment and realgar as escharotics and remedies for ulcers and abscesses (Fig. 1) (Jolliffe 1993; Hoonjan et al. 2018; Hughes et al. 2011; Bentley and Chasteen 2002; Waxman and Anderson 2001; Zhu et al. 2002; Riethmiller 2005). Aristotle and the Roman author Pliny the Elder (23–79 AD) both wrote on the medicinal properties of arsenicals (Fig. 1) (Jolliffe 1993; Gorby 1988). The Greek physician Galen (129–210 AD) recommended the use of arsenic sulfide to treat ulcers (Jolliffe 1993; Riethmiller 2005). The first book on Chinese traditional medicine, *Shen Nong Ben Cao Jing*, compiled in the Eastern Han dynasty (25–220 AD), traces the use of arsenic in traditional Chinese medicine as far back as 200 BC (Fig. 1) (Liu et al. 2008), which agrees with the fact that the Chinese Nei Jing Treaty (263 BC) recorded the use of arsenic pills for treatment of periodic fever (Hoonjan et al. 2018; Zhu et al. 2002; Chen and Chen 2017). Sun Si-Miao (581–682 AD), a Chinese physician called China's King of Medicine, used a combination of realgar, orpiment and ATO for treatment of malaria (Hoonjan et al. 2018; Zhu et al. 2002; Chen and Chen 2017). Shi-Zhen Li (1518–1593 AD), a Chinese physician in the Ming dynasty, wrote *Ben Cao Gang Mu*, or *Compendium of Materia Medical*, a major pharmacopoeia in
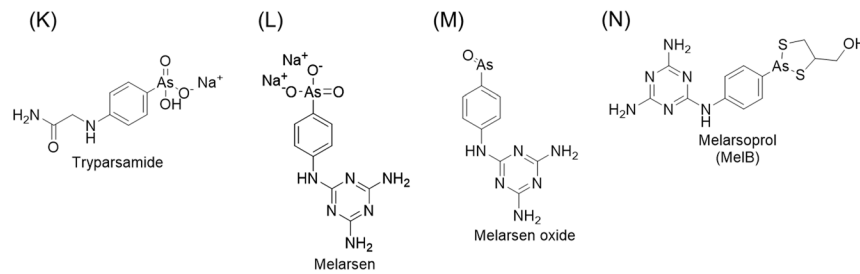
Chinese history, where he described the use of ATO as a remedy for various diseases (Zhu et al. 2002; Chen and Chen 2017; Gibaud and Jaouen 2010). In traditional Indian medicine, the three main arsenicals used in Ayurveda, an alternative system of medicine originating from the ancient Indian subcontinent several thousand years ago, are orpiment, realgar and ATO (Panda and Hazra 2012). In Arabia, Avicenna (980–1037 AD), a Persian physician, introduced the internal use of ATO for the treatment of fevers (Zhu et al. 2002). Paracelsus (1493–1541 AD), a Swiss physician recognized as the Father of Toxicology and Pharmacology, is known to have used elemental arsenic extensively (Fig. 1) (Jolliffe 1993; Hoonjan et al. 2018; Gorby 1988; Waxman and Anderson 2001; Zhu et al. 2002; Borzelleca 2000). He advocated for the use of minerals and chemicals, including arsenic, in medicine, emphasizing that the dosage makes the difference between a drug and a poison. In 1786 Thomas Fowler (1736–1801 AD), a British physician and pharmacist, reported the effects of a flavored solution of 1% potassium arsenite named "*liquor mineralis*" for malaria, remittent fevers, and periodic headaches (Fig. 1). This medicine, renamed "*Fowler's solution*", once introduced into the London Pharmacopoeia in 1809, became popular in Western countries throughout the Victorian Era as a main therapeutic option for a wide variety of ailments and diseases, including asthma, chorea, eczema, psoriasis, rheumatism, syphilis, tuberculosis and ulcers (Jolliffe 1993; Hoonjan et al. 2018; Gorby 1988; Hughes et al. 2011; Bentley and Chasteen 2002; Doyle 2009; Waxman and Anderson 2001; Zhu et al. 2002; Gibaud and Jaouen 2010; Thomas and Troncy 2009).

There is some concern over the present-day use of arsenicals in traditional medicine (Ernst 2002), leading to evaluation of the bioavailability of arsenic species in their prescriptions. In Indian traditional ayurvedic medicine, for example, a special subset of herbal medicines called *Rasa Shastra* involves intentional use of toxic elements including arsenic, which are believed to be converted into non-toxic forms called *bhasmas* via the preparation procedures. However, the bioaccessibility of arsenic in several traditional Indian medicines was suggested to lead to accumulation of arsenic above the acceptable daily limit if consumed at recommended doses (Koch et al. 2011). More recently a similar concern was raised about some traditional Chinese medicines (Liu et al.
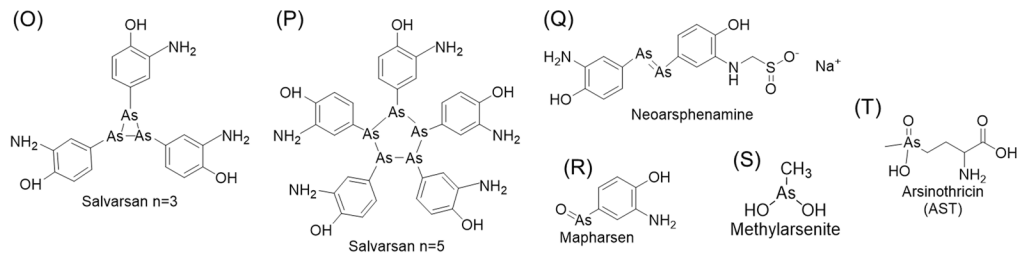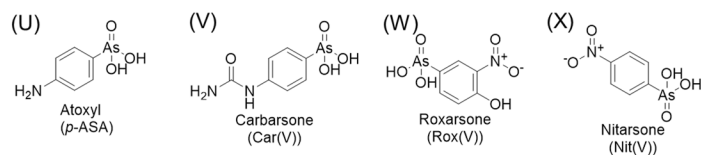
1. Anticancer chemotherapeutics

(A)

Arsenic trioxide
(ATO)

(B)

Realgar

(C)

2-(((4-(oxoarsanyl)phenyl)imino)methyl)phenol

(D)

2-methoxy-4-(((4-(oxoarsanyl)phenyl)imino)methyl)phenol

(E)

