Development of Hypoxia Selective Cytotoxins for Cancer Treatment: An Update

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Abstract: Increased attention has centered on exploiting hypoxia in tumors for targeting the design of selective antitumor agents. This review presents an update of the principal families of compounds under study and in clinical trials, such as *N*-oxide derivatives, nitro compounds and quinone derivatives. Especially promising for bioreductive activation is the reduction of some moieties able to trigger a mechanism that releases cytotoxic antitumor drugs. The most remarkable redox-activated triggers are presented, *N*-oxide, nitro, azido, quinone, metal ions, 1,2-benzisoxazolyl and sulfoxide moieties.

INTRODUCTION

Since the 1960s it has been known that necrotic regions in solid tumors are hypoxic [1-9]. These hypoxic regions may represent up to 20% of the total tumor mass [10]. The oxygen level in different kind of tumors can vary between 2 to 20 mm Hg in hypoxic zones compared with 30 to 50 mm Hg for surrounding normal tissues [11]. These hypoxic zones are generated because cancerous cells become relatively isolated from their blood supply due to their rapid growth and deficient vascularization. Hypoxic cells are very resistant to radiation damage, and the same diffusional limitations for oxygen also affect oberved reactivities of, cytotoxic drugs used traditionally in chemotherapy.

Chronic hypoxia is thought to give rise to a resistant subpopulation of potentially clonogenic cells which are not undergoing normal growth. Molecular oxygen is utilized in cells principally as a terminal electron acceptor in oxidative phosphorylation but also for a variety of dioxygen requiring processes including steroidogenesis and heme degradation. Flavoprotein containing dehydrogenases and oxidoreductases mediate the flow of electrons from reducing agents like the nicotinamide dinucleotide coenzymes, NADH and NADPH, to oxygen and other endogenous acceptor molecules. Various chemicals are also substrates for certain flavoprotein enzymes that ordinarily participate in intermediary metabolism [12]. Recently, it has also been recognized that hypoxia enhances tumor metastasis, transforming tumors to forms that are incurable by surgery [11]. This is associated with bad prognosis in cancer, so attacking hypoxic cells is important to avoid malignancy in tumors.

This common hypoxic feature of cancerous cells is being used for the development of distinctive therapies for treating cancer. On the basis of using prodrugs, capable of bioreduction under hypoxic conditions (bioreductive antitumour agents [13]) to active cytotoxins that damage cancerous cells, has led to hypoxia-selective cytotoxins as antitumor drugs. Oxygen reverts the bioreductive process in normal oxygenated tissues giving selectivity (Fig. (**1**)). The bioactivation depends on appropriate levels of reductase enzymes [14] and is governed by the redox properties of drugs. An interesting review was published by Wardman, establishing the importance of these physicochemical properties in the design of new drugs [15].

Bioreductive antitumor agents are prodrugs (Fig. (**1**)). That is, they are inactive in their own right, but are able to undergo metabolism to species that could damage biomolecules upon reduction [14,16,17]. Among the compounds described as bioreductive antitumor agents [18-21] have been *N*-oxide derivatives [22,23], nitro derivatives [24-26] and quinone derivatives [27]. On the other hand, the special conditions of the hypoxic tissues have also been employed as a strategy for delivering traditional antineoplastic agents (Fig. (**2**)) [14]. In this sense, a great number of hybrid compounds have been developed. *N*-oxide, nitro, azido, quinone and metal ion moieties have been employed as redox-activated trigger entities.

In this article an update on the medicinal chemistry of bioreductive activation strategies is reviewed.

1. CHEMICAL ENTITIES DEVELOPED AS BIOREDUCTIVE DRUGS

1.1. *N*-Oxide Derivatives

The *N*-oxide functional group is the result of the addition of an oxygen atom to the lone pair electrons on the nitrogen atom. Compounds containing this group are numerous and include the *N*-oxide of tertiary amines (aliphatic and aromatic) as bioreductive drugs, Fig. (**3**) [23]. The *N*-oxide moiety can be reduced in hypoxic conditions *via* one or two electrons by reductase enzymes yielding toxic species that damage cancerous cells. This process can be reversed by oxygen, producing the prodrug recycling and oxygen radicals (redox cycling). So, the selectivity arises from the difference in oxygen levels between solid tumors and normal tissues. The role of oxygen is crucial for selectivity, the toxic species generated in hypoxic environments must be more

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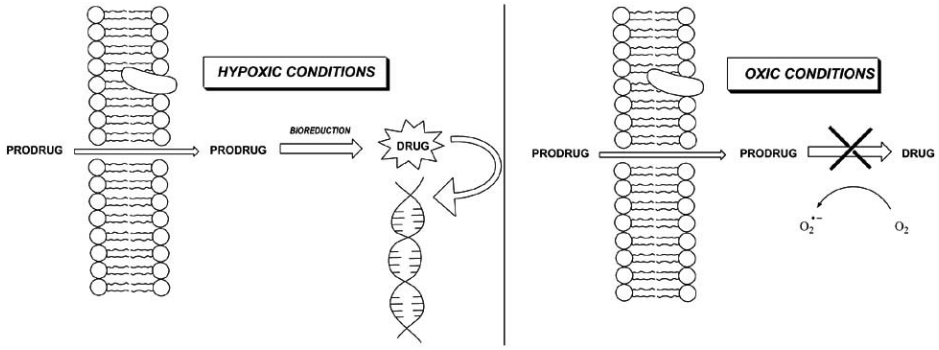


Fig. (1). Selectivity of hypoxic selective cytotoxins.

damaging than the amount of superoxide generated normally in oxygenated cells [14, 23].

