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Anticancer metal compounds in NCI's tumor-screening database: putative mode of action

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Abstract

Clustering analysis of tumor cell cytotoxicity profiles for the National Cancer Institute (NCI)'s open compound repository has been used to catalog over 1100 metal or metalloid containing compounds with potential anticancer activity. The molecular features and corresponding reactivity of these compounds have been analyzed in terms of properties of their metals, their associated organic components (ligands) and their capacity to inhibit tumor cell growth. Cytotoxic responses are influenced by both the identity of the metal and the properties of its coordination ligand, with clear associations between structural similarities and cytotoxicity. Assignments of mechanisms of action (MOAs) for these compounds could be segregated into four broad response classes according to preference for binding to biological sulfhydryl groups, chelation, generation of reactive oxygen species (ROS), and production of lipophilic ions. Correlations between specific cytotoxic responses and differential gene expression profiles within the NCI's tumor cell panel serve as a validation for candidate biological targets and putative MOA classes. In addition, specific sensitivity toward subsets of metal containing agents has been found for certain tumor cell panels. Taken together, our results expand the knowledge base available for evaluating, designing and developing new metal-based anticancer drugs that may provide the basis for target-specific therapeutics.
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Keywords: Data mining; NCI tumor screen; Metal compounds; Mechanism of action; Drug discovery; Cancer

1. Introduction

The importance of metal compounds in medicine dates back to the 16th century [1] with reports on the therapeutic use of metals or metal containing compounds in the treatment of cancer. Now the list of therapeutically prescribed metal containing compounds includes platinum (anticancer), silver (antimicrobial), gold (antiarthritic), bismuth (antiulcer), antimony (antiprotozoal), vanadium (antidiabetic) and iron (antimalarial). Metal ions are electron deficient, whereas most biological molecules (proteins and DNA) are electron rich; consequently, there is a general tendency for metal ions to bind to and interact with many important biological molecules. Metal ions also have a high affinity for many small molecules, e.g. O_2, that are crucial to life. These considerations alone have fueled much of the past and current interest in developing novel means to use metals or metal containing agents to modulate biological systems.

Biologically essential life processes requiring metals usually involve enzymatic, structural or reactive roles. Catalytic activities for an estimated 12% of all enzymes can be ascribed to metal centers. Metals act to bridge substrate to enzyme such that electrons are withdrawn from the metal and the excess local positive charge lowers the free energy of enzyme activation. Alternatively, structural metals function much like disulfide bonds to participate in the correct tertiary folding of proteins. Transition metals, on the other hand, are known to generate potentially harmful, but in some cases useful, reactive intermediates. General classes of metals can be loosely assigned according to their enzymatic, structural or reactive roles. Iron (Fe) and manganese (Mn) act as enzyme cofactors and catalyze

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redox reactions. Zinc (Zn) and calcium (Ca) provide, respectively, structural integrity, such as in zinc finger proteins, or flexibility, as in calmodulin, for many proteins. Fe plays a critical role in oxygen transportation and electron shuttling. Sodium (Na) and potassium (K) function as charge carriers. Magnesium (Mg) and manganese (Mn) function in hydrolysis and group-transfer. These diverse roles for metals provide many opportunities for biological modulation.

Metal binding substances[1] have furnished many useful drugs. These compounds mainly exploit metals' role in enzymatic activation or molecular structure. Organometallic compounds have achieved importance as enzyme inhibitors, partly due to their capacity to alter enzyme function by, for example, binding biological targets more strongly than metal free organic substrates [2]. Based on this feature, inhibition of metalloenzymes can be achieved by metal coordination to exogenous ligands or by chelation substitution or exchange. Precedence for chelation affecting biological activity existed over 50 years ago with the demonstration that the biological properties of oxine were linked to its ability to bind metal ions [3]. Metal complexes can also inhibit non-metalloenzymes by coordinating to their active site residues and sterically blocking substrate interaction, or coordinating to structurally important residues near the active site. A secondary effect of transition metals is to catalyze the generation of reactive oxygen species (ROS), the presence of which is believed to play important but poorly understood roles that can modulate drug-induced cytotoxic responses and affect cancer pathogenesis [1,4]. Metal-coordination is also one of the most efficient strategies in the design of repository, slow-release or long-acting drugs. Often overlooked, the property of metals to release therapeutic ligands has found utility, especially when ligands are not strongly bound, as exchange vehicles for alternative ligands within a targeted biological path [5]. While many metals are essential for all forms of life, their levels in normal homeostasis or therapeutic intervention must be strictly regulated because most are toxic in excess. As with all drugs, the use of metals in drug development will depend largely on understanding their MOA and selectively controlling their toxicity.

Historically, the antitumor potential of metal-containing agents, from early transition metals to main group elements, has been extensively evaluated. The specific case of organo-platinum compounds, such as cisplatin and its analogs [6], has generated high interest in discovering alternative platinum-containing complexes. To date, considerable evidence points to platinum's therapeutic efficacy being severely attenuated depending, for example, on its molecular configuration, as some *trans*-configured ligands exhibit greater cytotoxicity when compared to their *cis* counterparts. Of particular interest is that *trans*-platinum complexes show cytotoxicities in cisplatin resistant tumor cell lines [7].

In addition to selectively controlling their toxicity, metal-containing compounds must also have appropriate pharmacological properties. Many interesting antitumor active compounds have failed to reach clinical use due to poor physio-chemical properties, including insufficient water solubility, hydrolytic instability and the tendency to readily decompose when exposed to solvents, humidity, light or air. Difficulties in controlling selective toxicity or devising appropriate pharmacological properties have contributed to a general reluctance in the development of metal-based drugs. Consequently, despite the fact that metal complexes are often cytotoxic in vitro at a significantly lower dose than organic drugs, metal compounds are often not investigated further in tumor model systems. Another critical factor when determining therapeutic potential is the metal's oxidation state. Increased oxidation potential for most transition metals occurs with decreased ionic radius associated with higher valence. Toxicity, however, does not appear to relate simply to a higher or lower oxidation state, since chromium(VI) compounds are highly toxic when compared to chromium(III) compounds, whereas arsenite (As(III)) is more toxic than arsenate (As(V)). This brief introduction underscores the much-storied history of metals in clinical applications. The primary goal of the following analysis is to provide a comprehensive study of tumor cell cytotoxicity in light of many of the considerations discussed above as important for the discovery of novel metal-containing antitumor agents.

Our analysis focuses on the tumor cell line cytotoxicity data generated at the NCI for in vitro anticancer drug screening. These tumor cell lines reflect diverse cell lineages (lung, renal, colorectal, ovarian, breast, prostate, central nervous system, melanoma and hematological malignancies, referred to collectively as the NCI_{60}). Since its inception in 1990, cytotoxicity measures for over 40,000 compounds have been obtained that are publicly available. Using this database, our group has developed a suite of computational tools (http://spheroid.ncifcrf.gov), incorporating structural chemotypes as well as biological data into self-organizing maps (SOMs) [8]. SOM is a neural network-based, unsupervised learning algorithm [9] that has been widely used to organize high-dimensional data into lower dimensional space. This lower dimensional space can be organized into a two-dimensional, hexagonal lattice in such a way that the initial data points lying near each other are mapped to nearby locations on the SOM. This level of organization can be used as a tool to visualize complex dependencies

[1] A substance which binds metal is called a *ligand*, while their complex is called an *organo-metallic complex*. Metals bound to the elements N, O, or S can form a chelate ring that binds the metal more tightly when compared to the non-chelate form. The term *chelation*, coined by Morgan and Drew [156], is derived from *chela*, the crab's claw.

between and within different data sets. Here we apply the SOM method specifically to the tumor cell growth inhibition (GI_{50}) data available at the NCI. GI_{50} growth patterns have been found to be an information rich source for establishing a compound's MOA [8,10]. For each compound the SOM procedure uses the GI_{50} data for all cell lines to cluster similar response profiles into nodes. Each node is developed as the best characterization of the data vectors belonging to that node. The distinguishing feature of SOM with respect to hierarchical clustering methods is that, during the generation of the SOM, the developing cluster is established in the context of all clusters, rendering the division of the data into a multi-dimensional dendrogram where neighboring clusters can share common features. The visualization of the resultant clustering can be rendered as a two-dimensional map to emphasize connectedness between clustered nodes. A further characterization of the two-dimensional SOM is to establish larger regions of similarity between groups of nodes. In this case the information contained in the node representation is utilized to cluster similar nodes into a hierarchy of clades, the region is thus defined as a collection of similar clades, which represents the major response categories of the GI_{50} data. SOM clustering of the NCI_{60} data previously segregated compounds into six major response categories: mitosis (M), membrane function (N), nucleic acid metabolism (S), metabolic stress and cell survival (Q), and two unexplored regions P and R [8]. Each of these regions is further divided into a total of 51 clades (sub-regions; M_1–M_2, N_1–N_{12}, P_1–P_{14}, Q_1–Q_7, R_1–R_9, S_1–S_7). The compounds analyzed by Rabow et al. [8] included over 1100 compounds containing transition metals, main group metals, or metalloids (a total of 55 different elements). SOM clustering, based solely on differential cytotoxicity, found metal compounds exhibiting diverse cytotoxic response profiles (Fig. 1). Special cases are found where clusters with similar cytotoxic response profiles are comprised of structurally similar compounds.

Our analysis inspects metal containing compounds that have been clustered according to similarities in their GI_{50} response profiles. Compounds in these clusters are subsequently examined according to their reactivity and structural similarities (including properties of the metal alone, the ligands coordinated to the metal, and the coordination mode). The differential cytotoxic response profiles for selected compound sets are in turn correlated with the gene expression profiles generated from these same tumor cell lines. Based on these results the potential targets and possible MOAs of these metal compounds are proposed and discussed. Our results reveal a clear association between certain types of metals and cytotoxicity, as well as a perspective on the importance of the organic component in defining cytotoxicity, and the underlying mechanistic origins of the diverse cytotoxic responses associated with metal compounds. This perspective can also be used to help assign MOAs for non-metal-containing agents that show similar cytotoxic response profiles to those of metal-containing compounds. Taken together, our results expand the knowledge base needed for evaluating, designing and developing new metal-based anticancer drugs.

The subsequent analysis and results will be presented in two major sections. The first section surveys the chemical structural features of the metal compounds according to their appearance in the previously defined four SOM subsections (Q, S, N/P and M). Similarities in chemical features and consequent similarities in reactivity within each SOM region, together with literature support, form the basis for proposing putative MOAs. Metal type seems to play a predominant role in defining the cytotoxic response for some SOM regions (e.g., Q), whereas ligand properties appear to be the determining factor in others (e.g., S_1 and S_2). Cases also exist where cytotoxicity is dictated by the combined effect of the metal–ligand entity (e.g., N/P and M). These results will reveal a wide variety of metal complex chemotypes that affect a diverse array of molecular targets and biological pathways. The second section analyzes the genomic features uniquely associated with each SOM region, which implicate classes of genes representing various biological pathways that may be affected by drug insults. The examination of region specific gene-drug relationship in this section serves as additional support for the putative MOAs proposed in the first section and may also reveal novel targets and MOAs for metal-based drug molecules. The present study represents a comprehensive survey of metal compounds possessing anticancer activity viewed in terms of their chemical structural features, putative MOAs and correlated genomic features.

2. Compound structural features and putative MOA: heavy metal compounds preferably bind to sulfhydryl (SH) groups (Region Q)

The greatest number of metal containing compounds is found in the lower left corner of the SOM, designated as the Q-region (see Fig. 2, Panel A). The most predominant feature of these metal complexes is their high affinity for sulfur-containing ligands. Approximately 90% of these metal complexes consist of second- or third-row transition metals (Rh, Pd, Ag, Ir, Pt, Au, Hg, etc.), or heavy post-transition metals (Sn, Pb, Sb, Bi, etc.). Heavy metals are generally known as ''soft acids''; they tend to favor the formation of stable complexes with ''soft bases'' [11]. Thus, heavy metals favor ligand atoms of the second or later row of the periodic table (e.g., S, Se), as opposed to the atoms of the first row within the same group (e.g., O). The coordination preference of heavy metals for sulfur or selenium over oxygen can be attributed to the presence of considerable covalent character (π bonding from overlapping of the d-orbitals) in their metal–ligand bonds. This

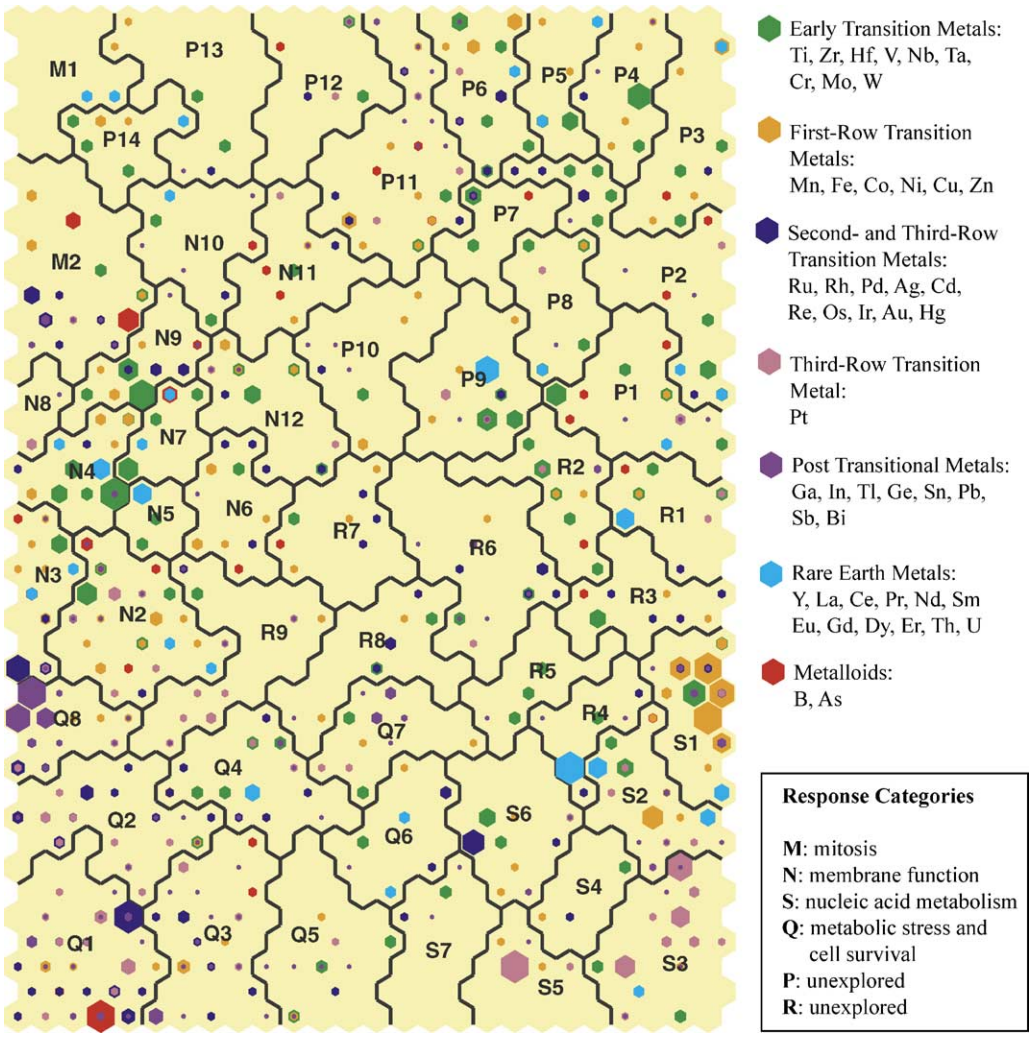
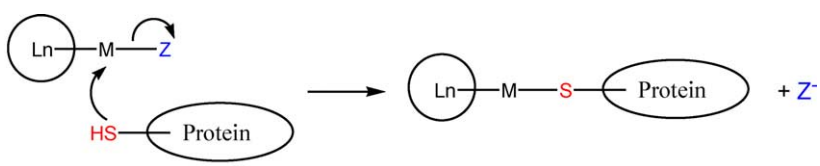


Fig. 1. Cytotoxicity measurements for nearly 40,000 small molecules screened against the NCI's tumor cell panel (NCI_{60}) were clustered into 1066 groups or nodes using a self-organizing map (SOM). These nodes could be separated into six major response categories (regions): mitosis (M), membrane function (N), nucleic acid metabolism (S), metabolic stress and cell survival (Q), and two unexplored regions P and R. Each of these regions is further divided into a total of 51 sub-regions (M_1–M_2, N_1–N_{12}, P_1–P_{14}, Q_1–Q_7, R_1–R_9, S_1–S_7), shown here by the black boundary lines. The agents analyzed include over 1100 compounds containing transition metals, main group metals or metalloids, exhibiting diverse cytotoxic response profiles. Locations of these metal and metalloid containing compounds are shown here as colored hexagons, where neighboring clusters identify compounds with similar cytotoxicity responses, and grouped according to the position of their metals in the periodic table. Hexagons are sized proportional to the number of compounds within each cluster. The dispersion of the metal containing compounds across the map indicates that these compounds affect a diverse array of biological targets and pathways. In addition, the specific groups of metals associated with specific cytotoxic responses indicate that these compounds have target specificity and cannot be associated with a single simplified toxic response. SOM regions populated by metal/metalloid containing compounds are Q (357, 9.09%), S (209, 8.06%), N (199, 5.71%), P (225, 3.05%), M (42, 3.70%) and R (124, 3.28%).

feature has relevance when assigning MOAs to molecular targets with varying sulfur or oxygen content.