Darinaparsin

(F)

4-(N-(S-glutathionylacetyl)amino) phenylarsenoxide (GSAO)

(G)

Arsenicin A

(H)

Compound 2

(I)

Compound 3

(J)

Compound 5

2. Antiparasitic agents

(K)

Tryparsamide

(L)

Melarsen

(M)

Melarsen oxide

(N)

Melarsoprol
(MelB)

3. Antibiotics/antimicrobials

(O)

Salvarsan n=3

(P)

Salvarsan n=5

(Q)

Neoarsphenamine

(R)

Mapharsen

(S)

Methylarsenite

(T)

Arsinothricin
(AST)

4. Synthetic aromatics in animal husbandry

(U)

Atoxyl
(p-ASA)

(V)

Carbarsone
(Car(V))

(W)

Roxarsone
(Rox(V))

(X)

Nitarsone
(Nit(V))

2018). To exploit the full potential of arsenic as medicine, therefore, further evaluation is required to develop regulations for the proper dosage of arsenic-containing traditional medicines.

Applications of arsenicals extend beyond drugs and poisons. They have been used in areas of agriculture, metallurgy, cosmetics, electronics semiconductor and other industrial uses (Bentley and Chasteen 2002). Monosodium methylarsenate (MSMA) and sodium dimethylarsenate (cacodylate) have been used as post-emergent herbicides on cotton fields and other non-food crops (Matteson et al. 2014). Although banned for general use by the USA by the Environmental Protection Agency (EPA), MSMA is still in limited use in the United States for cotton fields, new golf courses and highway medians, and it is still applied world-wide as an herbicide on rice, cotton, fruit trees and coffee in a number of countries around Asia.

Inorganic and organic arsenic-containing drugs

Development of arsenical drugs

In the modern era, the use of arsenicals as drugs has alternated between successes and failures. As described below, arsenical drugs can be generally grouped into inorganic, for example, ATO (Fig. 2A), and organic compounds, such as atoxyl (*p*-aminophenylarsenate or *p*-arsanilic acid (*p*-ASA)) (Fig. 2U). Atoxyl, the first effective artificial organoarsenic drug, was synthesized by the French scientist Antoine Béchamp (1816–1908 AD), in 1859 by heating a mixture of aniline and arsenic acid (Fig. 1) (Riethmiller 2005; Gibaud and Jaouen 2010). Its clinical effectiveness was not demonstrated until some forty years later, when the physicians Canadian Harold W. Thomas (1875–1931 AD) and Australian Anton Breinl (1880–1944 AD) at the Liverpool School of Tropical Medicine first used it in 1905 to treat human trypanosomiasis (Fig. 1) (Jolliffe 1993; Gibaud and Jaouen 2010). Although it causes optic atrophy due to its high arsenic content (Jolliffe 1993), the trypanocidal effects of Béchamp's atoxyl inspired Paul Ehrlich (1854–1915), the German Nobel Laureate

known as the Father of Chemotherapy, to initiate an extensive synthesis of organic arsenicals to find a drug against the syphilis spirochaete (Jolliffe 1993). Arsphenamine was the 606th aromatic arsenical he synthesized in 1910 (Fig. 1). Compound 606 was later called the *silver bullet* Salvarsan, the first effective chemotherapeutic drug for the treatment of syphilis (Jolliffe 1993; Gorby 1988; Hughes et al. 2011; Bentley and Chasteen 2002). The composition of Salvarsan was a question of debate for almost a century. In 2005, Nicholas and colleagues provided evidence based on electrospray ionization mass spectrometric data that Salvarsan in solution exists as cyclic species $(RA)_n$, with $n = 3$ (Fig. 2O) and $n = 5$ (Fig. 2P) (Lloyd et al. 2005). Like atoxyl, however, Salvarsan treatment was lengthy, and the side effects unpleasant. Less toxic derivatives such as neoarsphenamine (Neosalvarsan) (Fig. 2Q) and oxophenarsine hydrochloride (Mapharsen) (Fig. 2R) made treatment more bearable (Jolliffe 1993; Bentley and Chasteen 2002; Gibaud and Jaouen 2010). Ehrlich's work with Salvarsan ushered in the modern era of chemotherapy.

Arsenical anticancer chemotherapeutic agents

Arsenic trioxide (ATO)

Arsenicals have a long history of use as cancer chemotherapeutic agents. ATO (Fig. 2A) was a favorite compound in traditional ancient Chinese medicine for over 2000 years (Bentley and Chasteen 2002). ATO is an amphoteric oxide that readily dissolves in alkaline solutions. It was originally made from orpiment by roasting and purifying the smoke (Gibaud and Jaouen 2010). In 1878, the related formulation, Fowler's solution, was found to be effective for the treatment of leukemia, and, in addition, Fowler pastes were applied topically potentially for the treatment of skin and breast cancers (Hoonjan et al. 2018; Hughes et al. 2011; Waxman and Anderson 2001; Gibaud and Jaouen 2010). Arsenic therapy was the mainstay of antileukemia treatment until the advent of radiation therapy in the early twentieth century (Hoonjan, et al. 2018; Waxman and Anderson 2001). Despite its toxicity, arsenic remained in use in traditional Chinese medicine (Bentley and Chasteen 2002). Taking inspiration from this traditional medicine, investigators at Harbin Medical University showed that a solution of ATO produced complete remission of acute

promyelocytic leukemia (APL) in about two-third of patients in the 1970s (Fig. 1) (Zhu et al. 2002; Chen and Chen 2017). The ATO used in those clinical studies contained trace amounts of mercury, so it was possible that the anticancer effects were due to mercury rather than arsenic. Clinical trials with pure ATO began in 1994, and, by 1996, its effectiveness was confirmed in other countries. In 2003 ATO, marketed as Trisenox®, was approved by the U.S. Food and Drug Administration (FDA) for treatment of APL refractory to all-trans retinoic acid (ATRA) (Gibaud and Jaouen 2010). The revival of ATO for treatment of APL and other specific hematological malignancies has sparked renewed interest in arsenic-based drugs (Hoonjan et al. 2018; Hughes et al. 2011; Gibaud and Jaouen 2010).

