Mechanisms of Anti-Cancer Agents: Emphasis on Oxidative Stress and Electron Transfer

Peter Kovacic* and Jose A. Osuna, Jr.

Department of Chemistry, San Diego State University, San Diego, CA 92182-1030, USA

Abstract: A large body of evidence has accumulated indicating involvement of oxidative stress (OS) in the mode of action of various bioactive substances, including those of the immune system. The data for anticancer drugs (main and miscellaneous) are summarized herein. Although diverse origins pertain, reactive oxygen species (ROS) are frequently generated by redox cycling via electron transfer (ET) groups, such as quinones (or phenolic precursors), metal complexes (or complexors), aromatic nitro compounds (or reduced products) and conjugated imines (or iminium species). We believe it is not coincidental that these functionalities are frequently found in anticancer agents or their metabolites. Generally, the ET moieties display reduction potentials in the physiologically active range. Often ROS are also implicated in more traditional rationales, namely, enzyme inhibition, membrane or DNA insult, and interference with DNA or protein synthesis. A multi-faceted approach to mechanism appears to be the most logical. Significantly, the unifying theme of ET-OS also applies to other drug categories, as well as to toxins, carcinogens, hormones, and enzymes. Since this theoretical framework aids in our understanding of drug action, it can serve as a useful tool in the design of more active and safer pharmaceuticals.

INTRODUCTION

Various strategies have been used in drug design, including analogy, SAR, and more recently, combinatorial, as well as diverse other approaches. An understanding of drug mechanism can provide valuable insight in the search for more effective and safer pharmaceuticals. The electron transfer-oxidative stress (ET-OS) theory serves as a unifying theme that aids in our understanding the various ramifications of bioactivity.

During the past forty years, the ET-OS theory has arisen from research in many laboratories, including that of the principal author. It is intriguing that this thesis has found successful application to all of the principal drug categories, as well as to enzymes, hormones, and toxins. The ability of the comprehensive framework to aid in rationalizing the action of a wide variety of anticancer agents provides valuable insight for modern drug design. This review documents extensive evidence for the involvement of ET-OS in various main categories of anticancer drugs, as well as miscellaneous ones. In order to furnish a well-rounded perspective, other proposed modes of action are included. Since a broad approach is taken which is not in depth, only representative references are included, regrettably resulting in omission of important contributions.

*Address corresponding to this author at the Department of Chemistry, San Diego State University, San Diego, CA 92182-1030, USA; Tel: +1-619-5945595; Fax: +1-619-594-4634; e-mail: jgenoves@sundown.sdsu.edu

TENETS OF ET-OS THEORY

The preponderance of bioactive substances or their metabolites incorporate ET functionalities, which, we believe, play important roles in physiological responses. These main groups include quinones (or phenolic precursors), metal complexes (or complexors), aromatic nitro compounds (or reduced derivatives), and conjugated imines (or iminium species). In vivo redox cycling with oxygen can occur giving rise to OS through generation of ROS, as discussed in the Metabolism section. In some cases, ET results in interference with normal electron transport chains, e.g., in respiration. ET-OS, the focus of this review, can contribute to drug efficacy or can lead to undesirable toxicity. Alternatively, as discussed later in the text, ROS can arise in some instances by non-ET avenues.

Generally, active entities possessing ET groups display reduction potentials in the physiologically responsive range, i.e., more positive than –0.5 V. However, a correlation between reduction potential and activity is not always observed since important roles are played by other factors, such as, solubility, metabolism, diffusion, adsorption, site binding, cell permeability, and stereochemistry. Reduction potential is influenced by various factors including conformation which can differ in vitro vs. in vivo. Thus, electrochemistry, which has enjoyed relatively little attention, provides valuable insight in relation to mode of action.

1381-6128/00 $19.00+.00

Our theoretical framework incorporates several features common to most anticancer agents [1].

1. Binding to DNA by alkylation, intercalation, groove association, or complexation.

2. Existence of an ET entity present in the parent drug or a metabolite.

3. Formation of ROS or other radicals usually by ET involving oxygen.

4. ROS generation in close proximity to DNA giving rise to lethal effects, e.g., strand cleavage and base oxidation.

There is a plethora of experimental evidence supporting the ET-OS theoretical framework, including generation of the common ROS, lipoperoxidation, degradation products of oxidation, depletion of antioxidants (AOs), effect of exogenous AOs, DNA oxidation and cleavage products, as well as electrochemical data. This comprehensive, unifying mechanism is supported by the frequent observation that many ET substances display a variety of activities, e.g., multiple-drug properties, as well as toxic side effects.

In relation to the ET-OS approach, it is instructive to make comparison with phagocytosis, the natural defense against foreign organisms. Following engulfment, the invader is bombarded by a variety of ROS including superoxide, hypochlorous acid, hydroxyl radical, and nitric oxide. If anticancer drugs are subjecting tumor cells to ROS, then this scenario comprises another example of humans following in the footsteps of nature.

ET-OS is a broad approach that has stood the test of time. Although our focus is on this theory, it should be emphasized that bioactivity is quite complicated. Other well-supported, general proposals include enzyme inhibition, DNA and membrane damage, and interference with protein or nucleic acid synthesis. It is indicative that evidently ET-OS may play a role in a number of the alternate hypotheses. The most likely scenario is complementarity entailing multifaceted attack.

CHRONOLOGY

An inspection of the literature reveals little recognition of the early, interwoven history of the cancer and anticancer areas in relation to the OS theory. This concept for carcinogenesis was advanced by several groups [2-5] in the 1950s with Brues and Barron apparently being the first. Some corollaries were proposed including protection against cancer by antioxidants [4,5] and involvement of OS in anticancer drug action [5]. Elaboration of the ET-OS framework in the anticancer domain was reported by various groups beginning mainly in the 1980s [1,6-8]. The theory, however, has been accepted very slowly by various segments of the pharmacology community. For example, inspection of even recent medicinal chemistry texts reveals only meager treatment.

A baffling paradox enunciated by Haddow in the early years states that, generally, agents that are antineoplastic are also carcinogenic, and vice versa. Well-known examples are radiation and nitrogen mustards. If the premise is valid that both actions involve OS, then a reasonable rationale exists, which was advanced in 1959 [5] and elaborated in 1986 [1]. Also relevant is the observation that the incidence of second cancers is increased after treatment of primary ones with antitumor drugs [1,9,10]. Of course, specificity would be important in connection with a greater sensitivity of tumors to ROS and preferential binding to the cancer cells. Other mechanistic approaches appear plausible, such as interference with DNA [11] or enzyme action, but these could also entail OS.

METABOLISM

1. Generation of ROS

Hydrogen peroxide, a viscous, pale-blue, water-miscible liquid, is a stable nonradical molecule that easily migrates within and between cells in vivo. As a weak oxidant, hydrogen peroxide can lead to cellular depletion of ATP, GSH, and NADPH, as well as inducing rises in free cytosolic Ca^{2+} and activation of poly-ADP-ribose polymerase, events leading to apoptosis. As a powerful oxidant, hydrogen peroxide decomposes in vivo to the extremely reactive hydroxyl radical upon reduction by metals, such as iron and copper ions in the cytosol or bound to lipids, proteins, and DNA. If generated at very close proximity, hydroxyl radical rapidly oxidizes these essential cellular constituents, accounting for much of the damage. In general, any drug or biological process that generates superoxide can produce hydrogen peroxide by dismutation (Fig. (**1**)).

$$2O_2^{\bullet -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Fig. (1). Dismutation of superoxide.

Probably the most important biological sources of superoxide in eukaryotic cells are leakage from the electron transport chain of mitochondria and uncoupling of superoxide from cytochrome P450 reductase in the endoplasmic reticulum. Additional biological sources include activated phagocytic cells, oxidases such as xanthine and D-amino oxidases, auto-oxidation of glyceraldehyde, $FADH_2$ and adrenaline, and the release of superoxide from heme proteins. Many anticancer drugs are also capable of generating superoxide, typically by redox cycling with oxygen. These drugs contain electron-transfer entities that readily accept electrons from biological sources, followed by transfer to oxygen. Many of these drugs are the focus of this review.

Hydrogen peroxide rapidly decomposes to hydroxyl radical and hydroxide anion through metal ion catalyzed radical reactions, known as the Haber–Weiss or Fenton reaction. The latterreaction (Fig. (**2**)), despite the almost 120 years since its discovery, proceeds by an unknown mechanism in which the intermediates are still unidentified.

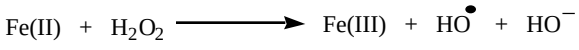


Fig. (2). The Fenton reaction.

In vitro, chromium, nickel, cobalt, titanium, and vanadium compounds can also participate in hydroxyl radical formation. However, in vivo, most attention has focused on ferrous and cuprous ions, in which the catalytic cycle is perpetuated by reduction of the oxidized metal ion by biologically available superoxide (Fig. (**3**)). Much evidence

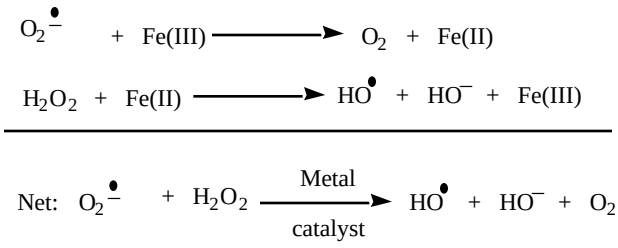


Fig. (3). Superoxide assisted Fenton reaction.

supports these roles of superoxide, most importantly the correlation between hydrogen peroxide cell killing sensitivity and level of endogenous or externally added superoxide dismutases (SOD).

Alternatively, hydroxyl radicals can be generated from hypochlorous acid by reaction with superoxide or metal ions, as with the Fenton case (Fig. (**4**)). Hypochlorous acid, formed from hydrogen peroxide by the enzyme myeloperoxidase (MPO) in activated neutrophils, is also a powerful oxidizing agent capable of attacking and inhibiting essential enzymes and other vital cellular constituents. Moreover, hydroxyl radical generation can be achieved through homolytic fission of the O-O bond of hydrogen peroxide induced byultraviolet radiation. This could conceivably happen in skin exposed to sunlight.

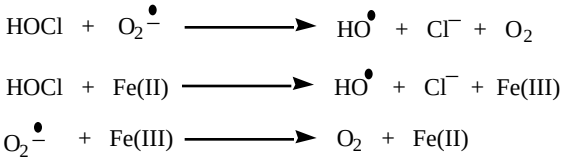


Fig. (4). Hypochlorous acid decomposition to hydroxyl radical.

Exposure of cells to hydrogen peroxide generates the multitude of products and damage patterns consistent with hydroxyl radical attack on lipids, proteins, and the sugars and bases of DNA. Examples include oxidation of various positions of purines and pyrimidines, with 8-hydroxyguanine usually being the focus of attention, and hydrogen atom abstraction at the sugar moieties giving rise to carbon-based radicals which in the presence of oxygen undergo a number of reactions, including C–C bond fragmentation, some of which result in single-strand breaks.

2. Oxidation of Phenols

Oxidation, often catalyzed by cytochrome P450 enzymes, is one of the most important reactions in biochemistry. This is a common pathway in drug

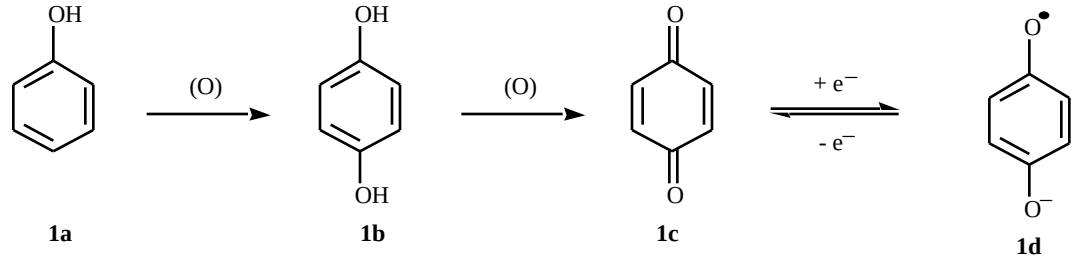


Fig. (5). Metabolic oxidation of phenol.

transformations resulting in activation, toxicity, and detoxification. The pathway for phenol is illustrated in Fig. (**5**).

When catechol is involved, then o-quinone results. An aspect that should be emphasized is the opportunity for redox cycling when semiquinone (**1d**) transfers an electron to oxygen with formation of superoxide. Ensuing generation of various ROS is described in the preceding section. Binding to biopolymers has been documented for a variety of physiologically active quinones.

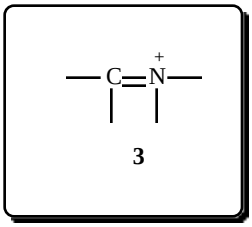
3. Reduction of Nitro Aromatics

The nitro compounds, both benzenoid and heterocyclic, comprise a diverse category in pharmacology, often accompanied by high toxicity. The reaction avenues entailing reductases have been extensively explored. A common sequence, outlined in Fig. (**6**), involves the following products: nitroso derivative (**2b**), radical (**2c**), hydroxylamine (**2d**), and primary amine (**2e**).

Redox cycling with generation of ROS can occur with participation of **2b**, **2c** and **2d**, and oxygen. Electrochemical studies demonstrate that generally **2b** displays a more positive reduction potential than **2a** [12]. Binding to biopolymers can occur with **2b** and **2d**.

4. Imine and Iminium Formation

Properly conjugated imines and iminium ions **3** can function as ET species, although this category is not as well recognized as the others.



3

Catalyzed oxidation of amines in vivo leads to these functionalities. In addition, imines are formed from uncatalyzed condensation of primary amines, e.g., in protein or amino acids, with carbonyl compounds. Familiar examples in this class are paraquat and flavins.

PART I: ET-OS AND TRADITIONAL MODES OF ACTION

The first portion of this section deals mainly with ET-OS, our focus of attention, whereas the more traditional mechanistic approaches are addressed thereafter. It is evident that ET-OS is pervasive, playing a role in the alternative modes. Although ROS usually arise from ET reactions, other sources can serve, such as radiation, peroxides, and enediynes. A logical conclusion is the involvement of a multi-pronged attack. Part II briefly provides outlines of more recent, novel probes.

RADIATION

This early, useful technique enjoys a consensus for a mechanism entailing ROS, thus serving as a foundation for the theoretical framework.

Radiation affects matter by donating energy, which varies widely, to the electrons or nuclei of constituent atoms or molecules. When radiation of relatively low energy, such as in the visible or ultraviolet range, impinges on matter, certain molecules (chromophores) absorb the incident radiation and promote an electron to a higher-energy orbital. This activation, which generates short-lived excited states with increased chemical reactivity, is known as photoexcitation. When high-energy radiation, such as x-rays, gamma rays, and particle radiation impinges, atoms and molecules are ionized resulting in either disruption of chemical bonds or the ejection of orbital electrons.

Over the last decade, considerable radiobiological data indicate that both excitation and ionizing radiation damage cells, primarily by generating ROS that attack critical cellular targets, such as, DNA, and to a lesser extent, cellular membranes and proteins. The most reactive and hazardous ROS is the hydroxyl radical, although peroxyl radicals, alkoxy radicals, superoxide, singlet oxygen and hydrogen peroxide are all known to induce cellular damage, directly or indirectly. ROS attack and damage DNA bases and sugars. In the case of hydroxyl radicals, damage occurs generally by two mechanisms: addition to double bonds or hydrogen abstraction, resulting in a wide variety of base alterations, in sugar-phosphate backbone strand breaks, and in DNA-based cross-links.

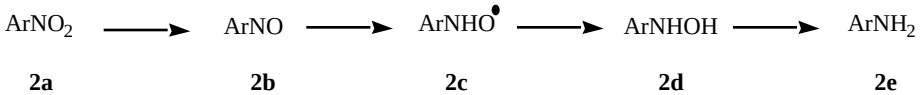


Fig. (6). Reduction sequence of aromatic nitro compounds.

Ultraviolet radiation (UV) can act directly or indirectly to damage DNA. Direct damage entails absorption by DNA bases to form photoproducts, some of which may be responsible for the cytotoxic effects. For example, cross-linked dimers of cytosine and thymine can arise, in addition to other adducts between DNA and proteins, and single- and double-strand breaks. If these altered bases are not repaired, mutation or death can result. Indirect action of UV radiation on cellular components includes OS generated through the formation of singlet oxygen, peroxyl radicals, and hydroxyl radicals. Ultraviolet radiation is known to homolytically cleave hydrogen peroxide to hydroxyl radicals [13]. Peroxyl radicals are formed from generated carbon-based radicals which react with molecular oxygen. These radicals are highly reactive and can participate in the initiation and promotion of free radical chain oxidation of membrane proteins and phospholipids. Alternatively, UV excited organic molecules can transfer energy directly to molecular oxygen, leading to the reactive singlet oxygen, which, in turn, can transfer energy to other biomolecules (quenching) increasing their chemical reactivity. Alternatively, it can react with biomolecules by a variety of other mechanisms. The most studied reactions are the pericyclic ones involving the carbon-carbon double bonds of biomolecules [13], resulting, for example, in endoperoxides, ene-reaction products, and dioxetanes. It is well known that singlet oxygen reacts with unsaturated membrane phospholipids by the ene reaction to give phospholipid hydroperoxides. In addition, DNA bases appear to be oxidized directly [14,15], e.g., adenine, guanine, and thymine in the presence of free radical scavengers, implicating singlet oxygen.

When biological materials directly absorb ionizing radiation, chemical bonds within the target molecules are broken. Unfortunately, knowledge of the chemical changes that occur when DNA absorbs ionizing radiation (direct effect) is very limited, and this effect is considered insignificant except in cases of high-energy particulate radiation. Because cells are 80% aqueous, the majority of the energy is absorbed by water, resulting in ionization to hydrogen atoms, solvated electrons, and, most importantly in terms of damage to DNA, hydroxyl radicals. It is estimated that about 65% of the damage is caused by hydroxy radicals [16] which are short lived and react very rapidly with each other or with surrounding molecules. For example, these species are estimated to have a lifetime of only one nanosecond with a diffusion range of up to 3.5 nanometers from the site of initiation [17]. Coupling produces hydrogen peroxide, which can regenerate hydroxyl radicals in a Fenton reaction. Solvated electrons react with molecular oxygen to form superoxide.

The critical target in radiotherapy is DNA, with the most lethal lesion being the double-strand break. Because ionizing radiation deposits much larger amounts of energy per absorption event than is needed for a single ionization of water, each absorption event ionizes multiple water molecules and generates a localized concentration of hydroxyl radicals. Since large amounts of water are intimately associated with DNA [18], these radicals can be in close proximity to DNA, giving rise to multiple and simultaneous attacks on the complementary strands. A minor percentage of these damaged sites can then undergo either oxidative fragmentation (sugar damage) or excision (removal of damaged bases by endonucleases), or both, giving rise to potentially lethal double-strand breaks.

