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Review

Some Applications of Electrochemistry in Biomedical Chemistry. Emphasis on the Correlation of Electrochemical and Bioactive Properties

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Essa revisão resume alguns dos aspectos mais relevantes na correlação entre processos e parâmetros eletroquímicos e atividades biológicas, relacionadas, principalmente, a doenças tropicais e câncer. Apesar da gama de possibilidades e da complexidade da química celular/tecidual/extracelular, é possível racionalizar o papel da eletroquímica em poucas bases teóricas, principalmente: transferência eletrônica – estresse oxidativo, geração eletroquímica *in situ* de agentes tóxicos diferentes das espécies oxigenadas reativas, interação com endobióticos, com ênfase particular em alquilação biorredutiva e substituição de endobióticos com função em reações de oxi-redução biológicas. O uso de métodos eletroquímicos para a obtenção de mecanismos de ação de drogas e na análise de eventos celulares é também apresentado. Nessas correlações, métodos e/ou parâmetros eletroquímicos exercem papel relevante, porém, não absoluto.

This review summarises some of the more relevant achievements in the correlation between electrochemical processes and parameters and bioactive properties, mainly related to cancer and tropical diseases. Despite the broad range of possibilities and the complexity of cell/tissue/extracell chemistry, it is possible to rationalise the role of electrochemistry in few basic theoretical frameworks, mainly, the one based on electron transfer-oxidative stress and *in situ* generation of toxic species, other than the reactive oxygen species; interaction with endobiotics, with emphasis on bioreductive alkylation and replacement of endobiotics with function in biological redox reactions. The use of electrochemical methods to obtain relevant informations about drugs' mechanism of action and analysis of cellular events is also presented. Electrochemical methods and/or parameters play essential but not absolute roles.

Keywords: oxidative stress, bioreductive alkylation, tropical diseases, quinones, nitroaromatics, N-oxides, electron transfer

1. Introduction

This review does not intend to cover exhaustively the many areas of research involved, but to provide some background regarding the significance of the electrochemical and electrosynthetic techniques in analysis of natural and synthetic products, in their relationship with bioactive properties, mainly antiparasitical and antitumour activities. In addition, it seeks to provide a brief electrochemical overview of the main classes of bioactive compounds possessing adequate electron transfer functionalities.

Electrochemical parameters do not give absolute correlation with biological activity data, due to the enormous complexity of the biomedical chemistry. Indeed, in a live host, this kind of relationship is always a complex outcome not usually dominated by a sole parameter. Caution must be always used in interpreting this kind of correlation. Many other important factors must also be considered in the mechanistic aspects of *in vivo* drug activity, *e.g.*, stereochemistry, diffusion, solubility, metabolism, membrane permeability, *etc.*[1] Other parameters, like bioavailability, partition coefficient and specific enzyme interactions, also play critical roles.

Mention will be made of examples where electrochemistry, dealing with different aspects of electron transfer (ET), contributes significantly to biomedical chemistry.

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Particular emphasis has been placed upon quinones and nitroaromatics, since the majority of studies reported in the literature concern those classes of compounds.

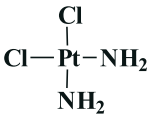
Many of the most important physiological processes are based on oxidoreduction chains involving numerous successive enzyme-catalysed processes. There is a set of similarities between electrochemical and biological reactions concerning electron transfer (ET) pathways, which are not duplicated in other chemical systems.[2]

Electrochemical studies should furnish an enormous amount of evidence regarding the mechanisms of biological electron-transfer processes.[2]

2. General Comments

This topic should begin with an interesting case of serendipity approaching electrochemistry and medicinal chemistry.

One of the most efficient antitumour agents for the treatment of testicular and ovarian tumours is *cis*-diaminedichloroplatinum(II) (**1**) and derivatives. Its discovery was fortuitous in the extreme, arising from research carried out on a platinum electrode to investigate the effects of an electric current on the growth rates of *E. coli*.[3,4] During the experiments, bacterial cell division was inhibited. Further research led to the discovery that the electrolysis product, **1**, was responsible for the activity.



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In general, electrochemistry has been used in processes where bioreduction or bio-oxidation is concerned. In its approach to biomedical chemistry, a large number of examples have been accumulated in the literature. They can be classified in common theoretical frameworks or as analytical tools to observe, prove and predict biological phenomena. In spite of the division into classes of drugs' mechanism of action, it should be emphasised that several factors may be operating in a multifaceted attack.[5]

The necessity to resemble biological conditions has fomented several discussions. The environment of the cell could be hydrophilic or lipophilic. The reduction/oxidation processes can be carried out in nonaqueous media resembling the situation in lipophilic systems or in aqueous media corresponding to situations in most biological fluids. Another important factor is related to the O_2 content of the cell. Some tissues, for example, solid tumours, contain regions of low oxygen tension (hypoxia) generally thought to arise as a consequence of a poor and disorganized blood supply. The O_2 tension influences deeply the outcome of biological electrochemical reactions,[6] as shown in the next sections. All those facts must be considered in the attempt to mimic biological environments.

Electroanalytical techniques, mainly polarography, cyclic, square wave and differential pulse voltammetry, coulometry, together with electron spin resonance (ESR) experiments are well described in a series of excellent books[7,8] and for non-specialists, a review appeared recently in the literature.[9]

The usual parameters normally obtained and employed, especially in cyclic voltammetry, the method most used, are the potentials of the oxidation (E_{pa}) and reduction (E_{pc}) peaks or E_{redox} ($E_{pc} + E_{pa}$)/2 (for reversible systems) or E_{pc}-$E_{pc/2}$ (for irreversible ones), the magnitude of the current function ($I_p/(\nu^{1/2} \times C)$) and the ratio between the anodic and cathodic currents I_{pa}/I_{pc}. The potential E_{redox} or similar parameters, $E_{1/2}$, in polarography, give a quantitative measure of the ease of reduction of an oxidant or electron acceptor, A, since the more positive the value of the potential of the couple E ($A/A^{\cdot-}$), the more powerful the oxidant. Similarly, the more negative the value of E ($A^{\cdot-}/A^{2-}$), the more powerful the reductant.[2,7-9]

2.1. General theories

2.1.1. Electron transfer (ET)-oxidative stress (OS) theory

Oxygen is required for many life-sustaining metabolic reactions. Oxygen and its activated intermediates, ROS (reactive oxygen species), however, may react with cellular components with resultant degradation or inactivation of essential molecules.[5,13]

Oxidative stress[5,10-13] is not simply an undesirable consequence of aerobic life (Scheme 1).[12] It also represents an important principle of the organism chemical defence against invaders, generated by neutrophilic granulocytes during phagocytosis.[10]

There is increasing evidence of ET-OS involvement in the mechanism of action of a wide variety of physiologically active materials.[5,10]

Exposure of cells to hydrogen peroxide generates a 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 pyrimidines (**2**) and purines (**3**, **4**), with 8-hydroxyguanine (**3**) 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

$$A + NADPH \xrightarrow{H^+} AH_2 + NADP^+$$

$$A \xrightarrow[H^+, e]{\text{enzyme}} AH^\bullet$$

$$AH_2 + O_2 \rightarrow AH^\bullet + HO_2^\bullet$$

$$AH^\bullet + O_2 \rightarrow A + HO_2^\bullet$$

$$HO_2^\bullet \rightleftharpoons O_2^{\bullet-} + H^+$$

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

$$2\,H_2O_2 \xrightarrow{catalase} 2\,H_2O + O_2$$

$$CMX\text{-}Fe\,(III) + O_2^{\bullet-} \longrightarrow CMX\text{-}Fe\,(II) + O_2$$

$$CMX\text{-}Fe\,(II) + H_2O_2 \longrightarrow CMX\text{-}Fe\,(III) + OH^\bullet + OH^- \text{ (Fenton Reaction)}$$

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + HO^- + HO^\bullet \text{ (Haber-Weiss Reaction)}$$

Hypochlorous acid decomposition to hydroxyl radical

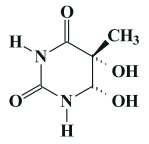
$$HOCl + O_2^{\bullet-} \rightarrow HO^\bullet + Cl^- + O_2$$

$$HOCl + Fe\,(II) \rightarrow HO^\bullet + Cl^- + Fe\,(III)$$

$$O_2^{\bullet-} + Fe\,(III) \rightarrow O_2 + Fe\,(II)$$

Scheme 1. Pathways related to oxidative stress (OS).[12] SOD = superoxide dismutase; CMX-Fe(III) = iron complexed with protein or ATP.

oxygen undergo a number of reactions, including C-C bond fragmentation, some of which result in single-strand breaks.[13] Being among the best hydrogen atom acceptors, hydroxyl radicals are prone to induce a large variety of biological mutations and to disrupt the cohesion of cellular membranes *via* radical peroxidation of cell bilipids. Severe oxidative stress can cause cell injury or death as a consequence of insufficient antioxidant potential.



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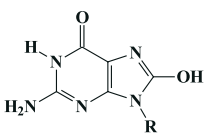

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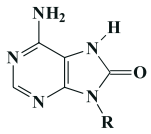

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Organisms use superoxide dismutase, catalase and glutathione peroxidase as protection against generation of reactive oxygen species.