The metal complexes clustered in the Q-region are generally comprised of one or more weakly coordinated or hydrolysable ligands (leaving groups, e.g., Cl^-, Br^-, $RCOO^-$, H_2O) (Table 1; Fig. 2, Panel A). These characteristics make the metal complexes susceptible to attack by biological nucleophiles, e.g. the sulfhydryl group (–SH) of a cysteine residue, to undergo nucleophilic ligand addition and/or substitution reactions (Eq. (1))



(1)

M = Metal; Z = Leaving group: Cl, Br, I, OAc, etc.; Ln = Ligands.

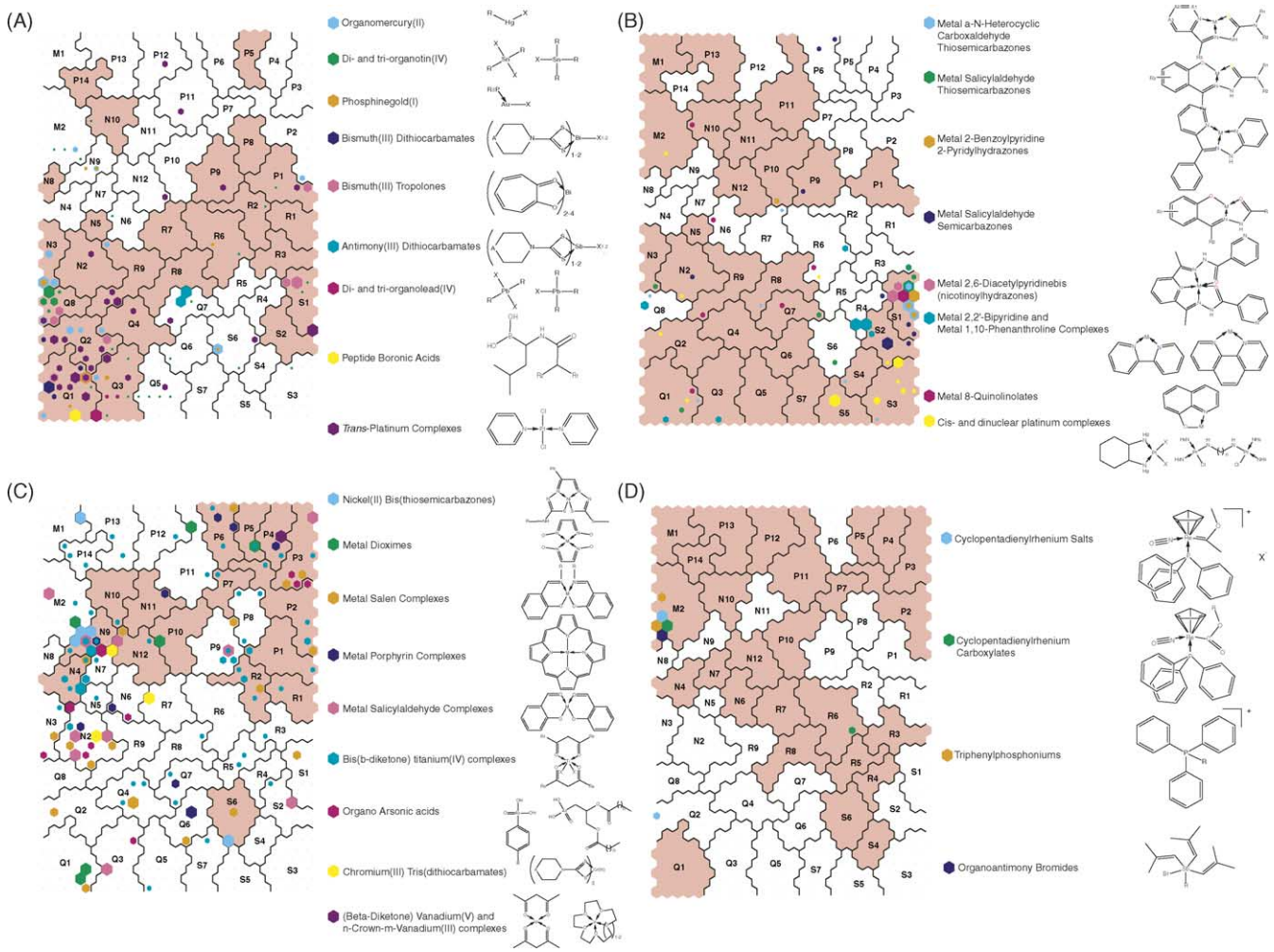


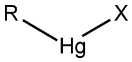
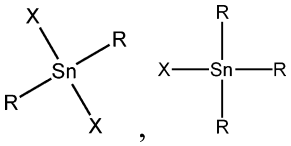
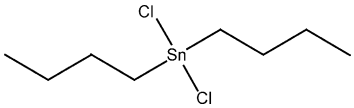
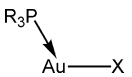
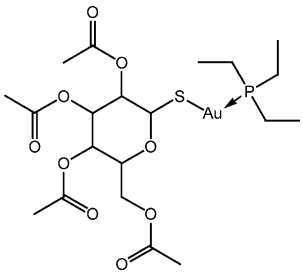
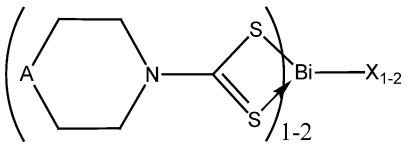
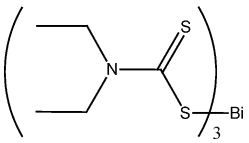
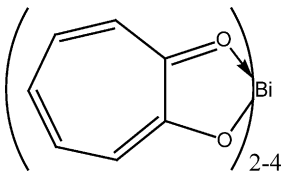
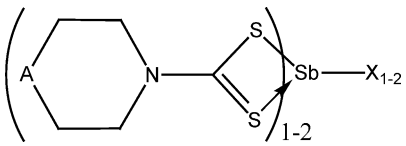
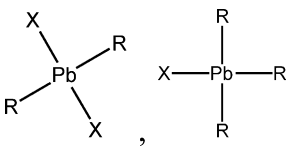
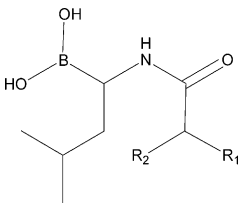
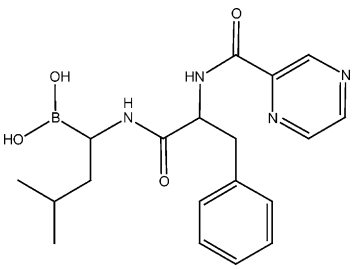
Fig. 2. Metal and metalloid containing compound motifs clustered in each SOM region. Colored hexagons correspond to clusters containing compounds within structural motifs listed in Tables 1–4. Hexagons are sized proportional to the number of compounds within each cluster in that region. Drug–gene relationships for these compounds were examined by correlative analysis between cytotoxic response and differential gene expression profiles within the NCI_{60}. This figure, and Fig. 4 highlight the SOM regions having the most extreme correlations between the constitutive gene expression profiles of different gene families and cytotoxicity profiles of compounds with different structural motifs. A general feature of the metal containing compounds is that different classes of metals show preferential activities against specific groups of gene products. Heavy metal compounds in Panel A interact with proteins characterized by sulfhydryl groups, chelation complexes in Panel B are associated with the S-region, i.e. DNA synthesis process; whereas the diverse group of metals in Panel C are associated with reactive oxygen species generation and oxidative stress. The compounds collated in Panel D, which are clustered in the M-region, are characterized by their ability to produce ionic/charged species, and are thought to act in mitochondrial processes. Backgrounds colored in rosy red indicate regions significantly ($p < 0.05$) correlated with the expressions of a specific class of genes. Panel A: structural motifs (Table 1) clustered in the Q-region show significant correlation with gene expression patterns from a family of cysteine or glutathione-dependent proteins (274 measurements). Panel B: structural motifs (Table 2) clustered in the S-region show significant correlation with gene expression patterns from a family of DNA-binding proteins (380 measurements). Panel C: structural motifs (Table 3) clustered in the N and P regions show significant correlation with gene expression patterns from a family of oxidoreductases (235 measurements). Panel D: structural motifs (Table 4) clustered in the M_2-region show significant correlation with gene expression patterns from a family of mitochondrial proteins (276 measurements).

where M is the metal, Z the leaving group and Cl, Br, I, OAc, etc. are the ligands.

2.1. Mercury (Hg)

Most of the organomercury(II) complexes (Table 1, Entry 1) are located at the top of the Q-region (see Fig. 2, Panel A). They are characterized by an extremely high affinity for -SH groups. Mercury ranks along with cadmium, lead, and zinc, as having a strong preference for binding sulfur over oxygen atoms based on a S–O affinity scale [12]. Hg(II) is known to form glutathione (GSH) complexes both in vitro and in vivo. Methylmercury–GSH complexes have been identified in different mammalian tissues, where tissue GSH levels are an important determinant of both renal and hepatic methylmercury uptake [13]. GSH has been suggested to play a role in protecting cells against mercury cytotoxicity due to its capacity to complex with heavy metals [14]. Hg(II) is also known to affect the activity of two key enzymes involved in GSH metabolism: γ-glutamyl-cysteine synthetase and GSH transferase [15].

Table 1
Structural motifs, known drug examples, and possible biological targets and modes of action (MOAs) for metal and metalloid containing compounds in the Q-region of the SOM

Compound motif	Known drug examples	Proposed targets
 1. Organomercury(II)		GSH metabolism
 2. Di-, and tri-organotin(IV)	 Di(*n*-butyl)dichlorotin	Glutathione *S*-transferase, caspase, ATPase, hexokinase, acetylcholinesterase
 3. Phosphinegold(I)	 Auranofin	AP-1 (*Jun* and *Fos*) and NF-κB, thioredoxin reductase
 4. Bismuth(III) dithiocarbamates	 Tris(diethyldithiocarbamato)-bismuth(III)	GSH metabolism, proteases, lipases, glycosidases, phospholipases
 5. Bismuth(III) tropolones		
 6. Antimony(III) dithiocarbamates		Glutathione reductase
 7. Di-, and tri-organolead(IV)		Glutathione *S*-aryltransferase, ATPase
 8. Peptide boronic acids	 PS-341	Proteasome

2.2. Tin (Sn)

The majority of organotin(IV) compounds (Table 1, Entry 2) display cytotoxicity profiles similar to those of the mercury compounds. This indicates that these tin compounds may share similar cellular targets as the mercury complexes. Di-*n*-butyltin dichloride, $(n\text{-Bu})_2SnCl_2$, is known to inhibit the glutathione *S*-transferase activity of cytosol supernatants from rat liver and thymocytes by binding to their –SH groups [16]; and trialkyl and triaryl tin compounds have been found to be effective inhibitors of rat liver glutathione-*S*-aryltransferase activity [17]. Tributyltin (e.g. $(n\text{-Bu})_3SnCl$) is known to inhibit caspase activity by binding to essential cysteine thiol groups [18].

Di- and tri-organotin complexes may also bind membrane proteins, glycoproteins, and other cellular proteins. Et_3SnBr can bind to ATPase and hexokinase [19], and $(n\text{-Bu})_2SnCl_2$ and $(n\text{-Bu})_3SnCl$ bind to ATPase and acetylcholinesterase within human erythrocyte membranes [20]. It has been suggested that interaction of dibutyltin with rat liver plasma membranes disturbs their structural and functional organization and leads to hepatotoxicity [20]. Some dialkyl, diphenyl, and dibenzyl tin compounds are also known to inhibit ATPase activity in a dose-dependent manner [21]. Overall, the organotin complexes bound to sulfur containing ligands appear to be more stable than those coordinated by oxygen or nitrogen containing ligands [22].

2.3. Gold (Au)

''Soft'' gold(I), a third-row late transition metal, binds only weakly to ''hard'' oxygen and nitrogen ligands; however, it exhibits a very high affinity for ''soft'' donor atoms and thus forms numerous complexes with sulfur, selenium, and phosphorus containing ligands. Approximately 80% of the phosphinegold(I) complexes (Table 1, Entry 3) are found in the Q and nearby SOM regions (see Fig. 2, Panel A). Given the range of sulfur-containing molecules with biological activity, many investigations have been carried out on the anti-tumor activity of phosphinegold(I) complexes directed at cysteine residues within critical in vivo putative targets [23,24]. Au(I)-based drugs were also shown to bind strongly and specifically to thiol groups in transcription factors such as AP-1 (*Jun* and *Fos*) and NF-κB [25,26], giving rise to the possibility that gold can affect transcription. Au(I)-containing compounds are potent inhibitors of several selenocysteine-containing enzymes. A series of organogold compounds, including the antirheumatic drugs auranofin (Table 1) and aurothioglucose (ATG), strongly inhibit human thioredoxin reductase in vitro [27] and murine thioredoxin reductase activity in vivo [28]. Auranofin and other Au(I) complexes have also been shown to inhibit the growth of cultured tumor cells in vitro and many have antimitochondrial activity [29]. Auranofin is known to be a potent inducer of mitochondrial permeability transition, through the inhibition of mitochondrial thioredoxin reductase [30].

2.4. Bismuth (Bi) and antimony (Sb)

A series of bismuth(III) dithiocarbamates (Table 1, Entry 4) and tropolone complexes (Table 1, Entry 5) are clustered together in the lower left edge of Q_1 (see Fig. 2, Panel A). Bismuth is the heaviest stable element in the periodic table. According to Pearson's hard-soft acid–base (HSAB) theory [11], Bi(III) has high affinity for oxygen, nitrogen and sulfur ligands, and is known to form stable carboxylate, aminocarboxylate, and thiolate complexes [24]. However, there is a wide range of thiobismuth compounds due to the thermodynamically favorable Bi(III)–S interaction. Bi(III) forms very stable complexes with cysteine and glutathione preferentially through sulfhydryl group binding only [31]. The MOA of some bismuth-containing drugs has been suggested to involve inhibition of enzymes such as proteases, lipases, glycosidases and phospholipases.[31] Antimony is located one position above bismuth in the periodic table, in the same group. Antimony(III) dithiocarbamates (Table 1, Entry 6), the Sb(III) analogs of bismuth(III) dithiocarbamates, are found in upper Q_7, located in two adjacent clusters. Trivalent anti-leishmanial antimonials inhibit both trypanothione reductase and the analogous mammalian enzyme, glutathione reductase [32]. A more detailed discussion on metal dithiocarbamate complexes is given in a later section.

2.5. Other heavy metals

Other heavy transition metal complexes found in the Q-region include complexes of second-row transition metals rhodium (Rh), palladium (Pd), silver (Ag), and third-row transition metals iridium (Ir) and platinum (Pt). These metals are all in a low ($\leq$2) oxidation state (e.g., Rh(I), Rh(II), Pd(II), Ag(I), Ir(I), Pt(II)), and are typical ''soft'' acids that have strong preference for ''soft'' bases such as sulfur and selenium. Rh(II) complexes of the type $[(RCOO)_4L_2Rh_2(II)]$ (where R = Me, Et, Ph or CF_3; L = H_2O or other solvent), which have shown antitumor activity against human oral carcinoma, Ehrlich ascites, L1210 and P388 cells [33], are known to irreversibly inhibit proteins with cysteines in or near their active site; the μ-acetates are sequentially displaced by cysteines to form the complex $[(Cys)_4(H_2O)_2Rh_2]$, whereas enzymes lacking essential sulfhydryl groups are not affected [34]. In each case, the rate of enzyme inactivation closely parallels the observed toxicity and antitumor activity of the Rh(II) complexes. Furthermore, enzymes demonstrated to be most sensitive to established sulfhydryl inhibitors are also most sensitive to Rh(II) carboxylate inactivation. Methionine also forms a complex with $[(CH_3CO_2)_4Rh_2]$ in which the sulfurs of two methionines coordinate to the axial

positions, and glutathione appears to form a bis-chelating complex formulated as $[(CH_3CO_2)_2(GS)_2(H_2O)_4Rh_2]$ [34].