Since ATO was approved as an effective drug for clinical treatment of hematological malignancies, including APL and multiple myeloma, its mechanism as anticancer agent has been under active investigation. The mechanism of action of ATO is not clear, and there are a number of potential targets. Like most trivalent arsenicals, it has the potential to bind to thiols in metabolites such as glutathione, vicinal thiol pairs in lipoamide and in proteins such as lipoamide dehydrogenase, inhibiting cellular energy production and increasing production of intracellular reactive oxygen species (ROS) (Carney 2008; Emadi and Gore 2010). ATO treatment results in demethylation of DNA, affecting the promoters of many genes and also binds to oncoproteins/transcription factors (Emadi and Gore 2010; Dawood et al. 2018; Huynh et al. 2019). These alterations affect multiple cellular processes in a variety of cancers, resulting in cell cycle arrest, apoptosis and mesenchymal to epithelial transition through a variety of molecular targets (Chen et al. 1997; Bao et al. 2016; Miller et al. 2002; Shao et al. 1998). The final outcome depends on the cell type as well as the concentrations of administration and duration of ATO exposure (Chen et al. 1997).

However, those are rather nonspecific effects of ATO and do not explain its selective ability to treat APL. APL is characterized by chromosomal translocation t(15;17) (q24;q21), which produces a fusion promyelocytic leukemia (PML) protein-retinoic acid receptor alpha (RARα) gene that is found in over 98% of patients (Borrow et al. 1990; de Thé et al. 1990; Golomb et al. 1980). The PML-RARα fusion gene consists of the PML gene on chromosome 15

and the RARα gene on chromosome 17. The production of the PML-RARα oncoprotein alters myeloid differentiation at the promyelocytic stage, leading to accumulation of immature cells (Grisolano et al. 1997). In addition, PML-RARα increases cell survival and increases proliferation of leukemic cells, resulting in progressive leukemogenesis (Grignani et al. 1993; Pandolfi 2001; Puccetti and Ruthardt 2004). PML-RARα appears to be a target of ATO, which binds to the PML-RARα oncoprotein in NB4 cells, a human APL cell line, and alters SUMOylation of the PML moiety, leading to protein degradation (Zhang et al. 2010). Although the effect of ATO on the PML-RARα leukemic stem cells appears to be mainly throught inhibition of proliferation (Testa and Lo-Coco 2015), this PML-RARα degradation is also thought to induce apoptosis or differentiation to myeloid cells, leading to a decrease in leukemic cells (Zhang et al. 2010; Rojewski et al. 2002).

Another putative target of ATO is the Wip1 phosphatase. ATO has been reported to activate the Chk2 and/or p38 MAPK apoptotic pathways in various chronic myelogenous leukemia cells (Giafis et al. 2006; Shim et al. 2002; Verma et al. 2002) as well as APL cells (Yoda et al. 2008) by inhibiting Wip1 phosphatase activity. Since expression of Wip1 is amplified in a number of cancers, including breast, papillary thyroid, colorectal and prostate cancers and other types (Emelyanov and Bulavin 2015; Li et al. 2002; Natrajan et al. 2009), ATO is potentially a therapeutic agent for other tumor types.

ATO may also be a treatment for other forms of leukemia via its function as a pro-oxidant factor, disrupting redox pathways in cancer cells. The combination of ATO with ascorbate (vitamin C), a dietary antioxidant that also possesses pro-oxidant activity in high concentrations (Kaźmierczak-Barańska et al. 2020), selectively killed blasts from APL patients and was also effective against approximately one-third of primary acute myeloid leukemia (AML) samples examined, presumably due to apoptosis induced by overproduction of ROS (Noguera et al. 2017). This pro-oxidant activity provides a rationale for testing the combination of ATO and ascorbate in advanced cases of AML and APL (Noguera et al. 2017).

Pin1, the peptidyl-prolyl *cis–trans* isomerase NIMA (never in mitosis A)-interacting 1, has been reported to be another target of ATO, enhancing its anti-cancer effects against multiple tumor types

(Kozono et al. 2018). Pin1 is a major regulator of cancer signaling networks. It catalyzes *cis–trans* isomerization at phosphorylated Ser/Thr–Pro motifs, resulting in changes of protein conformation, function and stability, which in turn activates numerous cancer-driving pathways. Pin1 is overexpressed in various cancers and cancer stem cells (Ayala et al. 2003; Bao et al. 2004; Luo et al. 2015; Rustighi et al. 2014; Wulf et al. 2004) and involved in regulation of more than 50 oncogenes and 20 tumor suppressor factors (Lu and Hunter 2014; Zhou and Lu 2016). ATO inhibits Pin1 via direct and noncovalent binding to the active site, inducing degradation of Pin1. Interestingly, the anticancer effects of ATO are indirectly enhanced by co-treatment with ATRA, another well-known Pin1 inhibitor, which increases cellular ATO uptake via induction of Aquaporin-9 (AQP9) expression, in addition to directly inhibiting and degrading Pin1 (Kozono et al. 2018).

However, a higher dose of ATO is required for the treatment of solid tumors compared to soft tumor hematologic malignancies, which raises concerns about toxicity. Methods to effectively deliver ATO to the cells without the accompanying toxicity are under development. For example, liposomal-encapsulated ATO delivered to HeLa cells, which are derived from human papillomavirus (HPV)-cervical carcinoma, effectively reduced levels of HPV-E6 proteins and induced apoptosis with reduced toxicity compared to free ATO. Encapsulation of ATO using this liposomal nanotechnology was shown to decrease membrane permeability to ATO by allowing its gradual release (Wang et al. 2016). The O'Halloran group developed a nanoparticulate formulation of ATO encapsulated in "nanobins" (liposomal vesicles) (Chen et al. 2006). The cytotoxicity of the encapsulated ATO was evaluated against a panel of human breast cancer cell lines and was found to be much less compared to the free ATO. In contrast, the nanobins potentiated the antitumor efficacy of ATO in vivo in an orthopic model of triple-negative breast cancer (Ahn et al. 2010). The group has also developed a synthesis method that combines ATO and cisplatin (*cis*-diamminedichloroplatinum(II)), a compound commonly used in the treatment of solid tumors, to form a stable aqueous complex, arsenoplatin, having a distinct biological activity from ATO and cisplatin individually (Miodragović et al. 2013). Arsenoplatin can be loaded in liposomal drug delivery systems

and has been shown to possess significant biological activity against several cancer cell lines. When compared to cisplatin, it showed greater activity in breast, leukemia, colon, and central nervous system cancer cell lines (Miodragović et al. 2019). Other systems have been investigated for the effective delivery of arsonium compounds in cancer therapeutics, such as the triphenylarsonium-functionalised gold nanoparticles (Lalwani et al. 2015). The gold nanoparticles are decorated with the triphenylarsonium groups to serve as potential nanocariers for intracellular therapeutics. The development of delivery systems for slow dosing with arsenical drugs can modulate toxicity, significantly expanding medical applications of arsenic.