The proposed mode of action for radiation-based therapies (still the most successful non-invasive means of treatment for most cancers) relies on the abundance of oxygen within the tissue of interest and generation of ROS.[13]

ET-OS represents a broad and unifying understanding of drug-action that can aid in the design of new drugs.[5,10]

2.1.2. Generation of toxic species different from ROS

2.1.2.1. The electrochemical treatment of tumours

Electrochemical treatment of tumours (EChT), which is also known as electrochemical therapy, is a therapy in which tumour tissue is treated with direct current through the use of electrodes placed inside the tumour or in its close vicinity.[14] When tissue is electrolysed, electrical energy is converted into chemical energy through electrochemical reactions at the electrodes.

Encouraging results from human clinical trials in China were recently presented, but the mechanism of destruction of tissues exposed to electrochemical treatment and the parameters important in the process are still uncertain, which partially have hindered its development as a clinically accepted therapy.[14] Still, there is no doubt that the electrochemical processes at the electrodes form locally destructive reaction products, which are transported into the tissue surrounding the electrodes. The electrogenerated products may also react with organic and inorganic tissue constituents, to potentially form new toxic products. The production of toxic electrolytic products may not fully explain the anti-tumour effects obtained in EchT studies. The electric field itself and the resultant extreme local pH changes influence both survival and proliferation of the cells, causing unphysiological conditions, responsible for the modification of the ion exchange across the cell membranes and vital protein denaturation.[14]

The choice of anode material determines the electrode reactions at the anode. If the anode material is electrochemically soluble, the major part of the anodic current will consist of metal dissolution. A small amount of the anodic current is transferred by oxidation and reduction of certain species already dissolved in the tissue. The electrode potential obtained will therefore dictate which species can be oxidised or reduced, at the electrode surface.[14] The choice of cathode material is less crucial than the choice of anode, since the cathode is generally more stable and the main reaction is quite exclusively water decomposition into hydrogen and hydroxyl ions.[14]

The anodic current density is a very important parameter. Different destructive effects are obtained depending on the magnitude of current density used during the treatment.

Electrode orientation, known as field configuration, is another important aspect to be considered. There exists an optimal distance between the electrodes. Several field configurations have been tried.[14] The anode is preferably placed in the tumour and the cathode in a blood vessel or in a fresh surrounding tissue.[14]

Mathematical models of these processes were developed and showed to be powerful tools in establishing a reliable dosage method, with the prediction of the tumour destruction produced through EchT.[14] Concentration profiles of substances dissolved in tissue and the potential profile within the tissue itself can be simulated as a function of time.

The main electrochemical reactions, if platinum is used in biological tissue, are chlorine and oxygen evolution, at the anode and hydrogen evolution at the cathode (Eqs. 1-3).

$$2\,H_2O \leftrightarrows O_2 + 4\,H^+ + 4\,e^- \quad \text{Eq. 1}$$

$$2\,Cl^- \leftrightarrows Cl_2 + 2\,e^- \quad \text{Eq. 2}$$

$$2\,H_2O + 2\,e^- \leftrightarrows H_2 + 2\,OH^- \quad \text{Eq. 3}$$

Consequently, the dominating reaction products that are locally destructive at the anode are hydrogen ions and various oxygen and chlorine containing species. Hydroxyl ions and molecular hydrogen are the destructive reaction products at the cathode (Eq. 3).

EChT provides a safe, simple and effective complementary treatment for patients with lung neoplasms, who are neither suitable for surgery nor responsive to chemo- or radiotherapy.[14]

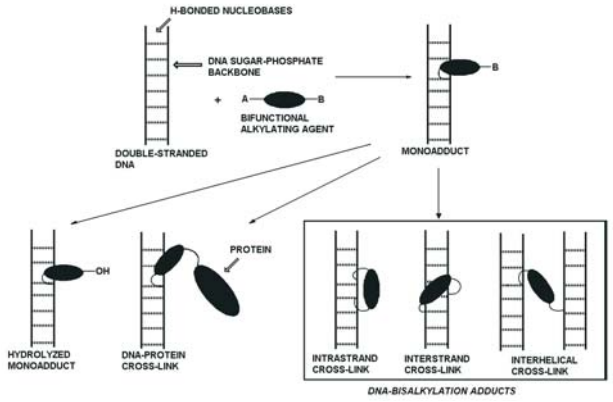
2.1.3. Interaction with endobiotics

2.1.3.1. Bioreductive alkylation

The vast majority of clinically employed alkylating agents behave as electrophilic traps for molecular nucleophiles. Such nucleophiles often include amino acids such as cysteine, lysine, tyrosine, and threonine. Additionally, the nucleobases of DNA and RNA represent likely targets[4] (Scheme 2). Pro-drugs are normally employed and activation occurs through redox processes.

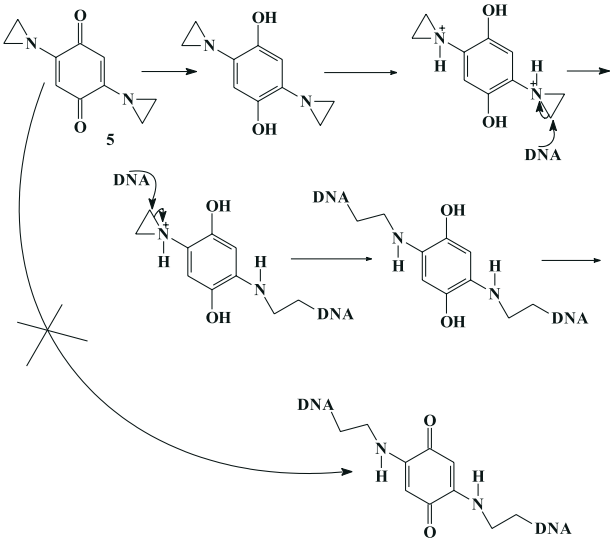
Concerning DNA as a target, it is generally agreed that the formation of interstrand cross-links represents by far the most toxic of all alkylating events (Scheme 2). DNA interstrand cross-linking agents comprise an extremely important class of clinical agents not only in the treatment of cancers, but also for diseases such as psoriasis and various anemias.[10]

The redox process is fundamental to the action, as exemplified for aziridinylquinones (**5**) (Scheme 3).[15] Reduction of the quinone results in the transformation of a non-aromatic quinone to the aromatic semiquinone or hydroquinone. The resulting altered electronic distribution no longer invokes conjugation of the nitrogen lone pair electrons with the respective carbonyls. As such, this substantially enhances the basicity of the aziridinyl



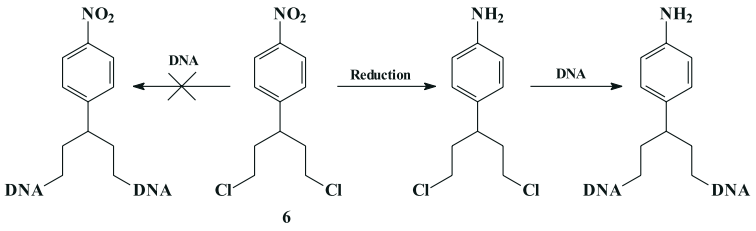
Scheme 2. Mechanistic pathway for DNA functionalization by interstrand cross-linking agents. A and B represent electrophilic moieties within the cross-linking agent of interest.[4]

nitrogens, thus, facilitating protonation of each tertiary amine. This activating process vastly enhances aziridine electrophilicity thus affording a species capable of facile DNA alkylation. Autooxidation back to the quinone is favoured over the parent hydroquinones due to the increased electron donation, as opposed to that of the ring-strained aziridinyl case.[15]



Scheme 3. Reductive mechanism of DNA interstrand cross-linking by the diaziridinylquinones (**5**). Mytomicin C-like prodrugs.[15]

Whether a one or two electron species reduced species (or some combination of both) is responsible for interstrand crossing is still an issue of some controversy. It is noteworthy, however, that the vast majority of the literature regarding these issues portrays the reduced intermediate as a fully reduced hydroquinone or some equivalent thereof.[4]



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Scheme 4. Reductive activation of nitroaniline chloro-mustards **6**.[4]

A scheme similar to Scheme 2 can be drawn for nitroaromatics, as exemplified by nitroaniline mustards (**6**) (Scheme 4).[4]

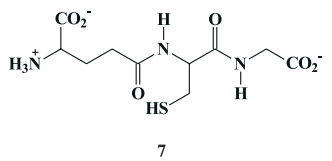
To look for long time cytoxicity of drugs, mainly nitroaromatic derivatives, electrochemical methodology has been considered a useful quantitative tool to study the *in situ* reactivities of electrogenerated intermediates toward endobiotics.[16]

The studies deal mainly with glutathione (**7**), other thiol derivatives and the nucleic acid-containing bases.

A quantitative procedure to calculate interaction constants between electrochemically generated nitro radical anions from extensively used drugs and xeno/endobiotics is provided through the use of cyclic voltammetry. The method was based on the decrease in the return-to-forward peak current ratio of the reversible system after the addition of endobiotics.[16] Adenine and guanine are susceptible to interaction with reduced nitroimidazoles.