2.6. Platinum (Pt)

The platinum complexes fall into two major, well-separated, groups on the GI_{50} SOM: compounds in the lower left corner (Q-region) (see Fig. 2, Panel A) and compounds in the lower right corner (S-region) (see Fig. 2, Panel B). The two groups of platinum complexes have very distinct features. Over 90% of the S-region platinum compounds are either cisplatin or its analogs, complexes that are "forced" to have a *cis* configuration (two of the coordinating atoms are from one bidentate ligand that forms a chelation ring with platinum) (e.g., oxaliplatin, carboplatin), or dinuclear cisplatin or carboplatin analogs (two platinum centers linked together by a long aliphatic chain) (see Fig. 3(b)). These features enable the S-region platinum complexes to form intrastrand or interstrand cross-links between two DNA bases, where a *cis*-configuration or a dinuclear complex linked by a flexible chain is essential.

In contrast, Q-region platinum complexes contain bulky ligands such as aromatic ring structures or trialkyl or triaryl phosphines (see Fig. 3(a)). Their steric hindrance makes a *trans*-configuration largely favorable and cross-linking of DNA by these platinum complexes impossible or unfavorable. In addition, as a "soft" metal, Pt(II) generally shows low affinity for "hard" (O, N, etc.) ligands and a preference for heavier/"softer" (S, Se, P, etc.) ligands that can form π-bonds. Therefore, Pt(II) complexes in general have a higher affinity for molecules containing thiol groups, such as cysteine, reduced glutathione, and methionine. Overall, the structural features of the platinum compounds in the Q-region appear to be more diverse than those found in the S-region, whose agents are specifically targeting nucleic acids [8].

Recently, some Pt(II) complexes have been found to efficiently inhibit the human thioredoxin reductase, a selenoenzyme, and strongly inhibit the proliferation of three different glioblastoma cell lines and two different head-and-neck squamous carcinoma cell lines [35]. Competition reactions have shown that the compound *trans*-[{E-HN:C(O-Me)Me}$_2$Pt(II)Cl$_2$] exhibits antitumor effects and binds preferentially to glutathione over 5'-GMP [36]. Cisplatin and its major metabolite, a GSH-platinum complex, have both been found to inhibit the intracellular activity of mammalian thioredoxin, thioredoxin reductase and glutaredoxin [37]. Oxaliplatin and analogs have been shown to form adducts with both GSH and GSH disulfide, forming thiolate-bridged dimeric complexes [38]. Platinum binding to thiol groups has usually been viewed as responsible for the toxic effects of these drugs; however, a growing body of research has pointed to more complex anticancer mechanisms other than DNA binding, involving interactions of platinum compounds with proteins and enzymes [39].

2.7. Lead (Pb) and boronic acids

Lead is a post-transitional metal, the heaviest element of group IVA in the periodic table. All the organolead compounds are located in the Q-region of the GI_{50} SOM (see Fig. 2, Panel A). Pb^{2+} has been shown to interact relatively strongly with both oxygen and sulfur ligands, and relatively weakly with nitrogen donor ligands [12]. Organolead ions, especially those originated from di- or tri-aryl lead compounds (e.g., Ph_2Pb^{2+}, Ph_3Pb^{+}) (Table 1, Entry 7),

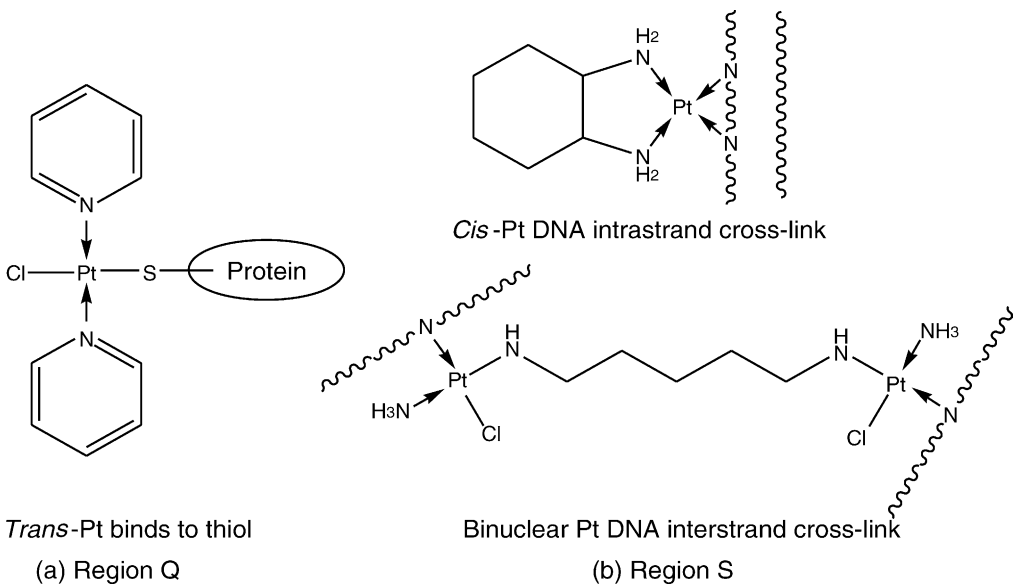


Fig. 3. Structural motifs of platinum complexes found in the NCI_{60} tumor cell screen. (a) Example of platinum complexes with bulky *trans*-configured ligands whose cytotoxic response profiles are mostly clustered in the Q-region. These compounds will most likely bind to cysteine sulfhydryl groups in proteins. (b) Examples of platinum complexes whose cytotoxic response profiles are clustered in the S-region. Top: platinum complexes with *cis*-configured ligands that form intra-strand DNA cross-links. Bottom: dinuclear platinum complexes that form inter-strand DNA cross-links.

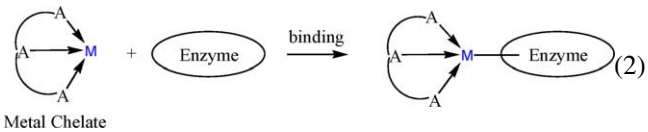
are "softer" than inorganic Pb^{2+} because their positive charge is distributed over their aromatic ligands. Trialkyllead compounds (R_3PbX) are known to disrupt cytoskeletal elements [40] and inhibit glutathione *S*-aryltransferase activity from rat liver [17]. These compounds also inhibit membrane bound Na^+–K^+-ATPase from *E. coli* and HeLa cells, and ATP-hydrolysis of the mitochondrial F0-F1-ATPase complex [41]. However, addition of high excess of GSH totally restores both enzyme activities [41].

Interestingly, most lead compounds have cytotoxicity response profiles very similar to those of boronic acids (Table 1, Entry 8). PS-341 and other boronic acid analogs are known to be highly specific proteasome inhibitors, which block the enzyme function through an electrophilic attack on the proteolytic subunit [42]. The very similar cytotoxicity profiles exhibited by the lead compounds and boronic acids is a strong indication that these two classes of agents may share common, specific molecular targets, different from the rest of the Q-region metal complexes. Boronic acids inhibit protease function by covalently binding to the threonine –OH groups in the active sites of the enzyme. Since lead has strong affinity for both O and S donor ligands, –SH, –OH and –COOH groups all have the capability of binding to lead. These unique properties of lead compounds make them potential proteasome inhibitors. Pb^{2+} is also known to bind to Ca^{2+} sites (composed solely of O donors) on proteins and act as inhibitors of calpain by competing for its Ca^{2+} binding sites [43].

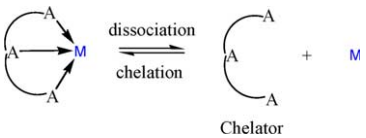
3. Compound structural features and putative MOA: chelators and metal complexes of chelators (sub-regions S_1 and S_2)

The metal compounds in the S-region fall into several major clusters. The compounds in sub-regions S_3 and S_5 are mainly clusters of platinum complexes. As mentioned earlier in the Q-region discussion, these compounds are either *cis*-configured or dinuclear platinum complexes with their primary MOA being the formation of intra- or inter-strand cross-links between DNA base pairs. S_1 and S_2 are the other two S sub-regions enriched with metal compounds. The key feature of these compounds is that they are mainly coordination complexes of first-row transition metals (Mn, Fe, Co, Ni, Cu, Zn) with strong chelating agents (especially tridentate ligands) (see Table 2; Fig. 2, Panel B). Not only are the metal chelation complexes mostly found in S_1 and S_2, but so are the chelating agents themselves. Chelators have received substantial attention as therapeutic agents because of their capability to alter the metabolism and homeostasis of such essential metals as iron, copper and zinc (see Refs. [44–46] for reviews on metal chelation therapies). Iron metabolism is altered in cancer; rapidly proliferating tumor cells show an increased requirement for iron. On the other hand, copper regulates growth factor production associated with angiogenesis and elevated serum copper levels have been observed in patients with several cancer types [45]. It is noteworthy that S_1 is the most copper compound-rich sub-region of the SOM, where 28 out of 68 copper complexes are located.

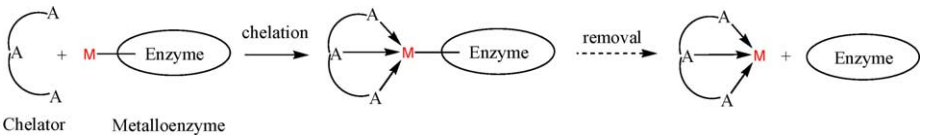
Chelating agents may interfere with the catalytic activities of metalloenzymes or metalloproteins by affecting their structural or catalytic metal cofactors. They could chelate (either bind to and/or remove) the functional metals in the enzymes or proteins (Eq. (4)), or sequester the non-protein bound metal ions and, thus, deprive the metalloenzymes of their metal supply (reverse reaction in Eq. (3)). MOAs for metal chelates may be more complex with a few possibilities shown in Eqs. (2)–(5). The metallo drug itself could bind to the enzyme's metal-binding sites (Eq. (2)). The metal compound could also act as a carrier of the chelator by dissociating, after entering the biological system, into a free metal and a chelator forward reaction in Eq. (3), which is then available for chelation of other biologically important metals (Eq. (4) and reverse reaction in Eq. (3)), or binding to other biological targets (Eq. (5)), respectively. The fact that chelating agents possess cytotoxic response profiles similar to their metal chelates, and that the metal type does not appear to significantly affect the activity of these metal chelates, indicate that either they are prodrugs and the active species are their metal chelates formed in situ, or the metal chelates serve as carriers for chelators which themselves are the active drug molecules.



(2)



(3)



(4)

(5)

M = Metal; A = Donor atom

3.1. Metal α-N-heterocyclic carboxaldehyde thiosemicarbazone complexes (M-N-HCATs; Table 2, Entry 1)

α-*N*-Heterocyclic carboxaldehyde thiosemicarbazones (*N*-HCATs) fall almost exclusively in the S-region (S_1, S_2, and bottom S_3) and have been studied extensively as therapeutic iron chelators and anticancer agents [44,46]. *N*-HCATs are tridentate chelators that bind transition metals through sulfur and nitrogen donors. *N*-HCATs also have relatively high affinity for other divalent essential metals including copper, zinc, and manganese as well as trivalent iron. *N*-HCATs are among the most potent known inhibitors of ribonucleotide reductase (RR), a strategic target for antitumor drug design due to its involvement in DNA synthesis [47]. Iron chelators are proposed to inhibit RR by chelating the iron required for synthesis and regeneration of the functional R2 subunits [47], short of complete extraction of iron from the enzyme. Early studies on structure–activity relationships of *N*-HCATs have shown antitumor activity directly correlated with their chelating ability [48]. Several *N*-HCATs, including Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazones, 3-AP), 5-HP (5-hydroxy-2-formylpyridine thiosemicarbazone) and 3-AMP (3-amino-4-methyl-pyridine-2-carboxaldehyde thiosemicarbazone), have been evaluated as antitumor drugs in clinical trials [47]. Triapine® is currently in phase I clinical trials [49].

The mechanism of RR inhibition by *N*-HCATs does not appear to be simple iron depletion, as is the case for other iron chelators. The iron or copper complexes of some *N*-HCATs also strongly inhibit RR. The MOA proposed is that the metal complexes inhibit RR by reductively quenching the tyrosyl radical of the R2 subunit near its catalytic center [50]. For *N*-HCATs, the iron chelate formed from chelation of intracellular iron has been suggested to be the actual active drug species [51]. However, more recent studies have raised questions concerning the universal validity of the assumption that RR is the sole target of *N*-HCATs and their metal chelates (M-*N*-HCATs), and suggested inhibition of other enzyme targets including DNA topoisomerase II, DNA polymerase α, dihydrofolate reductase, and IMP dehydrogenase [52,53]. DNA synthesis has thus been proposed as a major target of these complexes. M-*N*-HCATs induce cytotoxicity by affecting several metabolic pathways, including a reduction in de novo purine synthesis, although DNA intercalation is not significant. This is consistent with our earlier finding that the primary MOA of the S-region compounds is to affect nucleic acid metabolism [8].

3.2. Metal salicylaldehyde thiosemicarbazones (M-SATs; Table 2, Entry 2)

SATs, found in the border region of S_1 and S_2, are tridentate chelators with antiproliferative activity relatively selective for tumor cells; their MOA being due to chelation of intracellular iron [54]. No apparent correlation is observed between the iron-binding affinity of these chelators and their antiproliferative activity. Inhibition of DNA synthesis is, however, associated with high iron-chelation efficacy and antiproliferative activity for some chelators,

Table 2
Structural motifs and possible biological targets and modes of action (MOAs) for metal chelates clustered in SOM sub-regions S_1 and S_2

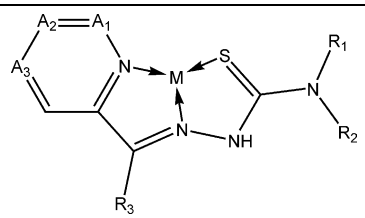
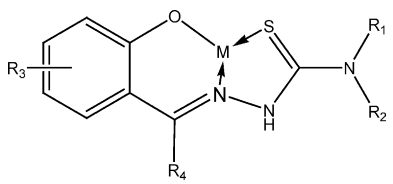
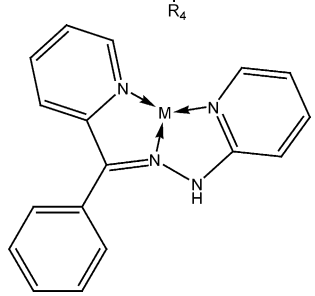
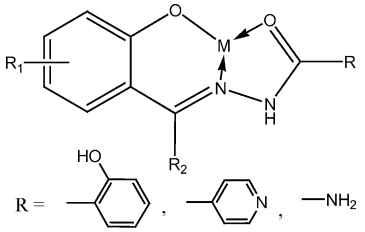
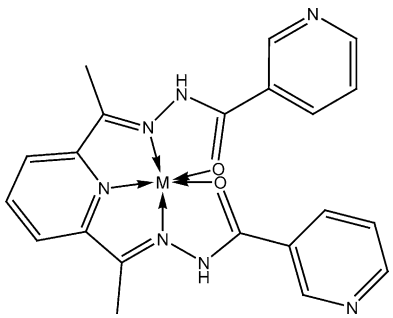
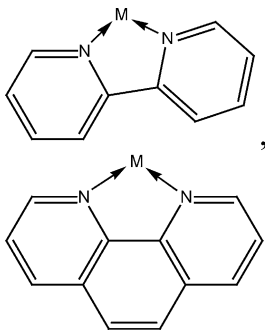
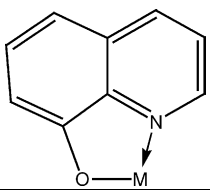
Compound name	Compound motif	Proposed targets
1. Metal α-*N*-heterocyclic carboxaldehyde thiosemicarbazones (M = Cu, Fe, Ni, Bi, Al; A_1 A_2, A_3 = N or C)		DNA synthesis; de novo purine synthesis; ribonucleotide reductase, DNA topoisomerase II, DNA polymerase α, dihydrofolate reductase, IMP dehydrogenase
2. Metal salicylaldehyde thiosemicarbazones (M = Cu, Ni, Zn, Pd)		DNA and purine syntheses; DNA polymerase α, PRPP amido transferase, IMP dehydrogenase, dihydrofolate reductase, TMP kinase; thymidylate synthetase
3. Metal 2-benzoylpyridine, 2-pyridylhydrazones (M = Mn, Fe, Co, Ni, Cu, Zn)		Tubulin polymerization

Table 2 (*Continued*)

Compound name	Compound motif	Proposed targets
4. Metal salicylaldehyde semicarbazones (M = Cu, Fe, Mn, Co, Ni, Zn)		DNA synthesis
5. Metal 2,6-diacetylpyridinebis(nicotinoyl hydrazones) (M = Ni, Cu, Zn)		HIV-1 integrase
6. Metal 2,2′-bipyridine and metal 1,10-phenanthroline complexes (M = La, Nd, Ce, Nb, Pt, Mo, Sn, Co, Ru)		DNA intercalation
7. Metal 8-quinolinolates (M = Ti, Sn, Ge, Ga, V, Bi)		Iron metabolism

but less correlation is found for others. The modest prevention of DNA synthesis by the unsubstituted SATs indicates that they have other MOAs besides inhibition of RR. Nickel(II) complexes of SATs are observed to be potent cytotoxic agents in human and rodent tissue cultured tumor cells [55]. These agents selectively inhibit L1210 DNA and purine syntheses, and DNA polymerase α, PRPP amido transferase, IMP dehydrogenase, dihydrofolate reductase, TMP kinase and thymidylate synthetase activities.