The most generally proposed result of double-strand breaks is chromosomal aberration [19], leading to loss of cell viability. Alternatively, cell death may be caused by depletion of critical cellular coenzymes consumed during the repair process. As part of the broad unifying theme of OS as an element in anticancer action, radiation chemistry comprises a well-established piece of the overall mosaic, as previously pointed out [1].

1. Radiation Sensitizers

The presence of hypoxic regions in tumors is a major factor limiting the efficacy of radiotherapy and many chemotherapeutic agents. Chemical agents that damage tumor cell DNA by free radical mechanisms play an important role in cancer chemotherapy [20]. Unfortunately, many cancers are resistant to radical-based DNA damage produced by radiotherapy [21] and by many chemotherapeutic agents [22] due to a subpopulation of solid tumor cells that are oxygen deficient (hypoxic) [23,24]. Under low oxygen concentrations (relative to normal cells), the cytotoxicity of a DNA-radical lesion is typically decreased because many DNA-radical lesions require prior reaction with molecular oxygen followed by fragmentation [25]. However, as per a proposal made many years previously [26], this unique feature of solid tumor cells has recently been used advantageously to selectively direct the action of some newly developed antitumor agents toward hypoxic tumor cells [27,28].

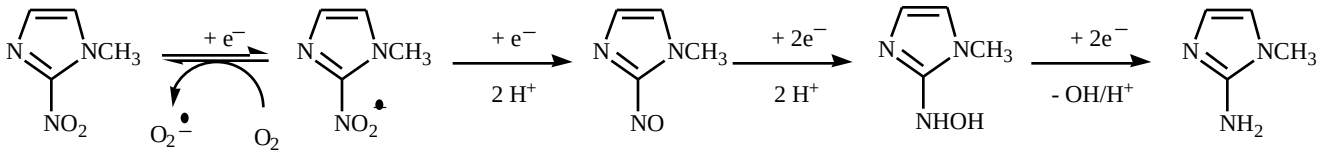


Fig. (7). Bioreductive pathway of N-methyl-2-nitroimidazole.

a. 2-Nitroimidazoles

Nitroheterocyclic compounds, in particular 2-nitroimidazoles, are a class of drugs that are capable of inducing a variety of biological effects, the majority of which are exacerbated in hypoxic cells. Consequently, 2-nitroimidazoles are currently being evaluated, after almost two decades of development, for the hypoxic treatment of solid tumors as radiosensitizing agents, as cytotoxins, and as molecular markers [29-31]. The biological properties of 2-nitroimidazole, as well as most other nitroheterocycles, are related to their electron affinity or, more precisely, to their one-electron reduction potential [32,33] (Fig. (**7**)). In general, the more positive the reduction potential of a compound the more toxic. This trend is observed because the more electron-affinic compounds are more easily reduced to their toxic species and are less sensitive to oxidation back to the parent compounds by molecular oxygen.

Originally, the interest in this class centered on their ability to selectively sensitize normally radioresistant hypoxic cells to the lethal effects of radiation through redox-mediated free radical mechanisms [34]. In two complementary mechanisms, 2-nitroimidazoles are suggested to act as true radiosensitizers by mimicking the damaging effects of molecular oxygen. In the first mechanism, the unaltered sensitizer, which is either present in or near DNA, traps electrons liberated by radiation [35,36]. Electron trapping influences the distribution of free radical centers on the DNA and, consequently, the extent of radiation damage. In the second mechanism, the sensitizer reacts directly with free radical centers on the DNA and, therefore, potentiates the lethal effects of ionizing radiation in hypoxic cells. This is supported by much evidence on the formation of DNA adducts following irradiation of DNA in the presence of radiosensitizers

By an alternative mechanism, 2-nitroimidazoles are metabolically reduced by intracellular enzymes in both hypoxic and aerobic cells in a series of stepwise one-electron transfers (Fig. (**7**)) [37]. Bioreductive drugs act as substrates for various intracellular nitroreductases present in almost all cells and comprise, in addition to the nitroheterocycles, aromatic and aliphatic N-oxides, and indolequinones. This general class is a rapidly developing field and many compounds are currently in clinical evaluation as hypoxic cytotoxins and hypoxic-region markers.

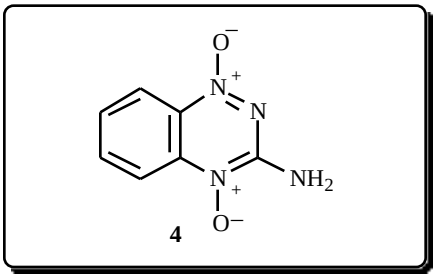
As illustrated in Fig. (**7**), under aerobic conditions, 2-nitroimidazole is first reduced to the nitro radical anion in a one-electron transfer. This radical anion is so reactive toward oxygen that it is rapidly oxidized back to the parent nitroimidazole, effectively leaving no substrate for the second electron-transfer step. This futile redox cycle is responsible for the hypoxic selectivity of 2-nitroimidazoles and is hypothesized as a mechanism for the formation of ROS derived from superoxide, such as hydrogen peroxide and hydroxyl radical, and, consequently, the aerobic toxicity [37].

Under hypoxic cellular conditions, the radical by further step-wise one-electron bioreduction leads to the nitroso, hydroxylamine, and amine derivatives in two-, four- and six-electron transfers, respectively [38], each of which has been implicated in the biological activity. Initial work in this field indicated that the hydroxylamine derivative was responsible for the selective hypoxic cytotoxicity through the formation of metabolites capable of binding with cellular macromolecules, such as proteins, and DNA [39-41], e.g., reaction with water to yield glyoxal which in turn can bind with nucleic acid bases following drug administration under hypoxic conditions. Glutathione, as the initial nucleophile, behaved similarly to water. More recent data, however, has shown that the nitroso derivative is also highly reactive and may be responsible for much of the hypoxic cytotoxicity [42,43] through reacting with and depleting intracellular sulfhydryls. Depletion of essential sulfhydryls is suggested to induce cell death through an apoptotic-like mechanism [30,44] and enhanced OS.

b. Tirapazamine

A novel class that exhibits selective toxicity toward hypoxic cells is the 1,2,4-benzotriazine 1,4-dioxides [45-47], an iminium type. Tirapazamine (3-amino-1,2,4-benzotriazine 1,4-dioxide, **4**), the lead member, exhibits superior selectivity to that of

many other hypoxic cytotoxins, such as, mitomycin C and misonidazole, and is currently undergoing phase II and III clinical trials for treatment of various cancers [48].



4

The DNA damage produced by tirapazamine is due to radical species generated by one-electron reduction. Reduction to the free radical **5** [49] is reported to occur by three mechanisms: (1) bioreduction by enzymatic reductases, such as, xanthine oxidase, cytochrome P450, and NADPH-cytochrome P450 oxidoreductase [50], (2)

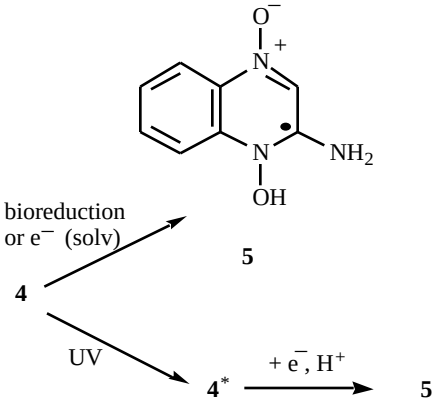


Fig. (8). One-electron reduction mechanisms of tirapazamine **4**.

photoexcitation by ultraviolet radiation followed by hydrogen atom abstraction [51], and (3) reduction by trapping of solvated electrons resulting from the effects of ionizing radiation on water (Fig. (**8**)).

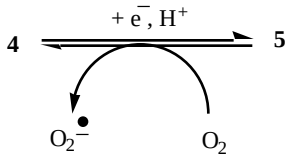


Fig. (9). Interconversion of **4** and **5**.

Under aerobic conditions, activated tirapazamine **5** is rapidly reoxidized by molecular oxygen to **4** with concomitant formation of superoxide and ROS [52,53] (Fig. (**9**)).

Rapid back-oxidation to compound **4** is thought to be the basis for the hypoxic selectivity of **4**. The cytotoxic effects of the ROS formed under aerobic conditions are significantly less than the activated species formed under hypoxic conditions. At low concentration of **4**, cellular enzymes, such as, superoxide dismutase, glutathione reductase, and catalase, rapidly mitigate the cytotoxicity of the low levels of superoxide and ROS formed [54]. Studies have correlated increased levels of these cellular enzymes with increased cell resistance under aerobic conditions. However, at higher concentrations, **4** does kill aerobic cells and is believed to be responsible for many clinically observed side effects. There is also evidence that the photoactivated species can transfer energy to molecular oxygen to form singlet oxygen that may also play a role in cytotoxicity.

Under hypoxic conditions, the radical species **5** is longer lived and hence freer to diffuse and interact with cellular macromolecules. A possible pathway for **5** to initiate DNA-strand damage under hypoxic conditions [50] involves direct abstraction of a hydrogen atom from deoxyribose of DNA. Considerable evidence now indicates that the enhanced hypoxic cytotoxicity of **4** stems from various roles it plays in the process of DNA damage [25,55]. In addition to initiating DNA-strand damage as described, **4** has also been shown to trap deoxyribosyl radicals by forming covalent adducts at the N-oxide oxygen. This adduct subsequently undergoes cytotoxic oxidative fragmentation to products similar to those from deoxyribose under aerobic conditions [56,57] (Fig. (**10**)). Therefore, the enhanced cytotoxicity of tirapazamine is due to its ability to generate free radicals on the deoxyribose moiety and then to act as surrogate for molecular oxygen by donating an oxygen atom to the deoxyribosyl radical.

c. Indolequinones

Quinones are a class of electron-affinic compounds that display a wide range of

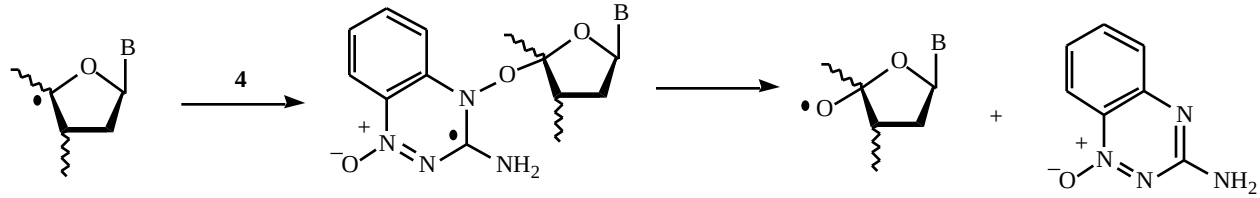
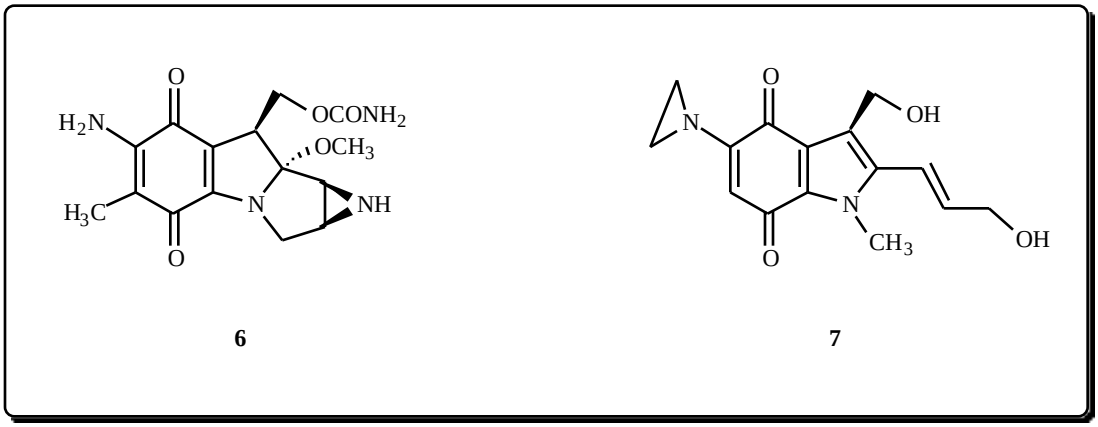


Fig. (10). N-Oxide-deoxyribosyl adduct and decomposition.

biochemical activity, including use as hypoxia selective cytotoxins for the treatment of cancer. Representative is the prototype indolequinone mitomycin C, a naturally occurring quinone derived from the *Streptomyces* species. Unfortunately, mitomycin C (**6**), despite its effectiveness in vitro, has not progressed to general clinical use, mainly because of its small margin of safety [58]. This has prompted the search for related compounds that are not only less toxic, but also exhibit higher activity. An example of an improved drug that shows these characteristics is the structurally related EO9 (**7**) [58].



Despite the relatively poor therapeutic indexes, this class has been effective in clinical trials in combination with radiotherapy and chemotherapy [59]. Because radiation will preferentially remove the aerobic tumors cells, administration of these potent hypoxia-selective agents before or after irradiation eliminates the fraction of extremely hypoxic, radioresistant cells, thereby aiding in the prevention of tumor regeneration. Results indicate that tumors treated with these two quinones in combination with radiation therapy have a better response than those exposed to radiation alone [34,60].

Two major factors control the cytotoxic selectivity: the relative cellular oxygen concentration and the type of reductive enzymes present. In aerobic cells, i.e., cells with oxygen tensions between 24 and 66 mmHg, the concentration of molecular oxygen is sufficient to lessen the cytotoxicity of indolequinones reduced by one-electron reductases, but not by two-electron reductases. Cytochrome P450 and DT-diaphorase are examples of one- and two-electron reductases, respectively, present in both normal and tumor tissue. The one-electron reduction product is the semiquinone and the two-electron product is the hydroquinone.

Semiquinones are extremely reactive toward back-oxidation by molecular oxygen, and therefore under aerobic conditions do not exist long enough for the drug to exert its cytotoxic effect. This rapid back-oxidation serves as the basis of the hypoxic selectivity. However, two-electron reductases reduce quinones directly to the hydroquinones, thus bypassing the semiquinone and the protection afforded by molecular oxygen. Hydroquinones are much less rapidly oxidized by oxygen [61] and exist long enough to exert their cytotoxic effects. Therefore, formation of relatively stable hydroquinones by two-electron reductases in normal cells is believed responsible for the low hypoxic-to-oxic cytotoxic differential observed for many quinone antitumor drugs.

Under anaerobic conditions in vitro, i.e., oxygen tension falling below 5 mmHg, both the semiquinone and hydroquinone are cytotoxic to a wide range of hypoxic tumor cells. Unfortunately, oxygen tension in most of these cells typically range between 5 and 10 mmHg which is sufficient to rapidly oxidize the semiquinone, thereby reducing the overall cytotoxicity of the drug due to more reliance on two-electron reduction to the hydroquinone. Attempts to decrease the low-oxygen sensitivity of the semiquinone has focused on increasing the electron-affinity of the indolequinones. Other bioreductive drugs, e.g., nitroimidazoles, have shown a direct correlation between increasing electron-affinity and decreased sensitivity of the radical anion to the oxygen concentration. Unfortunately, this lead proved unsuccessful since the compounds were still extremely sensitive to back-oxidation at oxygen concentrations similar to that of many hypoxic cells [61].

Activated indolequinones exert their cytotoxicity by OS through the formation of superoxide and hydrogen peroxide, and by alkylation and cross-

linking of DNA (Fig. (**11**)). OS is believed to play only a minor role in overall cytotoxicity [61].

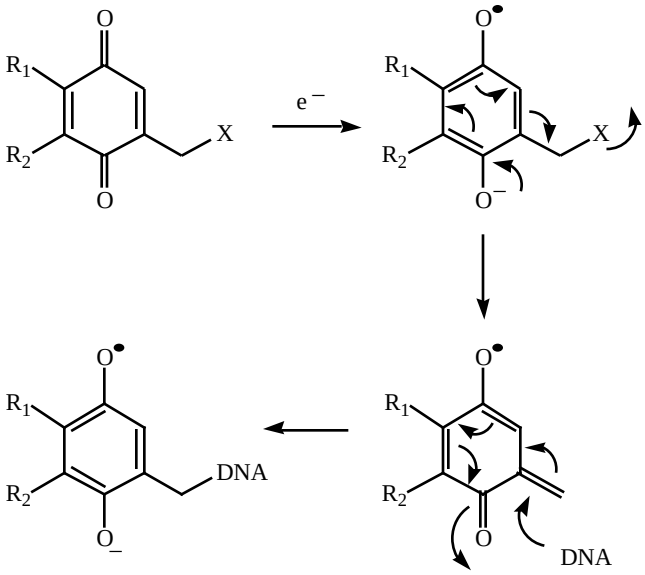


Fig. (11). Reductive alkylation of DNA by quinones.

Fig. (**11**) illustrates the generalized mechanism of alkylation of DNA with a simple quinone model. Upon activation of the appropriately substituted quinone, the semiquinone undergoes a series of electron transfers within its ring system, resulting in elimination with formation of an electrophilic site. Nucleophilic attack by guanine of DNA leads to binding by alkylation. In the case of mitomycin C, cross-linking can occur because mitomycin C generates two electrophilic sites upon activation—the first at a carbon bearing the fused aziridine ring and the second at the carbon bound to the carbamate moiety [59]. Elucidation of the mechanism of action has generated the concept of using these entities as hypoxia selective trigger molecules [59]. As illustrated in Fig. (**11**), activation of appropriately substituted quinones triggers the release of specific groups accompanied by electron rearrangement. If these entities are themselves cytotoxic, then the potential exists of designing more active indolequinone derivatives that release secondary active agents. Current research is investigating this possibility [62]. Mechanistic features are more broadly addressed in the Quinones section.

d. Miscellaneous Agents

Miscellaneous compounds or their metabolites generally fall into the typical ET categories: namely, benzenoid and heterocyclic nitro compounds, quinones, methotrexate, paraquat, actinomycin, adriamycin, phenothiazine, acridines, acridinium salts, and stable free radicals [63], as well as compounds of metals, e.g., Fe, Cu, Hg, Co, Ag, and Ru [64]. The arylidene cyclopentenediones represent an unusual category [65]. cis-Pt, an anticancer drug, is a particularly interesting member of this category. Sensitivity increases with decreases in GSH levels, just as with radiosensitivitygenerally [66]. The relatively weak electron affinity of the compound has been noted. However, the analogous $Cl_2Pt(OH)_2(H_2NPr\text{-}i)_2$ displays a much more positive reduction potential, within the physiological range, and it loses the chlorine ligands faster than cis-Pt. It is important to recognize that metabolic transformations and DNA binding might well appreciably alter the ET nature of the bound metal. This class has also been characterized as quasi-alkylating, which suggests that a charged metabolite may bind to DNA. Some investigators believe that ET is not involved [67] (see Metals).