In the case of nifedipine (**8**),[17] nitrendipine (**9**)[18] and flutamide (**10**),[16] results provide experimental proof of the significant reactivity of the nitro radical anions toward adenine and uracil and the ability of GSH (**7**) to behave as a scavenger of the generated nitroradical anion (Eq. 4). Those results emphasise the potential cytotoxicity of these drugs in mammalian cells during long period of treatment and the possible thiol-dependent reversal of cytotoxicity.

$$Ar\,NO_2^{\bullet} + RS^- + 2\,H^+ \rightarrow Ar\,NO + RS^{\bullet} + H_2O \qquad \text{Eq. 4}$$



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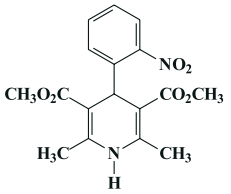

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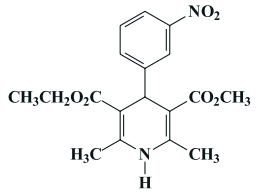

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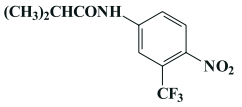

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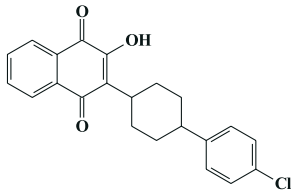
2.1.3.2. Replacement of endobiotics: antimetabolites

The "molecular sabotage", held mainly in the mitochondria chain electronic transport, held by endobiotics'structurally closed substances, mainly, quinones, interrupts the cell energy generation.[19] An interesting result is shown in the case of atovaquone (**11**), a coenzyme Q (**12**) analogue, a broad spectrum antiparasitic drug, which antimalarial activity involves indeed an interaction with cytochrome b, being as well an inhibitor of the ubiquinol oxidase activity of the cytochrome bc.[19] Atovaquone (**11**) inhibits mitochondrial electron transport and also depolarises malarial mitochondria with consequent cellular damage and death.



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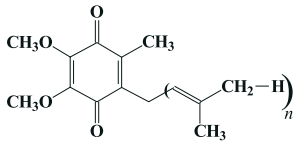

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2.2. Electrochemical tools

2.2.1. Electroanalytical studies of biologically active substances

One obvious application of electrochemistry is related to the electroanalytical studies of endobiotics and drugs, for quantification in biological liquids or other purposes. This has been published in excellent books[2,20] and will not be considered further. The presence of an electroactive group or its transformation from electroinactive ones is a pre-requirement. Electrochemical detectors for analytical methods, such as in HPLC and/or biosensors[20] have been extensively used and play important role in endobiotics' analyses.

2.2.2. Determination of drugs' mechanism of action

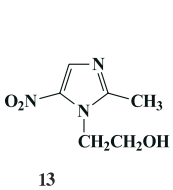
Electrochemical techniques have been used to clarify drugs' mechanism of action. The main contribution couples

electrochemical devices and methods used to analyse free radicals, mainly electron spin resonance spectroscopy.[21] The value of electrochemistry in studying these redox systems is that it is a relatively clean chemical system, it is relatively easy to control and can be studied in aprotic and aqueous solutions which allows one to evaluate the behaviour of free radicals generated in biological systems.[21]

Covalent modification of DNA by antineoplastic agents represents a potent biochemical lesion, which can play a major role in drug mechanism of action. The ability to measure levels of DNA covalent modifications in target cells *in vivo* may, therefore, be seen as the ultimate form of therapeutic drug monitoring. There is considerable enthusiasm over development of electrochemical biosensors for DNA hybridisation. The development of assay techniques that have the convenience of solid-phase hybridisation and are rapid, sensitive and readily multiplexed will have a significant impact on diagnostics and genomics.[22]

2.2.2.1. Selected examples

A newly developed DNA-modified glassy carbon electrode was used for the investigation of the electrochemical reduction of several important drugs, including metronidazole (**13**)[23] and chloramphenicol (**14**).[24] This methodology allows the discrimination of the reactive species among the possible intermediates, for example **14** or **15**.[24]



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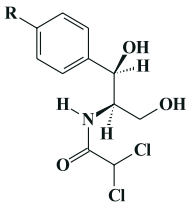
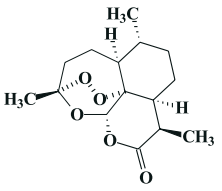


14 R = NO_2 15 R = NO

Human malaria, caused mainly by *Plasmodium falciparum*, is one of the most important parasitic infections of the planet. In the last 25 years, it has regained its former position as the greatest threat to the health and economic prosperity of mankind.[25]

The antimalarial action of artemisinin (**16**) appears to be mediated by a reaction with intraparasitic hemin with the subsequent formation of free radicals. Although the mechanism of action of artemisinin is not unequivocally known, it is believed to involve the breaking of the oxygen-oxygen bond by iron (II).[26]

Malaria parasites in the infected red cells are rich in hemin and the reaction of hemin with **16** accounts for the selective toxicity to the malaria parasite. Electrochemistry was used to investigate the interaction between hemin and artemether (**17**) so as to further reveal the antimalarial mechanism and to facilitate the development of its various derivatives. The results from cyclic voltammetry showed that hemin could electrocatalyze the reduction of **17**. Hemin-Fe(III) played the role as the catalyst in the process, making the reduction of **17** much easier. The hemin-catalyzed effect caused a 0.64 V positive shift of the peak potential. Such a large shift results from the decrease of the reduction activation energy of **17** and a resulting increase in the electron transfer rate.[26] Actually, hemin-Fe(II) was the substance, which directly acted with **17** in the catalytic process. For the process to occur *in situ*, there must be a donor, which transfers its electrons to artemether. Thiols, such as glutathione (**7**), which is present in malaria parasites in millimolar concentrations, may play this role.



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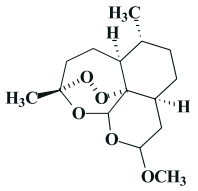

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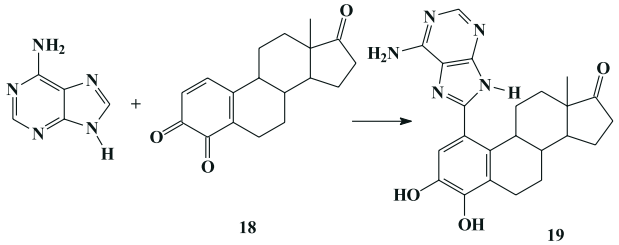
Interaction evidence can be also obtained through preparative electrolysis.

Estrogens generate electrophilic species that can covalently bind to DNA. The latter role is thought to proceed through catechol estrogen metabolites, which can be oxidised to *o*-quinones that bind to DNA. Synthesis and characterisation of the first estrogen nucleic acid adduct (**19**) obtained by intermolecular coupling of electrochemically reduced 3,4-estrone-*o*-quinone (**18**) and adenine was performed (Scheme 5).[27,28]



18 19

Scheme 5. Reductive coupling of **18** with adenine. Cathodic reduction (DMF-$LiClO_4$, Pt cathode, E = -0.5 V *vs.* SCE).[27,28]

2.2.3. In situ electroanalysis of cellular events

Living cells exchange informations through the emission of chemical messengers. The importance of such

messengers has been widely recognized by biologists.[29] However what is less understood is how these chemical messengers are released by the cell in its outer-cytoplasmic fluids, due mainly to the fact that those releases occur in the atto- or femtomole ranges which prevent the use of classical analytical methods.[29]

Ultramicroelectrodes may prove extremely useful for monitoring such events. They allow fundamental biological events to be monitored in real time at the single cell level.[29,30] Because an ultramicroelectrode, normally a platinized carbon fiber, may only probe a volume that is comparable to its own size, it behaves like a flux-microscope observing concentration changes in its vicinity. The acquired information is thus essentially dynamic. The film of extracellular fluid comprised between the cell and the electrode surfaces defines an artificial synaptic cleft of a few hundred femtoliters volume, in which the release of minute molecular amounts produces a sudden and important concentration rise.

One of the most impressive examples is related to the collection and examination of the very nature of the massive oxidative bursts produced by human fibroblasts when their membrane is locally depolarised by a puncture made with a micrometer sized sealed pipette. The electrochemical analysis of the response indicates that oxidative bursts consist of a mixture of a few femtomoles of highly cytotoxic chemicals: hydrogen peroxide, nitrogen monoxide and peroxynitrite, together with nitrite ions.[30]

Electrochemical methods also provide non-morphological observation methods for following cell health state and evaluating effectiveness of chemical compounds, including human mammalian tumour cells HL60 (the cell line of human leukaemia).[31]

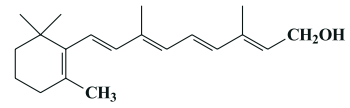
2.2.4. Correlation of electrochemical data and structural parameters

The comparison between electrochemical and bioactive properties, based upon the mentioned general theories (section 2.1), with evidence of relationship, carries a great significance, allowing the use of electrochemical parameters as direct evaluators of a biological activity. As the effect of substituent can be, generally, directly related to electrochemical parameters, the benefits of QSAR studies, finding mathematical forms for the relationship, bring additional relevance to the methodology.