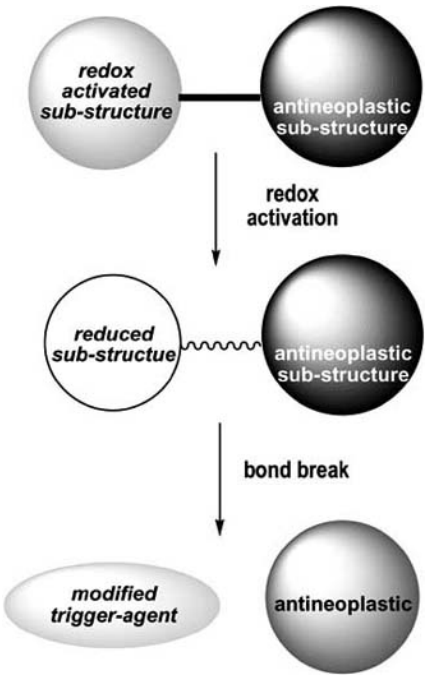


Fig. (2). Releasing process of antineoplastic drugs in hypoxic cells.

1,2,4-Benzotriazine 1,4-Dioxide

The first and most studied *N*-oxide derivative as a bioreductive drug was Tirapazamine (Tpz), 3-amino-1,2,4-benzotriazine 1,4-dioxide, **1** Fig. (**4**). This is currently in Phase II/III clinical trials in combination with radiotherapy and also with cisplatin-based chemotherapy [28-30]. The mechanism of action of Tpz has been extensively studied during the last decade. First, it was established that the main toxic species was the hydroxyl radical generated upon reduction *via* one electron (Fig. (**4**)) [23,31]. This hydroxyl radical abstracts a hydrogen radical from DNA, causing bond rupture leading to cell death. Recently, Denny and coworkers proposed that an alternative pathway is also possible because it was not clear that the released $^{\bullet}$OH, in the

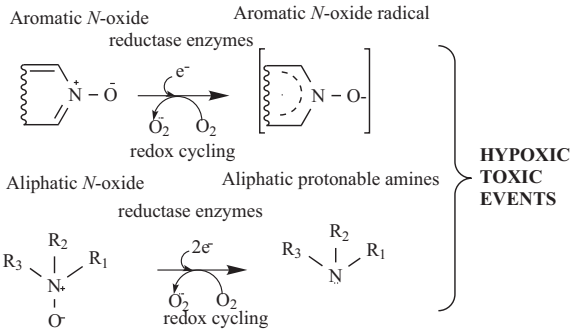


Fig. (3). Mechanism of prodrug activation of *N*-oxide of amines.

bioreduction process, was enough to produce the cellular damage (Fig. (**4**)). In consequence, the authors proposed that the production of the benzotriazinyl radical **IV**, from the protonated species **II**, is involved in this pathway (Fig. (**4**)). Like $^{\bullet}$OH, the benzotriazinyl radical **IV** also reacts with 2-deoxiribose, the well-established reaction that leads to DNA strand breaks causing cellular damage [32-36]. New insights in the mechanism of action of Tpz have demonstrated that it inhibits DNA replication [37], is a Topoisomerase II poison [38], and also is metabolized by cytosolic NOS II [39]

In order to complete the first Tpz-QSAR studies [40,41], Denny and coworkers recently described a full series of 1,2,4-benzotriazine 1,4-dioxide substituted in the benzo ring [42]. They found that the one electron reduction potential was the most important physicochemical property for hypoxia-related cytotoxicity. Weakly electron-donating benzo-substituents produce the most active derivatives, **2-7** (Fig. (**5**)), where the lipophilicity slightly affects the potency. Also, 5-substituted derivatives showed high aerobic cytotoxicity though low selectivity to hypoxic tumor (**8-10**, Fig. (**5**)).

Brown demonstrated that nuclear reductases are responsible for DNA damage caused by Tpz [43]. Taking into account this result, new approaches to improve Tpz activity were described [33,44,45]. The development of new derivatives targeting groups that could interact with DNA have been described (i.e. 11-13 (Fig. (**6**)). Chromophores like acridine were used to put the *N*-oxide moiety near to DNA, increasing the selective cell killing in hypoxic conditions.

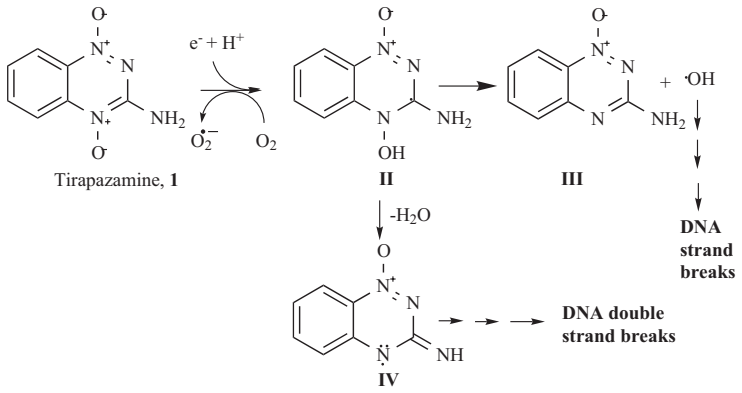
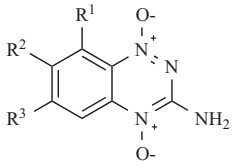


Fig. (4). Mechanism of Tpz selective cytotoxicity in hypoxia.

Therapeutic studies in different areas have also been performed with Tpz and analogues. For example, gene-directed enzyme prodrug therapy (GDEPT) has been applied with Tpz alone or combined with cisplatin and/or with radiotherapy [46]. In this approach, Tpz potentiated the radiotherapeutic outcome in overexpressing NADPH: cytochrome P450 reductase HT1080 fibrosarcoma producing tumor regression. Wilson and coworkers have described the multicellular resistance to Tpz due to restricted extravascular transport [47] and studies have included seventeen new Tpz analogues [48]. Tpz has been combined with radiation as a metastatic dissemination inhibitor [49], or combined with radioimmunetherapy [50], or with electric pulses [51].