3.3. Metal 2-benzoylpyridine 2-pyridylhydrazones (M-BPPHs; Table 2, Entry 3)

Ring substituted BPPHs, clustered together with their metal chelates (M-BPPHs) in sub-region S_1, are more potent than the RR inhibitor hydroxyurea against several cancer cell lines [56]. RR is not the primary target of these hydrazone derivatives [57]. The BPPH complexes of Mn(II), Fe(III), and Cu(II) have been studied as models for catechol 1,2-dioxygenase and catechol oxidase [58]. The Mn(II) and Cu(II) complexes catalyze the oxidative transformation of catechol to the corresponding *o*-benzoquinone in the presence of O_2. The Fe(III) complexes catalyze the aerobic oxidation of catechol to the intra-diol cleavage product. The Mn(II) complex of BPPH inhibits tubulin polymerization but fails to increase the mitotic index [59]. Analogs of the marine product discorhabdin, which inhibits DNA topoisomerase II and is active toward a human colon carcinoma cell line, share similar cytotoxicity profiles as the M-BPPHs [60].

3.4. Metal salicylaldehyde semicarbazones (Table 2, Entry 4)

Tridentate chelators salicylaldehyde benzoylhydrazones (R = aryl, SABHs), located in S_2, have been evaluated in vivo as iron-chelating drugs [61]. They appear to be potent inhibitors of DNA synthesis and cell growth in a variety of human and rodent cell lines grown in culture. When tested on certain cell lines, the copper complexes of these hydrazones show increased potency (up to 100-fold) compared with the metal-free organic ligands. Subsequently, salicylaldehyde acylhydrazones (R = alkyl, SAAHs) are found to be cytotoxic, again with their copper complexes showing enhanced activities [62]. QSAR studies have been conducted for SABHs, SAAHs, and their complexes with first-row transition metals including copper, iron, manganese, cobalt, nickel, and zinc [62]. The activities of these compounds have been found to be related to their solubility and lipophilicity, as well as the electronic properties of their benzoyl ring substituents and the charge density on the metal.

3.5. Metal 2,6-diacetylpyridinebis(nicotinoylhydrazones) (M-DAPNHs; Table 2, Entry 5)

DAPNH analogs and their coordination complexes with some first-row transition metals, clustered in sub-region S_1, have been reported to have antimicrobial and genotoxic activities [63]. The pentadentate ligands themselves are found to be HIV-1 integrase inhibitors [64]. These metal complexes have been suggested to serve as carriers and intracellular repositories for active ligands [63] which can chelate catalytic site metals (e.g., Mn) in HIV-1 integrase.

3.6. Metal 2,2′-bipyridine and metal 1,10-phenanthroline complexes (Table 2, Entry 6)

Bipyridine (*bpy*) and 1,10-phenanthroline (*phen*) are bidentate chelators with nitrogen donor ligands that have relatively high affinity for both copper and iron [65]. Derivatives of *bpy* and *phen* mainly cluster in the S-region, with the highest concentration in S_2. They exhibit similar cytotoxic profiles when compared to their metal complexes in S_2. The *phen* derivative, neocuproine, is widely used as a copper chelator [66], though it has similar affinity for Fe(II). The metal complexes of *bpy* and *phen* in S_2 and nearby SOM clusters are mainly those of trivalent lanthanide ions, although those of a few other metals such as Mo, Ru, Co, and Pt are also found within these clusters. In addition to the chelating ability, their extended aromatic ring system makes these ligands potential DNA intercalators. The metal complexes of *bpy* and *phen* derivatives show cytotoxicity responses similar to those of the Pt(II) complex of 2,2′,2″-terpyridine, which is involved in DNA intercalation and DNA topoisomerase I inhibition [67]. Many octahedral complexes of Ru(II), Re(I), Rh(III), and Co(III) have been shown to bind DNA intercalatively, including Ru(II) complexes of phenanthroline, quinonediimine and derivatives [68].

3.7. Metal 8-quinolinolates (M-8-QLs; Table 2, Entry 7)

Derivatives of the bidentate chelator with nitrogen and oxygen donor atoms, 8-hydroxyquinoline (8-HQ), are located mainly in S_2 and S_1. Their metal chelates, M-8-QLs, are primarily found in one cluster in S_1. 8-HQs display strong complexing ability with various metals and have high affinity for trivalent iron [69]. Antiproliferative effects and phenotypic alterations induced by 8-HQ in melanoma cell lines have been observed [70]. 5,7-Substituted derivatives of 8-HQ are known inhibitors of catechol *O*-methyltransferase [71]. Antitumor activities have been documented for M-8-QLs, such as those of gallium [72], tin [73] and bismuth [74].

In summary, the majority of compounds clustered in S_1 and S_2 are known chelating agents and their metal complexes, which support the MOA for many of these compounds as closely linked with their capacity for chelation of biologically important metals. The characteristics of these compounds appear to affect their targeting specificity and, consequently, their antitumor activity. In general, however, enzymes involved in DNA synthesis appear to be the major targets of these metal complexes.

4. Compound structural features and putative MOA: reactive oxygen species and oxidative stress (regions N and P)

The metal compounds clustered in regions N and P are the most diverse throughout the SOM in both structural types and reactivity. The cytotoxicity response profiles of the compounds in these two regions are the most dissimilar when compared to other SOM regions. Regions N and P are adjacent to each other on the SOM and many metal complex structural motifs are shared between these two regions (see Table 3; Fig. 2, Panel C). Nevertheless, metal compounds having the same structural motif still exhibit similar cytotoxicity response profiles in general.

Regions N and P are enriched with redox active metal complexes, e.g., complexes of V, Cr, Mn, Fe, Co, Ni, Cu. The common MOA of these complexes seems to originate from their ability to catalyze the generation of ROS that oxidatively modify cellular components (lipids, proteins, and DNA), disturb the redox balance in the cell, and/or interfere with the redox-related cellular signaling pathways. Multiple mechanisms may be involved in the production of ROS. Fenton-like reactions may be commonly associated with most membranous fractions as mitochondria, microsomes, and peroxisomes. Phagocytic cells may be another important source of ROS [75].

Table 3
Structural motifs and possible biological targets and modes of action (MOAs) for metal and metalloid containing compounds clustered in SOM regions N and P

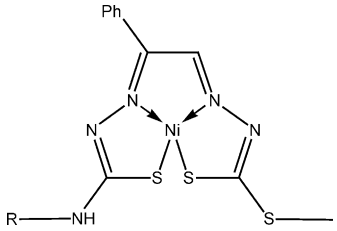
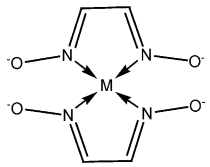
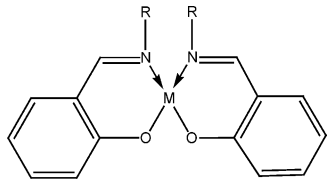
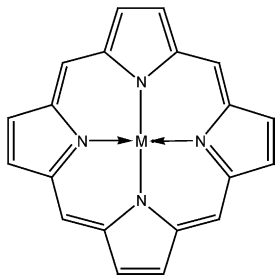
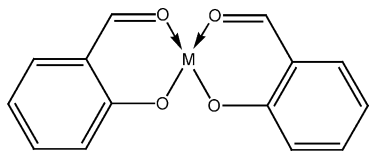
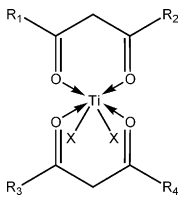
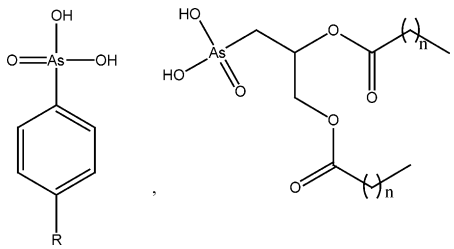
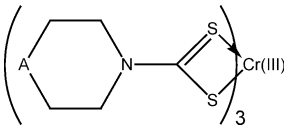
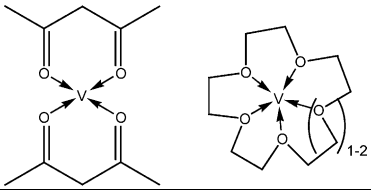
Compound name	Compound motif	Proposed targets
1. Nickel(II) bis(thiosemicarbazones)		IMP dehydrogenase
2. Metal dioximes (M = Co, Fe, Pd, Ti)		Autoxidation of NADH, coenzyme Q4H2, and cytochrome *c*; succinate-cytochrome *c* reductase
3. Metal salen complexes (M = Mn, Co, Cu)		ROS
4. Metal porphyrin complexes (M = Fe, Mn, Cu)		ROS, heme oxygenase
5. Metal salicylaldehyde complexes (M = Cu, Mn)		ROS
6. Bis(β-diketone) titanium(IV) complexes		ROS
7. Organo arsonic acids		ROS; pyruvate dehydrogenase; oxidative phosphorylation

Table 3 (*Continued*)

Compound name	Compound motif	Proposed targets
8. Chromium(III) tris(dithiocarbamates)		ROS
9. (β-Diketone) vanadium(V) and *n*-crown-*m*-vanadium(III) complexes (n = 15 or 18; m = 5 or 6)		ROS; protein tyrosine phosphatases, protein tyrosine kinases

4.1. Electron-transfer complexes and superoxide dismutase mimetics

Bis-chelate metal complexes of the general types [M–S_4] (Table 3, Entry 8), [M–O_4] (Table 3, Entries 5, 6,and 9), [M–N_4] (Table 3, Entries 2 and 4), [M–N_2S_2] (Table 3, Entry 1), and [M–N_2O_2] (Table 3, Entry 3), where M = Cr, Mn, Fe, Co, Ni, Cu, etc., have been long established as electron-transfer complexes (ETCs) [76]. Complexes of these types are mainly found in regions N and P. ETCs are capable of catalyzing ET reactions (redox reactions) and thereby may interfere with cellular redox-signaling pathways and oxidatively modify cellular components.

IMP dehydrogenase, which catalyzes the oxidation of inosine 5′-phosphate to xanthosine 5′-phosphate through the reduction of NAD^+ to NADH, has been proposed as a major target for complexes of the type [M–N_2S_2] (Table 3, Entry 1), where M = Cu(II), Ni(II), Zn(II), and Cd(II) [77]. These complexes are active against growth in a number of human tumor cell lines. Cobalt dioximes (Table 3, Entry 2) have been widely studied as coenzyme B_{12} models. Some of these complexes can interfere with biological electron transfer processes such as the autoxidation of NADH, coenzyme Q4H2, and cytochrome *c* [78]. The iron dioxime complexes have been found to inhibit succinate-cytochrome *c* reductase [79]. Cu(II)–dimethylglyoxime complexes exhibit superoxide dismutase-like activities and also behave as Fenton-type catalysts to generate hydroxyl radicals from the superoxide dismutation product, hydrogen peroxide (H_2O_2) [80].

$$2O_2{}^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

SOD1 : Cu/Zn, mainly located in cytosol SOD2 : Mn, mitochondria. (6)

$$H_2O_2 \xrightarrow{\text{CAT or peroxidases}} 2H_2O + O_2 \quad (7)$$

Superoxide dismutases (SODs) catalyze the dismutation of the superoxide radical ($O_2{\cdot}^-$) to H_2O_2 and molecular oxygen (O_2) (Eq. (6)), whereas catalase (CAT) and peroxidases catalyze the decomposition of H_2O_2 to water and O_2 (Eq. (7)). SODs, CAT, and peroxidases are important enzymes in a cell's defense against oxidative stress. ETCs that exhibit SOD-like activity often show antitumor activity. Fast proliferating cancer cells, which are active in energy metabolism, are under constant oxidative stress [81]. ETCs that catalyze $O_2{\cdot}^-$ dismutation will cause an increase in H_2O_2 concentration, which can react with cellular free iron and generate hydroxyl radicals (·OH) through the Fenton reaction (Eq. (8)). ·OH radicals are much more reactive and destructive than $O_2{\cdot}^-$, and are the major species that cause cellular damage. If the metals in these complexes are ''Fenton metals'' (e.g., Fe(II), Cu(I), Cr(II), Co(II), V(II), Ti(III)) themselves [82], then the metal complexes will dismutate $O_2{\cdot}^-$ in the first step of the reaction, and subsequently react with the reaction product H_2O_2 and produce ·OH radicals

$$\text{Fenton reaction} : \mathrm{Fe(II)} + \mathrm{H_2O_2} \rightarrow \mathrm{Fe(III)} + {}^{\cdot}\mathrm{OH} + \mathrm{OH^-} \quad (8)$$

Low molecular weight synthetic metal complexes that are SOD mimetics, including metal porphyrin complexes [83] and salen manganese complexes [84], have been developed, based on the implications of ROS in apoptosis and numerous pathological conditions. Metal salen complexes (Table 3, Entry 3) and porphyrin complexes (Table 3, Entry 4) are mainly located in regions N and P. Cobalt salen complexes are known as antioxidants against superoxide radicals [85]. Metalloporphyrin complexes of iron, manganese, and copper that exhibit SOD activity have been shown to possess antitumor activity and hence proposed as a new class of anticancer agents [86]. Conversion of intracellular $O_2{\cdot}^-$ to H_2O_2 by these complexes and subsequent Fenton-like reaction with H_2O_2 generating ·OH radicals is their proposed MOA.

Metalloporphyrins of metals such as Co, Zn, Cr, Mn, or Sn are known to function as competitive inhibitors of heme oxygenase [87]. The inducible isoform of heme oxygenase, HMOX1, is highly expressed in solid tumors in humans, including renal cell carcinoma [88] and prostate tumors [89], and animal models [90]. Inducible NO synthase (iNOS) is up-regulated in these models as well, to enhance exposure of tumor tissues to oxidative stress related to NO

and/or its reactive metabolites. Expression of HMOX1 is known to be induced by a variety of stress factors, including heat shock, UV irradiation, hydrogen peroxide, heavy metals, hypoxia, and NO. HMOX1 has been implicated to serve a key role in the tumor cell's adaptation to and/or defense against oxidative and other cellular stresses [91].

Bis-chelate Cu and Mn complexes of the type [M–O_4] also show SOD-like activity. Inhibitory effect of these compounds on lipid peroxidation has been observed initially, followed by an acceleration of lipid peroxide formation with time [92]. Both Cu(I) and Mn(II) are capable of catalyzing Fenton-like reactions, and the ·OH radicals thus produced may be responsible for the increased lipid peroxidation. These complexes are known to inhibit the growth of HCT-8 and HL-60 tumor cell lines. Examples of type [M–O_4] complexes found in N and P regions include the salicylaldehyde complexes of manganese and copper (Table 3, Entry 5). The first low molecular weight SOD mimetics described in literature are, in fact, copper complexes of the type [Cu–O_4] that show antitumor-promoting activity [93].

Thiram (tetramethylthiuram disulfide) and dimethyldithiocarbamic acid (DDC) have cytotoxicity response profiles similar to the SOD mimetic metal complexes. Thiram and DDC are known inhibitors of Cu/Zn superoxide dismutase (SOD1) and the proposed mechanism of enzyme inactivation is chelation of copper in the active site of SOD1 [94]. Thiram significantly is known to inhibit C6 glioma tumor development and reduce the metastatic growth of Lewis lung carcinoma.