Since the pioneer work by Zuman,[32] several quantitative structure-activity relationship studies have shown that *in vivo* or *in vitro* biological activities are dominated by the electronic properties of substituent groups, some referring to electrodonating effects, other to electronwithdrawing ones. Quantitative structure-electrochemistry relationships of aziridinylquinones (**5**) were established by Driebergen and coworkers.[33]

There is a wealth of prior experience in correlating polarographic data with Hammett and related substituent constants. This correlation can be used to extend limited experimental measurements and predict redox properties of a much wider range of compounds with considerable reliability. The prediction possibility facilitates the identification of quantitative relationships between structure and biological activity and aids in the design of more powerful derivatives.[34]

Two important fundamental quantities that can be obtained from half-wave reduction potentials in aprotic solvents and constantly used in biomedical chemistry are the ionization potential (IP) and electron affinity (EA). It is crucial to have reliable values of these quantities for endobiotics.[35] It is especially important for biologically significant molecules since few electron affinities have been measured. Chen and co-workers have calculated EA for a series of biologically active compounds, like vitamin A (**20**) and E (**21**), riboflavin (**22**) and others.[35]



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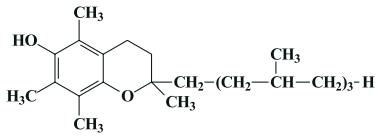

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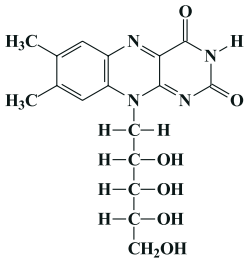

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3. Practical Problems in the Correlation: Choice of Experimental Conditions - Media, Electrode and Method

In the field of biomedical chemistry, some electrochemical experiments were either carried out in aqueous, aprotic or in solutions using mixtures, normally ethanol

or DMF in the presence of buffers. In simpler cases, the same overall trends for reduction/oxidation in aqueous solution hold as in the aprotic experiments. Prototropic effects in substituents provide the most marked exceptions to such generalizations.[34] Using mixed solvents, there is a possibility for studying the behaviour of the several classes of compounds in a situation which will be of more biological relevance than a purely aprotic medium.[36, 16-18] Also to mimic biological conditions, parallel experiments are held in the presence of different surfactants.[37] On the other hand, non-aqueous aprotic solvents should be better models of membrane environment in which peroxidation processes take place, because both superoxide anion radical and its conjugated acid, the hydroperoxyl radical, are virtually unstable in water and other protic solvents, owing to fast disproportionation.[38]

The reported experiments are normally performed under different conditions: supporting electrolyte, ionic strength and mainly different working electrodes. Potentials are frequently quoted without the appropriate reference electrode. Standardization would be extremely useful. Redox potentials (E_{redox}) obtained *vs.* reference redox systems as internal standards are free of liquid junction potentials and can therefore be used to compare data for a given redox system in different solvents. The use of only two reference systems and the knowledge of the potential differences between reference systems will yield potentials' data on a solvent independent scale.[39] In the absence of internal redox systems, interconversion of redox potentials can be used.[40]

Adsorption onto the surface is difficult to control, has negative effects on the reproducibility and should be avoided. Several techniques are available to decrease the problem of adsorption.[7,8]

Additionally, comparison with enzymatic systems would require knowledge concerning the transfer of 1, 2 or more electrons. Electrochemical studies in different media allow this comparison to be made.[41,42]

A good example of the usefulness of electrochemical methods in the analysis of neurotoxicity, with a more complete approach, is given by Livertoux and coworkers.[41] They proved that the superoxide production mediated by the redox cycling of endobiotics in rat brain microsomes was dependent on their reduction potential. The redox behavior of the assayed compounds (nitroaromatics, quinones and N-oxides) was studied in both phosphate (pH 7.0) buffer and in an unbuffered aprotic solvent, DMF. These assays were carried out because the NADPH-Cytochrome P-450 reductase membrane environment is both polar and lipophilic, as the flavoprotein is anchored to the phospholipid bilayer of the endoplasmic reticulum *via* a hydrophobic amino-terminal peptide, and is exposed to the cytoplasmic face of this membrane system.[41]

In some cases, the standard potential for reactions involving slow heterogeneous ET (dissociative electron transfer) is not determined easily using simple electrochemical methods since the direct reaction is subject to a large overpotential. As a result, reduction potentials measured from cyclic voltammetry are not themselves an accurate indication of the standard potential and cannot be used directly with the oxidation potential of donors to decide whether a particular ET will be feasible under physiological conditions. In those cases, thermochemical cycles are often used to estimate the standard reduction potential.[43] As an example, the reduction of the known antimalarial agent artemisinin (**16**) has been studied in N,N-dimethylformamide by cyclic voltammetry and other electrochemical techniques and has been determined for the first time to be -0.89 V vs. SCE. The thermochemical values determined are important to understand the biological activity of artemisinin (**16**) and to investigate its potential for undergoing electron-transfer-initiated processes with biological donors.[43]

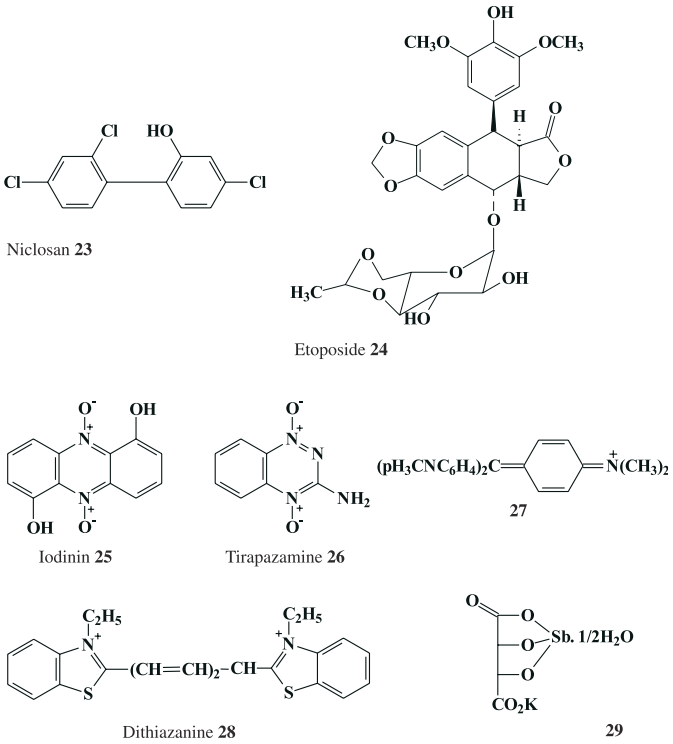
4. Main Classes of Compounds Biologically and Electrochemically Active

A wide range of extracted and synthesised drug molecules have electron transfer capabilities, which allow them to generate reactive oxygen species or to be considered as bioreductive alkylating agents.

The pharmacological and toxicological activity of a drug is in many ways the consequence of its metabolism. Many drugs, which do not contain ET functionalities, can be metabolised by various enzymes to agents capable of inducing ET in invasive organisms, or leading to undesirable side effects in the host.

There are several main classes of electron transfer agents, some of them already mentioned: nitroaromatics (**6**, **8-10**, **13-14**) (or their reduced forms, **15**), quinones (**5**, **11-12**) (or phenolic precursors **21, 23, 24**), aza compounds (or azo dyes), iminium ions [or imines, aromatic (**25, 26**) and aliphatic N-oxides, triarylmethane dyes (**27**), N-heterocyclic salts (**28**)] and metal complexes (**1**, **29**) or metal quelators. In the present case, emphasis will be held on nitroaromatics and quinones.

Significantly, a large number of physiologically active substances possess $E_{1/2}$ values greater than about -0.5 V *vs.* NHE, in the physiological active range, which can permit electron acceptance from biological donors or they can suffer metabolic changes, furnishing easily reduced derivatives.[5,10]



Since the efficiency of redox cycling agents depends upon the rate of catalytic turnover (Scheme 1), optimum activity would result when the one-electron redox potential of the agent is in-between that of cellular reductants, *ca.* -0.4 V *vs.* NHE, and that of the $O_2/O_2^{\cdot-}$, *ca.* -0.2 V *vs.* NHE, in an aqueous buffered medium, at pH 7.0, though this range could be extended somewhat by Nernstian effects of concentration and by kinetic effects of the rapid reoxidation of intermediate ion radicals.[44]

4.1. Nitroaromatics

Nitroaromatic compounds, $ArNO_2$, are a very important class of compounds which have been used extensively in the treatment of anaerobic infections and are under continuing investigation regarding their use in cancer therapy, acting as specific cytotoxins and markers for hypoxic regions in tumours.

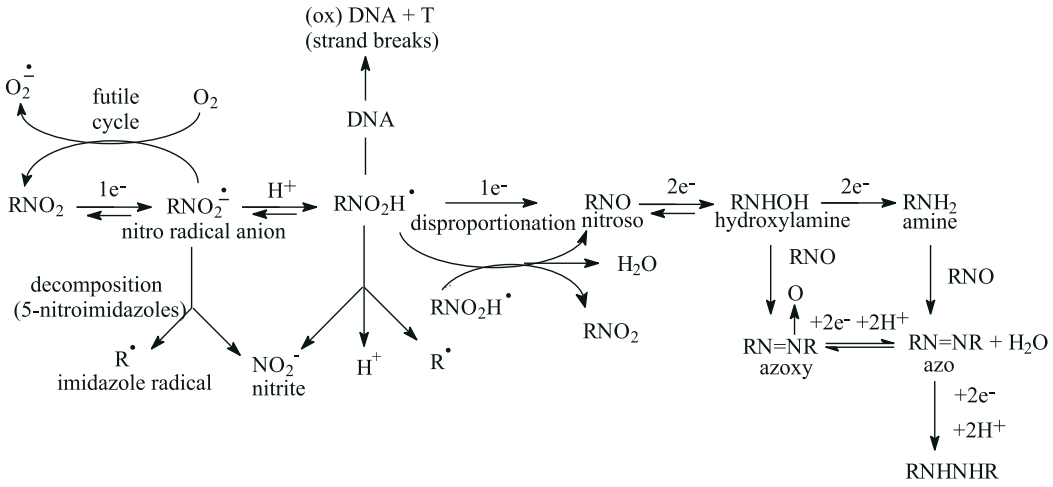
There is good evidence that some electrochemical properties of nitro compounds can be correlated with the pharmacological effects of these compounds. There is a direct proof that free-radical metabolites are involved in many applications.[45]

The most biologically relevant measure of nitro group reduction potential is that for the thermodynamically reversible addition of the first electron, E^1_7, obtained in aqueous-buffered medium and refered mainly to NHE. The one-electron reduction species, the radical anion, is very reactive towards oxygen and is oxidised to the parent molecule so efficiently that in the presence of oxygen, there is effectively no substrate for the second bioreductive step. This futile cycle generates reactive oxygen species (Scheme 1) without net accumulation of metabolites of the original compound and this is the basis for the anti-parasitic activity of some 2-nitroimidazoles. Although $E_{1/2}$ values determined in aqueous solutions by CV or polarography are not reversible reactions, and for nitroaromatics may involve the addition of up to 4 electrons, the first is usually the most difficult.[46]

In the absence of oxygen or under hypoxic conditions, the nitro radical anion is further reduced to the nitroso (2 e^-), hydroxylamine (4 e^-) and amine (6 e^-) (Eqs. 5-10). Scheme 6 exemplifies the several pathways related to the biological activity of nitroaromatic compounds, where reductive activation plays the major role.[45] Some of the mentioned metabolites are reactive and bind to various components of cells including macromolecules (Scheme 6).