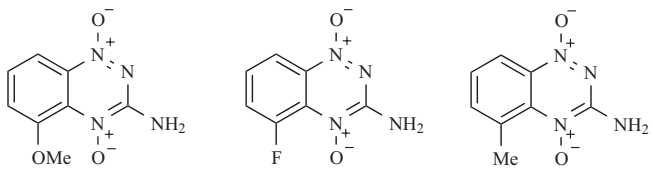
Other N-*Oxide Derivatives*

The Quinoxaline 1,4-dioxide (QdNO) system has been described as a pharmacophore for experimental bioreductive drugs but none of them have yet progressed to clinical studies [23]. In 1995 Monge and coworkers first reported QdNO derivatives as selective hypoxic cytotoxins. In particular, the cyano aminoalkyl derivatives (i.e. **14**, Fig. (**7**)) showed the most promising activities [52]. Recently, the authors have tested **14** against a panel of several tumor cell lines (Caco-2, MCF-7, HT-29 and Tk-10) and found the same profile of potency and selectivity as for non-tumor V79 cells [53]. Gali-Muhtasib *et al.* studied four new QdNO non-cyanoamine derivatives, the 2-benzoyl-3-phenyl derivatives



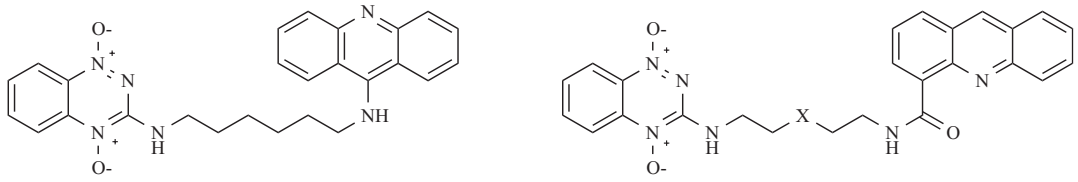
Ref.	-R[1]	-R[2]	-R[3]	HCR (SCCVII) [a]	*E* (1) (V) [b]	logP [c]
1	-H	-H	-H	258	-0.456	-0.33
2	-H	-H	-Me	166.7	-0.493	0.08
3	-H	-Me	-H	125.0	-0.474	0.14
4	-CF_3	-H	-H	112.5	-0.372	0.48
5	-Me	-H	-H	102.9	-0.510	0.24
6	-H	-OMe	-H	100.0	-0.494	-0.05
7	-H	-F	-H	70.3	-0.400	-0.28

[a] HCR: hypoxic cytotoxicity ratio, concentration required to reduce plating efficiency to 10% of controls in oxia divided the concentration required to reduce plating efficiency to 10% of controls in hypoxia. [b] One electron potential determined by pulse radiolysis in aqueous solution containing 2-propanol (0.1 M) buffered at pH 7.0. [c] Using the program ACDLogP v. 4.5.



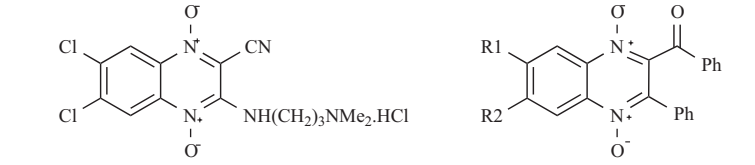
8, HCR= 0.83 **9**, HCR< 7.5 **10**, HCR< 10

Fig. (5). Benzotriazine derivatives and relevant biological and physicochemical properties.



11 -X-= -O-, **12**
-X-= -N(Me)-, **13**

Fig. (6). Benzotriazines target to DNA interacting agents.



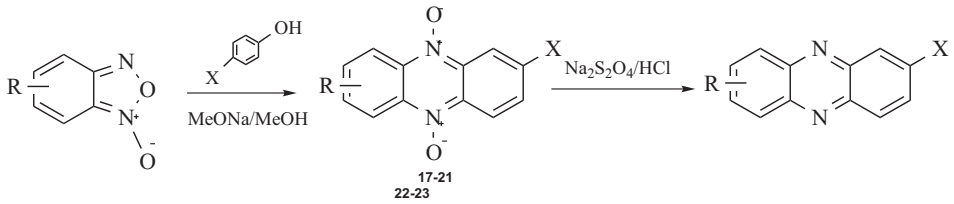
14 HCR=154.7(Caco-2); 116.7(HT-29); 97.7(MCF-7); 67.3(Tk-10); 250(V79)

R_1=R_2= H, **15** HCR= 40 (T-84)
R_1=R_2= Cl, **16** HCR= 100 (T-84)

Fig. (7). Most characteristic QdNO derivatives studied as bioreductive agents.

being the most active and selective in hypoxia (**15**, and **16**, Fig. (**7**)). They found the same activity profile as Monge and coworkers, the 6,7-dichloride QdNO derivative, **16**, being the most relevant. These QdNO derivatives also reduced the expression of HIF-1alpha (hypoxia inducible factor) and affected the cell cycle in the same manner [54,55]. QdNO **15** and **16** also had significant antiangiogenic [56] and apoptotic effects [57] and, combined with radiation in a mouse tumor model of lung carcinoma, resulted in an increase of necrosis. These results pointed to potential clinical applications. New 2-alkylcarbonyl and 2-benzoyl-3-trifluoromethyl-quinoxaline 1,4-di-*N*-oxide derivatives have been recently described as anticancer agents in oxia against a complete NCI screen panel of tumor cells, but no information on effects of hypoxia were reported [58].

Recently, phenazine 5,10-dioxide derivatives i.e. **17**-**23** (Fig. (**8**)), structurally related to QdNO and with potential π DNA-stacking properties, have been evaluated as selective hypoxia cytotoxins using a V79 clonogenic assay [59]. In general, the amino derivatives were more potent and selective than the hydroxyl derivatives, especially compounds **18**, **19**, and **21**. To gain insight to the mechanism of action deoxygenated phenazine derivatives were also tested. Except for derivative **23**, they were all inactive in hypoxic conditions as observed for other *N*-oxide derivatives. In



17-21
22-23

Ref.	R	X	SF hypox[a]	SF air[b]
17	H	NH_2	0	2
18	Cl	NH_2	0	0
19	Br	NH_2	0	12
20	Cl	OH	100	100
21	Br	OH	0	80
22	Cl	NH_2	100	0
23	Br	NH_2	0	0

[a]SF hypox= survival fraction in hypoxia at 20 μM. [b]SF air= survival fraction in air at 20 μM[d].