2. Photodynamic Agents

Photodynamic therapy (PDT) entails the combined use of light and photosensitizers to treat tumors in the presence of molecular oxygen. The photodynamic effect relates to the chemical action of the generated ROS on crucial cellular organelles and biomolecules, such as mitochondria [68], lipids, proteins, and nucleic acids [69-71].

PDT is a developing cancer treatment that seeks to destroy superficial tumors, thus sparing surrounding healthy tissue [72]. Solid tumors at almost every anatomical site have been treated by PDT; however, bladder, eye, skin [73], lung [74,75] and head and neck cancers appear to be the most responsive. PDT promises to be more selective than radio- and chemotherapy and can be applied to recurrent tumors that have already received maximal doses of conventional treatment and to otherwise untreatable cancers. In addition, PDT is only minimally toxic. Since photosensitizers lack toxicity in the absence of light, adverse reactions at other sites of drug accumulation are eliminated [76], and the drug-activating light (600–800 nm) is harmless in the absence of sensitizer. Some limitations to PDT include light-inaccessible tumors and large tumor masses. Development of better drugs and more convenient light sources in addition to improving modulation of drug-light conditions and light dosimetry [77] are the major topics of current research.

Tumor cell selectivity is achieved by the use of photosensitizers with higher affinity for cancerous tissue than for normal tissue, although targeting

mechanisms for many of the photosensitizers are unclear. Clinically, the most frequently used photosensitizers are the hematoporphyrin derivatives (HpD), namely, Photofrin I® and the purified version Photofrin II® [73]. Both are first generation photosensitizers composed of complex mixtures of water soluble monomeric and aggregated porphyrins. The active components of HpD consist of porphyrin aggregates covalently linked by ether and ester bonds [78]. Fig. (**12**) illustrates the general structure of the monomeric porphyrins comprising HpD.

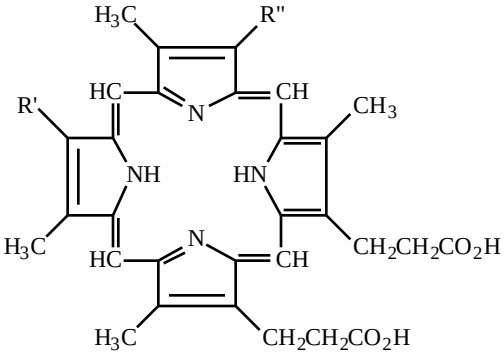


Fig. (12). Structure of hematoporphyrin.

Binding to low density lipoproteins (LDL) in the circulatory system is reported as the localization mechanism of injected HpD [78,79]. The adduct is then transferred to the surface of tumor cells containing elevated levels of surface-LDL receptors, thereby achieving selectivity.

The principal ROS implicated in the direct cytotoxic effects of PDT is singlet oxygen, although hydroxyl radicals are also involved, but to a lesser extent [80]. Since, PDT is oxygen dependent [81], the availability of oxygen is a rate-limiting factor for the production of singlet oxygen and the overall effectiveness in killing tumor cells [73]. Consequently, hypoxic, large tumors are typically excluded from therapy. PDT is classified as a type II photochemical reaction entailing the direct transfer of energy from a photoexcited photosensitizer to molecular oxygen with generation of singlet oxygen. In contrast, type I photochemical reactions comprise hydrogen atom or electron abstraction from substrates by the excited photosensitizer. Both the oxidized substrate and reduced photosensitizer can react with molecular oxygen to form hydroxyl and peroxyl radicals, respectively. Type I mechanisms may be responsible for the low levels of hydroxyl radicals present in PDT. Singlet oxygen is not a free radical, but is an electronically excited form of oxygen which is strongly electrophilic and reacts readily with a variety of cellular organelles and biomolecules, as described previously.

More recent evidence now implicates tissue microvasculature damage in tumor destruction by PDT [82,83]. Vascular changes induced by HpD are characterized by vessel constriction and blood flow stasis resulting in tumor hypoxia and nutrient deprivation [84,85]. For some photosensitizers, which act by the formation of singlet oxygen and are dependent on adequate oxygenation, vascular shutdown significantly reduces the efficacy of PDT [86].

Second generation photosensitizers are being developed that attempt to minimize side effects while maximizing tumor selectivity and yields of ROS generation by both type I and type II reactions, in addition to improving drug purity and decreasing host toxicity. The major side effect of HpD is prolonged accumulation of the drug in the skin, causing moderate to extreme skin sensitization to sunlight. Symptoms can range from slight erythema and edema to extensive skin damage and necrosis. Therefore, more tumor specific compounds with a higher rate of clearance from normal tissue are being sought. In addition, because HpD absorb poorly at wavelengths of maximal light penetration into tissue, photosensitizers with larger extinction coefficients are also being developed, requiring less drug and decreased amounts of light exposure for optimal response. Some of the next generation types which possesses many of these properties include the benzoporphyrin and phthalocyanine derivatives, chlorins, purpurine, and others, some of which are currently being tested in phase III trials for efficiency in treating tumors at various anatomical locations [73].

PEROXIDES

Since radiation research provides good evidence for crucial involvement of ROS, it is logical to expect peroxides to act as cytotoxins in a precursor role.

Better known for its effervescent antiseptic properties, hydrogen peroxide is also a powerful cytotoxin capable of killing many types of cancer cells. One of the earliest accounts of its use dates back to 1888 in JAMA. By the 1950s, the ability of ionizing radiation to destroy malignancy was well known, and its action was believed to occur

primarily through the generation of hydrogen peroxide and the highly oxidizing peroxyl and hydroxyl radicals, followed by subsequent DNA damage [87]. Consequently, researchers began investigating the carcinogenic and anticarcinogenic efficacy of hydrogen peroxide. These investigations determined not only that hydrogen peroxide caused cancer, producing melanonic tumors in fruit flies [88], but also that infusion and oral administration effectively treated rats with certain cancers, such as ascites sarcomas [89], lympoid tumors [90], and Walker 256 adenocarcinoma. In addition, it was found to improve the condition of human patients with very advanced inoperable tumors [91] and to increase the killing of cells from Hodgkin's disease in vitro [92]. Other similar studies, however, found little or no inhibitory effect on a variety of cancers [93]. The contradictory results are not surprising since the agent would be expected to have low specificity due to absence of binding, although selectivity was noted in some cases [94].

Cyclic, organic peroxides were shown to exhibit antitumor activity [95]. From a scrutiny of the literature, the American Cancer Society found no evidence that treatment with hydrogen peroxide or other "hyperoxygenation" compounds is safe or results in objective benefit [96].

ENEDIYNES

Interesting because of their unusual structure and mode of operation, this class fits the general framework, but the well-established reaction scheme entails carbon radicals which function per se or as precursors of ROS.

The term enediynes designates a class of complex and extremely potent antitumor antibiotics which contain at their core an unsaturated system **8** capable of cycloaromatization to a 1,4-benzenoid diradical **9** [97-99] (Fig. (**13**)).

The naturally occurring members, isolated from fermentation broth of microorganism, are structurally diverse and complex compounds, comprising multiple functional groups, each of which plays a specific role in the overall process of DNA damage. For instance, either a carbohydrate-based or intercalator functional group, or both, attach at specific base sequences within the minor groove, either by DNA–carbohydrate binding recognition or intercalation. In addition, a trigger initiates a cascade of rearrangement reactions terminating in cycloaromatization to the highly reactive **9**. The diradical, positioned adjacent to the deoxyribose backbone, abstracts hydrogen atoms from the sugar moiety (Fig. (**13**)), and in the presence of molecular oxygen, initiates oxidative degradation, concluding in single and double-strand cleavage.

In the absence of molecular oxygen, enediynes damage DNA by covalently linking the complementary DNA strands to the postactivated drug, forming DNA-drug interstrand cross-links and DNA-drug adducts at specific base sequences within the minor groove [100-102]. Therefore, enediynes are also of interest as cytotoxic agents for the treatment of large tumors where relatively anaerobic conditions prevail. Interstrand cross-links and drug monoadducts form in this scenario since the newly formed deoxyriboxyl radicals of each complementary strand do not react with oxygen to undergo oxygen-mediated degradation. Alternatively, in the absence of oxygen or a hydrogen donor, the sugar radicals likely react covalently with the unsaturated postactivated enediyne to form either interstrand cross-links or drug monoadducts. Enediyne drugs give different ratios of interstrand cross-links to drug monoadducts. The main features that seem to control the ratio are the relative reactivity of the radicals, proximity to the reaction site, and degree of exposure in the minor-groove. For example, neocarzinostatin mostly gives monoadduct under anaerobic condition and single-strand DNA cleavage under aerobic conditions. This suggests that one of the diradicals is more efficient in hydrogen abstraction. Therefore, quenching of the second radical before it has a chance to react is likely the reason for neocarzinostatin yielding a high ratio of drug adduct to cross-linked DNA under anaerobic conditions. In addition, the second

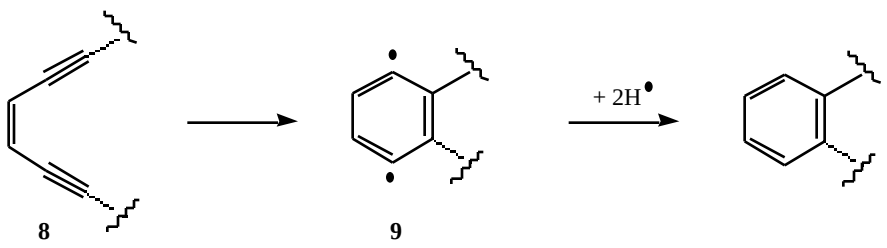


Fig. (13). Cycloaromatization of an enediyne.

radical is found to be more exposed in the activated drug–DNA complex, facilitating quenching. In contrast, C-1027 gives mainly the interstrand cross-link under anaerobic conditions and a substantial number of double-strand lesions under aerobic conditions, indicating that the drug is in closer proximity to the deoxyribose units. The nearness facilitates both hydrogen abstraction by **9** and covalent bonding of the deoxyriboxyl radical with the postactivated enediyne. Apparently, the drug–DNA complex is less exposed in the minor groove and, consequently, less susceptible to quenching by a hydrogen donor or molecular oxygen.

The intercalating functional group of many enediynes serves as a DNA-binding agent by interposing into the DNA base stack, thus positioning the crucial unsaturated core within the minor groove. There is evidence that the intercalator group also plays an important role in triggering cycloaromatization. An intriguing feature of the intercalating function of neocarzinostatin and dynemicin is the presence of either a quinone or hydroquinone, which are well-known ET agents.

METALS

Large numbers of metal-containing compounds possess activity including those of Pt, Fe, As, Ge, Ti, Sn, Ga, Ru, V, Mn, Co, Cu, Au, Rh, Mo, and Nb [103-105]. Both inorganic types and complexes with organic ligands are represented, with metals bearing higher oxidation states in most cases. In a number of investigations, ROS were reported along with the proposal of possible involvement in the lethal effect [1,105]. Further evidence for participation of metal drugs in ET derives from electrochemical studies of reduction potentials which generally gave quite positive values, thus permitting ET reactions in vivo. Compounds investigated included copper complexes [1,106,107], nickel salicylaldoximate [1], and metallocenes [106]. Recent reviews provide current information, including mechanistic aspects, on anticancer inorganic compounds: Pt [108], Fe [109], Au [110], Ga, Ru, Rh, V, Sn, and metallocenes [111]. Voluminous literature well documents the ET-OS role of metallic compounds in various drug classes, as well as toxins. Several of the more important drugs will be discussed in greater detail with focus on ET-OS.

1. Bleomycin [112,113]

Bleomycin, a clinically useful drug, is a glycopeptide produced by microorganisms. It is a powerful chelator, and the active form is the complex with Fe(II) which intercalates DNA by means of the bisthiazolyl entity. In the presence of molecular oxygen, polymer chain degradation occurs. Of the various possible ROS, a ferric peroxide evidently plays a key role, as well as the hydroxyl radical [114]. Apoptosis can be induced [112].

2. Arsenic Compounds

The most important one in this group is arsenic trioxide, the center of much attention. Various toxic effects have been reported involving apoptosis [115], mitochondria [115], tubulins [116], and DNA [117]. Adverse effects on antioxidant enzymes were noted in a study of liver injury [118]. In carcinogenic manifestation (Haddow's paradox), there is accompanying OS [119].

3. cis-Pt

One of the drugs in this category that has attracted considerable attention is cis-Pt. There is widespread consensus that activity results from intra- and inter-strand cross-links that bend and unwind DNA, resulting in attraction of certain proteins with resultant interference with excision repair and other vital DNA processes [120-122].

However, it appears that binding alone in not sufficient and that some mechanism which occurs after the Pt species attaches itself to DNA must account for the anticancer activity [123]. Since considerable evidence indicates that many other metals operate by ET-OS, it is reasonable to propose a similar mode of action in this case, as had been suggested previously based on various lines of evidence [1]. Since then, appreciable support has appeared for this thesis which has attracted little attention. Involvement of radicals and lipoperoxidation have been observed in various systems accompanied by decrease in antioxidant levels [124,125]. Superoxide arises from interaction with DNA [126]. Also, resistance to the drug is associated with increase in levels of glutathione [127]. Plausibility for this rationale is provided by recent reports that ROS are involved in the cytotoxicity which is enhanced by glutathione depletion [128,129]. In addition, cis-Pt is known to activate macrophages. Electrochemical data lend additional credence to this chemical viewpoint [1,130,131]. Some studies indicate a quite negative reduction potential for the cis isomer, whereas others, under different conditions, afford values in the physiological range. More negative values

pertain to the trans isomer, in line with the decreased activity. Metabolism to the diaquo form enhances electron uptake. The potential increases in the positive direction as the degree of ionization increases. In attachment to DNA, inner sphere complexes exhibit covalent binding, whereas outer sphere types are characterized by ionic bonds [132], which should influence ET properties.

There are additional relevant facets. The drug induces apoptosis which may be the culmination of the modes of action discussed. Radicals generated in the vicinity of DNA appear to be the mediators [133]. cis-Pt displays radiation sensitizer properties as discussed in that section. Free radical damage has been proposed for the nephrotoxicity which is ameliorated by antioxidants [1].

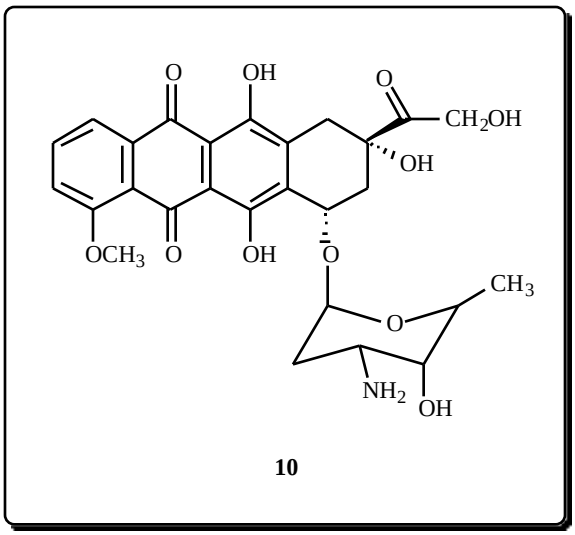
4. Metal Chelators

Anticancer activity is manifested by compounds which are well known chelating agents, such as 2,2´-bipyridyl, o-phenanthroline, hydroxyurea, diethyldithiocarbamate, and 8-hydroxyquinolines [106], as well as thiosemicarbazones [1]. Complexes with copper or iron salts gave relatively positive reduction potentials in the physiological range. Various lines of evidence pointed to generation of ROS. Bleomycin also falls into this category.

QUINONES

1. Anthracyclines (Anthraquinones)

The most common anthracycline antibiotics are doxorubicin (Adriamycin) (**10**) and daunorubicin (Daunomycin), which have clinical use.



10

Microorganisms served as the original source. Various modes of action have been advanced, but the subject remains controversial. These, the topics of a recent critical analysis by Gewirtz, will be summarized [134]. Of the multiple mechanisms cited, one involves interference with DNA synthesis, which may be a reflection of initial events, such as intercalation, enzyme inhibition, and OS. Inhibition of topoisomerase II (see Topoisomerase Inhibitors), the likely primary mode of action at in vivo concentration, appears to entail interaction with the enzyme-DNA complex followed by strand breaks. Resistant tumor cells possess reduced levels of topoisomerase II with accompanying decrease in the degree of cleavage. From many studies, there is difficulty in correlating cytotoxicity with extent of strand cleavage.

It is well established that anthracyclines possess the capacity to undergo redox cycling with generation of ROS. The process involves enzyme catalyzed one-electron reduction to the semiquinone radical which on interaction with oxygen generates superoxide. Various ROS have been detected, e.g., by ESR, including superoxide, peroxides, and hydroxyl radicals, and the ensuing step of lipoperoxidation was observed. Typical antioxidants, such as SOD, catalase, DMSO, and glutathione peroxidase proved to be effective scavengers for ROS. However, the reviewer states, "The unresolved question is whether free radicals are generated at clinical relevant concentrations of the anthracyclines and at normal (i.e., hypoxic) oxygen tension in the tumor cell and whether such free radicals could be responsible for anthracycline toxicity to the tumor." The conclusion was drawn that lipid peroxidation is not implicated in the antitumor effects. Various studies were cited indicating that free radicals do not contribute significantly to cytotoxicity. For example, ROS may be generated, but only at elevated drug concentrations. A persistent theme throughout the review was a cautionary note in drawing conclusions from experiments whose conditions do not closely emulate those pertaining to clinical practice. Others support the notion of a non-major role for free radicals [135].

In sharp contrast, Koch and co-workers in a 1998 review state unequivocally, "Induction of OS is responsible for most if not all biological activity" [136]. Production of ROS is well established, evidently from the typical redox cycling characteristic of quinones. Functionalities in the drug provide a site for chelation of iron, which should exert a favorable influence on the reduction potential. Oxy radicals generated in the proximity of

DNA possess the ability to induce strand cleavage. This scenario is buttressed by the observations that drug-resistant tumors contain lower levels of cytochrome P450 reductase which is involved in induction of OS, and higher levels of the antioxidants, glutathione peroxidase and SOD. Substantial evidence documented in our own review for pervasive involvement of OS in almost all anticancer categories should also be taken into account with regard to a possible unifying hypothesis. Also in some cases OS is difficult to detect.