In vivo, the reductive metabolism is carried out by widely distributed constitutive flavo-enzymes that are able to use nitroaromatics as alternative electron acceptors (flavine dependent reductases, cytochrome P_{450}).[41]

Several excellent monographs deal with the electrochemical behaviour of nitroaromatics.[47] Briefly, the reduction pathway of the nitrocompounds changes drastically with the medium and, in general, could be represented by the following equations and cyclic voltammograms (Figures 1 A-C).



Scheme 6. Reductive activation of nitroaromatic compounds.[45]

The reduction mechanism in aqueous media (Eqs. 5-10) is the same for a variety of structures and is dominated by the reduction of the nitro group. This is a complex process that can occur in two stages, initially by an irreversible 4-electron step to hydroxylamine (Eqs. 5-8), followed by a further reduction, in acidic medium, to the corresponding amine or ammonium salt (Eqs. 9-10, Figure 1 A). In aprotic media, there is a deep change in the redox mechanism (Figure 1 B, Eqs. 11-12). In several examples, the nitro radical anion formed is perfectly stable on the time-scale of the experiments, and a reversible system is observed, followed by an irreversible and more intense second wave. Sometimes, it is also possible to observe a reversible system, related to a second electron uptake by the nitro radical anion. The long-term stability of the electrochemically generated nitro radical anion in aprotic media leads to the opportunity of studying any chemical following reactions, especially with endobiotics.[16-18]

$$Ar\text{-}NO_2 + H^+ + e^- \rightarrow Ar\text{-}NO_2H^{\xi} \quad \text{Eq. 5}$$

$$Ar\text{-}NO_2H^{\xi} + H^+ + e^- \rightarrow H_2O + Ar\text{-}NO \quad \text{Eq. 6}$$

$$Ar\text{-}NO + H^+ + e^- \rightarrow Ar\text{-}NOH^{\bullet} \quad \text{Eq. 7}$$

$$Ar\text{-}NOH^{\xi} + H^+ + e^- \rightarrow Ar\text{-}NHOH \quad \text{Eq. 8}$$

$$Ar\text{-}NHOH + 2H^+ + e^- \rightarrow H_2O + Ar\text{-}NH_2^{+\bullet} \quad \text{Eq. 9}$$

$$Ar\text{-}NH_2^{+\bullet} + e^- \rightarrow Ar\text{-}NH_2 \quad \text{Eq. 10}$$

$$Ar\text{-}NO_2 + e^- \leftrightarrows Ar\text{-}NO_2^{\bullet -} \quad \text{Eq. 11}$$

$$Ar\text{-}NO_2^{\bullet -} + 3e^- + 4H^+ \rightarrow Ar\text{-}NHOH + H_2O \quad \text{Eq. 12}$$

In mixed aqueous-organic systems, intermediate behaviour is found (Figure 2 C), with decrease of current

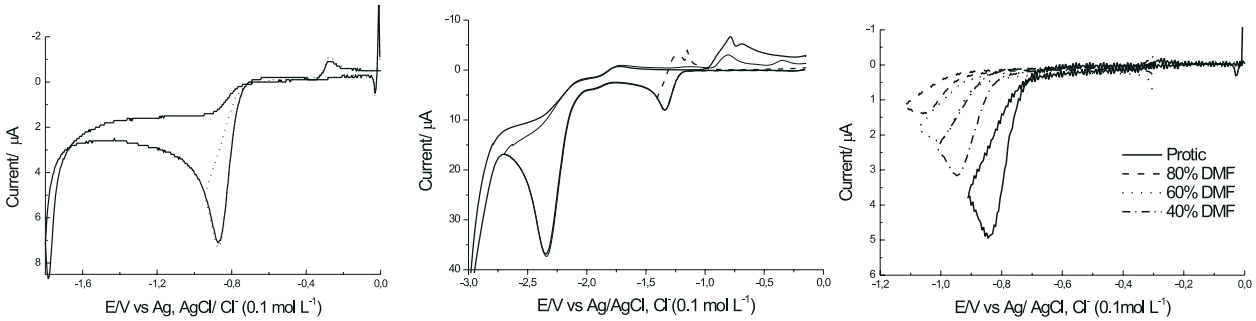


Figure 1. Representative cyclic voltammograms for the reduction of nitroderivative (2-trifluoromethyl-4-nitroaniline), c = 1 mmol L^{-1}. Hg electrode, sweep rate = 0.100 V s^{-1}. A: protic medium, phosphate buffer, pH 7; B: aprotic medium, DMF + TBAP 0.1 mol L^{-1}. E vs. Ag/AgCl, Cl^- 0.1 mol L^{-1}. C: protic medium, phosphate buffer, pH 7; addition of successive amounts of DMF.

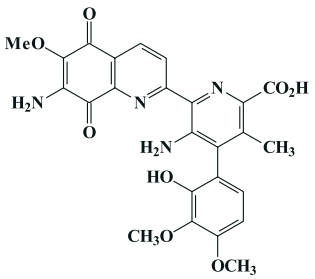
and potential of the first wave, in relation to the process in aqueous medium. The reversibility of the couple RNO_2/ $RNO_2^{\cdot-}$ increases with addition of organic solvents.[17,18]

4.2. Quinones

Numerous quinones play vital roles in the biochemistry of living cells and exert relevant biological activities. Their cytostatic and antimicrobial activities emerge due to their ability to act as potent inhibitors of electron transport, as uncouplers of oxidative phosphorylation, as intercalating agents in the DNA double helix, as bioreductive alkylating agents of biomolecules and as producers of reactive oxygen radicals, by redox cycling, under aerobic conditions. In all these cases, the mechanism of action, *in vivo*, requires the bioreduction of the quinones as the first activating step.[48,49]

In cancer chemotherapy, they are considered the second more important group.[48] The mechanism of action of quinoid anti-tumour agents have been thoroughly investigated. Under aerobic conditions, *i.e.*, in organs with sufficient blood supply, a one-electron reduction predominates, resulting in free-radical intermediates. This can cause additional damage to the DNA of the tumour cell, but, frequently, it also induces unwanted damage to normal cells, leading to serious side effects. An alternative pathway of activation involves a two-electron reduction of the quinone function, which may be followed by its inactivation through subsequent glucuronidation and/or sulfation or by the conversion of the hydroquinone into an alkylating intermediate, the quinone methide.[48] Such a pattern is believed to predominate under anaerobic conditions. Nevertheless, the electrochemical properties of the compounds are very important for its bioreductive activation, either to the semiquinone or to the hydroquinone.

It should also be remembered that several of the quinone-group containing antitumour antibiotics bind metal ions. The formed complex often play key roles in their biological activity, as shown in the case of streptonigrin (**30**), an aminoquinone, which ability to damage DNA was shown to be dependent on the binding of transition metal ions, as those of iron or copper.[5]



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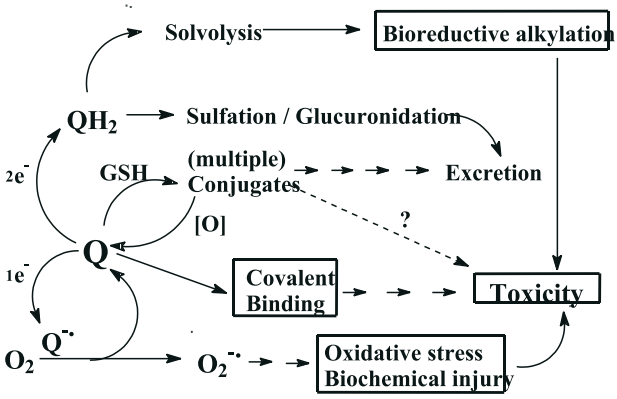
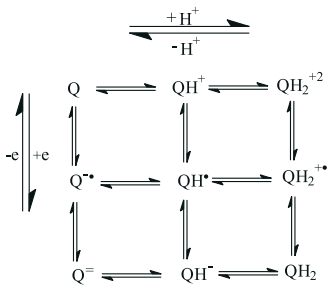
The metabolism of quinones by flavoenzymes that catalyse one-electron reduction is more closely related to the one-electron reduction potential of the quinones than to other structural or physicochemical parameters including lipophilicity. The lowest limit of the reduction potential for the metabolism by NADPH is close to the potentials of endogeneous carriers of the mitochondrial electron transport chain.[50]