The metabolic activity of cancer cells has been associated with uncontrolled cell proliferation and dysfunction of metabolic regulation mechanisms [81]. Low levels of free radicals accumulated in cancer cells are believed to stimulate cell proliferation and may contribute to tumor growth and drug resistance, whereas severe free radical accumulation leads to lethal damage [95]. Most cancer cells have an imbalance in anti-oxidant enzymes compared with normal cells [96]. In cancer cells, ROS levels can overwhelm the cell's anti-oxidant capacity, leading to irreversible damage and apoptosis [97]. In many instances, cells with increased complements of SODs prove to be hypersensitive to oxidative stress rather than being protected [98]. Reasons for the toxicity of high SOD levels are not clear; however, the compensatory effect of CAT suggests that overproduction of H_2O_2 from $O_2{\cdot}^-$ dismutation might be responsible [99]. An alternative explanation is that over-scavenging of $O_2{\cdot}^-$ by excess SODs may reduce radical chain termination and result in increased lipid peroxidation [100]. The overall intracellular balance of antioxidant enzymes may be more important in the cell's defense against oxidative stress than the contribution from any single component. Optimal protection is achieved only when an appropriate balance between the activities of these enzymes is maintained [98]. In general, the SOD mimetic metal complexes appear to act by perturbing cellular redox balance and by doing so contribute to cytotoxicity.

4.2. Bis(β-diketone) titanium(IV) complexes

Titanium (Ti) compounds are mainly (70%) located in sub-regions N_4, N_9 and P_9 on the GI_{50} SOM. Seventy-six percent of all Ti compounds are the bis(β-diketone)titanium(IV) complexes, which are also the most abundant metal complexes in the N and P regions (Table 3, Entry 6). Bis(β-diketone)titanium(IV) complexes are analogs of the titanium drug budotitane (*cis*-diethoxybis(-phenylbutane-1,3-dionato)titanium(IV)), which is the first non-platinum metal drug to reach clinical trials (phase I) [101]. The therapeutic target of budotitane derivatives is gastrointestinal tumors, but the MOA of titanium anticancer compounds is unknown.

The reduction potential of the Ti(IV)/Ti(III) redox couple is −0.055 V and the reduction potentials of cellular reductants, such as NADH, GSH, and ascorbate, range from −0.17 to −0.92 V. This means that the reduction of Ti(IV) to Ti(III) by cellular reductants is thermodynamically feasible. The redox potential for the $O_2/O_2{\cdot}^-$ couple is −0.33 V and for the $O_2{\cdot}^-/H_2O_2$ couple is +0.89 V; therefore, Ti(IV)/Ti(III) is also thermodynamically capable of catalyzing the dismutation of $O_2{\cdot}^-$ to O_2 and H_2O_2. Ti(III) is a Fenton metal as well and can activate H_2O_2 and generate ·OH [82]. Titanium compounds are thereby capable of initiating and sustaining a destructive catalytic cycle that produces ROS that can potentially cause cellular damage.

Titanium has a marked affinity for oxygen atoms; organic solutions of titanium complexes hydrolyze easily (budotitane in moist acetonitrile decomposes in a few seconds [101]). Complete hydrolysis of the β-diketone titanium complexes leads to total loss of ligands and formation of the water insoluble titanium dioxide (TiO_2). Supermolecular Ti–O chemistry may, therefore, contribute significantly to establishing the antitumor mechanism of the titanium compounds. Interestingly, TiO_2 itself has been shown to have antitumor activity. Ultrafine titanium dioxide (UF-TiO_2) particles have been shown to produce enhanced levels of ROS in human polymorphonuclear leukocytes and human or rat alveolar macrophages (AM) [102]. Exposure of UF-TiO_2 causes cytotoxicity in rat AMs, along with enhanced lipid peroxidation and increased rate of H_2O_2 generation [102]. Production of ·OH radicals has been observed in rat lungs exposed to UF-TiO_2 [103]. In a more recent report, iron-derived free radicals (ROS) have been implied as part of the mechanism of TiO_2-induced mitochondrial membrane potential changes, which in turn result in alveolar epithelial cell apoptosis [104]. Photoactivated TiO_2 particles significantly suppressed cancer cells in vitro and in vivo [105]. Several studies have provided unequivocal evidence for the

photoproduction of H_2O_2 by TiO_2 in aqueous solution, through the generation of $O_2{\cdot}^-$ under aerobic conditions [106]. SOD has been shown to enhance the efficiency of photocatalytic killing of cancer cells with TiO_2 particles, whereas the presence of ·OH and H_2O_2 scavengers, such as L-cysteine and catalase, hamper its antitumor activity [107].

In phagocytic cells, high levels of ROS are generated via activation of the NADPH oxidase immune reaction [75]. In the process of phagocytosis, electrons are transferred from NADPH to O_2, reducing it to $O_2{\cdot}^-$, which can subsequently dismutate to H_2O_2 and O_2 in the presence of H^+. Many research efforts have recently revealed that several cells, including vascular smooth muscle cells [108], fibroblasts (NIH 3T3) [109] and epidermoid carcinomas (A431), can induce transient ROS generation by growth factors and cytokines [110]. Specifically, NADPH oxidase-dependent ROS generation has been confirmed in non-phagocytic cells.

4.3. Arsenic compounds

Potassium arsenite ($KAs(III)O_2$) and organo arsonic acids (As(V); Table 3, Entry 7), mostly clustered in subregions N_2, N_7 and P_3, share similar cytotoxicity response profiles. Arsenic compounds may generate ROS during cellular metabolism. Production of ·OH radicals has been observed when organic arsenites (As(III)) (such as dimethylarsinic acid, which is a major metabolite of inorganic arsenicals) and arsenates (such as monomethylarsonic acid) are metabolized in rat liver resulting in oxidative stress [111]. Arsenic trioxide (As_2O_3) is also found to be very effective in the treatment of acute promyelocytic leukemia (APL) and is currently used as a treatment for certain forms of leukemia [112]. Arsenite alone has been shown to stimulate superoxide formation in vascular endothelial cells through activation of NADPH oxidase and increased extracellular accumulation of H_2O_2 [113]. A recent study [114] revealed that arsenite inhibits pyruvate dehydrogenase (PDH) activity in human leukemia cell line HL60 cells by elevating H_2O_2 production in mitochondria. This effect may cause accelerated production of hydroxyl radicals through the Fenton reaction that results in oxidative damage to PDH.

Arsenate (As(V)), a molecular analog of phosphate, enters the cell through phosphate transporters and inhibits oxidative phosphorylation [115]. Reduction of As(V) to As(III) is believed to be the first step in the metabolic toxification of As(V) in cultured cells, erythrocytes, and human liver cytosol. In addition, mitochondria have been proposed recently to function in reducing As(V) to As(III) [116]. Therefore, the cytotoxic MOA for the organo arsonic acids may involve a first step reduction to arsenites in mitochondria, and generation of ROS in the subsequent metabolism of arsenites. The long aliphatic tails on some of the arsonic acids may help their diffusion through cell membranes.

4.4. Chromium complexes

Most chromium complexes are located within and around regions N_2, N_6 and N_7. Both Cr(VI) and Cr(III) compounds cause increased production of ROS (by peritoneal macrophages and hepatic mitochondria) including $O_2{\cdot}^-$ and $^{\cdot}OH$ radicals, lipid peroxidation, DNA fragmentation and apoptotic cell death for in vitro and in vivo models [117]. Numerous studies have indicated that the cytotoxicity of Cr(VI) complexes originates from their cellular reduction to Cr(V) (e.g., by glutathione reductase in the presence of NADPH), which catalyzes the formation of ·OH radicals through a Fenton-like reaction [117]. Cr(II) is also known to catalyze Fenton-like reactions, and with appropriate ligands, the Cr(III)/Cr(II) redox couple can be positively shifted such that the intracellular reduction of Cr(III) to Cr(II) is possible. Association with ligands can also compensate the positive charge on the metal ion and help Cr(III) to pass through the plasma membrane.

Chromium(III) dithiocarbamates (Table 3, Entry 8) exhibit antimicrobial activities [118]. These complexes can exert their cytotoxicity in two ways: either through the chromium metal center or by providing dithiocarbamate ligands, which have high affinity for copper and can chelate the catalytic copper in the Cu/Zn enzyme SOD1. The reduction of chromium(III) tris(dithiocarbamates) gives chromium(II) bis(dithiocarbamates) [119]. The dithiocarbamate ligand released upon reduction of the chromium complex is then free to chelate other biological metal ions.

4.5. Vanadium compounds

The vanadium compounds map almost exclusively (9 out of 11) to the P region, mostly in the upper right corner. These compounds cover all three common oxidation states of vanadium, i.e., V(III), V(IV), and V(V). Simple oxovanadium compounds (e.g., sodium vanadate) as well as oxovanadium centers coordinated to organic ligands (Table 3, Entry 9) are known to mimic several biological functions of the regulatory peptide hormone insulin [120]. At physiological pH vanadium is found in the pentavalent oxidation state, which is the most stable form among its various oxidation states. Vanadyl(IV) is capable of undergoing spontaneous oxidation to vanadate(V) in vivo [121]. Oxovanadium compounds can activate H_2O_2 both in vitro and in vivo, forming peroxovanadium complexes [122].

Studies on the antitumor activities of vanadium compounds have been reviewed extensively [123]. Vanadium compounds seem to exert their antitumor effects mainly through inhibition of cellular protein tyrosine phosphatases (PTPs) and/or activation of protein tyrosine kinases (PTKs). Both effects activate signal transduction pathways, leading either to apoptosis and/or to activation of tumor suppressor genes. ROS generated by Fenton-like reactions

and/or during the intracellular reduction of V(V) to V(IV) by NADPH seem to contribute to the majority of vanadium-induced intracellular events. Peroxovanadates irreversibly inhibit PTPs by oxidizing the critical cysteine residue in their catalytic domain [124].

A uniform theme for the MOA of metal complexes in regions N and P appears to be oxidative stress from elevated hydrogen peroxide levels, which increases the production of hydroxyl radicals through Fenton or Fenton-like reactions, to subsequently cause enhanced lipid peroxidation and/or DNA damage.

5. Compound structural features and putative MOA: lipophilic ions (region M)

The organometallic complexes in the M region cluster almost exclusively in the lower half of sub-region M_2. A distinct feature of these complexes is their capability to produce ionic/charged species. The most representative class of complexes is a series of cyclopentadienylrhenium complexes that are either ionic compounds composed of the cationic $[(C_5H_5)(NO)(Ph_3P)ReL]^+$ moiety and one of the negatively charged counter ions, BF_4^- or PF_6^- (Table 4, Entry 1); or carboxylates of the type $[(C_5H_5)(NO)(Ph_3P)Re(-COOR)]$, where R = Me, Ph_3Ge, or Ph_3Sn (Table 4, Entry 2). The rhenium carboxylates are moisture sensitive and will easily hydrolyze to the anion $[(C_5H_5)(NO)(Ph_3P)Re(COO^-)]$ and cation R^+ in the presence of water [125]. Some ionic cyclopentadienylrhenium complexes have been tested against Ehrlich ascites tumor in mice and found to have a maximal cure rate of 100% [126].

Other agents clustered together with the rhenium species are mostly ionic compounds such as a series of triphenylphosphoniums ($[Ph_3PR]^+$, Table 4, Entry 3), tetraphenyl arsonium $[Ph_4As]^+$, and organoantimony bromides (Table 4, Entry 4) that can be easily hydrolyzed to give the cationic organoantimony moiety and Br^-. All these agents are lipophilic cations possessing a delocalized positive charge that can diffuse through cell membranes [127]. These compounds are generally referred to as DLCs, for delocalized lipophilic cations.

DLCs are concentrated into mitochondria by cells in response to their negative inside transmembrane potentials. DLCs selectively accumulate in carcinoma mitochondria because of their higher plasma and/or mitochondrial membrane potentials compared to normal epithelial cells, providing a basis for selective cancer cell killing [127]. Several of these compounds have already displayed some degree of efficacy as chemotherapeutic agents in vitro and in vivo. Preferential accumulation of phosphonium in non-small cell lung carcinoma in mice has been reported [128]. Cationic phosphonium salts selectively inhibit the growth of human pancreatic carcinoma-derived cells (PaCa-2) and Ehrlich Lettre Ascites cells (ELA) in vitro [129]. These agents are also shown to inhibit FaDu carcinoma cell growth by inhibiting mitochondrial respiration and ATP synthesis [130], and inducing lipid accumulation in the human breast cancer cell line DU4475 [131].

6. Correlations between cytotoxicity and gene expression

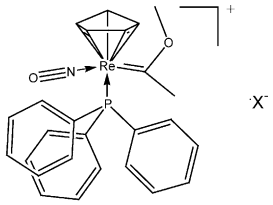
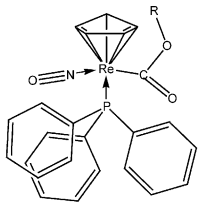
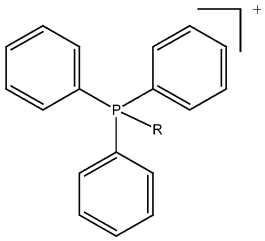
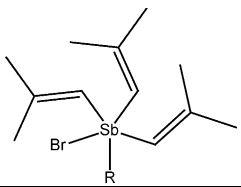
Approximately 10,000 constitutive gene expression measurements have been made available for each of the NCI_{60} tumor panel cells. Gene expression data complements the small molecule cytotoxicity data used previously to assign structure–activity relations, MOAs, and role of the cellular environment in cytotoxicity. The goal of a global drug–gene correlation analysis is to identify SOM regions having significant correlations ($p < 0.05$) with gene expression profiles (see Supplementary Information for global drug–gene correlation analysis methods). This procedure serves as an auxiliary method for validating a compound's MOA by identifying putative molecular targets (gene products) or biological pathways (many gene products) exhibiting correlated responses. Although simple one-to-one relationships between gene expression and cytotoxic responses are unlikely, the strongest correlations may indicate direct or indirect interactions between drugs and gene products.

6.1. Cysteine, glutathione-dependent protein activities in the Q-region (Fig. 2, Panel A)

The analysis of a set of 274 cysteine- or glutathione-dependent (GSH) protein gene expression profiles, which contain several gene classes, including disintegrins and metalloproteinases, alcohol dehydrogenases, calpains, caspases, cystatins, cathepsins, collagens, GSH peroxidases, GSH reductase, GSH synthetase, GSH *S*-transferases, metallothioneins, myosins, serine (or cysteine) protease inhibitors, tumor necrosis factor receptor superfamily members, thioredoxin reductases, ubiquitin-activating enzymes, ubiquitin-conjugating enzymes, ubiquitin specific proteases, and other cysteine-rich proteins, reveals that this class of genes is significantly correlated with cytotoxic response profiles in the bottom left corner (Q-region) and the middle portion (R-region) of the SOM. This result is consistent with the earlier finding that the Q-region is enriched with heavy metal complexes that have specifically high affinity for sufhydryl groups. A detailed discussion on gene classes showing strong correlations with Q-region cytotoxicity profiles can be found in a separate report [132].

The high numbers of heavy metal compounds in the Q-region and their preference for soft donor atoms (S or Se) suggest interference with cellular GSH or cysteine metabolism; making cysteine or GSH-dependent proteins and enzymes important targets for these compounds. On the other hand, strong inverse cytotoxicity-gene expression

Table 4
Structural motifs and possible biological targets and modes of action (MOAs) for organometallic compounds and phosphoniums clustered in the M_2 sub-region of the SOM

Compound name	Compound motif	Proposed targets
1. Cyclopentadienylrhenium salts	 X = BF_4^-, PF_6^-.	
2. Cyclopentadienylrhenium carboxylates	 R = Me, Ph_3Ge, Ph_3Sn.	Mitochondrial membrane permeability transition; mitochondrial electron transport phosphorylation
3. Triphenylphosphoniums		Mitochondrial respiration and ATP synthesis; acetylcholinesterase
4. Organoantimony bromides		

correlations are found with gene classes involved in cellular detoxification (e.g., thioredoxin and enzymes involved in GSH metabolism). This inverse correlation suggests a protective role for these enzymes and the potential for drug resistance to these agents. Metallothioneins (MTs) [133] are proteins particularly rich in cysteine residues (~30% of all MT amino acid residues) and have a high affinity for heavy metal ions such as Cd(II), Bi(III), Pt(II), Ag(I), Hg(II), Cu(I), and Zn(II). MTs are found preferentially in renal tissue where they play important roles in metal homeostasis, metabolism, and metal detoxification. MTs have also been found to play roles in cell proliferation and apoptosis, with MT over-expression associated with a high degree of tumor malignancy and poor chemotherapeutic outcome [133]. Three MT genes (MT1H, MT1L, MT2A) exhibit significant negative correlations with compounds in the Q-region. This negative correlation indicates that increased MT expression induces increased cellular resistance (insensitivity) to drug insults. MTs could provide cellular protection by sequestering and detoxifying heavy metal ions.