Fig. (8). Phenazine 5,10-dioxide derivatives and deoxygenated derivatives studied as bioreductive agents.

particular, deoxygenated analogues of **18** and **19**, **22** and **23**, were very toxic in oxic conditions. So, it could be proposed that **21** is a hypoxia-selective cytotoxin and compounds **18** and **19** are hypoxia-dependent triggering agents.

Other aromatic *N*-oxides have been described as bioreductive drugs. 1,2,4-Triazine mono and di-*N*-oxide derivatives have high potency but poor selectivity against hypoxic V79 cells. EPR studies were used to confirm the production of radical species in such compounds (**24**, Fig. (**9**)). The electrochemical behavior of these triazines showed a window of redox potential for active compounds, demonstrating the importance of this property for the design of new drugs [60-62]. Similarly, 1,2,5-oxadiazole *N*-oxide (furoxans) have also been described as potential hypoxic cytotoxins. A QSAR study showed the relevance of the electrophilic characteristics of the benzyl-like position to react with bio-nucleophiles. Also, selected furoxans were tested in two tumor animal models, with the 3-cyano derivative **25** (Fig. (**9**)) having the best pharmacological profile in agreement with its excellent nitric oxide releasing capacity [63-65], and were used as ^{99m}Tc-radiolabelled markers for hypoxia in tumors [66].

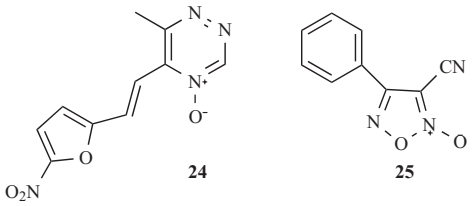


Fig. (9). 1,2,4-Triazine *N*-oxide and furoxan derivatives studied as potential bioreductive agents.

1.2. Nitro Compounds

Since the 1970s the nitro compounds, nitrofurans and especially nitroimidazoles, have been designed and used as radiosensitizers [67,68]. The radiosensitizers through bio-reduction affect the natural cellular detoxification processes, the response for radiotherapy in solid tumors. As with Tpz, development of new radiosensitizer nitroimidazoles targeting groups that could interact with DNA have been described. The best derivative is compound **26**, namely NLCQ-1, that contains a quinolinyl group as chromophore (Fig. (**10**)) [69]. This compound also shows good hypoxia-selective cytotoxicity. The 8-chloroquinolinyl group is a weak DNA-binding entity but in the hypoxic conditions of tumors the nitroso moiety increases DNA-binding properties of compound **26** (Fig. (**10**)).

Freeman and coworkers have described the use of the nitroimidazolyl moiety as a bioreductive pharmacophore that produces potential thymidine phosphorylase inhibitors in hypoxic conditions [70,71]. This enzyme is expressed in high levels in the hypoxic regions of many tumors and its inhibitors inhibit angiogenesis and metastasis and promote apoptosis [72]. Based on this principle, derivatives **28** and **29** (Fig. (**11a**)) were designed using as template the well known thymidine phosphorylase inhibitor **27**. Using docking analysis, the reduced products of **28** and **29** were studied as enzyme inhibitors, the results being concordant with experimental inhibitor potencies.

Recent descriptions of nitroimidazole derivatives refer to them as imaging agents under hypoxic conditions [73]. Maeda and coworkers have designed the nitroimidazole **32** using LUMO energies data as descriptors of bioactivity. Compound **32** is a mixed derivative between two well known imaging markers (**30** and **31**, Fig. (**11b**)). It presents adequate lipophilic properties and when ^{18}F-**32** was administrated together with hypoxia enhancing agents, hydralazine or nitro-*L*-arginine, it showed higher tumor accumulation than when it was administrated alone [74]. This fact highlights the role of hypoxia in ^{18}F-**32** accumulation.

Finally, nitro compounds have also been employed as hypoxia-selective triggers in traditional antineoplastic drugs (Fig. (**2**)). These kinds of compounds will be discussed ahead.

1.3. Quinone Derivatives

The most important quinone studied as a bioreductive agent has been the natural antibiotic mitomycin C (**33**, MMC) [14]. Recently, Tomasz and coworkers have described the synthesis and biological evaluation of dimers of MMC as polyfunctional DNA alkylators [75]. The dimers were less cytotoxic than MMC, but **34** (Fig. (**12**)) exhibited

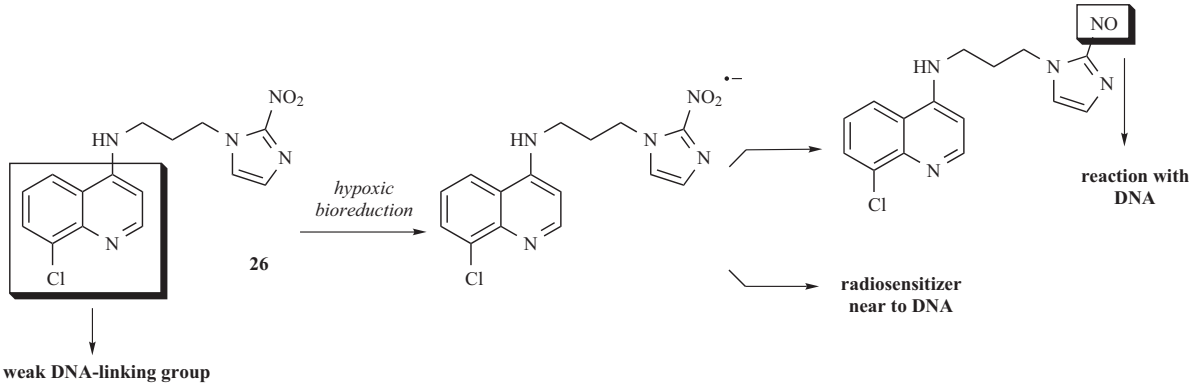


Fig. (10). NLCQ-1 bioactivation process.