Scheme 7 summarises the role of quinones in biological activities.[48]



Scheme 7. Biological fates of quinones.[48]

The electrochemistry of quinones has been extensively reviewed and is strongly dependent on the media and acid-base characteristics of the substrates and supporting electrolytes.[51,52] Depending on the media, it can be represented in the Scheme 8.[53] In aprotic solvent, the reduction is normally represented by two reversible monoelectronic waves, generating, after the first and second electron captures, the anion radical and dianion, respectively (Figure 2). In water, electrochemical reduction of quinones can be represented by a single two-electron wave (figure not shown) or it assumes greater complexity in that all the protonated forms of the various intermediates are possible.[34]



Scheme 8. Scheme relating redox and acid-base behaviour of quinones.[53]

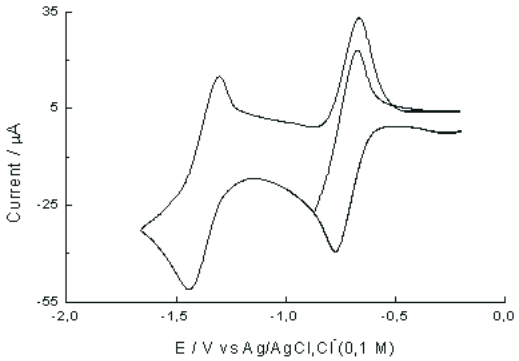


Figure 2. Representative cyclic voltammogram of 2,3-dimethyl-1,4-naphthoquinone, c = 2 mmol L^{-1}, Hg electrode, sweep rate = 1.0 V s^{-1}, DMF + TBAP 0.1 mol L^{-1}. E vs. Ag/AgCl, Cl^- (0.1 mol L^{-1}).

4.2.1. Selected examples concerning quinones

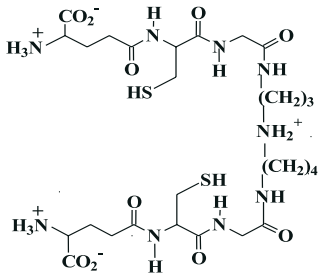
Chagas' disease is a long term debilitating disease caused by the flagellate protozoan *Trypanosoma cruzi*, transmitted by Triatomine insects and by blood transfusion. It is one of the most serious parasitic diseases of Latin America, with a social and economic impact far outweighing the combined effects of other parasitic diseases.[54]

A special feature of *T. cruzi* refers to its unique sensitivity to the action of intracellular generators of H_2O_2. *T. cruzi* possesses an original redox defence system, based upon trypanothione (**31**) and trypanothione reductase, a NADPH-dependent flavoprotein, which regenerates trypanothione (**31**) from its oxidised form (disulphide form). It lacks catalase and glutatione peroxidase, being substantially more sensitive to oxidative stress than their biological hosts.[54] H_2O_2 might be detoxicated in uncatalyzed reactions.

To date, Chagas' disease has defied all attempts to develop an efficient chemotherapy. Despite the recognition of the importance of redox cyclers as potent trypanocidal agents, few reports showed a possible correlation between redox potentials and trypanocidal activities. Several naphthoquinones (structures not shown) were assayed as trypanocidal and their E_{redox} were measured in aprotic medium, using Hg as the working electrode.[49] It was reasonable to suggest that it is more probable to find trypanocidal activity among the quinones presenting first reduction potential more positive than –0.72 V *vs.* SCE, especially if they are *ortho*-naphthoquinones.[49]

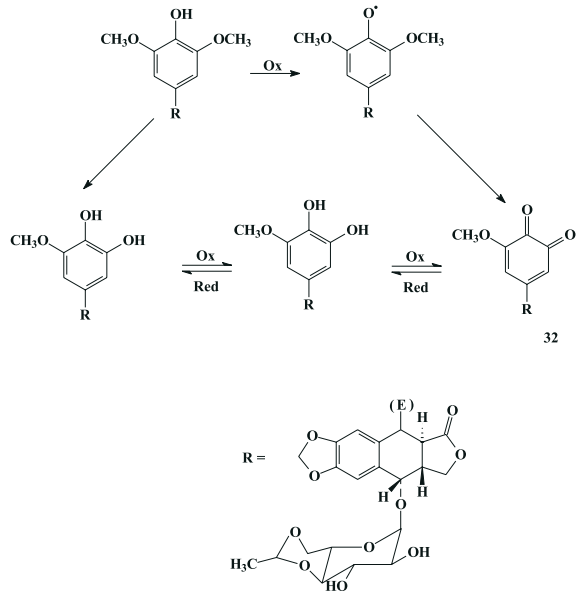
4.2.2. Quinones and quinonoids as metabolites

Phenols (**21, 24**) can be found in a wide variety of drug classes. Redox mechanisms leading to ROS are plausible since this functionality is readily converted to ET quinones, in an oxidatively activating process.[4,5,10,55]



Tripanothione **31**

As an example, etoposide (**24**), a widely used anticancer drug whose toxicity is associated with trapping of the topoisomerase II/DNA cleavable complex and formation of protein-DNA cross-links and nicked DNA, can be metabolised to several highly reactive products. Among them, an *ortho*-quinone (**32**) was shown to be a powerful inhibitor of topoisomerase II (Scheme 9).[56]



Scheme 9. Major oxido-reductive pathways of the metabolic transformations of etoposide (**24**) mediated by P450 monoxygenase and/or peroxidase. These transformations alter the pendant dimethoxyphenolic group.[56]

5. Electrochemistry and Cancer

5.1. Generalities

Electrochemistry has been used in cancer pharmacology in a variety of ways. This is the area where the majority of studies of correlation between electrochemical parameters and biological activities were performed. A recent review on the mechanism of anti-cancer agents with emphasis on electron transfer - oxidative stress appeared in the literature.[10]

In the case of cancer, biological activity, among other factors:

i) appears to vary with the tumour system. Hydrophilic drugs are used for aqueous tumour systems for the intracavitary therapy. On the other hand, selection of a lipophilic derivative with a low reduction potential for the continuous regional infusion of localised neoplasm may enhance tissue extraction, minimising the systemic toxicities.

ii) is related to the degree of cell and tissue oxygenation, as already commented. Solid tumours contain a proportion of cells, which are either transiently or chronically hypoxic. Because of their low proliferative activity and inaccessibility to blood-borne drugs, these cells represent a potential clinical problem in the chemotherapy of solid tumours. They are radioresistant and may contribute to local failure in radiotherapy. One method for overcoming this problem is the use of chemical radiosensitizers. The hypoxic microenvironment offers an attractive target. Drugs activated only in hypoxic regions may be truly specific for solid tumours.[4,57]

iii) depends on pH of the system. It is well recognised that pH plays a significant regulatory role in most cellular processes. There is an increased interest in transmembrane pH gradients, particularly with respect to tumour growth and response to therapy.[58] In hypoxic cells, the pH is lower than that generally found in adjacent well-oxygenated tissues. The H^+ concentration greatly influences the chemistry in these regions.

So, it is fundamental to analyse cytoxicity in anaerobic or aerobic (oxic/hypoxic) conditions and in several media. The same is true for electrochemical studies.

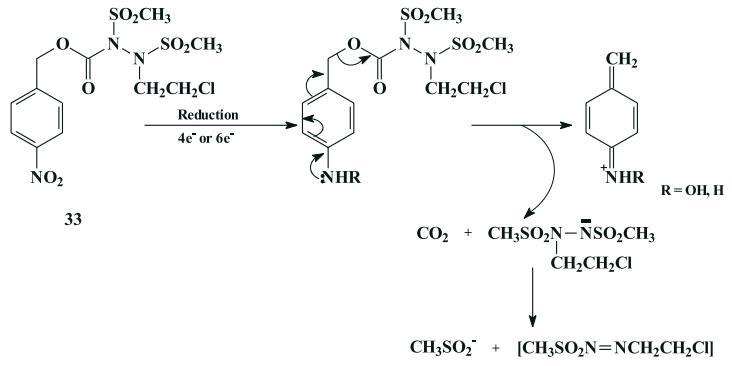
In the present case, emphasis will be held in hypoxia selective agents (HSA), once ET-OS was recently reviewed.[10]

Most HSAs rather than undergoing selective reduction, undergo reduction events in all cells, which are reversible in the presence of molecular oxygen (*via* superoxide generation). Hence, in the absence of O_2, the activated reduction product is long-lived enough to inflict macromolecular damage resulting in cytotoxicity. It has been demonstrated that agents possessing reduction potentials in the range -300 to -450 mV vs NHE are accessible to enzymatic reduction *in vivo*.[59] As said for other bioalkylating agents, there is an ideal redox potential. In spite of not being absolute, it appears that molecules with electron affinity less than -350 mV but more than -200 mV will show little activity against hypoxic cells *in vivo*. Molecules with low electron affinities are expected to be inadequately activated by bioreductive enzymes, while molecules with too high an electron affinity are expected to be rapidly metabolised and excreted.[60]

Ideally a hypoxic selective agent should be reduced more readily under more acidic conditions to exploit the differences. 70-80 mV change in redox potential leads to 10 fold change in the reduction rate. Two hypoxic active nitrobenzyloxycarbonyl derivatives of 1,2-bis (methylsulfonyl)-1-(2-chloroethyl) hydrazines (**33**) were studied by pulse polarography in different pH values and appear to be reduced more easily under acidic conditions than under neutral conditions (Scheme 10).[61]

While the potency of the bioreductive quinones varies with their redox potential, the direction and magnitude of the oxic/hypoxic differential cannot yet be predicted from the structures.[62] It should be stressed that a balance must be achieved between ease of reduction and oxygen reactivity so that hypoxia selectivity is maximised and tolerable aerobic toxicity achieved.