Realgar

Another form of inorganic arsenic, realgar (As_4S_4, red arsenic) (Fig. 2B), has been used as a therapeutic agent since the days of ancient China (Wu et al. 2011). Inspired by nano-drug, lately, realgar nanoparticles (an average particle size of < 100 nm) have been employed in studies rather than coarse realgar. This approach is adopted to overcome the problem of limited solubility of realgar particles in aqueous solutions, and to increase its bioavailability (Shi et al. 2016). Several in vitro studies demonstrated that realgar nanoparticles significantly decreased cell proliferation and promoted apoptosis in B16 melanoma cells (Zhao et al. 2010) and rat C6 glioma cells (An et al. 2011). Furthermore, in tumor-bearing C57BL/6 mice, transdermal delivery of the realgar nanoparticles markedly decreased the tumor volumes with little toxicity to the mice (Zhao et al. 2010). Recently the effect of realgar nanoparticles was compared with ATO against several multiple myeloma cell lines and primary cell lines from multiple myeloma patients (Cholujova et al. 2017). The realgar nanoparticles were prepared by milling realgar into nano-sized dimensions under high energy. Both forms of inorganic arsenic were cytotoxic, but the realgar nanoparticles were two- to four-fold more effective than ATO in the cell lines, xenograft and multiple myeloma patient-derived myeloma mouse models. Mechanistic studies showed that the effects of the realgar nanoparticles and ATO on the multiple myeloma models included pronounced apoptosis and G2/M cell cycle arrest. In this study, realgar nanoparticles but not ATO could significantly deplete

the amount and clonogenicity of multiple myeloma stem-like side population in bone marrow stromal cells. Also, there was synergistic anti-multiple myeloma activity when realgar and ATO were combined with lenalidomide or melphalan, both of which have been approved for treatment of multiple myeloma. In an attempt to increase the uptake of realgar and prolong the retention time in cancer cells, (-)-Epigallocatechin-3-gallate (EGCG), another natural medicine that inhibits cancer cell growth, was used as a drug carrier to encapsulate realgar nanoparticles (Fang et al. 2019). Compared with realgar nanoparticles, the EGCG-realgar nanoparticles significantly inhibited the proliferation of APL HL-60 cells. In a subcutaneous solid tumor model mice, EGCG-realgar nanoparticles decreased tumor volumes at an inhibitory rate of 60.18% at a dose of 70 mg/kg. More recently, the effect of realgar nanoparticles on lung cancer stem cell was also examined. The nano-realgar was shown to inhibit tumor growth both in vitro and in vivo by repressing metabolic reprogramming via downregulation of HIF-1α expression and PI3K/Akt/mTOR pathway (Yang et al. 2021).

Organoarsenicals

Organic arsenicals are under current examination for potential therapeutic use. Several synthetic organoarsenicals were tested for antitumor activity against HL-60 (leukemia), SGC 7901 (gastric cancer) and MCF-7 (breast cancer) human cancer cell lines (Fan et al. 2016). 2-(((4-(oxoarsanyl)phenyl) imino)methyl)phenol ($C_{13}H_{10}AsNO_2$) (Fig. 2C) and 2-methoxy-4-(((4-(oxoarsanyl)phenyl)imino)methyl) phenol ($C_{14}H_{12}AsNO_3$) (Fig. 2D) exhibited the highest growth inhibition of HL-60 cells, with IC_{50} values of 0.77 μM and 0.51 μM, respectively. Both induced apoptosis via oxidative stress in HL-60 cells (Fan et al. 2016). Another organoarsenical that is being evaluated for the treatment of solid tumors is the glutathione conjugate of DMAs(III), darinaparsin (L-γ-glutamyl-S-(dimethylarsino)-L-cysteinyl-glycine) (Fig. 2E). The injectable form of darinaparsin, SP-02L, is currently in phase 2 clinical trial in patients with relapsed or refractory peripheral T-cell lymphoma (https://clinicaltrials.gov/ct2/show/NCT02 653976). Analysis of data from two phase 1 clinical trials in Japan and Korea showed that darinaparsin has good potential efficacy and high safety profile

(Ogura et al. 2021). A related glutathione conjugate, 4-(N-(S-glutathionylacetyl)amino) phenylarsenoxide or GSAO (Fig. 2F), is in phase 1 clinical trial in patients with advanced solid tumors (Horsley et al. 2013).

Polyorganoarsenicals

Another class of organoarsenicals with potential clinical value is polyarsenicals. The first reported is arsenicin A (2,4,6-trioxa-1,3,5,7-tetrarsatricyclo [3.3.1.13,7] decane) ($C_3H_6As_4O_3$) (Fig. 2G), a natural product isolated from *Echinochalina bargibanti*, a marine sponge belonging to the class Demospongiae (Mancini et al. 2006). Arsenicin A has both antibiotic and anti-APL leukemia activity. It has a cage-like structure similar to the carbon structure in the diamond backbone adamantane $((CH)_4(CH_2)_6)$, in which the four methanetriyl carbon bridgeheads are replaced by arsenic and three methylene bridges are replaced by oxygen (Lu et al. 2012, 2010). The anti-proliferative activity of arsenicin A was examined in the PML-RARα-positive APL cell line NB4 (Lu et al. 2012). Arsenicin A exhibits a 21-fold greater anti-proliferative activity compared to ATO in NB4 cells. Using flow cytometry, arsenicin A was shown to induce cell death at a 27-fold lower concentration ($IC_{50} = 53$ nM) compared with ATO ($IC_{50} = 1440$ nM), and proliferative arrest at 20 nM compared with 790 nM for ATO (Lu et al. 2012).