Also noteworthy is that a series of ubiquitin specific protease genes (USPs) exhibit an overall positive correlation (average *Z*-score[2] = 0.30) with Q-region agents, with USP16 having the highest *Z*-score value of +2.77 and USP4 the second highest of +2.43. USPs are deubiquitinating enzymes (DUBs) that belong to the family of cysteine proteases. DUBs are involved in the regulation of the ubiquitin-dependent pathway and are associated with the 26S proteasome. DUBs are also implicated in various important biological pathways including cell growth and differentiation, development, oncogenesis, neuronal diseases, apoptosis, chromosome structure and transcriptional regulation [134]. The fact that the DUB gene family contains both oncogenes and tumor suppressor genes also makes them potential anticancer drug targets. The cyto-

[2] See Supplementary Information for method details.

toxic response profiles of the peptide boronic acid family (Table 1, Entry 8) of proteasome inhibitors [135] fall in the Q-region. This is consistent with the finding that the USPs, which are involved in regulating the ubiquitin-proteasome pathway, show significant positive correlation with Q-region agents and provide an indication that these compounds can interfere with USP activities. Boronic acids inhibit the chymotrypsin-like activity of the proteasome by covalently binding to the threonine residue located near the active site of the β-subunit. Compounds found in the Q-region that have sulfhydryl-targeting capability could inhibit USP activities by binding to the active site cysteine residues.

6.2. Iron- and copper-related protein activities in S_1 and S_2 (Fig. 4)

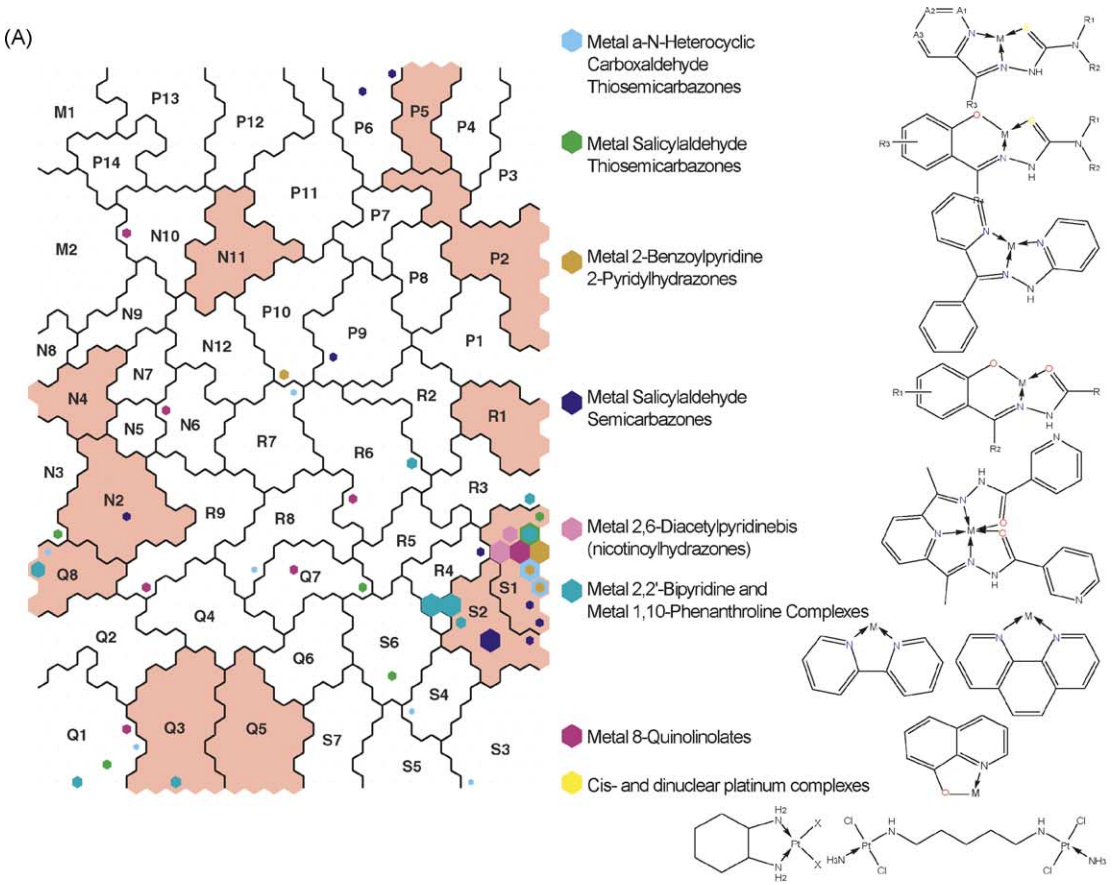
There are 38 copper-related and 93 iron-related genes (Cu or Fe-containing, binding, or transporting) with available microarray gene expression measurements. The copper-related genes show significant correlation with only agents whose cytotoxic response profiles fall in S_1, Q_7, and R_6 (Fig. 4, Panel B). This is consistent with the fact that the cytotoxic response profiles of most copper chelates fall in S_1. A large number of chelators in S_1 contain sulfur as donor atoms with high affinity for copper. Q_7 and R_6 contain bidentate chelators with two sulfur donor atoms. Proteins involved in copper homeostasis and enzymes utilizing copper as a cofactor are therefore potential targets of these copper-chelating agents. The average expression profile of the set of iron-related genes is significantly correlated with agents in both S_1 and S_2 (Fig. 4, Panel A). S_2 has considerably less sulfur containing agents than S_1, but more oxygen containing chelators, including the well-known iron chelators [46] desferrioxamine (DFO), 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone (NIH) and 1,10-phenanthroline. Chelators in both S_1 and S_2 have selective affinity for iron; iron homeostasis and iron-dependent enzymes may therefore be their potential targets.

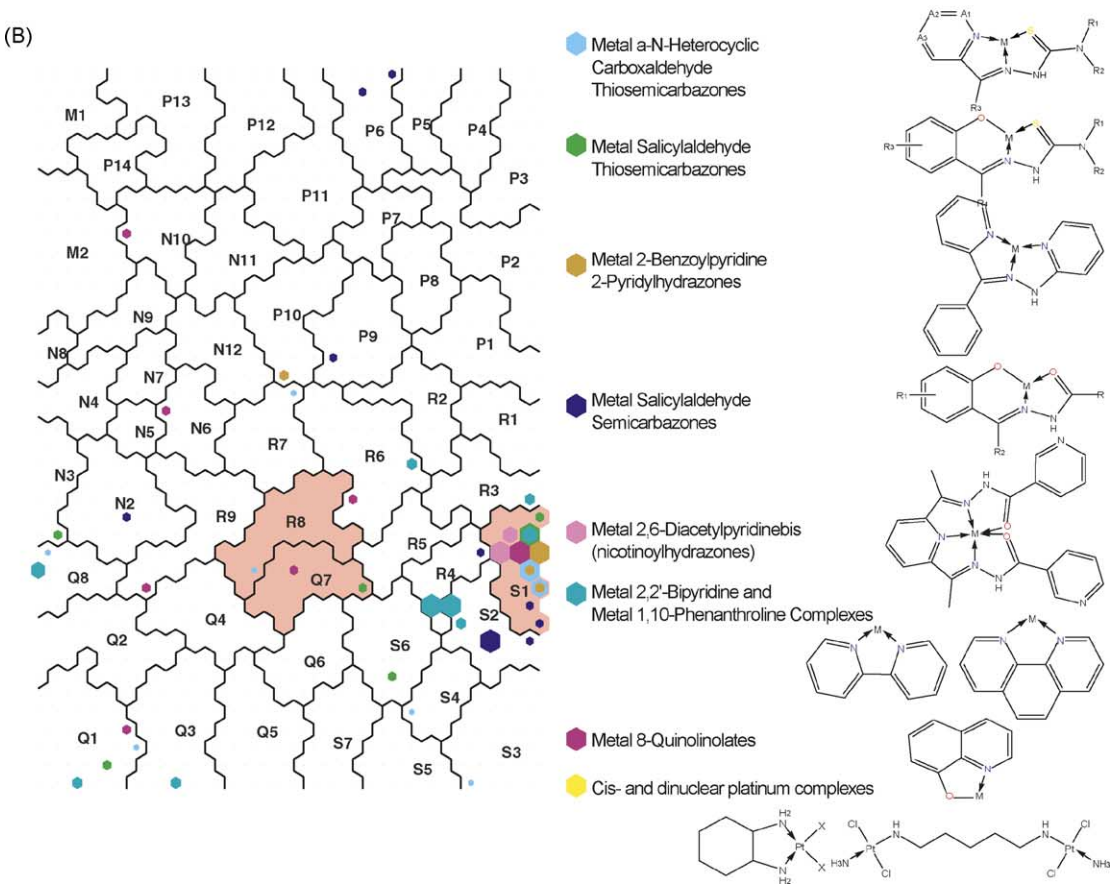
A number of iron–sulfur (Fe–S) proteins, which use Fe–S clusters as co-factors, show significant correlations with agents whose cytotoxic response profiles fall in S_1 and S_2. The iron-regulatory proteins (IRPs) post-transcriptionally control the expression of molecules that play essential roles in iron homeostasis. The IRPs bind to hairpin-loop structures called iron-responsive elements (IREs). Intracellular iron levels regulate the binding of the IRPs to the IREs via different mechanisms [136]. IRP1 binds a [4Fe–4S] cluster at high cellular iron levels with a loss of IRE-binding activity, whereas in the cells deprived of iron, the [4Fe–4S] cluster is not present and IRP1 can bind to the IRE. The IRP1 gene, ACO1 (aconitase 1), exhibits significant negative correlation with the cytotoxicity profiles for agents in S_1 and S_2 (average Z-score = −2.90). IRP2, on the other hand, does not contain a [4Fe–4S] cluster and is degraded in iron-depleted cells. The IRP2 gene, IREB2, in contrast to ACO1, shows a strong positive correlation with most agents in S_1 and S_2 (average Z-score = +1.93). Another Fe-S protein gene, NADH-ubiquinone oxidoreductase (NADH-coenzyme Q reductase), part of the mitochondrial complex I, also exhibits significant positive correlation (average Z-score = +2.07) with the S_1/S_2 compounds. Genes of other important iron-regulatory proteins found to have considerably strong correlations with agents whose cytotoxic profiles fall in S_1 and S_2 include the heavy chain of ferritin (FTH1, average Z-score = −1.50), transferrin receptor 2 (TFR2, average Z-score = 0.91), transferrin (TF, average Z-score = −0.83) and lactotransferrin (LTF, average Z-score = −0.83).

S_1 is the most copper compound-concentrated sub-region on the SOM; accordingly, significant correlations are observed for a number of copper binding and copper transporting proteins. Metallothioneins (MTs) can bind Cu(I) in vivo under physiological conditions in mammals and may play a role in copper detoxification. MT2A, the most highly expressed gene in humans, exhibits significant negative correlations (Z-score = −2.97) with compounds in S_1. Copper transporting proteins that show negative correlations with the S_1 region agents may participate in cellular resistance. These genes include the Cu(II)-transporting ATPase (ATP7A, Z-score = −1.55) and the solute carrier family 31 copper transporters (SLC31A1, Z-score = −0.96). Ceruloplasmin (Cp) [137] is a copper-binding protein primarily found in plasma. Cp has several important functions including copper transport and homeostasis, and angiogenic activity. Elevated serum copper levels have been found in several cancers and the degree of copper increase is associated with metastatic disease and the poorest prognosis. The increase in serum copper has been correlated to an increase in Cp concentration. A cytoprotective effect against the S_1 sub-region agents is also observed for the Cp gene (Z-score = −0.90). Increased cellular sensitivity is observed with increased expressions of the copper-containing amine oxidase (ABP1, Z-score = +1.70) and the copper-dependent dopamine β-monooxygenase (DBH, Z-score = +2.54), which could be potential targets for the S_1 region copper chelating agents.

6.3. Oxidoreductase activities in regions N and P (Fig. 2, Panel C)

Increased constitutive expression of genes involved in oxidoreductase activity is directly linked to a protective measure against drug insults that result in oxidative stress. The analysis of a set of 235 oxidoreductase genes with mRNA expression measurements reveals that the oxidoreductase genes show significant inverse correlations, compared to the complete set of ∼10,000 genes, within most of regions N and P (see Fig. 2, Panel C), largely overlapping with the sub-regions where the metal complexes are located. The average correlation of the oxidoreductase genes is up to ∼30% stronger than the average correlation


(A)
Metal a-N-Heterocyclic Carboxaldehyde Thiosemicarbazones
Metal Salicylaldehyde Thiosemicarbazones
Metal 2-Benzoylpyridine 2-Pyridylhydrazones
Metal Salicylaldehyde Semicarbazones
Metal 2,6-Diacetylpyridinebis (nicotinoylhydrazones)
Metal 2,2'-Bipyridine and Metal 1,10-Phenanthroline Complexes
Metal 8-Quinolinolates
Cis- and dinuclear platinum complexes
M1 M2 N1 N2 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11 P12 P13 P14
R1 R2 R3 R4 R5 R6 R7 R8 R9
Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8
S1 S2 S3 S4 S5 S6 S7


(B)
Metal a-N-Heterocyclic Carboxaldehyde Thiosemicarbazones
Metal Salicylaldehyde Thiosemicarbazones
Metal 2-Benzoylpyridine 2-Pyridylhydrazones
Metal Salicylaldehyde Semicarbazones
Metal 2,6-Diacetylpyridinebis (nicotinoylhydrazones)
Metal 2,2'-Bipyridine and Metal 1,10-Phenanthroline Complexes
Metal 8-Quinolinolates
Cis- and dinuclear platinum complexes
M1 M2 N1 N2 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11 P12 P13 P14
R1 R2 R3 R4 R5 R6 R7 R8 R9
Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8
S1 S2 S3 S4 S5 S6 S7

of other genes in these regions. This is rarely observed in any of the other SOM regions.

Among the oxidoreductases, superoxide dismutases (SODs) are responsible for scavenging superoxide radicals, whereas catalases and peroxidases are scavengers of H_2O_2 and other peroxo species. SODs, catalases, and peroxidases are important enzymes in a cell's defense against oxidative stress. Catalase exhibits protective properties (average *Z*-score = −0.81) against agents whose cytotoxic response profiles fall in the N and P regions. Catalase shows a particularly strong protective effect against agents in N_4 (*Z*-score = −2.21), P_3 (*Z*-score = −3.62) and P_4 (*Z*-score = −3.63), where some of the β-diketone titanium (Table 3, Entry 6), metal salicylaldehyde (Table 3, Entry 5), arsonic acids (Table 3, Entry 7) and all the vanadium complexes (Table 3, Entry 9) are located.

The selenoenzyme glutathione peroxidase (GPx) is a major peroxide scavenging enzyme. GPxs catalyze the reduction of hydroperoxides or peroxynitrite at the expense of GSH [138]. Four GPx genes, cellular (GPX1), gastrointestinal (GPX2), plasma (GPX3) and phospholipid hydroperoxidase (GPX4), all exhibit significant negative correlations (average *Z*-scores are −0.83, −1.30, −1.78 and −1.20, respectively) with the cytotoxic profiles of N- and P-region compounds. This is consistent with the earlier speculation that increased constitutive expression of these genes confers a protective measure against the agents in N and P that cause elevated levels of H_2O_2 and oxidative stress. Peroxiredoxins catalyze the reduction of H_2O_2 and organic hydroperoxides. In eukaryotic cells, they are both antioxidants and regulators of H_2O_2-mediated signaling [139]. The strong negative correlations of the cytoplasmic (PRDX1, *Z*-score = −106) and mitochondrial (PRDX3, *Z*-score = −1.61) peroxiredoxins with the cytotoxic response profiles of compounds in N and P reinforce the protective effect of peroxide scavengers against oxidative stress induced by these agents.

6.4. DNA binding and mitochondrial protein activities (Fig. 2, Panels B and D)

The S-region has previously been established to comprise compounds affecting the metabolism of nucleic acids [8]. In addition, enzymes involved in DNA synthesis are implied in earlier discussions as major targets of the metal chelates whose cytotoxic response profiles fall within subregions S_1 and S_2. To confirm this point, expression profiles of 380 DNA binding protein genes, including the families of transcription factors, histone family members, ribonucleoproteins, DNA ligases, minichromosome maintenance deficient proteins (MCMs), DNA and RNA polymerases, replication factors, regulatory factors, topoisomerases, zinc finger proteins and various other DNA binding proteins, have been analyzed for their gene expression correlations across the SOM. The gene expression profiles of these proteins are shown to be significantly correlated with all sub-regions of S (except for S_6) and all Q sub-regions (Fig. 2, Panel B). This provides additional evidence that DNA synthesis related proteins are possible targets of many S_1/S_2 region compounds. DNA replication and repair genes have previously been shown to be significantly correlated with Q-region response profiles using a different analysis procedure [132].