Very frequently, different oxygen concentrations for activation are required.[63] It was found that metronidazole (**13**) was only toxic at extremely low oxygen concentration, whereas nitrofurazone (**34**) was toxic at substantially greater concentrations of oxygen. This difference can be rationalised by the large variation in redox potential.[63]



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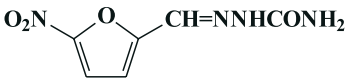
Scheme 10. Reductive cleavage of nitrobenzyloxycarbonyl derivatives **33**, generating a putative alkylating species.[61]



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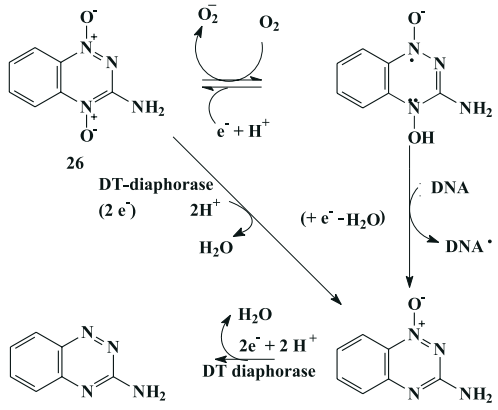
Further, since oxygen has a higher redox potential than most bioreductive drugs, it would be expected that compounds with redox potentials with substantially greater negativity than oxygen, would give up their electron (when reduced) to oxygen much more easily than compounds with redox potentials closer to oxygen.

The cytotoxic activity depends upon reduction of the nitro group, usually at low redox potentials, which are normally unattainable in well-oxygenated cells. The relative reduction rates in hypoxia or anoxia and under oxic conditions are the basis for their selective toxicity and therapeutic differential.[4]

Additionally, one of the most selective anti-cancer drugs, tirapazamine (**26**),[64] is an excellent substrate for various intracellular reductase enzymes, which can add a single electron to the molecule, thereby producing a free-radical intermediate (Scheme 11). In the presence of oxygen, this free radical is rapidly oxidized back to the parent molecule with the formation of a superoxide radical. However, in the absence of oxygen – the situation with hypoxic cells – this does not occur, and the highly reactive radical will remove hydrogen atoms from nearby macromolecules, possibly DNA, causing them a structural damage (Scheme 11).[57, 64]



Scheme 11. Major reductive pathway of the metabolic transformations of tirapazamine (**26**), mediated by reductases.[57]

6. Concluding Remarks

Electrochemical methods (analytical and preparative) and parameters can be widely used in Biomedical Chemistry, especially because they furnish an enormous amount of evidences regarding the mechanisms of biological electron-transfer processes.

The comparison between electrochemical and bioactive properties, based upon the general theoretical frameworks, as shown, carries a great significance, allowing the use of electrochemical parameters as direct evaluators of biological activities. The established relationship between the ease of reduction, represented by E_{pc}, $E_{½}$ or E_{redox} and/or electrochemical kinetic parameters (electron transfer rate constants) and biomedical properties shows the relevance of electrochemical studies as tools for the comprehension of drugs' mechanism of action against various diseases. As electrochemical parameters can be, generally, directly related to the effects of substituent, the benefits of QSAR studies, finding mathematical forms for the relationship, bring additional relevance to the methodology and allow prediction of biomedical properties.

One should not expect a general direct correlation between reduction potential and bioactivity. Caution must be always used in interpreting this kind of correlation. Many other important factors must be also considered in the mechanistic aspects of *in vivo* drug activity, *e.g.*, stereochemistry, diffusion, solubility, metabolism, membrane permeability, bioactivation and DNA binding. It is more probable to find correlation in case of ET-OS.

Discrepancy of results (absence of correlation) between electrochemical and other studies (enzymatic) could be explained by specific reactions catalysed by specific enzymes or by different biological pathways, not related to electron transfer.

Indeed, the number of physiologically active substances that possess E^1_7 values greater than about -0.5 V *vs.* NHE, in the physiological active range, which can permit electron acceptance from biological donors is significant. When the potential for a given drug is too low or two high, they can be modified *in vivo* to adequately potential-driven metabolites, that are the useful agents. Electrochemistry is also used to follow this chemical transformation.

The versatility of the electrochemical methodology allows to mimic the multitude of biological environments: the conditions can be widely varied in the attempt to resemble them. Different ranges of pH, oxygen content in the electrochemical cell and solvents of diverse properties can be used. However, standardization is urgently required, in terms of methods, electrodes, supporting electrolytes, *etc.*, to allow a more general use of the already available data.

In electrochemistry, considerable progress has recently been made in the development of new and rather sophisticated techniques, as exemplified in the present article.

The field of Biomedical Chemistry will, naturally, take advantage of this progress.

7. Glossary of Terms

Antimetabolites – Structural analogues of normal metabolites that are required for cell function and replication. They work by interacting with cellular enzymes and therefore stopping the cell from making, for example, the extra DNA necessary for replication.

Apoptosis – A genetically encoded program of cell death that can be activated under physiological conditions like hypoxia and may be an important safeguard against tumour development.

Bioreductive alkylation – The term used to describe the effect of those compounds, which express their mode of action as alkylating agents, but do so subsequent to their reduction *in vivo*.

Electron transfer (ET) – An electron is physically transferred between two dissimilar species resulting in chemical change. According to Molecular Orbital Theory, oxidation of a species occurs by removing one electron from the highest occupied molecular orbital (HOMO) and reduction by addition of an electron to the lowest unoccupied molecular orbital (LUMO). Thus, if the ET reactivity (ionisation potential, electron affinity, anodic and cathodic half-wave potentials, ET rate constants, *etc.*) of a series of compounds is determined, it often correlates well with the HOMO or LUMO energy coefficients, as calculated by simple or advanced MO theory.[65]

Hypoxia – Occurs in many common pathological conditions. Blood supply to the hypoxic region delivers less oxygen than in normal cells. Hypoxia in human tumours has also been associated with malignant progression and formation of metastases. It is the major physiological difference between tumours and normal tissues, and hence, it constitutes a very attractive target for selective therapy. It results from an inadequate and disorganized tumour vasculature, and hence an impaired oxygen delivery.

Pro-drugs – Compounds that are inactive in themselves, but which are converted by chemical or enzymatic means to an active drug.

Radiosensitizers – Compounds that selectively increase the radiosensitivity of hypoxic cells while leaving oxygenated cells unaffected.

Redox cyclers – Compounds that cause oxidative stress by transferring electron to O_2, the initial structure being regenerated.

Oxidative stress – The set of intracellular or extracellular conditions that leads to the chemical or metabolic generation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals, singlet oxygen, lipid hydroperoxides or related species.

Oxidatively activated agents – The term is used to describe the effect of those compounds, which express their mode of action, generally as alkylating agents, but do so subsequently to their oxidation *in vivo*.

Topoisomerase II – An essential nuclear enzyme whose major function is to regulate the topological state of DNA during replication and chromosome condensation and segregation. It does this by catalysing the transient cleavage of one DNA double helix, passage of an intact DNA strand through the break and resealing of the broken DNA strand. It is also the key cellular target for a number of clinically important anticancer drugs including etoposide and the anthracyclines doxorubicin and daunorubicin.

Xenobiotics — Substances which are foreign to the particular biological system under study.

List of symbols and abbreviations

EA: electron affinity
E_{redox} (E_{pc} + E_{pa})/2: redox potential
E^1_7: first reduction potential, in aqueous medium, at pH 7
$E_{1/2}$: measured half wave potential
E_{pa}: anodic peak potential
E_{pc}: cathodic peak potential
$E_{pc/2}$: cathodic peak potential at half peak height (I = Ip/2)
GSH: glutathione
I: current
IP: ionisation potential
I_{pa}/I_{pc}: ratio between anodic and cathodic peak currents
ν½: square root of scan rate
n: number of electrons
NHE: normal hydrogen electrode
QSAR: Quantitative Structure-Activity Relationship
SCE: saturated calomel electrode

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References

1. Kunz, K.R.; Iyengar, B.S.; Dorr, R.T.; Alberts, D.S.; Remers, W.A.; *J. Med. Chem*. **1991**, *34*, 2281.
2. Dryhurst, G. In *Electrochemistry of Biological Molecules*; Academic Press; New York, 1977, ch. 1, pp.1-5.