Five arsenicin A analogs were synthesized, and their activity was evaluated in vitro against a full panel of human cancer cell lines from the National Cancer Institute (NCI-USA) (Mancini et al. 2017). Three of these compounds, designated **compound 2** (9,10-dimethyl-2,4,6,8-tetraoxa-1,3,5,7,-tetraarsatricyclo[3.3.1.13,7]decane) ($C_4H_8As_4O_4$) (Fig. 2H), **compound 3** (2,4,6,8-tetraoxa-1,3,5,7-tetraarsa-adamantane) ($C_2H_4As_4O_4$) (Fig. 2I), and **compound 5** (an isomer of Arsenicin A) (Fig. 2J), showed significantly higher cytotoxicity against the various cancer cell lines than ATO. **Compound 2** was particularly effective in inhibiting growth of solid tumor cell lines of colon cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. Two sulfur-containing derivatives, arsenicin B and arsenicin C, also possess antibiotic activity against human pathogens. Although less potent than arsenicin A against leukemia cells, these sulfur-containing polyarsenicals

have especially potent antimicrobial activity against *Staphylococcus aureus*, a major human pathogen with growing resistance to conventional antibiotics (Tähtinen et al. 2018). These findings lend new perspectives on the development and use of polyorganoarsenicals as therapeutics.

Arsenical antiparasitic agents

Tryparsamide (*p*-glycineamidophenylarsonate) (Fig. 2K), developed by Walter A. Jacobs and Michael Heidelberger at the Rockefeller University in 1919, is acknowledged as the first effective arsenical therapeutic agent against Gambian sleeping sickness. That disease is the slow-progressing form of human African trypanosomiasis (HAT) and is caused by *Trypanosoma brucei gambiense*, which is endemic in western and central Africa, especially in the late stage of the infection (e.g. neurological stage through central nervous system invasion) (Gibaud and Jaouen 2010). Although this drug was widely used from the early 1920's, its use waned in the 1940's due to the spread of resistant strains. In the 1940s, Ernst A. H. Friedheim improved the treatment of trypanosomiasis with the introduction of melaminophenyl arsenicals (Fig. 1), although toxicity was still reported (Gibaud and Jaouen 2010). Melarsen (4-(4,6-diamino-1,3,5-triazin-2-yl)amino]phenylarsenate) (Fig. 2L), the first melaminophenyl arsenical that Friedheim synthesized in 1939, was less active than tryparsamide. In contrast, melarsen oxide (Fig. 2M), the reduced form of melarsen and the first trivalent organoarsenical used against trypanosomes, was very effective against both early (hemolymphatic) and late (neurologic) stages, yet it exhibited high toxicity (Friedheim 1948). Friedeim combined dimercaprol or BAL (British anti-Lewisite), the counteract compound for Lewisite, the trivalent organoarsenical-based chemical weapon first used in World War I (Peters et al. 1945), with melarsen oxide to produce the drug melarsoprol (MelB or arsobal) (Fig. 2N) (Friedheim 1949). Melarsoprol is 100-fold less cytotoxic and 2.5-fold less trypanocidal compared with melarsen oxide (Fairlamb and Horn 2018). It was introduced into clinical use in 1949 for use in African countries to treat Gambian sleeping sickness. Melarsoprol can cross the blood–brain barrier (Sekhon 2013) via the P2 adenosine transporter (TbAT1) (Carter and Fairlamb 1993; Mäser et al. 1999) and aquaglyceroporin 2 (TbAQP2) (Alsford

et al. 2012; Baker et al. 2012). However, a serious side effect of melarsoprol is reactive encephalopathy, which occurs in about 10% of patients (Blum et al. 2001; Pepin and Milord 1991). Even so, its ability to cross the blood–brain barrier into the cerebrospinal fluid made it especially useful for treatment of second stage Gambian sleeping sickness, when the trypanosome enters the central nervous system (Colotti et al. 2018; Rodgers et al. 2011). Given the absence of effective alternatives, the World Health Organization (WHO) recommends its use as the only chemotherapeutic for the second stage of the faster-progressing form of human African trypanosomiasis caused by *Trypanosoma brucei rhodesiense,* which is more common in southern and eastern Africa (Büscher et al. 2017). Melarsoprol is a prodrug, and the active form of the drug is melarsen oxide (Fig. 2M). This trivalent form of melarsen (Fig. 2L) can be detected in ceresbrospinal fluid 1 h after injection (Keiser et al. 2000). Melarsoprol is rapidly broken down mainly into melarsen oxide, perhaps enzymatically (Fairlamb and Horn 2018). As a trivalent organoarsenical, melarsen oxide has high affinity for thiols and forms a stable adduct with the parasite's alternative to glutathione, trypanothione. Reduction of free cytosolic trypanothione inhibits trypanothione reductase, the parasite enzyme that contributes to cytosolic redox balance (Cunningham et al. 1994; Fairlamb et al. 1989). In addition, melarsen oxide causes rapid lysis of *Trypanosoma brucei* in vitro (Van Schaftingen et al. 1987). Beginning in the 1990s, resistance to melarsoprol became widespread (Brun et al. 2001). Melarsoprol resistance in clinical isolates (Graf et al. 2013; Pyana Pati et al. 2014) is predominantly related to mutations in the parasite *TbAQP2* gene (Munday et al. 2015). Mutations in this aquaglyceroporin, which is involved in uptake of melarsoprol, include deletions (Baker et al. 2012) or rearrangements with *TbAQP3* to form a chimeric *AQP2-3* gene (Munday et al. 2014). Resistance to melarsoprol in human African trypanosomiasis patients has led to a decrease in the use of this arsenical drug (Fairlamb and Horn 2018). With the development of newer drugs and antibiotics, interest in arsenic-based drugs gradually waned mainly due to their low therapeutic index.

Antiviral arsenic agents

In addition to the use of arsenicals for control of pathogens and as cancer chemotherapeutics, their potential as antiviral agents is also under investigation. ATO has been shown to inhibit Hepatitis C Virus (HCV) replication at submicromolar concentrations (Hwang et al. 2004). The concentrations that gave 50% inhibition of replication (EC_{50}) without causing cellular cytotoxicity are 0.35 and < 0.2 μM, when determined by a reporter-based HCV replication assay and by RT-qPCR analysis, respectively. The anti-HCV activity of ATO was also demonstrated using an engineered cell line-based assay system that constitutes all steps in the full cycle of HCV infection and replication, where ATO at 0.3 μM abolished the HCV signal, while high concentrations of interferon-α, an antiviral cytokine used for the treatment of chronic hepatitis C, only minimally suppressed the viral signal. In a follow-up study, treatment of HCV-infected cells with 1 μM ATO, which effectively inhibited the HCV RNA replication without exhibiting cytotoxicity, led to depletion of intracellular glutathione and an increase in superoxide anion radicals (Kuroki et al. 2009). The anti-HCV activity of ATO was inhibited in the presence of *N*-acetyl-cysteine, an antioxidant and glutathione precursor. These results suggest that ATO exerts its effect against HCV by modulating the intracellular glutathione redox system and oxidative stress. These findings demonstrate the potential of ATO for the development of potent antiviral agents against HCV and related viruses.