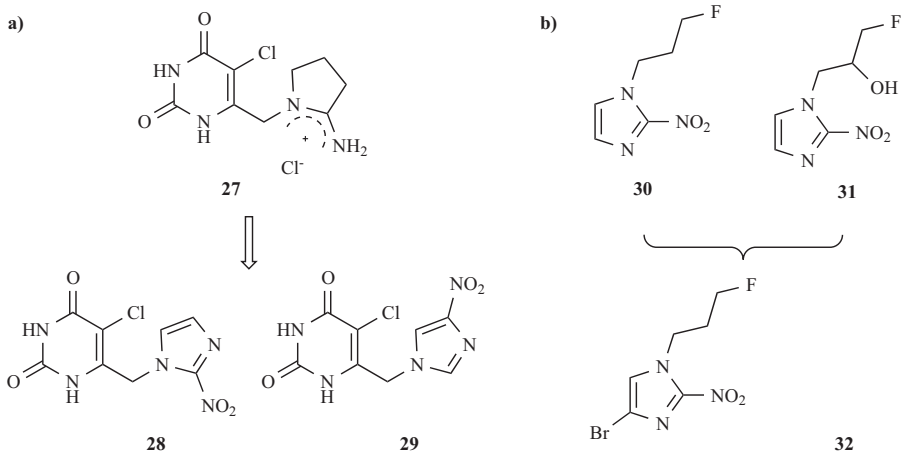


Fig. (11). Nitroimidazole derivatives developed as hypoxic therapeutic agents.

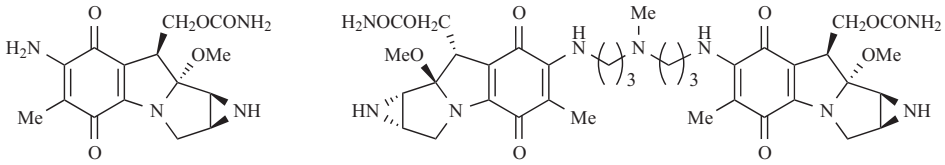


Fig. (12). MMC and the most relevant dimer developed.

much greater DNA cross-linking efficacy and was highly cytotoxic to all sixty human tumor cell cultures of the NCI screen panel. On the other hand, the cytotoxicity to EMT6 tumor cells is enhanced under hypoxic conditions by this dimer.

As in the case of nitro compounds, the most recent descriptions about quinone derivatives refer to them as hypoxia-dependent releasing agents of traditional antineoplastic drugs (Fig. (**2**)). Such compounds are discussed next.

1.4. Cytotoxin-Releasing Compounds

Currently, a great deal of research is concentrated on the design and development of prodrugs capable of releasing cytotoxins by bioreduction. From a structural viewpoint, these prodrugs consist of three motifs: a *redox-activated trigger* pharmacophore that is selectively reduced under hypoxia, a *cytotoxin* which is the active drug component responsible for cell killing, and a *linker moiety* which transmits the triggering event to the cytotoxin (for example, by fragmentation or through an electronic change) [11]. In the majority of the cases, a benzylic or a benzylic-like moiety has been employed as *linker* which, after the reduction of the *trigger* pharmacophore in an aromatic system, yields a quinone-like system (Fig. (**13**)). Traditionally antineoplastic agents have been employed as the *cytotoxin* in these kinds of compounds.

The most remarkable *redox-activated trigger* pharmacophores are *N*-oxide, nitro, azido, quinone and metal ion moieties. Also, benzisoxazole heterocycles have been

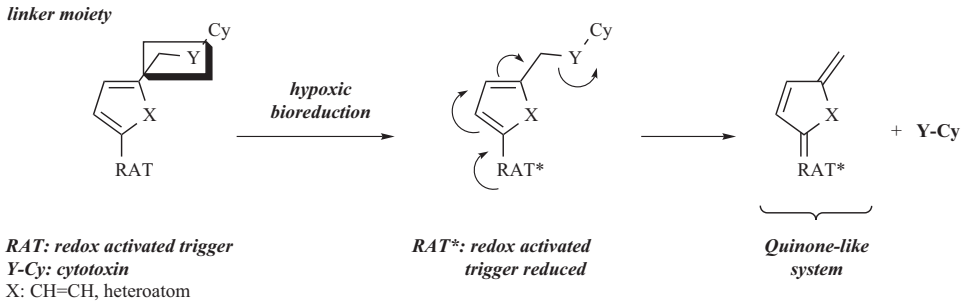


Fig. (13). Mechanism of redox activation of cytotoxins.

investigated as new redox-activated releasing groups in the last few years.

N-*Oxide Moiety as Redox-Activated Trigger*

AQ4N, an aliphatic diamine *N,N'*-dioxide derivative (**35**, Fig. (**14**)), is the most studied cytotoxin-releasing compound derived from *N*-oxide. AQ4N is not toxic but, after a four-electron reduction, the resulting AQ4 (**36**) acts as a topoisomerase II poison [23,76]. Most recently, AQ4N has completed a phase I clinical trial and demonstrated an interesting drug profile.

Nitro and Azido Moieties as Redox-Activated Triggers

The oldest example in the group of nitro derivatives as cytotoxin-releasing agents is SN 23862 (**37**, Fig. (**15a**)). In this case, the bioreduction changes the nucleophilic capacity of the tertiary amine which, after reduction of the 2-nitro moiety, reacts yielding the DNA-linking aziridinyl system [11,77]. This agent and analogues have been applied in GDEPT studies using *E. coli* nitroreductase enzymes as bioactivation system [78,79]. In the last few years, this kind of reduction has been employed in the design of new cytotoxin-releasing compounds. Fig. (**15b**) shows examples

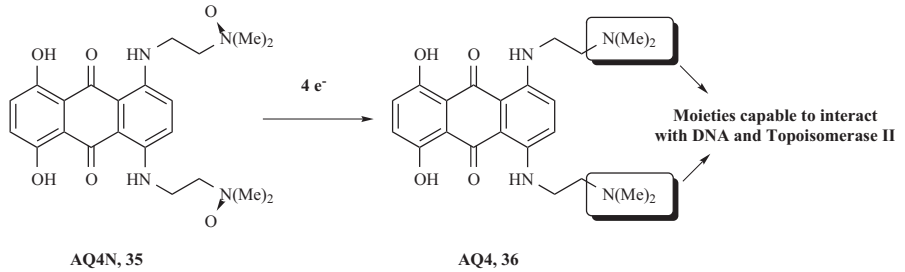


Fig. (14). Bioreduction of AQ4N to AQ4 *via* four electrons.