3. Rosenberg, B.; VanCamp, L.; Krigas, T.; *Nature* **1965**, *205*, 698.
4. Rajski, S.R.; Williams, R.M.; *Chem. Rev.* **1998**, *98*, 2723 and references therein cited.
5. Kovacic P.; Becvar, L.E.; *Curr. Pharm. Des.* **2000**, *6*, 143 and references therein cited.
6. Höckel, M.; Schlenger, R.; Mitze, M.; Schaffer, U.; Vaupel, P.; *Seminars in Radiation Oncology*, **1996**, *6*, 3.
7. Bard, A.J.; Faulkner L.R. In *Electrochemical Methods, Fundamentals and Applications*; John Wiley and Sons; New York, 1980.
8. Lunte, S.M.; Lunte, C.E.; Kissinger, P.T.; Heineman, W.R. (Eds.) In *Laboratory Techniques in Electroanalytical Chemistry*, Marcel Dekker: New York, *2 ed*.,1996.
9. Bernd, S.; *Curr. Org. Chem.* **1999**, *3*, 171.
10. Kovacic, P.; Osuna Jr., J.A.; *Curr. Pharm. Des.* **2000**, *6*, 277 and references therein cited.
11. Chow, C.K.; *Free Rad. Biol. Med.* **1991**, *11*, 215.
12. Lown, J.W.; *Acc. Chem. Res.* **1982**, *15*, 381.
13. Parrish, J.A.; Fitzpatrick, T.B.; Tanenbaum, L.; Pathak, M.A.; *N. Engl. J. Med*. **1974**, *291*, 1207.
14. Nilsson, E.; von Euler, H.; Berendson, J.; Thörne, A.; Wersäll, P.; Näslund, I.; Lagerstedt, A-S.; Narfström, K.; Olsson, J.M.; *Bioelectrochem.* **2000**, *51*, 1 and references therein cited.
15. Mayalarp, S.P.; Hargreaves, R.H.J.; Butler, J.; O´Hare, C.C.; Hartley, J.A.; *J. Med. Chem.* **1996**, *39*, 531.
16. Núñez-Vergara, L.J.; Farias, D.; Bollo, S.; Squella, J.A.; *Bioelectrochem*. **2000**, *53*, 103.
17. Núñez-Vergara, L.J.; Navarrete-Encina, P.A.; Ortiz, M.E.; Bollo, S.; Squella, J.A.; *Chem.-Biol. Interact.* **1996**, *101*, 89.
18. Núñez-Vergara, L.J.; García, F.; Domínguez, M.M.; de la Fuente, J.; Squella, J.A.; *J. Electroanal. Chem.* **1995**, *381*, 215.
19. Syafruddin, D.; Siregar, J.E.; Marzuki, S.; *Mol. Biochem. Parasitol.* **1999**, *104*, 185.
20. Turner, A.F.P.; Karube I.; Wilson, G.S.; In *Biosensors: Fundamentals and Applications*; Oxford University Press; Oxford, 1987.
21. Gutierrez, P.L.; Nguyen, B. In Electrochemistry in Cancer: Applications to Pharmacological Studies of Quinone-containing Antitumour agents. In *Redox Chemistry and Interfacial Behavior of Biological Molecules;* Dryhurst, G.; Niki, K., Ed.; Plenum Press; New York, 1988, p. 369.
22. Thorp, H.H.; *Trends in Biotechnology* **1998**, *16*, 117.
23. Brett, A.M.O.; Serrano, S.H.P.; Gutz, I.; La-Scalea, M.A.; *Bioelectrochem. Bioenerg.* **1997**, *42*, 175.
24. Tocher, J. H.; Edwards, D.I.; Thomas, A.; *Internat. J. Rad. Onc. Biol. Phys*. **1994**, *29*, 307.
25. Vroman, J.A.; Alvim-Gaston, M.; Avery, M.A.; *Curr. Pharm. Des.* **1999**, *5*, 101.
26. Chen, Y.; Zhu, S. M.; Chen, H.Y.; *Acta Chim. Sin.* **1998**, *56*, 925.
27. Abul-Hajj, Y.J.; Tabakovic, K.; Tabakovic, I.; *J. Am. Chem. Soc*. **1995**, *117*, 6144.
28. Shen, L.; Pisha, E.; Huang, Z.; Pezzuto, J.M.; Krol, E.; Alam, Z.; van Breemen, R.B.; Bolton, J.L.; *Carcinogenesis* **1997**, *18*, 1093.
29. Amatore, C.; Arbault, S.; Bruce, D.; de Oliveira, P.; Erard, M.; Sojic, N.; Vuillaume, M.; *Analusis*. **2000**, 28, 506.
30. Amatore, C.; Arbault, S.; Bruce, D.; de Oliveira, P.; Erard, M.; Vuillaume, M.; *Faraday Disc.* **2000**, *116*, 319.
31. Feng, J.; Ci, Y-X; Lou, J-L.; Zhang, X-Q.; *Bioelectrochem. Bioenerg*. **1999**, *48*, 217 and references therein cited.
32. Zuman, P. In *Substituents Effects in Organic Polarography*; Plenum Press; New York, 1967, Chs. I, II and VIII, pp.1-21; 23-41; 273-308.
33. Driebergen, R.J.; Moret, E.E.; Janssen, L.H.M.; Blauw, J.S.; Holthuis, J.J.M.; Kelder, S.J.P; Verboom, W.; Reinhoudt, D.N.; van der Linden, W.E.; *Anal. Chim. Acta* **1992**, *257*, 257.
34. Wardman, P. In *Selective Activation of Drugs by Redox Processes*, eds. G. E. Adams A. Breccia, E. M. Fielden and P. Wardman, Plenum Press, New York, 1990, pp. 11-24.
35. Chen; E.S.D.; Chen, C.M.; Sane, N.; Shulze, S.; *Bioelectrochem. Bioenerg*. **1999**, *48*, 69.
36. Tocher, J.H.; Edwards, D.I.; *Free Rad. Res. Comm.* **1988**, *4*, 269.
37. Dogovic, N.; Sladic, D.; Gasic, M. J.; Tabakovic, I.; Davidovic, A.; Gunic, E.; *Gazzetta Chimica Italiana*, **1991**, 121, 63.
38. Ossowski, T.; Pipka, P.; Liwo, A.; Jeziorek, D.; *Electrochim. Acta* **2000**, *45*, 3581.
39. Gritzner, G.; *J. Electroanal. Chem*. **1983**, *144*, 259 and references therein cited.
40. Wardman, P.; *J. Phys. Chem. Ref. Data*. **1989,** *18*, 1637.
41. Livertoux, M-H.; Lagrange, P.; Minn, A.; *Brain Res*. **1996,** *725*, 207.
42. Black, S.; Coon, M. J.; *J. Biol. Chem.* **1982,** *257*, 5929.
43. Donkers, R.L.; Workentin, M.S.; *J. Phys. Chem.* **1998**, *102*, 4061
44. Clarke, E.D.; Goulding, K.H.; Wardman, P.; *Biochem. Pharmacol*. **1982**, *31*, 3237.
45. Tocher, J.H.; *Gen. Pharmac*. **1997**, *28*, 485 and references therein cited.
46. Palmer, B.D.; Wilson, W.R.; Cliffe, S.; Denny, W.A.; *J. Med. Chem*. **1992**, *35*, 3214 and references therein cited.
47. Lund, H. In *Organic Electrochemistry*, Lund, H.; Baizer, M.M., Eds.; Marcel Dekker, Inc.; New York, 1991, pp 401-432.
48. Monks, T.J.; Hanzlik; P.; Cohen, G.M.; Ross, D.; Graham, D.G.; *Toxicol. Appl. Pharmac.***1992**, *112*, 2.
49. Goulart; M.O.F.; Freitas, L.R.; Tonholo, J.; de Abreu, F.C.; Raslan, D.S.; Starling, S.; Zani, C.L.; Oliveira, A.B.; Chiari, E.; *Bioorg. Med. Chem. Lett.* **1997**, *7***,** 2043 and references therein cited.
50. Pisani, D.E.; Elliott, A.; Hinman, D.; Aaronson, L.; Pardini, R.; *Biochem. Pharmacol.* **1986**, *35*, 3791.

51. Chambers, J. Q. In *The Chemistry of Quinonoid Compounds*; Patai, S., Ed.; John Wiley & Sons Ltd; N. York, **1974**, p. 737-791
52. Chambers, J. Q. In *The Chemistry of Quinonoid Compounds*; Patai, S., Rapport, Z., Eds.; John Wiley & Sons Ltd; N. York, 1988, p. 719-757
53. Jeftic, L.; Manning, G.; *J. Electroanal. Chem.* **1970**, *26*, 195.
54. Schirmer, R.H.; Müller, J.G.; Kraut-Siegel, R.L.; *Angew. Chem. Int. Ed. Engl*. **1995**, *34*, 141.
55. Zhang, F.; Dryhurst, G.; *J. Electroanal. Chem*. **1995**, *398*, 117.
56. Gantchev, T.G.; Hunting, D.J.; *Mol. Pharmacol*. **1998**, *53*, 422.
57. Sinhababu, A K.; Thakker, D. R.; *Adv. Drug Delivery Rev*. **1996**, *19*, 241.
58. Mason, R.P.; *Curr. Med. Chem.* **1999**, *6*, 481.
59. Tannock, I. F.; *Br. J. Radiol.* **1972**, *45*, 515.
60. Kelson, B.; McNamara, J.P.; Pandey, A.; Ryan, K.J.; Dorie, M.J.; McAfee, P.A.; Menke, D.R.; Brown, J.M.; Tracy, M.; *Anti-Canc. Drug Des.* **1998**, *13*, 575.
61. Shyam, K.; Penketh, P.G.; Shapiro, M.; Belcourt, M.F.; Loomis, R.H.; Rockwell, S.; Sartorelli, A.C.; *J. Med. Chem.* **1999**, *42*, 941.
62. Rockwell, S.; Sartorelli, A.C.; Tomasz, M.; Kennedy, K.A.; *Canc. Metastasis Rev.* **1993**, *12*, 165.
63. Stratford, I. J.; Workman, P.; *Anti-Canc. Drug Des.* **1998**, *13*, 519.
64. Brown, J.M.; *Mol. Med. Today* **2000**, *6*, 157.
65. Eberson, L.; *Electron Transfer in Organic Chemistry*, Springer Verlag, Berlin, 1987.

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