Viral latency has been recognized as the major source of viral rebound in human immunodeficiency virus-1 (HIV-1) infections after discontinuation of antiretroviral therapy (ART) (Siliciano and Siliciano 2000). There is, therefore, a need to render the latent HIV-1 susceptible to eradication. One way to provide drug access is by reactivation of viral replication. ATO has been reported to activate latent HIV-1 in the Jurkat T cell line in a process that involves the nuclear factor kappa B (NF-κB) signaling pathway (Wang et al. 2013). Similarly, inorganic sodium arsenite was shown to reactivate gene expression and viral replication of the latent genome of herpes simplex virus type 1 (HSV1) (Preston and Nicholl 2008). These results suggest that inorganic arsenicals may be able to enhance ART. Recently the ability of ATO in combination with ART to regulate

viral reservoirs in primary CD4+T lymphocytes of HIV-1-infected patients and simian immunodeficiency virus (SIV)-infected Chinese rhesus macaques was examined (Yang et al. 2019). ATO significantly increased the levels of cell-associated RNAs in resting CD4+T cells from both HIV-1-infected patients and SIV-infected macaques in a dose-dependent manner. Using chronically SIV-infected macaques, ATO in combination with ART delayed viral rebound, decreased SIV integrated DNA in CD4+T cells and restored CD4+T cell counts in vivo. In contrast, there was a rebound in the control group treated with ART alone in an average interval of 22 days after discontinuation of therapy. Furthermore, SIV-specific immune responses against the multiple SIV antigens increased after treatment with ATO. The use of ATO as a latency-reversing agent (LRA) in combination with combined ART (CART) is currently under investigation in a clinical trial ("The Effect of ATO on Eliminating HIV-1 Reservoir Combined with CART" 2019). https://clinicaltrials.gov/ct2/show/NCT03980665

ATO has been reported to exhibit potent inhibition of human adenovirus infection in vitro (Hofmann et al. 2020). PML nuclear bodies, otherwise referred to as PML oncogenic domains, are IFN-inducible nuclear structures that participate in cellular processes including apoptosis, senescence and antiviral defense. Infection with human adenovirus reorganizes the dot-like PML nuclear bodies into track-like structures, impairing their function. This aberrant PML nuclear body phenotype is observed in acute PML cells. In vitro treatment of APL cells with ATO at micromolar concentrations produced significant anti-adenovirus activity. This activity was partly due to the ability of ATO to induce oxidation of PML nuclear bodies before multimerization by the virus, reconstituting the usual dot-like structure and restoring the antiviral function of PML nuclear bodies in the cells of APL patients' cells (Hofmann et al. 2020).

The effectiveness of arsenic-based drugs in virus-associated cancers has also been reported (Kchour et al. 2013). In patients with human T-cell leukemia virus type 1 (HTLV-1) associated adult T-cell leukemia/lymphoma (ATL), ATO in combination with IFN-α and zidovudine, an FDA-approved nucleoside reverse-transcriptase inhibitor (NRTI) class antiretroviral drug, improved the cytokine gene expression profile by a shift from an initial

immunosuppressive-like state (T_{reg} (T regulatory)/(Th2 phenotype) to an immunocompetent-like state (Th1 phenotype) after 30 days of treatment. This shift is possibly the result of the enhanced immune response leading to eradication of ATL cells and control of infections caused by opportunistic pathogens. These results support suggestions on the use of ATO to treat immune disorders (Wang et al. 2019; An et al. 2020).

Epstein–Barr virus (EBV), the first identified human oncogenic virus, is associated with various malignancies, including carcinomas (e.g. nasopharyngeal carcinoma) and lymphomas (e.g. Burkitt's lymphoma). In a study of the role of PML nuclear bodies in EBV latency, treatment with low dose ATO disrupted PML nuclear bodies, leading to induction of EBV lytic proteins and increased susceptibility of the virus to ganciclovir, an approved FDA drug for the treatment of EBV-associated disorders (Sides et al. 2013). Low concentrations of ATO (0.5—2 nM) were shown to inhibit expression of EBV lytic genes Zta, Rta and BMRF1, promoting cell death in various EBV-positive latency cells (Mutu, Akata, BX-1, Cl13 and JY) in a dose-dependent manner. A synergistic effect was observed with ganciclovir, specifically in EBV-positive cells. These effects were reversed in the presence of a proteasome inhibitor, which suggests that ATO-mediated inhibition of EBV lytic genes occurs via the ubiquitin pathway, promoting ubiquitin conjugation and proteasomal degradation of EBV genes (Yin et al. 2017). Induction of cell death by ATO was also observed in P3HR1 cells, another EBV-positive latency cell line, yet it occurs via autophagy. With this cell line, treatment with sodium arsenite also leads cell death but via a different mechanism, caspase-dependent apoptosis (Zebboudj et al. 2014). These results demonstrate that ATO and sodium arsenite have the potential to be therapeutic agents for EBV-associated lymphoma.

A recent in silico study identified darinaparsin (Fig. 2E) as a potent inhibitor of the RNA-dependent RNA polymerases of SARS-CoV-2. The drug inhibited the 3C-like protease and papain-like protease that are necessary for formation of the viral replication complex (Chowdhury et al. 2020). These results suggest that, in addition to its anticancer activity (Bansal et al. 2015; Mann et al. 2009; Tian et al. 2012), darinaparsin has the potential to be repurposed against

the novel coronavirus that is responsible for the current global pandemic.

Arsenical natural products antibiotics

Selman Waksman, the Russian-Ukrainian-born American microbiologist, defined the term 'antibiotic' as "*a chemical substance, produced by microorganisms, which has the capacity to inhibit the growth of and even to destroy bacteria and other micro-organisms"*' (Waksman 1947). In 1952, Waksman was awarded the Nobel Prize in Physiology or Medicine for his discovery of the aminoglycoside antibiotic streptomycin, a natural product produced by the soil bacterium *Steptomyces griseus* that gave the organism a growth advantage over other soil bacteria. In this section two organoarsenicals with antimicrobial activity, methylarsenite (MAs(III)) and arsinothricin (AST), will be described. Both are natural products produced by soil bacteria to kill other bacteria, meeting Waksman's definition of an antibiotic (Li et al. 2021).