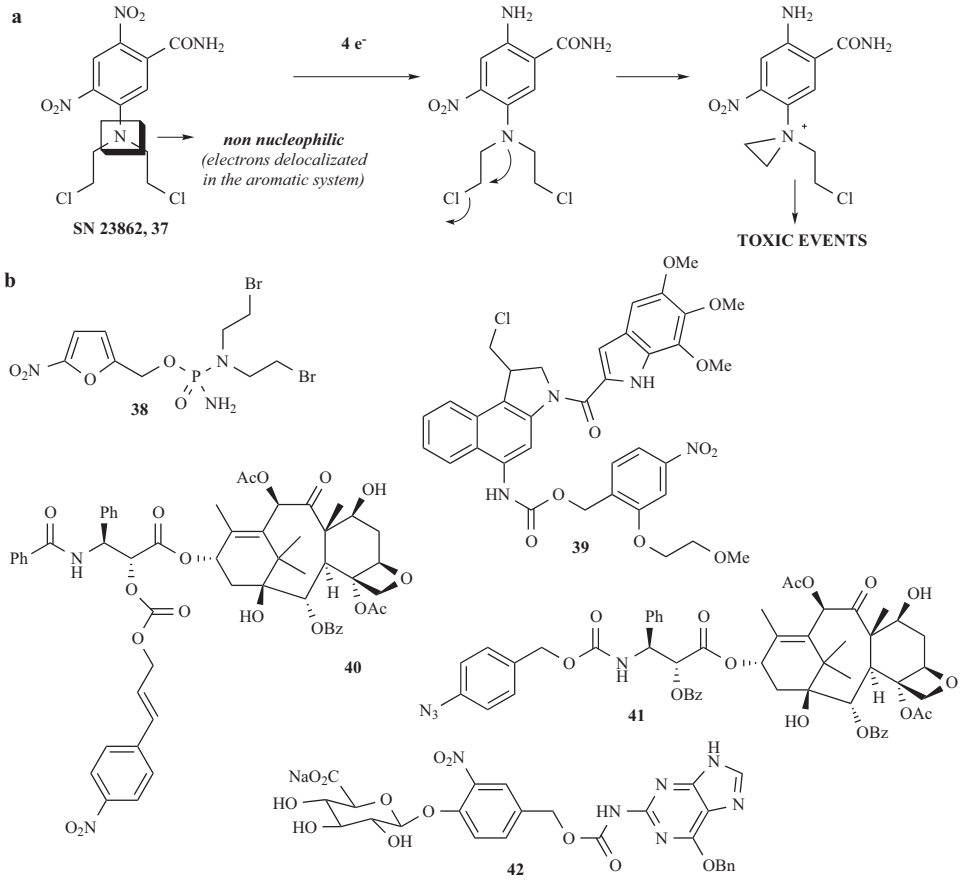


Fig. (15). Examples of nitro and azido derivatives with redox releasing capacity.

of these potential drugs where benzylic-like moiety acts as *linker* [14,80,81]. In these examples, alkylating agents derived from phosphoramides (**38**), minor groove alkylating agents derived from 5-aminobenz[*e*]indoline (**39**), and microtubules destabilizing agents derived from taxol (**40** and **41**), were used as *cytotoxins*. The kinetics of spontaneous fragmentation of carbamate *linker* moiety after bioreduction depend on the electronic characteristics of the benzyl ring. Thus, it was demonstrated that electron donating groups in the *ortho* position, as in compound **39**, accelerate the breakup of the *linker* [82,83]. Compound **41** is an example where the azido group acts as *redox-activated trigger*.

A new approach involves hybrid compounds (i.e. **42**, Fig. (**16**)) that combine a *redox-activated trigger* with a β-glucuronidase-cleavable moiety that, upon biotransformation, releases DNA-repair inactivators (i.e. O^6-benzylguanine) [84]. β–Glucuronidase is a lysosomal hydrolase overexpressed by tumor cells and released from necrotic tumor cells, transforming it into an excellent target with improved bioactivity [85].

Quinone Moiety as Redox-Activated Trigger

Fig. (**17**) shows approaches based on quinone moiety. An early example utilized an aziridinylindolequinone (EO9, **43**), whose reduction increased the basicity of the aziridinyl nitrogen and facilitated the nucleophilic attack on this heterocycle. The indolequinones are bioreductively activated by NAD(P)H:quinone oxidoreductase-1 (NQO1) and by DT-diaphorase (DTD) [86,87].

To improve the quinone's capacity to be substrate of DTD, a simple 1,4-benzoquinone has been described (compound **44** (Fig. (**17**)) [87]. It presents good *in vitro* and *in vivo* behavior and is currently in phase I clinical trial. A series of 1,4-naphthoquinone has also been developed in order to enhance EO9 poor pharmacokinetic properties, i.e. **45** [88]. Compound **45** (Fig. (**17**)) presents good substrate specificity for NQO1 and good pharmacokinetic profile. Also, the quinone pharmacophore has been attached to phosphoramide mustards through a benzylic-like *linker* (Fig. (**13**)) to act as redox cytotoxin-releasing moieties. In this sense, compound **46** is the best derivative reported by Hernick and Borch (Fig. (**17**)) [89].