The authors demonstrated that the action of **10** is more complicated. Oxidation of the keto side chain by hydrogen peroxide generates formaldehyde which subsequently effects covalent bonding of the drug via its amino group with the 2-amino moiety of a DNA guanine. In essence, the anthracycline is also functioning as an alkylating agent. They also demonstrated that synthetic anthracycline-formaldehyde conjugates, precursors for formaldehyde generation, can circumvent multi-drug resistance, thus having a potential for use in treatment of resistant cancers. Another advantage is that OS is not required for formaldehyde generation. These formaldehyde conjugates are orders of magnitude more cytotoxic that the parent substance.

The operation of a multifaceted attack, in addition to those above, was addressed. Alkylation is accompanied by intercalation and also hydrogen bonding to another DNA strand. About 15 years ago, it was reported that interaction of anthracyclines with DNA can be mediated by topoisomerase II. Increased stability conferred by the covalent DNA-drug link and intercalation may favor interaction with the enzyme, thus enhancing cytotoxicity.

Membrane effects are considered to play a role, e.g., in the topoisomerase case. Drug binding to the cell membrane apparently affects signal transduction. The various insults may ultimately terminate in the observed apoptosis.

Adverse side effects, in particular cardiotoxicity, limit the usefulness of this class. There is general consensus that induction of OS is the principal causative factor. The lower level of antioxidants in heart cells is a contributing aspect.

Other reports also address diverse mechanistic aspects [137-140]. It is evident that many avenues are being followed in the mechanism, features of which are still controversial and remain to be elucidated. Finally, it is significant that this drug category also displays antibiotic action which has been associated with an OS-ET pathway (phagomimetic action) [141].

2. Mitomycins (Naphthoquinones)

This category resembles the anthracyclines in the disagreement pertaining to important mechanistic features of cytotoxicity. Mitomycin C (MMC) (**6**) a clinically important drug, belongs to the class of bioreductive agents which undergo enzymatic activation prior to generation of alkylating entities and has already been discussed in the context of radiation sensitizers.

Prior literature centers attention on DNA adducts of MMC entailing inter- and intra-strand cross-links and mono- and di-alkylation [142,143] (see Radiation Sensitizers). Although redox cycling can occur leading to ROS, the authors claim general acceptance of the view that this aspect comprises a minor influence on cancer cell lethality. A recently modified type consists of a dimeric mitomycin incorporating a disulfide linkage, a structural feature which may permit DNA cross-linking reactions to proceed more effectively [144]. An investigation of various naphthoquinones showed that all underwent redox cycling at similar rates, but differed widely in their ability to arylate [145]. The authors concluded that arylation is a major factor in cytotoxicity since this property was in accord with the order of toxicity.

In contrast, studies revealed an excellent correlation between tumor cell kill and formation of ROS [146]. Also, drug covalently bound to DNA remained redox active with generation of oxy radicals. Conceivably, a similar scenario might pertain to tumor cells. Various mechanisms may be operating with OS playing a lesser role in hypoxic neoplasms. Additional support for the OS approach comes from the relationship between reduction potential and cytotoxicity for a series of mitomycins [147].

Results from cytotoxicity investigations of varied naphthoquinones led to the conclusion that several modes of action may pertain, including OS, depending upon substituents [148]. Additional examples in this category are the 2-phenylnaphthoquinones developed in a program oriented to topoisomerase II inhibitors that mediate DNA cleavage. Phenolic substituents can improve activity, presumably by way of hydrogen bonding or metal chelation [149]. We wish to point out that the inserted metal is a potential ET function, and

that phenols are metabolic precursors of ET quinones. Other agents in this category displayed reduction potentials conducive to ET in vivo**.**

Streptonigrin, a quinoline analog of naphthoquinone [59], is believed to act by causing DNA strand scission, in common with related compounds [150]. Transition metal ions appear to play an important role in complexation and DNA intercalation. Indolequinones are addressed in the Radiation Sensitizers section.

We surmise that a number of mechanisms are involved in the quinoidal class, including ET. The presence of ET functions, quinone and iminium from alkylation (see Alkylating Agents) lend credence, in addition to the pervasive involvement of these pathways with other anticancer agents. Various types of radicals may participate, depending in part on oxygen concentration. Very small amounts of drug should suffice since ET is a catalytic process.

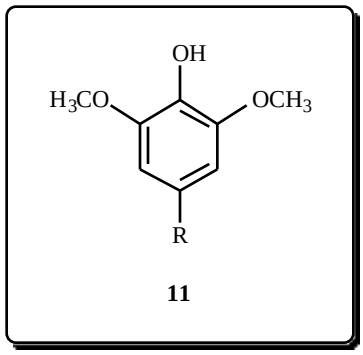
PHENOLS AND AROMATIC ETHERS

Phenols can be found in a wide variety of drug classes. The naturally occurring compounds are usually formed by aromatic hydroxylation. Redox mechanisms leading to ROS are plausible since this functionality is readily converted to ET quinones. In recent years marine organisms have yielded hundreds of physiologically active phenolic substances, mainly falling in the antimicrobial and antitumor classes [151]. Those that were cytotoxic could be grouped predominately into the following phenolic categories: p-keto, tyrosines, prenylated, and polynuclear heterocycles. Some were of the hydroquinone or catechol type which are semiquinone precursors.

Diverse types of phenols exhibit antineoplastic behavior, many being naturally occurring polyphenols [152,153], usually incorporating the catechol group. These, found in fruits, vegetables, and plants, are usually addressed as antioxidants. Proposed antitumor mechanisms include production of ROS [154,155] by redox cycling, e.g., by quinone metabolites. Some phenols are treated in the Antiestrogen section. Etoposide has been a focus of attention.

Etoposide

This drug, whose core is shown in **11**, is a semisynthetic analog of the antitumor antibiotic podophyllotoxin [107].



11

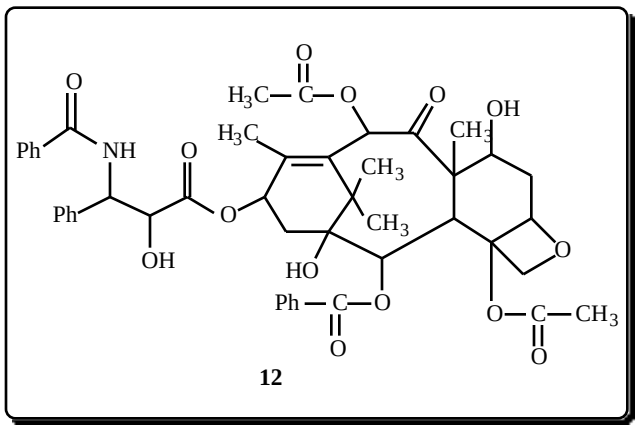
It is well established that the drug is metabolically activated by oxidation and demethylation to an o-quinone derivative, a functionality well known for redox cycling. The presence of oxygen contributes to cytotoxicity, and DNA cleavage is inhibited by radical scavengers. Other investigations have supported involvement of ROS [156]. Also, the model 3-methoxy-o-quinone exhibited a comparatively positive reduction potential making for facile electron uptake [107]. Additional possible contributors to activity comprise topoisomerase II inhibition, DNA intercalation, minor groove binding [157], and apoptosis [158]. It is significant that the quinone metabolite inhibits topoisomerase II more effectively than the parent [159]. Metals may play a role in ET [107], for example, via complex formation with the intermediate catechol [156,160]. Since aromatic ethers undergo dealkylation, as in this case, these compounds can serve as quinone precursors. Methylene acetals of catechols are not unusual in drug structures.

PACLITAXEL (TAXOL)

Although paclitaxel (**12**), a welcome relatively new addition to the pool of weapons in the battle against cancer, has undergone intensive investigation, much additional work is needed in order to elucidate the more detailed aspects of its operation. This class has attracted considerable attention related to clinical effectiveness, SAR, mechanistic aspects, metabolism, and synthetic analogs. A multiplicity of pathways appears to play a role in cytotoxicity with the relative importance of each not completely elucidated. The activities include apoptosis, microtubule arrest, cell cycle disruption, DNA fragmentation [161], inhibition of DNA synthesis [162], interference with membrane function [163], topoisomerase inhibition, and induction of NO production [164].

It is challenging to attempt a fit of **12** at this early stage into our mechanistic framework

entailing ET-OS. Oxidative stress may well play a role since **12** enhances the generation of superoxide by macrophage [165]. The effect was abolished by prior treatment with reducing agents acting as radical scavengers. Fixation to DNA occurs by groove binding [162]. The essential oxetane ring [166] may participate via hydrogen bonding to ether oxygen or conformational features associated with the rigid heterocycle [167]. We suggest that the strained ether may undergo ring opening leading to covalent alkylation of DNA bases (see Alkylating Agents). Metabolic investigations reveal oxidation as the main pathway. Benzenoid rings are transformed to phenols [168,169] which are common precursors of ET quinones. Some of the essential substituents bear aromatic rings [166,167]. Various sites in the drug are potential precursors for oxidative generation of 1,2-dicarbonyls. It is not well known that this functionality can operate in vivo in ET reactions. For example, biacetyl behaves as a radiation sensitizer [170], most of which act by electron affinity (see Radiation Sensitizers). The -ketoacetate region is a conceivable forerunner, as is the 1,2-diol generated by oxidation at C-6 [168,169]. As discussed elsewhere in this review, conjugated imines fall into the ET category and may arise non-enzymatically by condensations of carbonyl with pri-amino groups of protein. In this connection, a synthetic paclitaxel analog bearing an -ketoimine structure displayed good activity, accompanied by DNA fragmentation [171]. The abundance of ligands should make **12** an attractive candidate for metal chelation leading to ET capability.



12

Nonataxel, a nonaromatic mimic of the parent, exhibits appreciably higher activity, indicating a non-essential role for the benzenoid portions [172]. Common pharmacophores, the carbamate and two ester moieties of nonataxel, were identified for several structurally dissimilar natural cytotoxins.

MISCELLANEOUS AGENTS

This literature contains huge numbers of substances in this category, many of which also display various other types of drug activity. Only a small number have been selected as representative.

1. Hydroxyurea

This drug behaves as a ribonucleotide reductase inhibitor and exerts a myelosuppressive effect [173]. Other modes of action may be involved, e.g., indirectly by interaction with metal to form complexes capable of redox cycling [106]. Also, this simple molecule is known to undergo metabolism to nitric oxide (NO) [174], which can generate OS [175]. Hydroxyurea has also been used for treatment of HIV infections and sickle cell anemia [173].

2. Quinolones

Quinolones, particularly the fluorinated ones, have attracted much attention because of their broad spectrum potency and in vivo efficacy as antimicrobial agents [176]. Various modes of action have been demonstrated including antimitotic and inhibition of DNA gyrase and topoisomerase II [176,177]. Recent research revealed anticancer action [177]. Since this class exhibits powerful chelating ability, the resulting metal complexes, which exhibit favorable reduction potentials, could well play a role in the activity by way of OS [178].

3. Nitric Oxide

Nitric oxide, a fairly recent addition to the category of physiologically important agents [175], has been found to display anticancer activity [179]. Some lymphoma cells are sensitive to its presence. Nitric oxide generates OS, for example, as an important component in phagocytosis [175].

4. Aromatic Nitro Compounds

These include important chemotherapeutic agents [180], such as the radiation sensitizers discussed elsewhere in the review, which usually contain simple heterocycles. More complicated members incorporate acridine [181] and benzothiazoloquinolinium [1]. Benzenoid derivatives have also been investigated [182]. Mechanistic discussion of the various drugs addresses the aspect of interaction with DNA and

nitro group reduction. As pointed out in the Metabolism section, some of the intermediates have the potential for redox cycling.

5. Conjugated Quinolines

Camptothecin, the most important member of this group, is primarily associated with topoisomerase inhibition. Based on the term nucleic acid, it is reasonable to imagine protonation of quinoline nitrogen in the DNA region yielding a conjugated iminium entity. Also relevant is the observation that tumor cells are more acidic than normal ones [183]**.** At acid pH, the drug displays a reduction potential capable of ET in vivo [184]**.** The conjugated, planar, fused-ring system is one of the essential elements for activity. Structurally related compounds, including Dup 785 [184]**,** revealed relatively positive reduction potentials in most cases [185].

6. Phenothiazines

This class, which displays a variety of bio-activities, e.g., anticancer [186], includes chlorpromazine as a prominent member. Szent-Gyorgyi was a pioneer in proposing ET as a component of its drug role in the CNS [187]. Evidently, the heterocycle interferes with energy production and induces autoxidative cell injury in connection with cytotoxicity [188]. Relatively stable radical cations are generated by one-electron oxidation [189].

7. Gossypol

The substance, a cottonseed oil component, is a highly conjugated biscatechol aldehyde that displays a variety of activities including cytotoxicity. The action has been attributed to free radical generation [190] and to respiratory uncoupling [191]. Many aspects of gossypol in vivo behavior fit into our mechanistic framework [192]. It is known to produce ROS which could induce oxidative injury and a compromised antioxidant defense system. Many ET functionalities are present in the compound and its metabolites, which could play a role. The reduction potential of -0.45 V is conducive of ET. DNA strand cleavage involving ROS occurs in the presence of copper.

8. Vinca Alkaloids

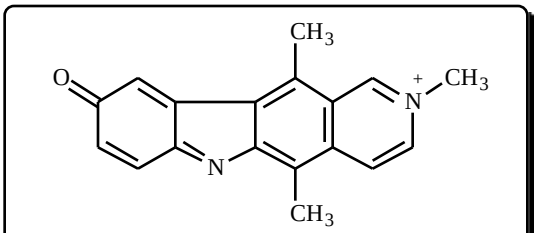
This indole category is represented by vincristine and vinblastine which have served in neoplastic treatment. Metabolic oxidation of vindoline generates a potential ET agent in the form of a conjugated iminium [193] whose electronic reduction product might also benefit energetically from transannular delocalization. It is significant that free radical generation and increased lipoperoxidation have been reported [194]. Activity has also been attributed to metaphase arrest [195] and tubulin interaction [196].

9. Antibiotics

Considerable attention has been devoted to the antitumor antibiotic class which includes streptonigrin, CC-1065, anthracyclines, aureolic acids, bleomycin, mithramycin, actinomycin, and enediynes [141,197]. Unfortunately, clinical use is limited by high toxicity. A number of factors contribute to activity, including ET-OS [141,192]. Some of these drugs are examined in greater depth in other sections.

10. Iminium Species

The ellipticines can serve to illustrate [1]. Various derivatives, as well as metabolites, incorporate conjugated iminium and the quinone-imine functionality, e.g., **13**.



13

Electrochemical data demonstrate the ability of the hydroxylated metabolite to function as an ET entity. Also, there are reports of DNA binding, generation of ROS, and DNA cleavage. Other examples, include rhodamine 123 [198] and iminium arising from condensation of methotrexate and -difluoromethylornithine with enzymes [199] (see Enzyme Inhibitors). Iminium species are formed from attack of DNA bases by alkylating drugs, which is treated in another portion of our review. The imine function is present in oximes and semicarbazones.

11. Hydrazines

Carcinogenic and anticancer activity, mostly the former, are exhibited by members of this class.

Procarbazine is an example of a substance possessing both characteristics [200]. Hydrazines are known to generate ROS species [201] and to increase lipoperoxidation [202]. Metabolic studies performed with monosubstituted derivatives support the route shown in Fig. (**14**) [203-205].

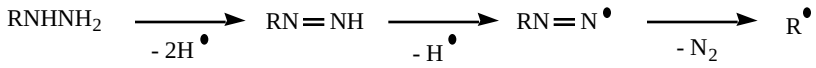


Fig. (14). Metabolism of hydrazines.

In an oxygen environment, the carbon radical readily serves as precursor for ROS which could be responsible for the observed DNA scission [206]. This sequence bears analogy to that described in the Peroxides section. Alternatively, evidence exists for a DNA alkylating pathway [204].

12. N-Nitroso Compounds

Although this category is better known for its carcinogenicity, several members have found use in cancer chemotherapy [207]. Metabolic studies point to intermediacy of a diazohydroxide which, per se or as the derived ester, can be converted to a DNA alkylator in the form of a carbocation (Fig. (**15**)) [208]. Preferred sites of methylation in DNA were found to be N-3 of adenosine and N-7 of guanosine giving rise to iminium species [209].

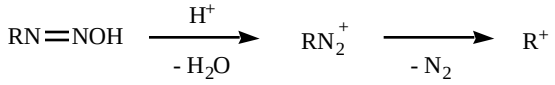


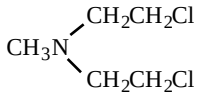
Fig. (15). Metabolism of N-nitroso compounds.

ALKYLATING AGENTS

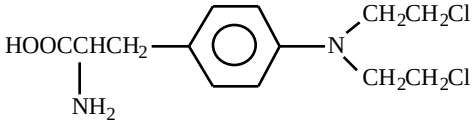
Alkylating agents were developed from sulfur mustard, the infamous "mustard gas" of World War I which exerted its lethal effect by killing various cells [210]. These lesions indicated that the toxin had a profound effect on rapidly dividing cells, suggesting that such compounds may act as potential antitumor agents. In fact, by 1931 sulfur mustard had been injected in chemotherapy directly into tumors in humans [211], but the practice was abandoned when toxicity proved to be prohibitive. At the conclusion of World War II, clinical trials performed during the war using the less reactive nitrogen mustards were declassified and published [212,213]. These agents, which reportedly kill rapidly dividing cells by alkylating cellular constituents, were found to induce temporary remission of Hodgkin's disease and acute leukemia. Despite their limited success, these trials spurred the development of less toxic and more potent alkylating derivatives, marking the beginning of modern cancer chemotherapy. Now, almost five decades later, alkylating agents are one of the more effective classes in the treatment of cancers, such as Hodgkin's and non-Hodgkin's lymphomas, lung, breast, ovarian, and brain. Representative compounds of the five more important classes are illustrated in Fig. (**16**).

Alkylating agents are drugs and prodrugs that irreversibly bind covalently to DNA through direct interaction between the drug's electron-deficient sites and DNA's electron-rich centers, such as amine, hydroxyl, and phosphate groups. The agents are non-specific and rather randomly attack DNA, RNA, enzymes, and proteins, although only DNA alkylation is reported as cytotoxic. These reactions occur either unimolecularly (SN1) or bimolecularly (SN2), depending on the type of alkylating agent. Unimolecular agents are characterized by prior formation of carbocation intermediates that rapidly react with nucleophiles at a rate dependent on formation of the ionic intermediate. Aromatic nitrogen mustards (Fig. (**16**)) react primarily by this route. Electronegative atoms or structural features that increase the reactivity of the carbon center, such as halogens and strained rings, characterize the bimolecular types. These agents are typified by the ethylene imines, methane sulfonic acid esters and alkyl nitrogen mustards which generate aziridinium intermediates (Fig. (**16**)). Rates of reaction depend on the concentration of both the alkylating agent and nucleophile.