Mitochondria have become major targets of anticancer research due to their critical role in the regulation of several aspects of cell biology such as energy production, molecular metabolism, redox status, calcium signaling and apoptotic cell death [140,141]. Mitochondria are major generators of reactive oxygen species (ROS). Considerable interest exists in utilizing the therapeutic potential of manipulating mitochondrial regulation of apoptosis by enhancing mitochondrial membrane permeability. A set of 276 mitochondria-related gene expressions is retrieved and analyzed across the SOM. This set of genes includes the families of ATP-binding cassettes, acyl-coenzyme A dehydrogenases, aldehyde dehydrogenases, ATP synthases, cytochrome *c* oxidases, cytochromes, mitochondrial ribosomal proteins, NADH ubiquinone dehydrogenases (complex I), pyruvate dehydrogenases, solute carrier families (mitochondrial carriers), succinate dehydrogenase complexes, succinate-CoA ligases, ubiquinol-cytochrome *c* reductases, and other mitochondrial regulatory proteins.

The 276 gene expression profiles exhibit significant correlations with compounds whose cytotoxicity profiles fall in the M region and most of the R, P, and N regions (Fig. 2, Panel D). This is consistent with the finding that cytotoxic activities of metal/metalloid compounds, whose response profiles are clustered in regions N and P, originate from their ability to generate ROS and disturb cellular redox balance. This is also an indication that the mitochondria may be potential targets for some of these compounds. The lipophilic ions in M_2 (see Table 4; Fig. 2, Panel D) have been shown to selectively accumulate in carcinoma mitochondria and cause mitochondrial toxicity, which may contribute to the strong correlation of these compounds with observed mitochrondrial protein gene expressions. The M region agents show an overall positive

Fig. 4. Metal containing compound motifs mostly clustered in SOM sub-regions S_1 and S_2. Colored hexagons correspond to clusters containing compounds within structural motifs listed in Table 2. Hexagons are sized proportional to the number of compounds within each cluster. Backgrounds colored in rosy red show regions significantly ($p < 0.05$) correlated with expressions of a specific class of genes. Copper chelating agents are associated with genes involved in copper homeostasis and enzymes containing copper as a cofactor, and iron-related/dependent genes are correlated with chelators that have a selective affinity for iron. Panel A: structural motifs clustered in sub-regions S_1 and S_2 show significant correlation with gene expression patterns from a family of iron-dependent proteins (93 measurements). Panel B: structural motifs clustered in sub-region S_1 show significant correlation with gene expression patterns from a family of copper-dependent proteins (38 measurements).

correlation with the NADH ubiquinone dehydrogenases (NDUFs, complex I) (average *Z*-score of 15 NDUF genes is +1.00), which means that increased expression of NDUFs causes increased cellular sensitivity to drug insults. Even though little is known about the R-region agents, cytotoxic responses of well-known antitumor compounds and inhibitors of mitochondrial complex I (e.g., acetogenins [142]) fall in the R-region. The significant correlations between mitochrondrial gene expressions and R-region agents support the mitochondrial targeting capability for these agents.

The redox control of mitochondrial permeability transition, which plays a critical role in mitochondrial regulated apoptosis, may depend upon the mitochondrial thioredoxin reductase/thioredoxin system [30]. Auranofin and other phosphinegold(I) drugs (see Table 1, Entry 3), whose cytotoxic profiles fall mainly into sub-region Q_1, have been shown to induce mitochondrial permeability transition through the inhibition of mitochondrial thioredoxin reductase [30]. This finding is consistent with the observation that mitochondrial gene expressions are mostly associated with compounds in sub-region Q_1, and provides a linkage among these compounds and the thioredoxin reductase/thioredoxin system, mitochondrial permeability transition and drug cytotoxicity. Thioredoxin reductase/thioredoxin gene expressions and Q-region agents have been discussed in greater detail in a separate report [132].

The gene-drug correlation analyses presented here provide a global view of how clustering according to cytotoxic response profiles may be used to propose MOAs related to different gene classes or protein families. These analyses also provide additional support for the speculated MOA of drugs in each different SOM region based on their structure and reactivity. These results may serve as a guide for discovery of drugs that target specific biological pathways.

7. Role of metal versus coordination ligand in dictating cytotoxicity response

SOM clustering analysis of the differential cytotoxic response profiles of over 1100 metal/metalloid compounds segregates them into four broad classes, each consisting of specific structural motifs and possessing a variety of MOAs: heavy metal complexes that have high affinity for sulfhydryl groups (Q), metal chelates (S_1/S_2), metal/metalloid compounds that can induce oxidative stress (N/P), and metal/metalloid compounds that can produce lipophilic ions (M_2). In addition, the global differential gene expression analysis reveals classes of genes that are significantly correlated to drugs that fall within these specific MOA classes. The cytotoxic activity of a metallo drug is shown to be determined by both the type of metal and its organic component, i.e. the ligand of the metal. In many cases, one appears to be the dominating factor over the other.

The metal compounds in the Q-region are structurally diverse (see Table 1); however, they exhibit similar cytotoxic response profiles because they all have one property in common, that is, they are all complexes of heavy metal atoms. The type of the metal is therefore the dominating factor in determining the cytotoxicity of these compounds. The metal complexes in S_1 and S_2, on the other hand, show close similarities in both structure and cytotoxic response profiles. They are all chelation complexes of chelating agents (see Table 2) that share clear structural similarities; the type of metal is, however, varied. In this case, it appears that the cytotoxicity is determined primarily by the organic component of the complex (the ligand) and the type of metal is only playing a minor role. The metal/metalloid compounds in regions N and P are the most structurally diverse in both the type of metal and coordination ligands (see Table 3). The metal and the ligand are therefore both important factors in dictating cytotoxic response. The cytotoxic activity of the M-region metal compounds appears to originate from their ability to form lipophilic charged species (see Table 4). These metals are generally well shielded by large lipophilic ligands and the metal type does not seem to play as crucial a role in cytotoxicity.

Cytotoxic response profiles of complexes of early transition metals, such as Ti, V, and Cr, which have high affinity for oxygen donor atoms, are clustered mostly in regions N and P (see Fig. 1). Compounds of middle or late first-row transition metals, such as Mn, Fe, Co, Ni, Cu, and Zn, which can have preferential affinity for either oxygen, nitrogen, or sulfur donor atoms depending on the oxidation state of the metal, are clustered most tightly in S_1, S_2, and less so, in N and P. Compounds of second- and third-row transition metals and post transitional metals, which have high affinity for soft donor atoms such as sulfur and selenium, are mostly found in the Q-region, and secondarily in R. Compounds of platinum, a third-row transition metal, are mostly found in either Q or S (mostly S_3 and S_5) depending on the ligand configuration of the compound, which may dictate whether the primary drug cytotoxicity is from targeting sulfhydryl groups (Q) or cross-linking DNA (S).

7.1. Fe versus Cu: ligand property dictates target selectivity

S_1 and S_2 contain mostly chelating agents and chelates of various metal complexes. Metallo enzymes or proteins, especially iron- and copper-dependent proteins, appear to be the major targets of these compounds. Iron is the most abundant transition metal in the human body, which contains approximately 4.2 g based on a standard body mass of 70 kg [143]. On the other hand, copper forms the most stable coordination complexes within the first-row divalent transition metals according to the Irving-Williams order [144]. Iron is an essential element involved in oxygen delivery, oxygen sensing, electron transfer, energy metabolism, and DNA synthesis. Iron metabolism is altered in

cancer. A majority of studies have identified perturbations consistent with an increased requirement of iron for rapidly proliferating tumor cells. Copper, on the other hand, is known to regulate growth factor production associated with angiogenesis [45]. Abnormally high serum copper levels have long been observed in solid tumors and copper depletion therapy has shown encouraging results in inhibiting angiogenesis and/or tumor growth [145]. Extensive research efforts have thus been dedicated to the design of iron or copper chelators as anticancer therapeutic agents [44,46,65,145,146].

Interestingly, compounds in S_1 and compounds in S_2 can be differentiated roughly based on whether iron or copper is their major target (S_1: copper; S_2: iron). Chelators that contain sulfur donor atoms have a selectively higher affinity for copper over iron (up to 100 times) whereas chelators with oxygen donor atoms generally have a higher affinity for Fe(III) over Cu(II) or Cu(I). S_1 and S_2 contain a similar number of compounds, 361 in S_1 and 350 in S_2; however, S_1 is more enriched in sulfur containing compounds than S_2 (S_1: 47%; S_2: 33%) and S_2 is more enriched in oxygen containing compounds than S_1 (S_1: 58%; S_2: 85%). Also, S_1 has slightly more nitrogen containing compounds than S_2 (S_1: 92%; S_2: 88%). Nitrogen has a measure of softness between oxygen and sulfur, but still has higher affinity for copper than iron [65]. This clearly indicates that the chelating agents in S_1 are more likely to target copper whereas those in S_2 are more likely to target iron. Desferrioxamine (DFO), the most widely used iron chelator that uses hydroxamate oxygen donor atoms, is located in S_2. The cytotoxic profile of neocuproine, a selective Cu(I) chelator with nitrogen donor atoms [66], falls expectedly into S_1.

7.2. Type of metal dictates cytotoxicity response

7.2.1. Metal complexes of 2,2′-bipyridine (bpy) and 1,10-phenanthroline (phen) based ligands

The metal complexes with *bpy* or *phen* derived ligands (see Table 2, Entry 6) are very similar structurally; however, their cytotoxic response profiles are clustered into two major groups, R_4/S_2 and N/P. The compounds found in R_4/S_2, which are generally associated with nucleic acid targeting agents, are composed mainly of metals that do not generate ROS (e.g., trivalent rare earth metals). Their cytotoxic response profiles are very similar to those of a 2,2′,2″-terpyridine platinum complex, which is well known to be involved in DNA intercalation and topoisomerase I inhibition [67]. Many octahedral complexes of Ru(II), Re(I), Rh(III), and Co(III) have been shown to bind DNA intercalatively, including Ru(II) complexes of *phen*, quinonediimine and their derivatives [68].

The *bpy* or *phen* complexes found in N or P, in contrast, are composed mainly of redox-active metals (e.g., Cu), which can catalyze ROS-generating reactions and cause oxidative damage to cellular components. The *phen* complexes of Cu(II) are known to induce significant cellular oxidative stress and promote membrane lipid peroxidation [147]. They can catalyze air oxidation of NADH, hexahydroubiquinol and cytochrome *c*, inhibit NADH oxidation and oxidative phosphorylation, and interfere with mitochondria respiratory activity [148]. Evidence has been presented in support of an electron transfer mechanism for the antitumor *bpy* and *phen* complexes of copper and iron [149]. Metal-free *bpy* and *phen* ligands are mostly found in S and R as general chelating agents.

7.2.2. Tropolone complexes (Table 1, Entry 5)

Tropolones are bidentate chelating agents with two oxygen donor atoms. The antitumor activity of tropolone derivatives has been related to iron chelation in the active site of ribonucleotide reductase [150]. Tropolone is best known as one of the most potent and selective inhibitors of tyrosinase [151], an oxidase that utilizes two copper ions as cofactors, with tropolones acting to chelate the copper ions required by the enzyme. Tropolone is also known to inhibit phenoloxidase, another copper enzyme [152]. The cytotoxic response profiles of metal-free tropolones and some tropolone complexes of Bi(III) and rare earth metals fall expectedly in the S_1 region where the chelating ability of the ligand is probably the predominant factor in dictating cytotoxicity. Most of the heavy metal tropolone complexes (e.g., those of Bi, Sn, Tl), however, fall in the Q region, where the type of the metal, rather than the chelating ability of the ligand, plays the dominant role in determining the compound's cytotoxicity response. All tropolone metal complexes are structurally very similar.

7.2.3. Metal dithiocarbamates

The metal complexes of dithiocarbamates are similar structurally but exhibit different cytotoxic response patterns depending on the type of metal. The metal-free dithiocarbamate ligands are bidentate ligands with sulfur donor atoms and show similar cytotoxic response to their metal complexes. Dithiocarbamates can act in two different ways: they can either react with sulfhydryl groups and form disulfide bonds, or chelate metals, especially copper, because of its high affinity for sulfur-containing ligands. The reactivity of a series of *N*,*N*-diethyl-dithiocarbamates (sulfenamide type: $R_1R_2N(C=S)SNR_3R_4$) has been tested against cysteine and GSH [153]. Some of the most reactive compounds showed moderate to powerful tumor growth inhibitory properties against several cancer cell lines in vitro. Another series of dithiocarbamates (disulfide type: $R_2N(C=S)SS(C=S)NR_2$) have been found to inactivate a zinc finger protein by chemically modifying the cysteine groups (forming disulfide bonds) in the zinc-binding domains [154]. The other well-known biological activity of dithiocarbamates (disulfide type and free type: $R_2N(C=S)S^-$) is copper chelation, which has been discussed earlier in the section on regions N and P.

A dual cytotoxic activity is also observed for the metal complexes of dithiocarbamates: they can act either through the metal, or by providing the dithiocarbamate ligands. The cytotoxic response profiles of the dithiocarbamate complexes with redox active metals (Table 3, Entry 8), such as Cr and Co, are clustered mostly around the N region where redox activities are considered the primary cause of cytotoxicity, whereas those of the heavy metal complexes (Table 1, Entries 4 and 6), such as Bi and Sb, are mainly located in the Q-region. The metal dithiocarbamates can also act by providing their dithiocarbamate ligands, which themselves can chelate biologically important metals such as copper. A number of dithocarbamate complexes, including those of copper, are found in S_1, where copper chelation is a major contribution to drug cytotoxicity.

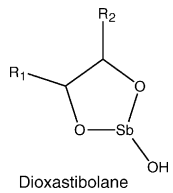
7.3. Ligand dictates cytotoxicity response

7.3.1. Antimony compounds

Three different classes of antimony compounds, with three different types of ligands (organic components), show anticancer activity. These three compound classes are structurally distinct and exhibit different cytotoxic responses as well. The cytotoxic profiles of the organoantimony bromides (Table 4, Entry 4) are grouped in M_2 because of their ability to hydrolyze and yield the charged lipophile $[R_4Sb]^+$. The identity of the metal itself is not as important in determining the cytotoxic activity of the compound as a whole. The antimony in these compounds is Sb(V) and they are true organometallic compounds with direct metal–carbon bonds.

The second class of antimony compounds is the antimony dithiocarbamates (Table 1, Entry 6) whose cytotoxic response profiles fall in the Q-region. These are coordination compounds of Sb(III) where the dithiocarbamate ligands could dissociate in solution, giving free dithiocarbamates and the Sb(III) ion. Both antimony and the dithiocarbamate ligands are capable of reacting with biological sulfhydryl groups, where the metal could bind to cysteine residues and the dithiocarbamates could form disulfide bonds. Free dithiocarbamates can also act as chelators of biologically important metals, including copper and zinc.

The third class of antimony compounds is the dioxastibolanes (or cyclic antimonates) (see common structural motif shown below), which are coordination compounds of Sb(III) or Sb(V) with galactonic acid (stibogluconates) or tartaric acid (antimony tartrates). Galactonic acid and tartaric acid are bidentate chelating agents with oxygen donor atoms. There are three examples of antimony compounds of this class, which exhibit dissimilar cytotoxic response profiles located in regions N, P, and Q. Stibogluconates and antimony tartrates have been used as anti-infective agents and have been shown to bind to the two sulfhydryl groups of trypanothione [155].