Metal ion Moiety as Redox-Activated Trigger

Metal ions with an adequate reduction potential have been described as carriers and cytotoxin-releasing moieties. The main idea is that after the reduction of metals, biologically or by radiation, the electronic structures of the complexes change and the cytotoxins are released (Fig. (**18a**)). Denny and Wilson described the first example using

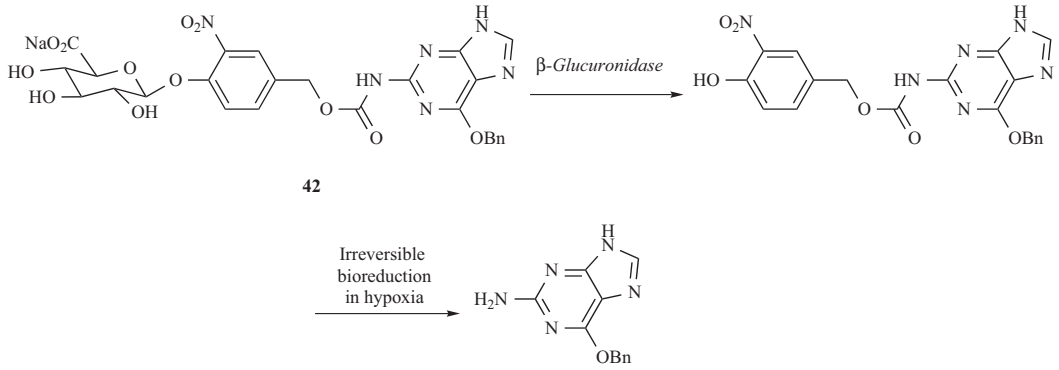


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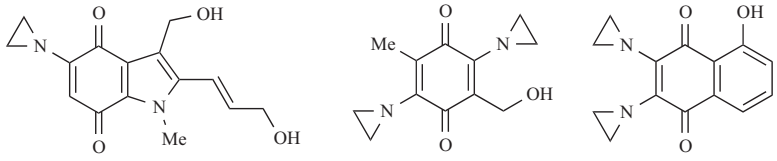
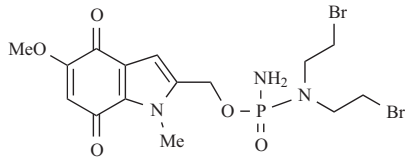
Fig. (16). Site specific hybrid compound with DNA-repair inactivation activity.



EO9, 43 **44** **45**



46

Fig. (17). Examples of quinone derivatives with redox releasing capacity.

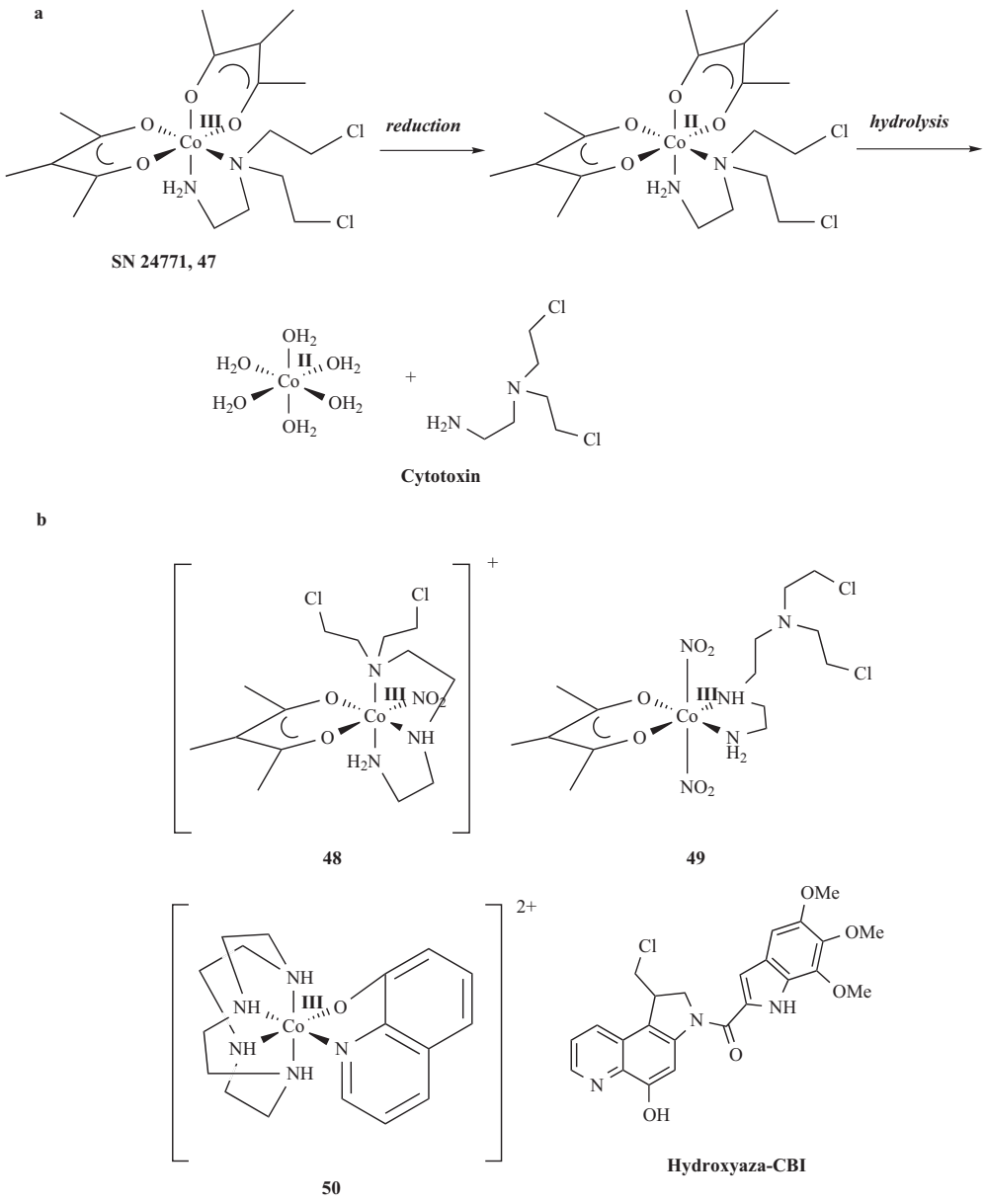


Fig. (18). Examples of Co(III) complexes developed as cytotoxin triggers.