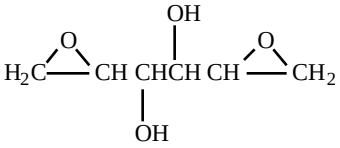
Because cell division in cancerous cells is more prevalent than in normal ones, alkylating agents have some selectivity; however they also have many serious side effects. Cell kill is accomplished by disruption of DNA function, such as unwinding, replication, and transcription, and by alteration of DNA structure, i.e., deguanylation [214] and mispairing of bases. Differences in cytotoxicity result primarily from pharmacokinetic factors, such as lipid solubility, membrane transport, detoxification reactions (including hydrolysis), and enzymatic processes capable of repairing altered regions [215]. As supported by quantum mechanical calculations [216], the N-7 of



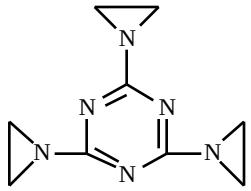
Alkyl nitrogen mustard
(Mechlorethamine)



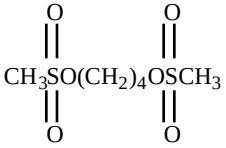
Aromatic nitrogen mustard
(Melphalan)



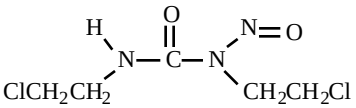
Epoxide
(Dianhydrogalacticol)



Ethyleneimine
(Azididine)



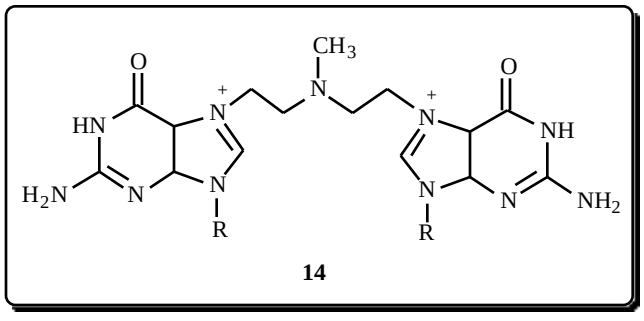
Methane sulfonic acid ester
(Busulfan)



Nitrosourea
(Carmustine)

Fig. (16). Representative DNA alkylating agents.

guanine is the most reactive, principal site of alkylation. Attack at the N-1 and N-3 of adenine and cytosine and the O-6 of guanine also occurs, but to a lesser extent. Reactivity of these nucleophiles is strongly controlled by steric, electronic, and hydrogen bonding effects. Bifunctional alkylating agents, those with two electrophilic sites, are more effective than monofunctional ones in that they can generate intra- and inter-strand cross-links, in addition to binding DNA to nucleoproteins and phosphate anions. Structure **14** illustrates a cross-linked DNA through N-7 alkylation of guanine bases by mechlorethamine.



14

In addition to altering the structure and function of DNA through binding, alkylating agents have also been proposed to induce oxidative damage to DNA [217]. These drugs are known to generate OS [217-219], but there is a paucity of information as to how this occurs. As represented in **14**, alkylation of DNA at the imine nitrogen of purine forms a conjugated iminium ion, which is a less well-known ET species. Reduction of the iminium ion, most likely by an adjacent base, gives the iminium radical which can then transfer the electron to molecular oxygen to produce superoxide (Fig. (**17**)), followed by subsequent metabolism to hydrogen peroxide and the highly damaging hydroxyl radical. As a result, redox cycling of the iminium ion incorporated into DNA might then cause significant cleavage.

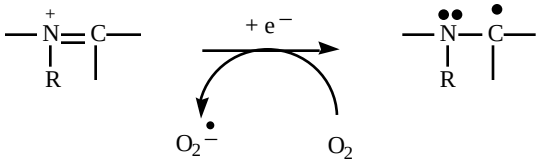


Fig. (17). Redox cycling of iminium ions.

A second possible source of oxidative damage by alkylation of DNA stems from base radical cations (holes) formed by single-electron reduction of the iminium ions. These species have been extensively studied in an effort to elucidate the factors that control the rate of migration. The rate differences observed may reflect the types and spacing of bases present in the various oligomers

employed. The reactive intermediates have been found to "hop" along the π-stack of DNA base pairs until terminated at sites of low oxidation potential, usually a guanine of a guanine-guanine nucleotide sequence [220,221]. Once trapped the guanine radical cation readily hydrates forming 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxoG), a product which is a common marker for monitoring DNA damage and a precursor to the onset of tumor formation [222]. Moreover, 8-oxoG is of even lower oxidation potential than guanine, thereby sensitizing DNA to further oxidative damage in a similar fashion or by ionizing radiation and ROS[223,224]. For example, 8-oxoG is highly reactive toward singlet oxygen [225]. Secondary oxidation results in lesions presumed to contain apurinic sites. Alternatively, the cation radical might conceivably interact with oxygen to generate alkyl peroxyl radicals capable of rendering insults to the chain. Many of these studies involved DNA oligomers with well-known electron-transfer species tethered or intercalated, such as quinones [224,226,227], riboflavin [228], and transition metals [229], in addition to ionizing radiation [230], and photoexcitation. We proposed in 1986 [1] that iminium ions generated by alkylation may also effect ET in the π-stack culminating in oxidative damage to DNA.

ENZYME INHIBITORS

1. Topoisomerase Inhibitors

In 1971, topological control of DNA was established when the enzyme topoisomerase I was isolated and found to convert a highly twisted closed form of DNA to a less twisted state. However, it was not until the 1980s that topoisomerases were recognized as the primary targets for a number of clinical anticancer drugs [231]. Since then, the field has been under intense scrutiny, progressing to a period of major discovery with the development of new and more effective topoisomerase-targeted agents [232].

Topoisomerase I and II, vital enzymes found in all living organisms, are essential in maintaining the integrity of separated DNA during metabolic processes, such as replication, transcription, and maintenance of structure. Several steps are performed by the enzymes in their interaction with DNA, including binding, cleavage, strand passage, and religation. Both enzymes effect scission by forming a reversible covalent bond between a phosphodiester linkage of the substrate and a tyrosine residue of the enzyme. This bond, the hallmark of topoisomerases, establishes the "cleavable complex." With the strand cut, whole single- or double-stranded DNA passes through the break, reducing the DNA linking number (the number of times DNA strands cross each other when projected onto a plane). After strand passage, the broken ends are rejoined without alteration of the original nucleotide sequence, followed by enzyme release.

Type I topoisomerases generate transient single-stranded breaks in DNA that allow passage of single-stranded DNA, and they not only help to remove excessive positive and negative supercoiling of DNA, but also aid in control of knotting and unknotting during replication and transcription. Moreover, their activity is independent of ATP and relatively independent of cell cycle phase. In contrast, type II topoisomerases generate transient double-stranded breaks that allow passage of double-stranded DNA, as well as cantenation and decantenation. In addition, type II topoisomerases are dependent on both ATP and the phase of cell growth. Enzyme levels are reported to increase at least 10-fold as cells change from a quiescent to a proliferating state.

Topoisomerases are major targets for several groups of clinically important antitumor drugs which can be classified into two groups. One category, termed topoisomerase poisons, inhibits by stabilizing the cleavable complex. The cleaved DNA is unable to reseal in the stabilized moiety, comprising drug, DNA, and enzyme. Subsequent adverse responses include initiation of damage-induced signaling, cell cycle arrest and initiation of apoptosis. Poisons that target topoisomerase II are diverse in structure, comprising both DNA intercalators and nonintercalators, and include anthracyclines (doxorubicin and daunomycin), acridines, (amascrine), epipodophyllotoxins (etoposide), and actinomycins [233,234]. The anthracyclines and epipodophyllotoxins are in clinical use. Type I topoisomerases are inhibited by nonintercalating camptothecin and by indolocarbazoles, which are at present in preclinical use.

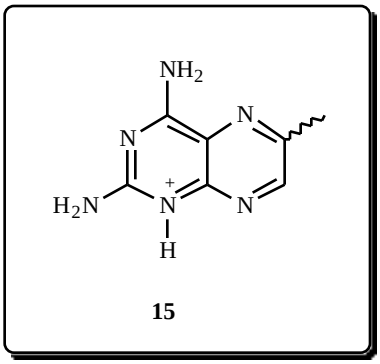
A more recent group of inhibitors function catalytically. Unlike the poisons, this class lacks the ability to stabilize the cleavable complex. Instead, they presumably function by interfering with other steps of the catalytic cycle, such as sequestering the enzymes and preventing them from performing essential functions, namely enzyme binding to DNA [235], involvement of ATP [236], and DNA breakage and rejoining [237]. Despite the fact that

the drug mechanisms are not clearly understood, there is therapeutic value since multi-drug resistance appears to be decreased [238,239]. Drugs in this category include merbarone [240], chloroquine [241], quinobenoxazine [242], and bisdioxopiperazines [243], only to name a few.

It is well established that many of these topoisomerase inhibitors also kill tumor cells by a variety of other mechanisms. For example, adriamycin, one of the more well known anthracyclines, can kill tumor cells by intercalating between DNA bases, by forming reactive DNA alkylating species, by inactivating cellular enzymes, or, most notably, by generating ROS through redox-cycling of its p-quinone or hydroquinone moieties [244] (see Quinones). Etoposide is another topoisomerase inhibitor that may generate ROS by redox-cycling of its metabolite with molecular oxygen [245,246] (see Phenols and Aromatic Ethers). The combination of enzyme inhibition and subsequent ET by the binder may be more common than currently recognized. Also, inhibition of many other enzymes by cytotoxins has been reported.

2. Dihydrofolate Reductase Inhibitors [199]

A focus of attention on the antineoplastic agent methotrexate (MTX) is inactivation of dihydrofolate reductase (DHFR). Considerable light has been shed on the nature of MTX-DHFR interaction which entails tight binding from protonation of the drug at N-1of the pteridine by acid residues to yield **15**.

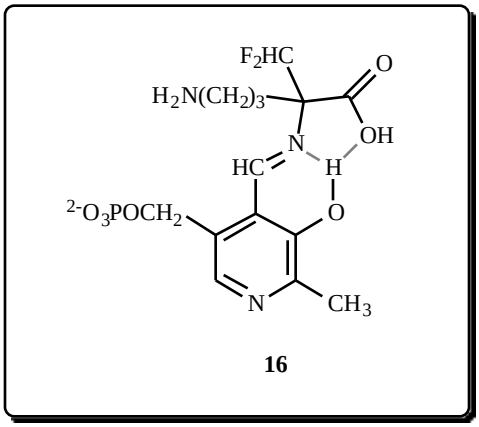


15

Reduction potentials of protonated MTX were relatively positive, in the quinone range. From x-ray data, MTX in the bound complex is in close proximity to NADPH which might then function as an electron donor in ET. Other pertinent observations include lipid peroxidation and lessening of cytotoxicity by the reducing agent ascorbate.

3. Ornithine Decarboxylase Inhibitors [199]

-Difluoromethylornithine (DFMO) apparently exerts its anticancer effect by inhibition of this enzyme which plays a role in polyamine biosynthesis. The enzyme, which is pyridoxal phosphate dependent, is believed to yield on interaction with DFMO the conjugated iminium **16**.



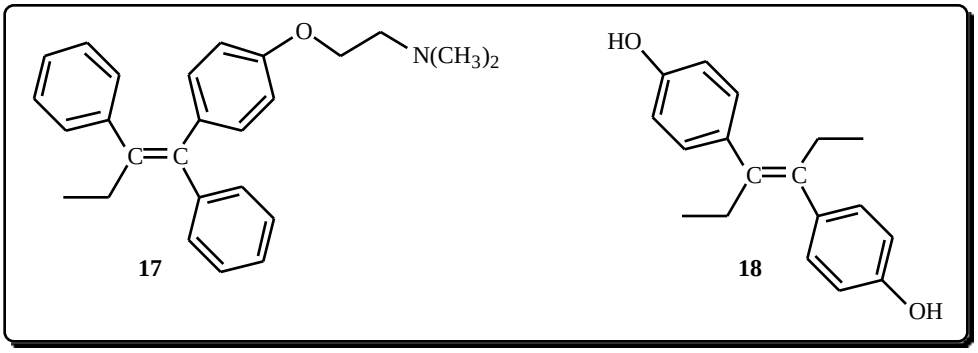
16

Reduction potentials were similar to those of quinones, which are well-known redox cyclers. Protonation of the pyridine nitrogen yields a second iminium species, thereby increasing ease of reduction. The drug potentiates DNA strand scission which is commonly associated with ROS. Also, the agent is a radiosensitizer, a property commonly associated with electron affinity (see Radiation Sensitizers). DFMO displays other drug properties that might involve ET.

INTERCALATORS

Intercalators are a major class of clinically important DNA-interactive drugs which include the anthracyclines, acridines, actinomycins, mithramycins, and bleomycin [214]. These agents contain flat aromatic or heteroaromatic moieties which bind to DNA by inserting between the stacked base pairs of the double helix. Intercalation is an energetically favorable process in which the principal driving forces are stacking and charge-transfer reactions [247]. Vertical separation of the base pairs occurs, distorting the regular helical structure and unwinding it at the site of binding. Consequently, there is interference with the action of DNA-binding enzymes, such as topoisomerases and polymerases.

Although controversial, evidence suggests, however, that for many of these agents intercalation alone is not sufficient [248] and that damage to



DNA by ET mechanisms may contribute [249,250]. The principal ET mechanism responsible for much of the damage to DNA is redox cycling of the drug with oxygen to form superoxide and, consequently, the highly reactive hydroxyl radical. It is significant that many of the intercalating agents contain well-known ET entities, such as p-quinone of the anthracyclines, p-iminoquinone of the actinomycins, aromatic nitro group of nitroacridine derivatives, and iron ion bound to bleomycin and anthracyclines.

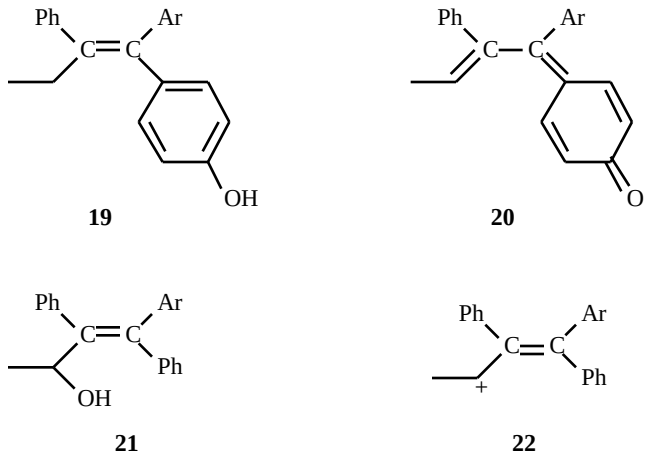
An alternative ET mechanism entails electron transfer from DNA bases to intercalated drug, a process reported as a major driving force for intercalation [214]. The process forms the drug radical anion and base radical cation. The radical anion may generate ROS by redox cycling with oxygen, and the base radical cation can migrate along the DNA helix until it is trapped by reaction with oxygen or water, resulting in oxidative damage to DNA [226,251]. Certain intercalated anthraquinone derivatives upon activation with ultraviolet light function by this process [224]. Thus, this category represents another example of complementarity of ET-OS [1] with a traditional approach, namely intercalation (see Alkylating Agents).

MINOR GROOVE BINDERS

The three main classes are CC-1065, distamycins, and pyrrolobenzodiazepines [252]. For some in this category, alkylation is part of the binding process [253,254]. Although these binders have attracted considerable attention, in part due to good activity, high toxicity presents a practical hurdle.

ANTIESTROGENS

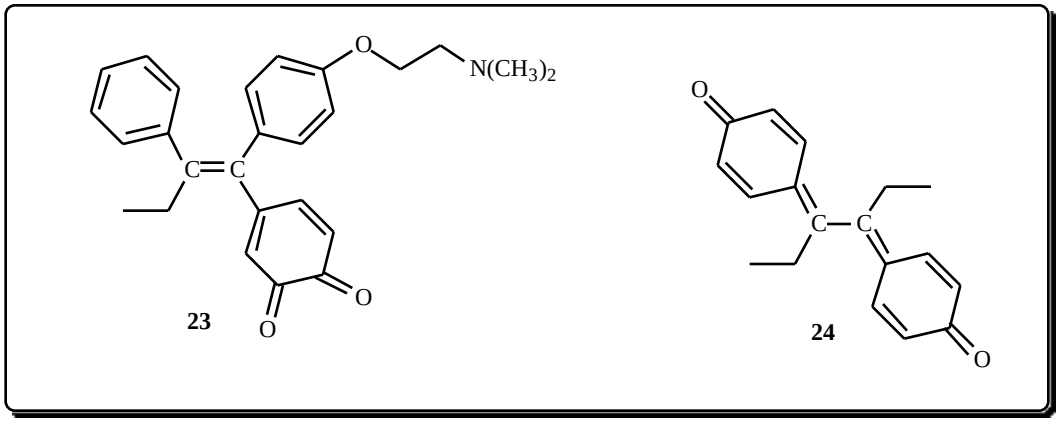
Carcinomas of the sex organs, such as ovaries, breast, prostate, and testes, exhibit the hormonal requirements of the tissue from which they arose. Therefore, one reasonable therapeutic approach has been the use of antagonists that inhibit biosyntheses of the required hormones [113]. Two common non-steroidal antagonists are tamoxifen **17** and diethylstilbesterol **18**. These agents, despite recent findings that they increase the incidence of endometrial cancer in patients [255,256] and cause liver cancer in animals [257], are currently used widely as primary treatments against advanced breast cancer and as adjuvant therapy against early stage breast cancer following surgery or radiation therapy.



Ar = p-$C_6H_4OCH_2CH_2N(CH_3)_2$

Fig. (18). Tamoxifen metabolites.

Compounds **17** and **18** undergo extensive metabolism in vivo yielding numerous products [258-260], many of which are capable of either binding covalently with DNA or generating ROS. These active metabolites are believed to contribute to the associated genotoxicity (Fig. (**18**)). There are two major pathways of metabolic activation of **17** to DNA alkylating species, one involving 4-hydroxylation to **19** with subsequent oxidization to the electrophilic quinone **20**. The other entails α-hydroxylation to **21** followed by activation to the stabilized conjugated carbocation **22**. Covalent adducts of compounds **20** and **22** with the



exocyclic nitrogen of deoxyguanosine of DNA have been isolated and deemed responsible for initiation or promotion of various carcinomas (see Alkylating Agents).

In addition, **17** and **18** are also metabolized to quinones which are well-known ET functionalities [261,262]. Like other quinones (see Metabolism), the extended o-quinone **23** and p-quinone **24** are capable of bioreduction to semiquinones which subsequently reduce oxygen to superoxide, followed by generation of the DNA damaging hydroxyl radical. Hence, this section represents another example of admixture entailing traditional and ET-OS modes.