Dioxastibolane

Even though all three classes of these compounds contain antimony, they exhibit distinct cytotoxic response profiles, due apparently to the differences in the nature of their coordination ligands. Other good examples of complexes containing the same metal but exhibiting different cytotoxic responses are the platinum complexes. Their cytotoxic response profiles fall either in the Q or S region, depending on the ligand type and configuration. The structural difference between the two types of platinum compounds is evident in that compounds in Q have bulky

Table 5
NSC number, metal type, SOM location, common name and therapeutic category of metal containing compounds in the SOM clustering that also appear in the Know Drugs database (Leadscope®)

NSC	Metal	SOM region	Drug name	Therapeutic category
NSC625324	Ag	Q_2	Silver sulfadiazine	Anti-infective
NSC313981	Au	Q_1	(Triethylphosphine)gold chloride	Antirheumatic
NSC321521	Au	N_3	Auranofin	Antirheumatic
NSC 118051	Bi	Q_1	Tris(diethyldithlocarbamato)bismuth(III)	Immunologic adjuvants; antiviral
NSC 192965	Ge	N_{10}	Spirogermanium	Antimalarials; antineoplastic
NSC241240	Pt	S_3	Carboplatin	Antineoplastic
NSC255917	Pt	S_5	*Cis*-dichloro(l,2-diaminocyclohexane)platinum(II)	Antineoplastic
NSC265459	Pt	S_5	*Cis*-dichloro(1,2-diaminocyclohexane)platinum(II)	Antineoplastic
NSC265460	Pt	S_5	*Cis*-dichloro(l,2-diaminocyclohexane)platinum(II)	Antineoplastic
NSC266047	Pt	S_5	1,2-Diaminocyclohexane platinum(II) malonate	Antineoplastic
NSC266046	Pt	S_5	Oxaliplatin	Antineoplastic
NSC363811	Pt	S_5	Tetraplatin; dexormaplatin	Antineoplastic
NSC363812	Pt	S_5	Tetraplatin; dexormaplatin	Antineoplastic
NSC363813	Pt	S_5	Tetraplatin; dexormaplatin	Antineoplastic
NSC256927	Pt	S_2	Iproplatin	Antineoplastic
NSC41809	Sb	S_6	Anthiolimin	Antheimintics, schistosomicides
NSC2604	Sn	Q_8	Dibutyldichlorotin	Antineoplastic and immunosuppressive
NSC68093	Zn	Q_7	Zinc pyrithione	Keratolytic

trans-configured ligands and compounds in S have *cis*-configured ligands or dinuclear platinum complexes. The basis for selective targeting lies in finding the right metal and ligand combination.

8. Known drugs containing metal ions

A wide variety of metal-based drugs have been approved by the FDA for clinical use or evaluated in clinical trials. The Known Drugs database compiled by Leadscope® contains ~13,000 compounds reported in the period of January 1982 to December 2002, 1080 of which contain metal ions or metalloids. Forty-four different metal/metalloid elements, ranging from alkali (Li, Na, K) and alkaline earth metals (Mg, Ca) to various transition metals (Ag, Au, Cd, Co, Cr, Cu, Fe, Hg, Ir, Mn, Mo, Nb, Ni, Pd, Pt, Re, Ru, Ta, Ti, V, W, Zn, Zr), heavy post transitional metals (Bi, Ga, Ge, Pb, Sb, Sn, Tl), other main group metal (Al) and metalloids (As, B, Se, Si), to rare earth metals (Ce, La, Nd, Sm) are found among these compounds. One hundred and forty-one of these metal-based drugs (twelve of which belong to the "antineoplastic and immunosuppressive" therapeutic category), covering seventeen different elements, have, at some time, been approved by the FDA for over-the-counter or clinical use.

Out of the metal compounds in the NCI_{60} cell screen that exhibit differential anticancer activity, only 18 compounds (not including those of alkali or alkaline earth metals, and Si or Se) appear in the Leadscope® Known Drugs database (see Table 5). The location of these known metal drugs on the SOM is shown in Fig. 5. Ten of the 18 drugs, not surprisingly, are those of platinum and are analogs of cisplatin. These platinum-based known drugs are targeting

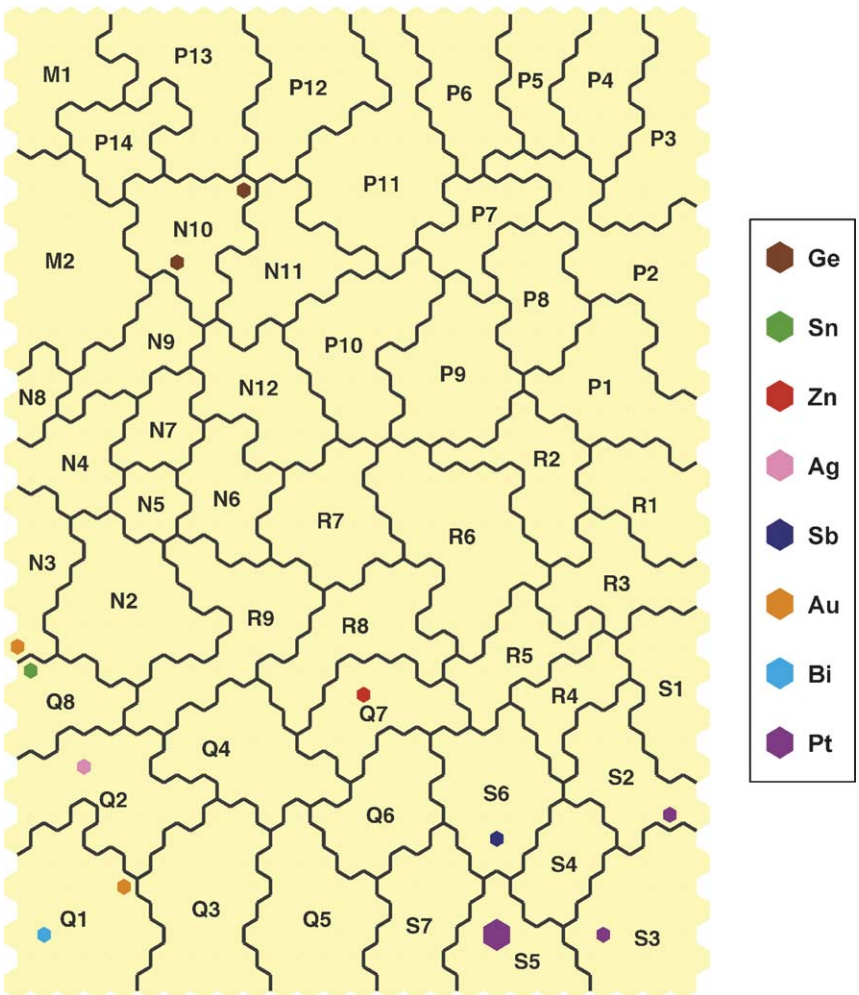


Fig. 5. Metal containing compounds in the SOM that also appear in the Leadscope® Known Drugs database. The Known Drugs database compiled by Leadscope® contains ~13,000 compounds reported in the period of January 1982–December 2002, 1080 of which contain metal ions or metalloids. Out of the metal compounds in the NCI_{60} cell screen that exhibit differential anticancer activity, 18 (not including those of alkali or alkaline earth metals, and silicon or selenium) are found in this database, including those of germanium (Ge), tin (Sn), zinc (Zn), silver (Ag), antimony (Sb), gold (Au), bismuth (Bi) and platinum (Pt). The largest group of drugs contain platinum and have a well known mechanism of action as DNA cross linkers; whereas the other compounds are associated with different biological responses. Further understanding of these mechanisms of action may lead to improvement in their therapeutic utility. Colored hexagons correspond to clusters containing these compounds, which are also listed in Table 5. Hexagons are sized proportional to the number of compounds within each map position and colored according to metal type.

DNA and therefore clustered exclusively in the S-region of the SOM. The other eight drugs include compounds of Ag, Au, Bi, Ge, Sn, and Zn, with the cytotoxic response profiles for six of these drugs scattered throughout the Q-region and remaining two drugs falling into the N-region. These results suggest that further understanding of MOAs for metal/metalloid compounds may lead to improvements in their therapeutic potential. The development of metal-based anticancer drugs has mostly focused on those of platinum, especially analogs of cisplatin, and on DNA targeting. Compounds of many metal elements have long been used as drugs in various therapeutic categories and many new metal compounds have been discovered to function in tumor cell killing using MOAs other than DNA targeting. The historical focus on platinum in anticancer drug research and development may be expanded to include alternative metal compounds that share similar cytotoxic response profiles in addition to novel compounds that exhibit markedly different cytotoxic profiles.

9. Cellular sensitivity

The metal/metalloid compounds in the NCI_{60} cancer cell screen database display unique cytotoxicity response profiles compared to non-metal compounds. The intra-tumor panel average cytotoxicity profiles for GI_{50} measurements of all screened compounds, all metal compounds, and screened compounds that also appear as antineoplastic or immunosuppressive agents in the Leadscope® Known Drugs database are shown in Panel A of Fig. 6. These histograms are ordered from left to right to describe tumor

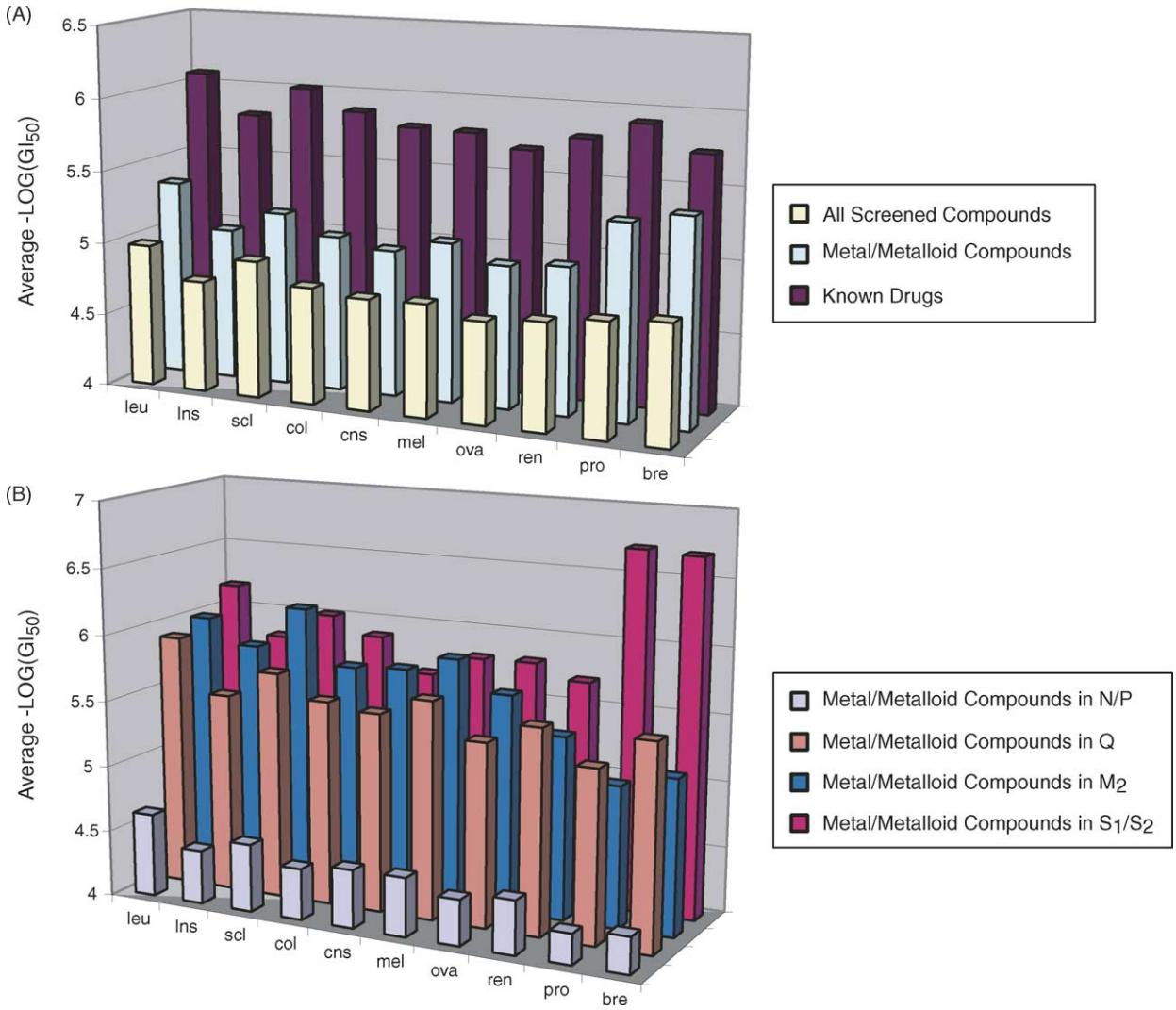


Fig. 6. Histograms for the average GI_{50} cytotoxic responses. Histograms are ordered from left to right to describe tumor panels for leukemia (leu), non-small cell lung cancer (lns), small-cell lung cancer (scl), colon (col), CNS, melanoma (mel), ovarian (ova), renal (ren), prostate (pro) and breast (bre) cancer. Each histogram represents the average GI_{50} value of individual tumor cells. Panel A: All compounds used to construct the SOM (~22,000) (front), metal and metalloid containing compounds (~1100) (middle), and compounds appearing as antineoplastic or immunosuppressive agents in the Leadscope® Known Drugs database (~300) (back). Panel B: metal and metalloid containing compound motifs clustered in SOM regions N and P (front), Q (second layer), M_2 (third layer), and S_1 and S_2 (back). The characterization of different compound classes associated with specific cancer etiologies indicates a rich complexity in cellular response and the possibilities for tumor-specific therapies.

panels for leukemia (leu), non-small cell lung cancer (lns), small-cell lung cancer (scl), colon (col), CNS, melanoma (mel), ovarian (ova), renal (ren), prostate (pro) and breast (bre) cancer. Each histogram represents the average GI_{50} value of individual tumor cells for each compound category. Larger GI_{50} values (higher bars) represent more sensitive responses and smaller GI_{50} values (lower bars) represent less sensitive responses. The histograms clearly show that this set of tumor cells, on average, is most sensitive to the known drugs (known drugs versus metal compounds: $p = 1.09 \times 10^{-7}$; known drugs versus all screened compounds: $p = 7.38 \times 10^{-13}$), but are more sensitive to metal compounds than ordinary screened agents (metal or non-metal containing) (metal compounds versus all screened compounds: $p = 1.35 \times 10^{-5}$). The high sensitivity of cancer cells to known drugs is not surprising because drug potency is one of the most important criteria when selecting drugs for clinical evaluation. The generally high potency of metal-based agents is intriguing and provides a good basis for their further development as anticancer drugs. The average sensitivity of the ten tumor panels in the NCI_{60} to known drugs and all screened compounds is nearly uniform except that the leukemia (leu) and small cell lung cancer (scl) panels are slightly more sensitive than the others; however, the breast (bre), prostate (pro) and leukemia (leu) sets are, on average, distinctly more sensitive to the metal/metalloid compounds than the other seven cell types ($p = 1.44 \times 10^{-4}$). This preference may be exploitable when designing therapies to treat cancers in these tissues.

Panel B of Fig. 6 illustrates the average cytotoxicity profiles for GI_{50} measurements in metal/metalloid compound motifs clustered in different regions (N/P, Q, M_2, S_1/S_2) of the SOM. The NCI_{60} tumor cell panels are the least sensitive, on average, to the metal compounds with cytotoxicity profiles clustered within regions N and P (compound motifs shown in Table 3). Nearly similar sensitivities are found for metal compounds clustered in regions Q and M_2. Uniform sensitivity is found for Q-region agents (compound motifs shown in Table 1), with the leukemia (leu) and small-cell lung cancer (scl) panels slightly more sensitive than the other panels. Small-cell lung cancer (scl) cells show the most sensitivity to the M_2 compound motifs (Table 4) with leukemia (leu) a close second. Prostate (pro) and breast (bre) cancer cell types are the least sensitive to the M_2-region agents. In contrast, the breast (bre) and prostate (pro) panels are apparently more sensitive to the metal compounds clustered in regions S_1 and S_2 (Table 2) than the other tumor panels ($p = 1.83 \times 10^{-4}$). These two tumor cell panels show even higher sensitivity, 15% on average, to the S_1/S_2-region metal compounds than to the Leadscope® known antineoplastic drugs. Evidence for panel-specific sensitivity for subsets of metal containing agents is an indication of the complexities of cellular responses to diverse chemical types. These differences, when cataloged as reported here, may provide the basis for tumor-specific therapies.

10. Summary

SOM clustering of the metal/metalloid compounds in the NCI_{60} cancer cell screen segregates these agents into four broad proposed MOA classes. Global drug–gene correlation analysis provides additional support for these classes. The cytotoxic activity of a metal complex is found to be dictated by both the identity of the metal and the organic components (ligands) that are bound to the metal and, in many cases, one could be the dominating factor over the other. Target specificity can be achieved by finding the right metal–ligand combination. A comparison between the screened compounds and a database of known drugs reveals the application status of metal-based drugs in clinical use and provides a basis for developing new metal drug candidates. The SOM investigation of anticancer metal compounds provides insights into the potential targets and MOA of non-metal drugs that share similar cytotoxic response profiles, and also novel metal- or non-metal-based anticancer drug candidates. In addition, specific sensitivity toward subsets of metal containing agents has been discovered for certain tumor cell panels. These differences can provide a basis for the development of tumor-specific cancer therapies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.toxlet.2004.12.014.

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