Methylarsenite (MAs(III)): a primordial antibiotic

Highly toxic MAs(III) (Fig. 2S) is produced by methylation of inorganic As(III) by the enzyme As(III) *S*-adenosylmethionine (SAM) methyltransferase, which is termed ArsM in microbes and AS3MT in animals (Dheeman et al. 2014; Qin et al. 2006). The *arsM* gene is considered to be one of the most ancient *ars* genes according to molecular clock analyses, arising at least 3 billion years ago (Chen et al. 2017; Chen and Rosen 2020). Thus, environmental arsenic methylation was widespread nearly a billion years before the Great Oxidation Event (GOE), when oxygen accumulated in the atmosphere. In the original anoxic atmosphere, trivalent MAs(III) would be stable. Since the ArsM product MAs(III) is considerably more toxic than the substrate As(III), methylation has been proposed to be an activation process, generating the primordial antibiotic MAs(III), which gave producers a competitive growth advantage over sensitive microbes during the Archean era (Li et al. 2016). Further methylation generates nontoxic volatile trimethylarsine (TMAs(III)), which may have functioned as a primitive mechanism for self-protection by the MAs(III)-producing microbes. After the GOE, MAs(III) would have been unstable in

air, oxidizing to relatively nontoxic methylarsenate, MAs(V). Filling an ecological niche, other aerobic bacteria evolved the ability to reduce pentavalent MAs(V), regenerating the MAs(III) antibiotic (Yan et al. 2019; Yoshinaga et al. 2011). The genes involved in MAs(V) reduction have not yet been identified, but this reaction now gives extant reducing microorganisms an advantage over MAs(III)-sensitive bacteria in microbial communities (Chen et al. 2019). Trivalent arsenicals such as MAs(III) are toxic in part due to their affinity for thiols groups in proteins and other cellular metabolites (Shen et al. 2013). Since MAs(III) can react with a large number of molecules, no single target can be assigned for its mechanism of action that applies in every cell.

However, one target for the antibiotic action of MAs(III) was recently identified in *Shewanella putrefaciens* 200 (Garbinski et al. 2020). MAs(III), but not inorganic As(III), effectively inhibits the enzyme MurA (uridine diphosphate (UDP)-*N*-acetylglucosamine enolpyruvyl transferase), a cytoplasmic enzyme involved in the synthesis of the key precursor of the peptidoglycan, UDP-*N*-acetylmuramate (UNAM) (Barreteau et al. 2008). Only prokaryotes utilize peptidoglycan as an essential structural component of the cell wall, which makes it a singular target for antibacterial therapy in gram-negative and gram-positive pathogenic bacteria (Du et al. 2000; Raz 2012; Sonkar et al. 2017; Vollmer et al. 2008). Fosfomycin ($C_3H_7O_4P$), the only clinically approved antibiotic that acts against MurA, inhibits MurA by alkylation of the highly-conserved catalytic cysteine residue in the active site (Baum et al. 2001). However, the conserved cysteine is often replaced by an arspartate in MurA orthologs from various pathogens such as *Mycobacterium tuberculosis*, contributing to their intrinsic fosfomycin resistance (De Smet et al. 1999). MurA from *S. putrefaciens* 200 has the conserved catalytic cysteine and is sensitive to fosfomycin, while its Cys-to-Asp mutant is resistant to fosfomycin but remained sensitive to MAs(III), indicating that the two compounds have different mechanisms of action. MAs(III) represent a new area for the development of novel compounds for combating the threat of antibiotic resistance (Garbinski et al. 2020). For MAs(III) to exert its antibiotic action, it first must enter sensitive cells. How do arsenicals in general and MAs(III) in particular get into and out of cells? The aquaglyceroporin GlpF facilitates uptake As(III)

and Sb(III) into cells of *Escherichia coli* (Meng et al. 2004; Sanders et al. 1997). Uptake of MAs(III) by GlpF has not been studied, but other AQPs facilitate its movement into and out of cells. The aquaglyceroporin AqpS from *Sinorhizobium meliloti* was recently demonstrated to conduct both MAs(III) and MAs(V) (Chen et al. 2021). Heterologous expression of the related mammalian aquaporin AQP9 in *Saccharomyces cerevisiae* resulted in three-fold more MAs(III) accumulation than inorganic As(III) (Liu et al. 2006a, b). In addition, inorganic As(III) is transported by sugar permeases, including yeast hexose (Hxt) transporters (Liu et al. 2006a, b) and plant inositol permeases (Duan et al. 2016). The mammalian glucose permease GLUT1 has been shown to transport MAs(III) as well as As(III) (Liu et al. 2006a, b). However, it is not clear if bacterial sugar transporters also transport arsenicals. In response to the high toxicity of MAs(III), bacteria adapted by developing resistance mechanisms (Chen and Rosen 2020). One of the most common mechanisms of bacterial resistance to antibiotics is to pump it out of the cells (Jia et al. 2019). Two MAs(III) efflux permeases are ArsP (Chen et al. 2015a, b) and ArsK (Jia et al. 2019; Shi et al. 2018). Other mechanisms that confer resistance to MAs(III) are the C–As bond lyase ArsI, which demethylates MAs(III) to As(III) (Pawitwar et al. 2017; Yoshinaga and Rosen 2014), and methylarsenite oxidases such as ArsH, ArsU and ArsV that oxidize MAs(III) to MAs(V) (Chen et al. 2015a, b).

Arsinothricin (AST), a pentavalent organoarsenical antibiotic

Arsinothricin (2-amino-4-(hydroxymethylarsinoyl) butanoate, or AST) (Fig. 2T) is a newly identified broad-spectrum organoarsenical antibiotic (Nadar et al. 2019). AST was first discovered as a natural product synthesized by the rice rhizosphere bacterium *Burkholderia gladioli* strain GSRB05 (Kuramata et al. 2016). AST is a non-proteinogenic analog of both glutamate and the arsenic mimetic of L-phosphinothricin (2-amino-4-(hydroxymethylphosphinyl) butanoate or PT), the antibiotic moiety of a *Streptomyces* antibiotic prodrug phosphinothricin tripeptide (PTT) or bialaphos (Nadar et al. 2019; Kuramata et al. 2016). AST inhibits the growth of *M. bovis* BCG, the attenuated etiological agent of bovine tuberculosis, which is closely related to *M. tuberculosis,* the cause

of human tuberculosis, and one of the WHO-designated priority pathogens carbapenem-resistant *Enterobacter cloacae*, whereas it exhibits low cytotoxicity on human monocytes. AST is chemically unrelated to other organoarsenicals and is a promising candidate to usher in a new class of antimicrobial agents (Nadar et al. 2019). MAs(III) and other trivalent arsenicals exert their toxicity through reaction with thiols. In contrast, AST is a pentavalent organoarsenical, and pentavalent arsenicals have low reactivity with thiols. Even though other pentavalent arsenicals are relatively benign and less toxic, AST is as effective an antimicrobial as MAs(III) and is 15-fold more effective as an antimicrobial than PT. PT and AST act by inhibition of glutamine synthetase (GS), a central enzyme in nitrogen metabolism. The likely mechanism of action is by mimicking the γ-glutamyl phosphate intermediate in the glutamine synthetase catalytic pathway (Nadar et al. 2019; Suzol et al. 2020).