ANTIMETABOLITES

Antimetabolites, structural analogs of normal metabolites, interfere with the formation or utilization of metabolites, thereby disrupting cellular functions, including replication. The most common targets of those used in the treatment of cancer are DNA and RNA. These false building blocks interfere with nucleic acids either by inhibiting enzymes required for synthesis or by causing DNA destabilization and dysfunction through incorporation. The most utilized ones can be divided into 4 groups: the antifolates (methotrexate), the fluoropyrimidines (5-fluorouracil), the pyrimidine analogs (cytarabine and 5-azacytidine), and the purine analogs (6-thioguanine and 6-mercaptopurine). Although they are chiefly used for the management of leukemias and lymphomas, the fluoropyrimidines are also effective against various solid tumors, such as cancers of the head and neck, breast, and stomach.

Most antimetabolites require conversion to their respective mononucleotides for pharmacological activity. However, oxidative metabolism of some may also generate species with ET potential. For example, the 6-thiopurines are oxidized to their respective purinic 6-sulfenic and 6-sulfinic acids [263,264]. These species, examined electrochemically, were found to possess reduction potentials near the physiological range [1,265], suggesting that ROS may be generated via redox cycling with oxygen. The photochemical behavior of 6-mercaptopurine and some of its metabolic oxidation products has also been investigated. Their photoexcited states were found to reduce oxygen to superoxide, thereby implicating sunlight in the high incidence of skin cancer in patients receiving 6-mercaptopurine in the form of the pro-drug azathioprine [266]. Finally, the thiyl radical can combine with oxygen to generate various ROS which may be more reactive than their carbon-based counterparts with accompanying biological significance [267-269].

APOPTOTIC AGENTS

For many years, the actions of cytotoxic drugs were ascribed to their ability to damage the chromosomes of tumor cells [270]. However, over the last decade, evidence now shows that the mechanism of many of these agents entails apoptosis [271,272] (programmed cell death), a process in which cells self-destruct when triggered by appropriate internal or external stimuli, and it is distinguished from necrosis (accidental cell death) mainly by morphological and biochemical features. The stimuli involved are as disparate as activation of the tumor suppressor gene p53 by genotoxic agents [273], heat shock [274], hypoxia [275], radiation [276,277], DNA-damaging antitumor drugs [276], and ROS [278-281]. Apoptotic features include cell shrinkage, loss of cellular contact with neighbors, nuclear membrane blebbing, chromatin aggregation, and DNA fragmentation. However, the dichotomy between apoptosis and necrosis has been lessened recently by studies showing many mechanistic and

biochemical similarities [282], suggesting that the two likely represent extremes of a range of possible cell death initiators [283].

As a normal physiological event, apoptosis is crucial to healthy development of organisms by offsetting cell proliferation. Most importantly, the process removes cells that are no longer needed within the body, for example, in embryonic development and in daily maintenance of mature organisms. It is also responsible for the elimination of cells that are a threat to the health of the organism, such as those that have mutated or are harboring viruses. However, various phenomena can disturb the regulation of apoptosis, resulting in either too much or too little cell death. Deregulation can cause many types of developmental defects and a variety of diseases, namely, cancer, autoimmune types, and neurodegeneration [284]. Moreover, insensitivity to induction of apoptosis may also result in cancer resistance to antitumor treatments [272]. For example, many types of cancers reveal aberrations in the tumor-suppressor p53 gene that is known to induce apoptosis arising from excessive DNA damage. Therefore, aberrant p53 cannot induce cell death after exposure to DNA-damaging therapies and drugs.

Almost all types of cancer treatments have been shown to kill tumor cells by inducing apoptosis [271,272]. Literature reports include radiation, adriamycin, 5-fluorouracil, and etoposide. Many of these presumably function by the generation of ROS. Support for the role of ROS stems from studies showing OS in apoptotic cells, from induction by hydrogen peroxide [285,286], superoxide [287], hydroxyl radicals [285], and lipid peroxides [288]. In addition, apoptosis also has been reported to occur through depletion of intracellular antioxidative enzyme levels, i.e., glutathione, catalase and superoxide dismutase, thus reducing the ability of the cell to scavenge and detoxify ROS [289,290]. Likewise, various antioxidative agents, for example, butylated hydroxyanisole and α-tocopherol, have shown the capacity to inhibit OS-induce apoptosis in UV-irradiated cancerous cells [276].

There are three functionally different phases in the process of apoptosis, in which ROS are suggested to play a role [284]. In the first (induction), an extracellular stimulus triggers the event. Although controversial, triggering by ROS conceivably occurs by uncoupling of electron transport from ATP within mitochondria. In the second (effector), which appears to be a central coordinator, induction leads to the opening of the mitochondrial pores facilitating the diffusion of low molecular weight compounds between the intermembrane space and the cytosol [291]. This phase is characterized by disruption of the membrane electric potential [292], depletion of reduced glutathione [293], release of cytochrome c and apoptosis induction factor (AIF), and the excessive generation of ROS [292,293]. If unchecked, the release of ROS and the other species into the cytosol accelerates the process and acts as a positive-feedback loop. This results in a self-amplifying cycle: the all-or-nothing switch of apoptotic cell death. During the final phase, ROS and the other agents that were released into the cytosol during the effector phase [294,295] either directly or by activation of various proteolytic enzymes, i.e., caspase, produce the characteristic morphological and biochemical degradation processes. Thus, the omnipresent ROS species evidently can play significant roles at various stages leading to apoptosis.

PART II: NEWER APPROACHES

This review would be incomplete without mention of the important aspect of multi-drug resistance, which was first observed some time ago and has escalated in recent years. The problem is reaching crisis proportions in the anti-infectious drug area. Contributing factors are mutation of cancers and infectious agents, as well as drug misuse. It is imperative that a multi-pronged attack be waged including classical screening, mechanism-based approaches, and, importantly, the exploration of novel avenues. In addition, more research is needed on the nature and functioning of cancer cells. This portion briefly deals with some newer approaches.

ANTIANGIOGENESIS

In 1971, the proposal that tumor growth could be stopped by inhibiting its ability to generate new blood vessels [296] followed from the finding that thalidomide, the notorious sedative banned in the 1950s because it caused birth defects when taken during pregnancy, inhibited embryonic blood vessel formation. Now decades later, this proposal has developed into a major strategy of cancer research that is beginning to provide drugs which are not only effective against a wide variety of tumors in animal studies [297], but also exhibit minimal side effects and drug resistance. Currently, more than 20 agents are in human clinical trials.

Angiogenesis, the formation of blood vessels to host tissue, occurs only rarely in normal adults, excluding wound repair and the female reproductive cycle, but is critical for growth and spread (metastasis) of solid tumors. Current antiangiogenesis agents block tumor recruitment of endothelial cells by inhibiting specific molecules that activate new blood vessel formation, such as endothelial growth factors released by the tumor, or by disrupting endothelial cell function, e.g., through release of metalloproteases into the surrounding tissue. Of considerable recent interest is the isolation of two proteins—angiostatin and endostatin—produced by primary tumors, that inhibit angiogenesis in other tumors. When administered together, the combination was found to eradicate all forms of cancer tumors in mice, with no apparent side effects. Clinical trials are underway.

It is quite interesting that a nitrocatechol derivative potentiates antiangiogenic activity, and D-penicillamine, a strong metal chelator, inhibits neovascularization [297]. Both compounds are potential ET agents.

ANTISENSE AGENTS

Some of traditional chemotherapy brings about the dysfunction of proteins critical to the survival or proliferation of the tumor. In contrast, antisense cancer therapy targets the formation of proteins by blocking the transmission of genetic information between the nucleus and protein production sites [298]. Because the therapy acts at the genetic level, it has the potential of being highly specific with low toxicity. However, problems with agent delivery and degradation have temporarily hampered the realization of their full potential.

Antisense agents are synthetic oligonucleotides of 15-30 units specifically sequenced complementary (antisense sequence) to the unit of mRNA responsible for translation of the target protein (sense sequence). These agents act by binding specifically to their mRNA complement, resulting in a mRNA-drug duplex that is unable to translate the protein. Inhibition is usually due to blocking of ribosome movement along the mRNA or to increases in the rate of degradation by ribonucleases. The resulting loss either kills the cell or inhibits cell proliferation.

ET and ROS do not play a role in the general action of antisense agents. However, a few attempts have been made to enhance their efficacy by attaching to the terminus or middle of the chains intercalating agents [299] that possess well-established ET moieties, such as anthracyclines [300]. Designed to increase the binding constant of the oligonucleotides, the ET agent can also inflict damage by generating ROS.

CELL SIGNAL TRANSDUCTION

This topic has enjoyed a flood of attention in recent years. The rapidly expanding body of knowledge holds promise for the development of new cancer treatments. A possible point of attack might be the initial interaction between the growth factor and its cognate receptor at the tumor cell surface [301]. In consideration of our mechanistic framework, it should not be surprising that an extensive review documents the interaction of free radicals with cell signaling pathways and points out how this plays an important role [302].

GENE THERAPY [303,304]

This concept for the amelioration or cure of a genetic disease involves recombination DNA technology, introduction of DNA in functioning form into mammalian cells, and repair or correction of defective genes. In cancer, the gene that normally suppresses abnormal cell proliferation fails to do so. Use in clinical research is expanding rapidly, and application to cancer therapy has been in the forefront in early trials. However, at this beginning stage there are questions about technique, effectiveness, and safety.

ACKNOWLEDGEMENT

Peter Kovacic acknowledges the contributions of his coworkers cited in the references.

LIST OF ABBREVIATIONS

ADP = Adenine dinucleotide phosphate

AIF = Apoptosis induction factor

AOs = Antioxidants

ATP = Adenosine triphosphate

CNS = Central nervous system

DFMO = -Difluoromethylornithine

DHFR = Dihydrofolate reductase

DMSO = Dimethyl sulfoxide

DNA = Deoxyribonucleic acid

ET-OS = Electron transfer-oxidative stress

ESR = Electron spin resonance

$FADH_2$ = Flavin adenine dinucleotide

GSH = Glutathione

HIV = Human immunodeficiency virus

HpD = Hematoporphyrin derivative

LDL = Low density lipoproteins

MMC = Mitomycin C

MPO = Myeloperoxidase

MTX = Methotrexate

NADPH = Nicotinamide adenine dinucleotide phosphate

NO = Nitric oxide

8-OxoG = 7,8-dihydro-8-oxo-2′-deoxyguanosine

OS = Oxidative stress

PDT = Photodynamic therapy

ROS = Reactive oxygen species

RNA = Ribonucleic acid

SAR = Structure activity relationship

SN1 = Unimolecular nucleophilic substitution

SN2 = Bimolecular nucleophilic substitution

SOD = Superoxide dismutase

UV = Ultraviolet

V = Volts

REFERENCES

[1] Kovacic, P.; Ames, J.R.; Lumme, P.; Elo, H.; Cox, O.; Jackson, H.; Rivera, L.A.; Ramirez, L.; Ryan, M.D. *Anti-Cancer Drug Des.*, **1986**, *1*, 197.

[2] Brues, A.M.; Barron, E.S.G. *Annu. Rev. Biochem.*, **1951**, *20*, 343.

[3] Holman, R.A. *Lancet*, **1956**, *ii*, 519.

[4] Harman, D. *J. Gerontol.*, **1956**, *11*, 298.

[5] Kovacic, P. *Ohio J. Sci.*, **1959**, *59*, 318.

[6] Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*, Clarendon Press: Oxford, **1985**, pp. 306-313.

[7] Emanuel, N.M.; Bogdanov, G.N.; Orlov, V.S. *Russ. Chem. Rev.*, **1984**, *53*, 1121.

[8] Lown, J.W. *Acc. Chem. Res.*, **1982**, *15*, 381.

[9] Huang, C.C.; Han, C.S.; Yue, X.F.; Shen, C.M.; Wang, S.W.; Wu, F.G.; Xu, B. *J. Natl. Cancer Inst.*, **1983**, *71*, 841.

[10] Harris, C.C. *J. Natl. Cancer Inst.*, **1979**, *63*, 275.

[11] Waring, M.J. *Annu. Rev. Biochem.*, **1981**, *50*, 159.

[12] Kovacic, P.; Kassel, M.A.; Feinberg, B.A.; Corbett, M.D.; McClelland, R.A. *Bioorg. Chem.*, **1990**, *18*, 265.

[13] Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*, Oxford University Press: New York, **1999**, pp. 531-533.

[14] Wei, H.; Cai, Q.; Rahn, R.; Zhang, X. *FreeRadical Biol. Med.*, **1997**, *23*, 148.

[15] Doetsch, P.W.; Zastawny, T.H.; Martin, A.M.; Dizdaroglu, M. *Biochemistry*, **1995**, *34*, 737.

[16] Roots, R.; Okada, S. *Radiat. Res.*, **1975**, *64*, 306.

[17] Ward, J.F.; Webb, C.F.; Limoli, C.L.; Milligan, J.R. In *Ionizing Radiation Damage to DNA: Molecular Aspects*; Wallace, S.S.; Painter, R.B., Eds.; Wiley-Liss: New York, **1990**, pp. 43-50.

[18] Qu, X.; Chaires, J.B. *J. Am. Chem. Soc.*, **1999**, *121*, 2649.

[19] Hall, E.J.; Cox, J.D. In *Radiation Oncology, Rationale, Techniques, and Results*; Cox, J.D., Ed.; Mosby: St. Louis, **1994**, pp. 3-66.

[20] Remers, W.A. In Wilson and Gisvold's Textbook of Organic, Medicinal and Pharamaceutical Chemistry, Delgado, J.N.; Remers, W.A., Eds., Lippincott, New York, **1991**, pp. 313-355.

[21] Gatenby, R.A.; Kessler, H.B.; Rosenblum, J.S.; Coia, L.R.; Moldofsky, P.J.; Broder, G.J.; Hartz, W.H. *Int. J. Radiat. Oncol. Biol. Phys.*, **1988**, *14*, 831.

[22] Kennedy, K.A. *Anti-Cancer Drug Res.*, **1987**, *2*, 181.

[23] Hockel, M.; Knoop, C.; Shlenger, K.; Vorndran, B.; Baubmann, E.; Mitze, M.; Knapstein, P.G.; Vaupel, P. *Radiotherapy Oncol.*, **1993**, *26*, 45.

[24] Nordsmark, M.; Overgaard, J.; Overgaard, M. *Radiotherapy Oncol.*, **1996**, *41*, 31.

[25] Daniels, J.S.; Gates, K.S.; Tronche, C.; Greenberg, M.M. *Chem. Res. Toxicol.*, **1998**, *11*, 1254.

[26] Lin, A.J.; Cosby, L.A.; Shansky, C.W.; Sortorelli, A.C. *J. Med. Chem.*, **1972**, *15*, 1247.

[27] Denny, W.A.; Wilson, W.R. *Cancer Metastasis Rev.*, **1993**, *12*, 135.

[28] Workmann, P.; Stratford, I.J. *Cancer Metastasis Rev.*, **1993**, *12*, 73.

[29] Overgaard, J. *Oncol. Res.*, **1994**, *6*, 509.

[30] Brezden, C.B.; McClelland, R.A.; Rauth, A.M. *Biochem. Pharmacol.*, **1994**, *48*, 361.

[31] Koch, C.J.; Evans, S.M.; Lord, E.M. *Br. J. Cancer*, **1995**, *72*, 869.

[32] Ings, R.M.J.; Mc Fadzean, J.A.; Ormerod, W.E. *Biochem. Pharmacol.*, **1974**, *23*, 1421.

[33] Declerq, P.J.; Deranter, C.J. *Biochem. Pharmacol.*, **1986**, *35*, 59.

[34] Adams, G.E. *Radiat. Res.*, **1992**, *132*, 129.

[35] Adams, G.E.; Cooke, M.S. *Int. J. Radiat. Biol.*, **1969**, *15*, 457.

[36] Al-Kazwini, A.T.; O'Neill, P.; Adams, G.E.; Fielden, E.M. *Radiat. Res.*, **1990**, *121*, 149.

[37] Brezden, C.B.; Horn, L.; McClelland, R.A.; Rauth, A.M. *Biochem. Pharmacol.*, **1998**, *56*, 335.

[38] Hodgkiss, R.J. *Anti-Cancer Drug Des.*, **1998**, *13*, 687.

[39] Whitmore, G.F.; Varghese, A.J. *Biochem. Pharmacol.*, **1986**, *35*, 97.

[40] Varghese, A.J.; Whitmore, G.F. *Cancer Clin. Trials*, **1980**, *3*, 43.

[41] Varghese, A.J.; Whitmore, G.F. *Cancer Res.*, **1980**, *40*, 2165.

[42] Noss, M.B.; Panicucci, R.; McClelland, R.A.; Rauth, A.M. *Biochem. Pharmacol.*, **1988**, *37*, 2585.

[43] Gip, J.J.; McClelland, R.A.; Mulcahy, R.T. *Biochem. Pharmacol.*, **1991**, *42*, S127.

[44] Berube, L.R.; Farah, S.; A., M.R.; Rauth, A.M. *Biochem. Pharmacol.*, **1991**, *42*, 2153.

[45] Siim, B.G.; Van Zijl, P.L.; Brown, J.M. *Br. J. Cancer*, **1996**, *73*, 952.

[46] Zeman, E.M.; Brown, J.M.; Lemmon, M.J.; Hirst, V.K.; Lee, W.W. *Int. J. Radiat. Oncol. Biol. Phys.*, **1986**, *12*, 1239.

[47] Zeman, E.M.; Baker, M.A.; Lemmon, M.J.; Pearson, C.I.; Adams, J.A.; Brown, J.M.; Lee, W.W.; Tracy, M. *Int. J. Radiat. Oncol. Biol. Phys.*, **1989**, *16*, 977.

[48] Brown, J.M.; Siim, B.G. *Semin. Radiat. Oncol.*, **1996**, *6*, 22.

[49] Priyanarsini, K.; Indira, K.; Tracy, M.; Wardman, P. *Free Radical Res.*, **1996**, *25*, 393.

[50] Daniels, J.S.; Gates, K.S. *J. Am. Chem. Soc.*, **1996**, *118*, 3380.

[51] Daniels, J.S.; Chatterji, T.; MacGillivray, L.R.; Gates, K.S. *J. Org. Chem.*, **1998**, *63*, 10027.

[52] Brown, J.M. *Br. J. Cancer*, **1993**, *67*, 1163.

[53] Elwell, J.H.; Siim, B.G.; Brown, J.M.; Brown, E. *Biochem. Pharmacol.*, **1997**, *54*, 249.

[54] Sies, H. Angew. Chem., Int. Ed. Engl., **1986**, 25, 1058.

[55] Jones, G.D.D.; Weinfeld, M. *Cancer Res.*, **1996**, *56*, 1584.

[56] Stubbe, J.; Kozarich, J.W. *Chem. Rev.*, **1987**, *87*, 1107.

[57] Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Giraud, L.; Imwinkelried, P.; Muller, S.N.; Schwitter, U. *J. Am. Chem. Soc.*, **1995**, *117*, 6146.

[58] Stratford, I.J.; Workman, P. *Anti-Cancer Drug Des.*, **1998**, *13*, 519.

[59] Jaffar, M.; Naylor, M.A.; Robertson, N.; Stratford, I.J. *Anti-Cancer Drug Des.*, **1998**, *13*, 593.

[60] Haffty, B.G.; Son, Y.H.; Sasaki, C.T.; Papac, R.; Fischer, D.; Rockwell, S.; Sartorelli, A. *Int. J. Radiat. Oncol. Biol. Phys.*, **1993**, *27*, 241.

[61] Everett, S.A.; Naylor, M.A.; Nolan, J.; Patel, K.B.; Wardman, P. *Anti-Cancer Drug Des.*, **1998**, *13*, 535.

[62] Moody, C.J.; Swann, E. *Farmaco*, **1997**, *52*, 271.

[63] Shenoy, M.A.; Singh, B.B. *Int. J. Radiat. Biol.*, **1985**, *48*, 315.

[64] Teicher, B.A.; Holden, S.A. *Radiat. Res.*, **1987**, *109*, 58.

[65] Koike, H.; Hori, H.; Inayama, S. *Biochem. Biophys. Res. Commun.*, **1988**, *155*, 1066.

[66] Nias, A.H. *Int. J. Radiat. Biol.*, **1985**, *48*, 297.

[67] Chibber, R.; Stratford, I.J.; O'Neill, P.; Sheldon, P.W.; Ahmed, I.; Lee, B. *Int. J. Radiat. Biol.*, **1985**, *48*, 513.

[68] Chatterjee, S.R.; Kamat, J.P.; Shetty, S.J.; Banerjee, S.; Srivastava, T.S.; Devasagayam, T.P.A. *Radiat. Phys. Chem.*, **1997**, *49*, 135.

[69] Salet, C.; Moreno, G. *J. Photochem. Photobiol.*, **1990**, *5*, 133.

[70] Shulok, J.R.; Wade, M.H.; Lin, C.W. *Photochem. Photobiol.*, **1990**, *51*, 451.

[71] Salet, C.; Moreno, G.; Atlante, A.; Passarella, S. *Photochem. Photobiol.*, **1991**, *53*, 391.

[72] Henderson, B.W.; Dougherty, T.J. *Photochem. Photobiol.*, **1992**, *55*, 145.

[73] Fuchs, J.; Theile, J. *Free Radical Biol. Med.*, **1998**, *24*, 835.

[74] Kato, H. *J. Photochem. Photobiol.*, **1998**, 42, 96.

[75] Sutedja, T.G.; Postmus, P.E. *J. Photochem. Photobiol.*, **1996**, *36*, 199-204.

[76] Gomer, C.J.; Rucker, N.; Ferrario, A. *Cancer Res.*, **1986**, *46*, 3348.

[77] Krueger, T.; Ris, H.-B.; Giger, A.; C.K., L.; Stewart, J.C.M.; Althaus, U.; Altermatt, H.J. *Br. J. Cancer*, **1999**, *79*, 1061.

[78] Pandey, R.K.; Dougherty, T.J. *Photochem. Photobiol.*, **1988**, *47*, 769.

[79] Jori, G.; Beltramini, M. *Cancer Lett.*, **1984**, *24*, 291.

[80] Weishaupt, K.R.; Gomer, C.J.; Dougherty, T.J. *Cancer Res.*, **1976**, *36*, 2326.

[81] Chapman, J.D.; Stobbe, C.C.; Arnfield, M.R.; Santus, R.; Lee, J.; McPhee, M.S. *Radiat. Res.*, **1991**, *126*, 73.

[82] Fingar, V.H.; Wieman, T.J.; Wiehle, S.A. *Cancer Res.*, **1992**, *52*, 4914.

[83] Henderson, B.W.; Waldows, S.M.; Mang, T.S. *Cancer Res.*, **1985**, *45*, 572.

[84] Henderson, B.W.; Finger, V.H. *Cancer Res.*, **1987**, *47*, 3100.

[85] Star, W.M.; Marijnissen, H.P.A.; Van den Berg-Blok, A.E. *Cancer Res.*, **1986**, *46*, 2532.

[86] Foster, T.H.; Gao, L. *Radiat. Res.*, **1992**, *130*, 379.

[87] Kindzelskii, A.L.; Petty, H.R. *Mutat. Res.*, **1999**, *426*, 11.

[88] Plaine, H.L. *Genetics*, **1955**, *40*, 268.

[89] Makino, S.; Tanaka, T. *Gann*, **1953**, *44*, 39.

[90] Hollcroft, J.W.; Lorenz, E. *Proc. 2nd Nat. Cancer Conf.*, **1952**, 582.

[91] Holman, R.A. *Nature*, **1957**, *179*, 1033.

[92] Samoszuk, M.K.; Rietveld, C.; Gidanian, F.; Petersen, A. *Cancer*, **1989**, *63*, 2111.

[93] Sugiyra, K. *Nature*, **1958**, *182*, 1310.

[94] Mavier, P.; Guigui, B.; Preaux, A.M.; Rosenbaum, J.; Lescs, M.C.; Zafrani, E.S.; Dhumeaux, D. *Hepatology*, **1988**, *8*, 1673.

[95] Konishi, T.; Matsugo, S. *Biochim. Biophys. Acta*, **1988**, *967*, 267.

[96] Murphy, G.P. *CA Cancer J. Clin.*, **1993**, *43*, 47.

[97] Nicolaou, K.C.; Smith, A.L. *J. Med. Chem.*, **1996**, *39*, 2103.

[98] Langley, D.R. In Borders, D.B.; Doyle, T.W. *Enediyne Antibiotics as Antitumor Agents*, Marcel-Dekker: New York, **1995**, pp. 239-279.

[99] Dedon, P.C.; Goldberg, I.H. *Chem. Res. Toxicol.*, **1992**, *5*, 311.

[100] Kappen, L.S.; Goldberg, I.H. *Biochemistry*, **1999**, *38*, 235.

[101] Xu, Y.Z., Y.; Goldberg, I.H. *J. Am. Chem. Soc.*, **1997**, *119*, 1133.

[102] Xu, Y.; Zhen, Y.; Goldberg, I.H. *Biochemistry*, **1997**, *36*, 14975.

[103] Kratz, F.; Schutte, M.T. *Canc. J.*, **1998**, *11*, 176.

[104] El-Naggar, M.M.; El-Waseef, A.M.; El-Halafawy, K.M.; El-Sayed, I.H. *Cancer Lett.*, **1998**, *133*, 71.

[105] Clarke, M.J.; Limauro, S. In *Handbook of Metal -Ligand Interactions in Biological Fluids: Bioinorganic Chemistry*; Berthan, G., Ed.; Marcel-Dekker: New York, **1995**, pp. 1010-1018.

[106] Kovacic, P.; Popp, W.J.; Ames, J.R.; Ryan, M.D. *Anti-Cancer Drug Des.*, **1988**, *3*, 205.

[107] Kovacic, P.; Ames, J.R.; Ryan, M.D. In *Redox Chemistry and Interfacial Behavior of Biological Molecules*; Dryhurst, G.; Niki, K., Eds.; Plenum Press: New York, **1988**, pp. 345-357.

[108] Jamieson, E.R.; Lippard, S.J. *Chem. Rev.*, **1999**, *99*, 2467.

[109] Clausen, C.A.; Long, E.C. *Chem. Rev.*, **1999**, *99*, 2797.

[110] Shaw, C.F. *Chem. Rev.*, **1999**, *99*, 2589.

[111] Clarke, M.J.; Zhu, F.; Frasca, D.R. *Chem. Rev.*, **1999**, *99*, 2511.

[112] Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*, Oxford University Press: New York, **1999**, pp. 711-715.

[113] Gringauz, A. *Introduction to Medicinal Chemistry: How Drugs Act and Why*, Wiley & Sons: New York, **1997**, pp. 128-130.

[114] Rashid, R.; Langfinger, D.; Wagner, R.; Schuchmann, H.-P.; Von Sonntag, C. *Int. J. Radiat. Biol.*, **1999**, *75*, 101.

[115] Kroemer, H.T.G. *J. Natl. Cancer Inst.*, **1999**, *91*, 743.

[116] Li, Y.M.; Broome, J.D. *Cancer Res.*, **1999**, *59*, 776.

[117] Gebel, T.; Birkenkamp, P.; Luthin, S.; Dunkelberg, H. *Anticancer Res.*, **1998**, *18*, 4253.

[118] Wang, A.; An, Y.; Gao, Z.; Wang, Y. *Zhongguo Gonggong Weisheng Xuebao*, **1998**, *17*, 86.

[119] Ahmad, S.; Anderson, W.L.; Kitchin, K.T. *Cancer Lett.*, **1999**, *139*, 129.

[120] Ohndorf, U.-M.; Rould, M.A.; He, Q.; Pabo, C.O.; Lippard, S.J. *Nature*, **1999**, *399*, 708.

[121] Chang, L.-C.; Sheu, H.-M.; Huang, Y.-S.; Tsai, T.-R.; Kuo, K.-W. *Biochem. Pharmacol.*, **1999**, *58*, 49.

[122] Takahara, P.M.; Rosenzweig, A.C.; Frederich, C.A.; Lippard, S.J. *Nature*, **1995**, *377*, 649.

[123] Stinson, S. *Chem. Eng. News*, **1992**, *70*, 19.

[124] Wachowicz, B.; Kustron, J. *Cytobios*, **1992**, *70*, 41.

[125] Uslu, R.; Bonavida, B. *Cancer*, **1996**, *77*, 725.

[126] Masuda, H.; Tanaka, T.; Takahama, U. *Biochem. Biophys. Res. Commun.*, **1994**, *203*, 1175.

[127] Shellard, S.A.; Hosking, L.K.; Hill, B.T. *Cancer Res.*, **1991**, *51*, 4557.

[128] Giyajima, A.; Nakashoma, J.; Yoshioka, K.; Tachibana, M.; Tazaki, H.; Murai, M. *Br. J. Cancer*, **1997**, *76*, 206.

[129] Miyajima, A.; Nakashima, J.; Tachibana, M.; Nakamura, K.; Hayakawa, M.; Murai, M. *Jpn. J. Cancer Res.*, **1999**, *90*, 565.

[130] Nias, A.H.W. *Int. J. Radiat. Biol.*, **1985**, *48*, 297.

[131] Llopis, J.F.; Colom, F. In *Encyclopedia of Electrochemistry of the Elements*; Bard, A.J., Ed.; Marcel-Dekker: New York, **1976**, pp. 180, 183, 185.

[132] Aletras, V.; Hadjiliadis, D.; Hadjiliadis, N. *Epitheor. Klin. Farmakol. Farmakokinet.*, **1995**, *13*, 153.

[133] Ikeda, K.; Kajiwara, K.; Tanabe, E.; Tokumaru, S.; Kishida, E.; Masuzawa, Y.; Kojo, S. *Biochem. Pharmacol.*, **1999**, *57*, 1361.

[134] Gewirtz, D.A. *Biochem. Pharmacol.*, **1999**, *57*, 727.

[135] Tarasiuk, J.; Tkaczyk-Gobis, K.; Stefanska, B.; Dzieduszycka, M.; Priebe, W.; Martelli, S.; Borowski, E. *Anti-Cancer Drug Des.*, **1998**, *13*, 923.

[136] Taatjes, D.; Fenick, D.J.; Gaudiano, G.; Koch, T.H. *Curr. Pharm. Des.*, **1998**, *4*, 203.

[137] Weiss, R.B. *Semin. Oncol.*, **1992**, *19*, 670.

[138] Doroshow, J.H. *Anthracyclines and Anthracenediones*, Lippincott-Raven: Philadelphia, **1996**, 409-434.

[139] Conie, M.-S. *Br. J. Cancer*, **1999**, *79*, 1090.

[140] Carney, J.M. *Pharmacol. Tox.*, **1998**, *83*, 62.

[141] Gutteridge, J.M.C.; Quinlan, G.J.; Kovacic, P. *Free Radical Res.*, **1998**, *28*, 1.

[142] Tomasz, M.; Palom, Y. *Pharmacol. Ther.*, **1997**, *76*, 73.

[143] Cummings, J.; Spanswick, V.J.; Tomasz, M.; Smyth, J.F. *Biochem. Pharmacol.*, **1998**, *56*, 405.

[144] Wang, S.; Kohn, H. *J. Med. Chem.*, **1999**, *42*, 788.

[145] Karczewski, J.M.; Peters, J.G.P.; Noordhoek, J. *Biochem. Pharmacol.*, **1999**, *57*, 27.

[146] Sinha, B.K. *Chem.-Biol. Interact.*, **1989**, *69*, 293.

[147] Pan, S.-S.; Gonzalez, H. *Mol. Pharmacol.*, **1990**, *37*, 966.

[148] Noda, Y.; Kawazol, Y.; Hakura, A. *Biol. Pharm. Bull.*, **1997**, *20*, 1250.

[149] Chang, H.-X.; Chou, T.-C.; Savaraj, N.L.; Liu, L.F.; Yu, C.; Cheng, C.C. *J. Med. Chem.*, **1999**, *42*, 405.

[150] Harding, M.M.; Long, G.V. *Curr. Med. Chem.*, **1997**, *4*, 405.

[151] Higa, T. *Bioorg. Mar. Chem.*, **1991**, *4*, 33.

[152] Ryahovskaya, T.V.; Ushbaeva, G.G.; Zhemaletdinov, F.G. *Rastit. Resur.* , **1989**, *25*, 249.

[153] Zhao, J.; Wang, J.; Chen, Y.; Agarwal, R. *Carcinogenesis*, **1999**, *20*, 1737.

[154] Hodnick, W.F.; Roettger, W.J.; Kung, F.S.; Bohmont, C.W.; Pardini, R.S. *Prog. Clin. Biol. Res.* , **1986**, *213*, 249.

[155] Galati, G.; Chan, T.; Wa, B.; Obrien, P.J. *Chem. Res. Toxicol.*, **1999**, *12*, 521.

[156] Sinka, B.K. *Chem. -Biol. Interactions*, **1989**, *69*, 293.

[157] Zhu, X.-K.; Guan, J.; Tachibana, Y.; Bastow, K.F.; Cho, S.J.; Cheng, H.-H.; Cheng, Y.-C.; Gurwith, M.; Lee, K.-H. *J. Med. Chem.*, **1999**, *42*, 2441.

[158] Zhao, E.G.; Song, Q.; Cross, S.; Misko, I.; Lees-Miller, S.P.; Lavini, M.F. *Int. J. Cancer*, **1998**, *77*, 755.

[159] Gantchev, T.G.; Hunting, D.J. *Biochem. Biophys. Res. Commun.*, **1997**, *237*, 24.

[160] Sinha, B.K.; Antholine, W.M.; Kalyanaraman, B.; Eliot, H.M. *Biochim. Biophys. Acta*, **1990**, *1096*, 81.

[161] Fan, W. *Biochem. Pharmacol.*, **1999**, *57*, 1215.

[162] Krishna, A.G.; Kumar, D.V.; Khan, B.M.; Rawal, S.K.; Ganesh, K.N. *Biochim. Biophys. Acta*, **1998**, *1381*, 104.

[163] Sonee, M.; Barron, E.; Yarber, F.A.; Hamm-Alvarez, S.F. *Am. J. Physiol.* , **1998**, *275*, 1630.

[164] Kirikae, T.; Ojima, I.; Ma, Z.; Kirikae, F.; Hirai, Y.; Nakano, M. *Biochem. Biophys. Res. Commun.*, **1998**, *245*, 698.

[165] Pae, H.-O.; Chang, C.-D.; Yoo, J.-C.; Kwak, H.-J.; Lee, S.-J.; Kook, Y.-A.; Park, R.-K.; Chung, H.-T. *Immunopharmacol. Immunotoxicol.* , **1998**, *20*, 27.

[166] Liang, J.; Kingston, D.G.I.; Chordia, M.D.; Jagtap, P.G.; Shen, Y.-C.; Long, B.H.; Fairchild, C.R.; Johnston, K.A. *J. Org. Chem.*, **1999**, *64*, 1814.

[167] Gunatilaka, A.A.L.; Ramdayal, F.D.; Sarragiotto, M.H.; Kingston, D.G.I.; Sackett, D.L.; Hamel, E. *J. Org. Chem.*, **1999**, *64*, 2694.

[168] Haiqing, Y.; Kingston, D.G.I. *Tetrahedron Lett.*, **1998**, *39*, 4967.

[169] Monsarrat, B.; Chatelut, E.; Royer, I.; Alvinerie, P.; Dubois, J.; Dezeuse, A.; Roche, H.; Cros, S.; Wright, M.; Canal, P. *Drug Metab. Dispos.*, **1998**, *26*, 229.

[170] Jacobs, G.P. *Int. J. Radiat. Biol.*, **1986**, *49*, 887.

[171] Distefano, M.; Scambia, G.; Ferlini, C.; Gallo, D.; De Vincenzo, R.; Filippini, P.; Riva, A.; Bombardelli, E.; Mancuso, S. *Anti-Cancer Drug Des.*, **1998**, *13*, 489.

[172] Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S.B.; S.D., K.; Danishefsky, S.J. *Proc. Natl. Acad. Sci.*, **1999**, *96*, 4256.

[173] Srinivas, R.V.; Fridland, A. *Curr. Opin. Anti-Infect. Drugs*, **1999**, *1*, 213.

[174] Sato, K.; Akaike, T.; Sawa, T.; Miyamoto, Y.; Suga, M.; Ando, M.; Maeda, H. *Jpn. J. Cancer Res.*, **1997**, *88*, 1199.

[175] Kovacic, P. *Bioelectrochem. Bioenerg.*, **1996**, *39*, 155.

[176] Kidwai, M.; Misra, P.; Kumar, R. *Curr. Pharm. Des.*, **1998**, *4*, 101.

[177] Xia, Y.; Yang, Z.-Y.; Morris-Natshke, S.L.; Lee, K.-H. *Curr. Med. Chem.*, **1999**, *6*, 179.

[178] Ames, J.R.; Kovacic, P.; Ryan, M.D. *Free Radical Biol. Med.*, **1986**, *2*, 377.

[179] Tanaka, J.; Koshimura, K.; Murakami, Y.; Tsumori, M.; Kato, Y. *Biomed. Res.*, **1998**, *19*, 415.