Recently the biosynthetic gene cluster for biosynthesis of AST was identified (Galván et al. 2021). An *ars* operon consisting of three genes, *arsQML*, was identified in the draft genome sequence of *B. gladioli* GSRB05, the AST producer. These three genes were shown to encode genes for the synthesis of AST and for its efflux from the cells. The *arsL* gene encodes a non-canonical radical SAM enzyme that transfers the 3-amino-3-carboxypropyl group from SAM to inorganic arsenite, forming hydroxyarsinothricin (2-amino-4-(dihydroxyarsinoyl)butanoate, or AST-OH), the precursor of AST. The *arsM* gene product, an As(III) SAM methyltransferase, methylates AST-OH, producing AST. Finally, *arsQ* encodes an efflux permease that extrudes AST from the cells, both protecting the producing cells from its own product and releasing AST into the extracellular milieu, allowing it to exert its antibiotic action (Galván et al. 2021). For AST to be a useful antibiotic, it must be available in sufficient quantities for clinical trials and for further drug development. Recently, a semi-synthetic method was reported in which D,L-AST-OH is chemically synthesized and then enzymatically methylated by ArsM to produce D,L-AST (Suzol et al. 2020).

Paul Ehrlich, the father of modern drug chemotherapy who synthesized the antimicrobial organoarsenical salvarsan, prophesied that drug resistance follows the drug like a faithful shadow (Ebrahim 2010). This has proven true for nearly every antibiotic and antimicrobial, and resistance to AST has already arisen.

AST is inactivated by acetylation of α-amino group by the enzyme ArsN1. The *arsN1* gene is found in *ars* operons, suggesting that resistance to AST probably arose soon after the evolution of its synthesis. ArsN1 is highly selective and has higher affinity for AST than structurally related PT (Nadar et al. 2019). The *arsN1* gene is widely distributed in bacteria, which implies that AST is also produced by many environmental bacteria. Even so, AST still has a future as an antibiotic. First, AST can be used in combination with ArsN1 inhibitors that can be predicted from the crystal structure of AST-bound ArsN1. Second, the chemical synthesis of AST can be used to produce modified derivatives with higher inhibition of GS or that evade ArsN1 acetylation. These inhibitors and derivatives will improve the clinical utility of this promising new class of antimicrobial drugs.

Synthetic aromatic arsenicals in animal husbandry

Although their medicinal uses waned after the advent of penicillin in the early 1940s, synthetic aromatic arsenicals have been repurposed for use in animal husbandry. Four pentavalent aromatic arsenicals were extensively used in the poultry and swine industry in the US since the mid-1940's and played significant roles as feed additives for improvement of weight gain, feed efficiencies and pigmentation, as well as prevention and treatment of parasitic infectious diseases until banned in the mid-2010's. Atoxyl (*p*-ASA) (Fig. 2U), the first organoarsenical drug for human trypanosomiasis, was repurposed for poultry and swine to promote growth and prevent or treat dysentery (Sharma and Anand 1997). Carbarsone (4-carbamoylaminophenylarsenate or Car(V)) (Fig. 2V), the carbamoylated *p*-ASA(V) derivative originally introduced in 1931 for the treatment of human protozoal infectious diseases trichomoniasis and amebiasis, was later restricted to application with turkeys to improve weight and control blackhead disease, a protozoan disease caused by *Histomonas meleagridis* (Hoekenga 1951; McDougald 1979; Radke 1955; Sasaki et al. 1956; Worden and Wood 1973). The other two are nitroaromatic pentavalent arsenicals, roxarsone (4-hydroxy-3-nitrophenylarsonate or Rox(V)) (Fig. 2W) and nitarsone (4-nitrophenylarsenate or Nit(V)) (Fig. 2X) that were exclusively used

for animal husbandry. Rox(V) was used for poultry to promote growth, treat coccidiosis, an intestinal protozoan parasitic disease caused by *Eimeria tenella*, as well as prevent gastrointestinal tract infections. Although mostly excreted unchanged from the animals, administered organoarsenical drugs were shown to increase the level of inorganic arsenic species in the chicken breasts (Liu et al. 2016). Roxarsone and nitrasone have been banned for nearly two decades by the European Union, in 2014 and 2015, respectively, by the FDA (https://www.fda.gov/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/ucm257540.htm), and more recently banned in China (Hu et al. 2019), although compliance is difficult to enforce. Several countries including Malaysia, Canada and Australia followed this move, yet their use is still allowed in countries such as Argentina, Brazil, Chile, Mexico and Vietnam (Hu et al. 2019). Nit(V) was the last drug in use in the United States to prevent and treat blackhead disease in poultry, and currently there are no efficacious drugs for this serious avian disease, raising a concern in poultry industry (https://www.fda.gov/animal-veterinary/resources-you/blackhead-disease-poultry).

Future perspectives

The major drawback of the use of arsenic in medicine is its toxicity. Therefore, there is a need to employ current advances in science to develop new generation arsenicals that can make up for the shortcomings of currently used arsenic-based drugs. Development of future arsenical drugs will build on the chemistry and properties of arsenic-based drugs already proven to be effective. Before advancements in scientific research, most arsenic-based drugs throughout history were marketed and used without rigorous clinical trials or understanding of their mechanisms of action. This lack of scientific rigor may have been responsible for the disuse of arsenic-based drugs in the late 1900s. The re-emergence of arsenic as a frontline treatment for APL shows the potential for development of new arsenicals with higher therapeutic efficacy and lower toxicity.

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