[180] Tocher, J.H. *Gen. Pharmacol.*, **1997**, *28*, 485.

[181] Ferguson, L.R.; Denny, W.A. *Mutat. Res.*, **1991**, *258*, 123.

[182] Alvarez-Lueje, A.; Pena, C.; Nunez-Vergara, L.; Squella, J.A. *Electroanalysis*, **1998**, *10*, 1043.

[183] Lown, W.J. *Molecular Aspects of Anti-Cancer Drug Action*, Macmillan: London, **1983**, p. 274.

[184] Kovacic, P.; Ames, J.R.; Grogan, J.W.; Hazra, B.; Ryan, M.D. In *Redox Chemistry and Interfacial Behavior of Biological Molecules*; Dryhurst, G.; Niki, K., Eds.; Plenum Publishing: New York, **1988**, pp. 295-307.

[185] Crawford, P.W.; Foye, W.O.; Ryan, M.D.; Kovacic, P. *J. Pharm. Sci.*, **1987**, *76*, 481.

[186] Hait, W.N.; Lee, G.L. *Biochem. Pharmacol.*, **1985**, *34*, 3973.

[187] Karreman, G.; Isenberg, I.; Szent-Gyorgyi, A. *Science*, **1959**, *130*, 1191.

[188] Jones, G.R.N. *Tumori*, **1985**, *71*, 563.

[189] Vincow, G. *Radical Ions*, Interscience: New York, **1968**, p. 188.

[190] Grankvist, K. *Int. J. Biochem.*, **1989**, *21*, 853.

[191] Mc Sheehy, P.M.J.; Bashford, C.L. *Biochem. Soc. Trans.*, **1988**, *16*, 616.

[192] Kovacic, P.; Becvar, L.E. *Curr. Pharm. Des.*, **2000**, *6*, 143-167.

[193] Sariaslani, F.S.; Duffel, M.W.; Rosazza, J.P. *J. Med. Chem.*, **1985**, *28*, 629.

[194] Sariaslani, F.S.; Eckenrode, F.M.; Beale, J.M.; Rosazza, J.P. *J. Med. Chem.*, **1984**, *27*, 749.

[195] Jordan, M.A.; Thrower, D.; Wilson, L. *Cancer Res.*, **1991**, *51*, 2212.

[196] Sangeetha, P.; Das, U.N.; Koratkar, R.; Suryaprabha, P. *Free Radical Biol. Med.*, **1990**, *8*, 15.

[197] Remers, W.A. *The Chemistry of Antitumor Antibiotics*, Wiley & Sons: New York, **1988**.

[198] Crawford, P.W.; Lumme, P.; Elo, H.; Ryan, M.D.; Kovacic, P. *Free Radical Res. Commun.*, **1987**, *3*, 347.

[199] Kovacic, P.; Ames, J.R.; Taylor, E.C.; Ryan, M.D. *J. Pharm. Sci.*, **1988**, *77*, 999.

[200] Sieber, S.M.; Correa, P.; Dalgard, D.W.; Adamson, R.H. *Cancer Res.*, **1978**, *38*, 2125.

[201] Weglarz, L.; Bartosy, G. *Free Radical Biol. Med.*, **1991**, *11*, 149.

[202] Kamsiah, K.; Nafeeza, M.I. *Asia Pac. J. Pharmacol.*, **1995**, *10*, 59.

[203] Albano, E.; Tomasi, A.; Iannone, A.; Goria-Gatti, L.; Vannini, V.; Dianzani, M.U. In *Eicosanoids, Lipid Peroxidation and Cancer*; McBrien, D.C.H.; Slater, T.F., Eds.; Springer-Verlag: New York, **1988**, pp. 273-280.

[204] Gamberini, M.; Cidade, M.R.; Valotla, L.A.; Armelin, M.C.; Leite, L.C. *Carcinogenesis*, **1998**, *19*, 147.

[205] Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine,* Oxford University Press: New York, **1999**, pp. 493-494.

[206] Runge-Morris, M.; Wu, N.; Novak, R.F. *Toxicol. Appl. Pharmacol.*, **1994**, *125*, 123.

[207] Magee, P.N. In *Free Radicals, Lipid Peroxidation, and Cancer*; McBrien, D.C.H.; Slater, T.F., Eds.; Academic Press: New York, **1982**, pp. 354-357.

[208] Lijinsky, W. *Chemistry and Biology of N-Nitroso Compounds,* University Press: New York, **1992**, pp. 106, 252.

[209] O'Driscoll, M.; Macpherson, P.; Xu, Y.-Z.; Karran, P. *Carcinogenesis*, **1999**, *20*, 1855.

[210] Krumbhaar, E.B.; Krumbhaar, H.D. *J. Med. Res.*, **1919**, *40*, 497.

[211] Adair, F.E.; Bagg, H.J. *Ann. Surg. (Chicago)*, **1931**, *93*, 190.

[212] Gilman, A.; Philips, F.S. *Science*, **1946**, *103*, 409.

[213] Rhoads, C.P. *J. Am. Med. Assoc.*, **1946**, *131*, 656.

[214] Silverman, R.B. *The Organic Chemistry of Drug Design and Drug Action,* Academic Press: London, **1992**, p. 220.

[215] Harris, A.L. *Cancer Surv.*, **1985**, *4*, 601.

[216] Pullman, A.; Pullman, B. *Int. J. Quantum Chem., Quantum Biol. Symp.*, **1980**, *7*, 245.

[217] Kovacic, P.; Crawford, P.W.; Ryan, M.D.; Nelson, V. *Bioelectrochem. Bioenerg.*, **1986**, *15*, 305.

[218] Mizumoto, K.; Glascott, P.A.; Farber, J.L. *Biochem. Pharmacol.*, **1993**, *46*, 1811.

[219] Higashi, K.; Inoue, N.; Tanaka, J. *Res. Commun. Chem. Pathol. Pharmacol.*, **1988**, *61*, 281.

[220] Holmlin, R.E.; Dandliker, P.J.; Barton, J.K. *Angew. Chem., Int. Ed. Engl.*, **1997**, *36*, 2714.

[221] Prat, F.; Houk, K.N.; Foote, C.S. *J. Am. Chem. Soc.*, **1998**, *120*, 845.

[222] Chen, K.C.; Cahill, D.S.; Kasai, H.; Nishimura, S.; Loeb, L.A. *J. Biol. Chem.*, **1992**, *267*, 166.

[223] Cullis, P.M.; Malone, M.E.; Merson-Davies, L.A. *J. Am. Chem. Soc.*, **1996**, *118*, 2775.

[224] Gasper, S.M.; Schuster, G.B. *J. Am. Chem. Soc.*, **1997**, *119*, 12762.

[225] Sheu, C.; Foote, C.S. *J. Am. Chem. Soc.*, **1995**, *117*, 6439.

[226] Breslin, D.T.; Schuster, G.B. *J. Am. Chem. Soc.*, **1996**, *118*, 2311.

[227] Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K. *J. Am. Chem. Soc.*, **1995**, *117*, 6406.

[228] Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.*, **1992**, *114*, 9692.

[229] Murphy, C.J.; Arkin, M.R.; Jenkins, Y.; Ghatlia, N.D.; Bossmann, S.H.; Turro, N.J.; Barton, J.K. *Science*, **1993**, *262*, 1025.

[230] Meggers, E.; Michel-Beyerle, M.E.; Giese, B. *J. Am. Chem. Soc.*, **1998**, *120*, 12950.

[231] Rothenberg, M.L. *Ann. Oncol.* , **1997**, *8*, 837.

[232] Osheroff, N. *Biochim. Biophys. Acta*, **1998**, *1400*, 1-2.

[233] Chen, A.Y.; Liu, L.F. *Annu. Rev. Pharmacol. Toxicol.*, **1994**, *34*, 191.

[234] Froelich-Ammon, S.J.; Osheroff, N. *J. Biol. Chem.*, **1995**, *270*, 21429.

[235] Sorensen, B.S.; Sinding, J.; Andersen, A.H.; Alsner, J.; Jensen, P.B.; Westergaard, O. *J. Mol. Biol.*, **1992**, *228*, 778.

[236] Andoh, T.; Ishida, R. *Biochim. Biophys. Acta*, **1998**, *1400*, 155.

[237] Wang, J.C. *Adv. Pharmacol.*, **1994**, *29A*, 1.

[238] Chen, M.; Beck, W.T. *Cancer Res.*, **1993**, *53*, 5946.

[239] Jensen, P.B.; Sorensen, B.S.; Sehested, M.; Demant, E.J.F.; Kjeldsen, E. *Biochem. Pharmacol.*, **1993**, *45*, 2025.

[240] Drake, F.H.; Hofmann, G.A.; Mong, S.M.; Bartus, J.O.; Hertzberg, R.P.; Johnson, R.K.; M.R., M.; Mirabelli, C.K. *Cancer Res.*, **1989**, *49*, 2578.

[241] Jensen, P.B.; Sorensen, B.S.; Sehested, M.; Grue, P.; Demant, E.J.F.; Hansen, H.H. *Cancer Res.*, **1994**, *54*, 2959.

[242] Permana, P.A.; Snapka, R.M.; Shen, L.L.; Chu, D.T.W.; Clement, J.J.; Plattner, J.J. *Biochemistry*, **1994**, *33*, 11333.

[243] Tanabe, K.; Ikegami, Y.; Ishida, R.; Andoh, T. *Cancer Res.*, **1991**, *51*, 4903.

[244] Keizer, H.G.; Pinedo, H.M.; Schuurhuis, G.J.; Joenje, H. *Pharmacol. Ther.*, **1990**, *47*, 219.

[245] Mans, D.R.A.; Retel, J.; van Maanen, J.M.S.; Lafleur, M.V.M.; van Schaik, M.A.; Pinedo, H.M.; Lankelma, J. *Br. J. Cancer*, **1990**, *62*, 54.

[246] Sinha, B.K. *Chem. -Biol. Interactions*, **1989**, *69*, 293.

[247] Neidle, S.; Abraham, Z. *Crit. Rev. Biochem.* , **1984**, *17*, 73.

[248] Hernandez, L.; Chology, W.M.; Hudson, E.A.; Resau, J.H.; Pauly, G.; Michejda, C.J. *Cancer Res.*, **1995**, *55*, 2338.

[249] Pindur, U.; Haber, M.; Sattler, K. *J. Chem. Ed.*, **1993**, *70*, 263.

[250] Corbett, A.H.; Osheroff, N. *Toxicol.*, **1993**, *6*, 585.

[251] Ly, D.; Kan, Y.; Armitage, B.; Schuster, G.B. *J. Am. Chem. Soc.*, **1996**, *118*, 8747.

[252] Baraldi, P.G.; Caccairi, B.; Guiotto, A.; Romagnoli, R.; Zaid, A.N.; Spalluto, G. *Farmaco*, **1999**, *54*, 15.

[253] Hurley, L.H.; Needham-VanDevante, D.R. *Acc. Chem. Res.*, **1986**, *19*, 230.

[254] Smaill, J.B.; Fan, J.-Y.; Denny, W.A. *Anti-Cancer Drug Des.*, **1999**, *13*, 857.

[255] Cuenca, R.E.; Giachino, J.; Arrendondo, M.A.; Hempling, R.; Edge, S.B. *Cancer*, **1996**, *77*, 2058.

[256] Curtis, R.E.; Boice, J.D.; Shriner, D.A.; Hankey, B.F.; Fraumeni, J.F. *J. Natl. Cancer Inst.*, **1996**, *88*, 832.

[257] Williams, G.M.; Iatropoulos, M.J.; Djordjevic, M.V.; Kaltenberg, O.P. *Carcinogenesis*, **1993**, *14*, 315.

[258] Beland, F.A.; McDaniel, L.P.; Marques, M.M. *Carcinogenesis*, **1999**, *20*, 471.

[259] Rajaniemi, H.; Rasanen, I.; Koivisto, P.; Peltonen, K.; Hemminki, K. *Carcinogenesis*, **1999**, *20*, 305.

[260] Sanchez, C.; Shibutani, S.; Dasaradhi, L.; Bolton, J.L.; Fan, P.W.; McClelland, R.A. *J. Am. Chem. Soc.*, **1998**, *120*, 13513.

[261] Dehal, S.S.; Kupfer, D. *Cancer Res.*, **1995**, *56*, 1283.

[262] Epe, B.; Schiffmann, D.; Metzler, M. *Carcinogenesis*, **1986**, *7*, 1329.

[263] Abraham, R.T.; Benson, L.M.; Jardine, I. *J. Med. Chem.*, **1983**, *26*, 1523.

[264] Doeer, I.L.; Wempen, I.; Clarke, D.A.; Fox, J.J. *J. Org. Chem.*, **1961**, *26*, 3401.

[265] Dryhurst, G. *J. Electrochem. Soc.*, **1969**, *116*, 1357.

[266] Moore, D.E.; Sik, R.H.; Bilski, P.; Chignell, C.F.; Reszka, K.J. *Photochem. Photobiol.*, **1994**, *60*, 574.

[267] Harman, L.S.; Mottley, C.; Mason, R.P. *J. Biol. Chem.*, **1984**, *259*, 5606.

[268] Sevilla, M.D.; Becker, D.; Yan, M. *Int. J. Radiat. Biol.*, **1990**, *57*, 65.

[269] Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*, Oxford University Press: New York, **1999**, pp. 70-72.

[270] Epstein, R.J. *J. Clin. Oncol.*, **1990**, *8*, 2062.

[271] Wyllie, A.H.; Kerr, J.F.R.; Currie, A.R. *Int . Rev. Cytol.*, **1980**, *68*, 251.

[272] Fisher, D.E. *Cell*, **1994**, *78*, 539.

[273] Bates, S.; Vousden, K.H. *Curr. Opin. Genet. Dev.*, **1996**, *6*, 12.

[274] Wang, H.; Li, J.; Bostock, R.M.; Gilchrist, D.G. *Plant Cell*, **1996**, *8*, 375.

[275] Mills, E.M.; Gunasekar, P.G.; Pavlakovic, G.; Isom, G.E. *J. Neurochem.*, **1996**, *67*, 1039.

[276] Verhaegen, S.; McGowan, A.J.; Brophy, A.R.; Fernandes, R.S.; Cotter, T.G. *Biochem. Pharmacol.*, **1995**, *50*, 1021.

[277] Gorman, A.; McGowan, A.; Cotter, T.G. *FEBS Lett.*, **1997**, *404*, 27.

[278] Buttke, T.M.; Sandstrom, P.A. *Immunol. Today*, **1994**, *15*, 7.

[279] Jacobson, M.D. *Trends Biochem. Sci*., **1996**, *21*, 83.

[280] Slater, A.F.G.; Nobel, C.S.I.; Orrenius, S. *Biochim. Biophys. Acta*, **1995**, *1271*, 59.

[281] Mignotte, B.; Vayssiere, J.-L. *Eur. J. Biochem.*, **1998**, *252*, 1.

[282] McConkey, D.J. *Toxicol. Lett.*, **1998**, *9*, 157.

[283] Leist, M.; Nicotera, P. *Biochem. Biophys. Res. Commun.*, **1997**, *236*, 1.

[284] Jabs, T. *Biochem. Pharmacol.*, **1999**, *57*, 231.

[285] Jacobson, M.D.; Raff, M.C. *Nature*, **1995**, *374*, 814.

[286] Zettl, U.K.; Mix, E.; Zielasek, J.; Stangel, M.; Hartung, H.P.; Gold, R. *Cell Immunol.*, **1997**, *178*, 1.

[287] Jabs, T.; Dietrich, R.A.; Dangl, J.L. *Science*, **1996**, *273*, 1853.

[288] Sandstrom, P.A.; Tebbey, P.W.; Van Cleave, S.; Buttke, T.M. *J. Biol. Chem.*, **1994**, *269*, 798.

[289] Kane, D.J.; Srafian, T.A.; Anton, R.; Gralla, E.B.; Valentine, J.S. *Science*, **1993**, *262*, 1274.

[290] Zucker, B.; Hanusch, J.; Bauer, G. *Cell Death Differ.*, **1997**, *4*, 388.

[291] Zamzami, N.; Susin, S.A.; Marchetti, P.; Hirsch, T.; Gomez-Monterrey, I.; Castedo, M.; Kroemer, G. *J. Exp. Med.*, **1996**, *183*, 1533.

[292] Zamzami, N.; Marchetti, P.; Decaudin, D.; Macho, A.; Hirsch, T.; S.A., S.; Petit, P.X.; Mignotte, B.; Kroemer, G. *J. Exp. Med.*, **1995**, *182*, 367.

[293] Garcia-Ruiz, C.; Colell, A.; Mari, M.; Morales, A.; Fernandez-Checa, J.C. *J. Biol. Chem.*, **1997**, *272*, 11369.

[294] Liu, X.; Kim, C.N.; Yang, J.; Jemmerson, R.; Wang, X. *Cell*, **1996**, *86*, 147.

[295] Yang, J.; Liu, X.; Bhalla, K.; Kim, C.N.; Ibrado, A.M.; Cai, J.; Peng, T.I.; Jones, D.P.; Wang, X. *Science*, **1997**, *275*, 1129.

[296] Folkman, J. *N. Engl. J. Med.*, **1971**, *285*, 1.

[297] Folkman, J.M. In *Biological Therapy of Cancer*; DeVita, V.T.; Hellman, S.; Rosenberg, S.A., Eds.; J.B. Lippincott: New York, **1991**, pp. 743-753.

[298] Cohen, J.S. In *Biological Therapy of Cancer*; DeVita, V.T.; Hellman, S.; Rosenberg, S.A., Eds.; Lippincott: New York, **1991**, pp. 763-775.

[299] Helene, C.; Toulme, J.J. In *Oligodeoxynucleotides*, Cohen, J.S., Ed., Boca Raton, FL, **1989**, pp. 137-172.

[300] Mori, K.; Subasinghe, C.; Cohen, J.S. *FEBS Lett.*, **1989**, *249*, 213.

[301] Tronick, S.R.; Aaronson, S.A. In *The Molecular Basis of Cancer*; Mendekohn, J.; Howley, P.M.; Israel, M.A.; Liotta, L.A., Eds.; Saunders: Philadelphia, **1995**, pp. 117-140.

[302] Kass, G.E.N. In *Free Radical Toxicology*; Wallace, K.B., Ed.; Taylor and Francis: Washington D.C., **1997**, pp. 349-374.

[303] Tolstoshev, P.; Anderson, W.F. In *The Molecular Basis of Cancer*; Mendelsohn, J.; Howley, P.M., Eds.; Saunders: Philadelphia, **1995**, pp. 534-557.

[304] Gringauz, A. In *Anticancer Drugs and Their Mechanisms of Action*; Wiley & Sons: New York, **1997**, pp. 684-685.