Co(III) as the coordinating metal (SN 24771, **47**, Fig. (**18a**)) and the stability of the reduced Co(II)-complex was the tool to promote cytotoxin-triggering [90-93]. Apart from Co(III), other metal ions have been assayed: Cr(III), Cu(II), Pt(IV) and Ru(III). The most recent results of Denny and Wilson have focussed on the synthesis of new Co(III) and Cr(III) complexes. In one approach, they employed a triamine mustard as a metal-binding ligand and found that when the mustard was coordinated as a tridentate ligand, **48** (Fig. (**18b**)), the complexes were less toxic in oxia than the mustard-bidentate complexes, **49** (Fig. (**18b**)), and less toxic than the parent cytotoxin. Due to metal complexation the nitrogen has decreased nucleophilicity and is less reactive against DNA or biomolecules, conferring selectivity [94]. Compound **48** possesses good hypoxia-dependent behavior. On the other hand, they developed Co(III) and Cr(III) complexes with a model ligand, 8-hydroxyquinoline, derived from the minor groove alkylating agent hydroxyaza-CBI (Fig. (**18b**)). These compounds, i.e. **50** (Fig. (**18b**)), designed as radiation-activated prodrugs differ from SN 24771 in that their reduction provides stable Co(II) products [95]. The complexes resulted in 1000-fold less potent activity than the free ligand as inhibitors of cell proliferation, indicating that the metal ion masks this adverse effect.

Fig. (**19**) shows approaches based on Cu(II) complexes. Lin and Ho proposed that Tpz-Cu(II) complex, **51** Fig. (**19a**)), could act as an agent for tumor therapy with a dual mechanism of action if radioactive copper isotopes were used [96]. In the same sense, QdNO was coordinated to Cu(II) yielding complexes with a 2:1 ratio (QdNO:metal ion) (i.e. **52**, Fig. (**19a**)). The hypoxia-dependent cytotoxicity of these complexes was maintained relative to the free ligands, whereas they were only weakly cytotoxic under well oxygenated conditions [97]. Another important group of Cu(II) complexes have been studied by Blower and coworkers [98,99] and by Stratford and coworkers [100]. The former authors have studied the mechanism of hypoxia selectivity of related compound of *bis*(thiosemicarbazone)-Cu(II) complexes (i.e. Cu(II)ATSM, **53**, Fig. (**19b**)), well known agents for imaging hypoxic tissue. Also, they prepared nitrogen mustard complexes of Cu(II) analogous to Co(III) complexes developed by Denny and Wilson. Stratford and coworkers synthesized macrocyclic nitrogen mustard Cu(II) complexes resulting in compound **54** which was selective for hypoxia, possessing an adequate reversible redox potential near to -0.37 V with respect to NHE. Other macrocycles assayed as ligands produced Cu(II)-complexes with low aqueous stability and so low selectivity for hypoxic conditions.

The reported Pt(II)-drugs with high reactivity and poor biological stability have been converted to Pt(IV)-complexes as potential prodrugs. In this higher oxidation state this metal ion could be considered as a redox-activated trigger. Pt(IV)-complexes could be activated upon reduction by endogenous biomolecules (glutathione, ascorbate and protein sulfhydryls) to the cytotoxic Pt(II)-complexes (Fig. (**20**)) [101]. So, in a rich reductive media of hypoxic tumors, this redox property could confer selectivity. The same idea is proposed for Ru(III)-complexes which could produce the cytotoxic Ru(II)-complexes after bioreduction [102].

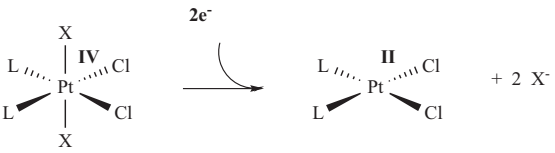


Fig. (20). Bioactivation of stable Pt(IV) to high reactive Pt(II) complexes.

Other Moieties as Redox-Activated Triggers

Kwon and coworkers have described original approaches looking forward to novel moieties as redox-activated triggers. One of the most recent descriptions is the use of 1,2-benzisoxazole system to release phosphorodiamides after bioreduction [102]. This aromatic system, present in the anti-epileptic Zonisamide, has been previously described as susceptible to reductive metabolism [103,104]. 1,2-Benziso-xazole was attached to the cytotoxin *via* a hydrocarbonated linker, yielding compounds that are NADPH-dependent metabolized in both hypoxic and oxic conditions (i.e. **55**, Fig. (**21a**)). The ketone metabolite, detected in this study, allows release of the cytotoxin *via* a base-catalyzed β–elimination (Fig. (**21a**)). Kwon and coworkers have also studied the capability of sulfoxide and sulfone moieties to act as redox-bioactivated triggers [105,106]. Fig. (**21b**) highlights oxic toxicity related to the sulfur oxidation state (see compounds **56-58**, Fig. (**21b**)), with sulfone the less active in oxic conditions.

2. CONCLUDING REMARKS

The information amassed to date from the development of new bioreductive drugs in hypoxia, with respect to selective cytotoxicity, biochemical pathways and mechanism of action has resulted in clinical development of some compounds as antineoplastic compounds. However, other strategies such as hypoxia activated gene therapy [107-109],

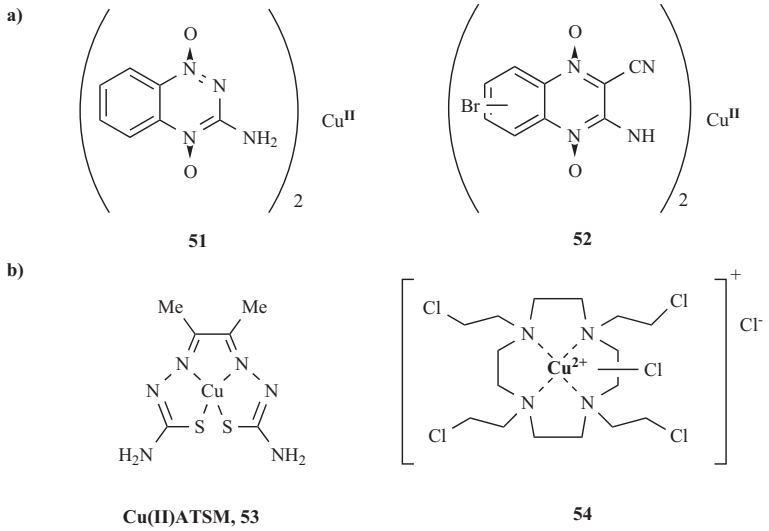


Fig. (19). Cu(II) complexes studies in tumor hypoxia field.

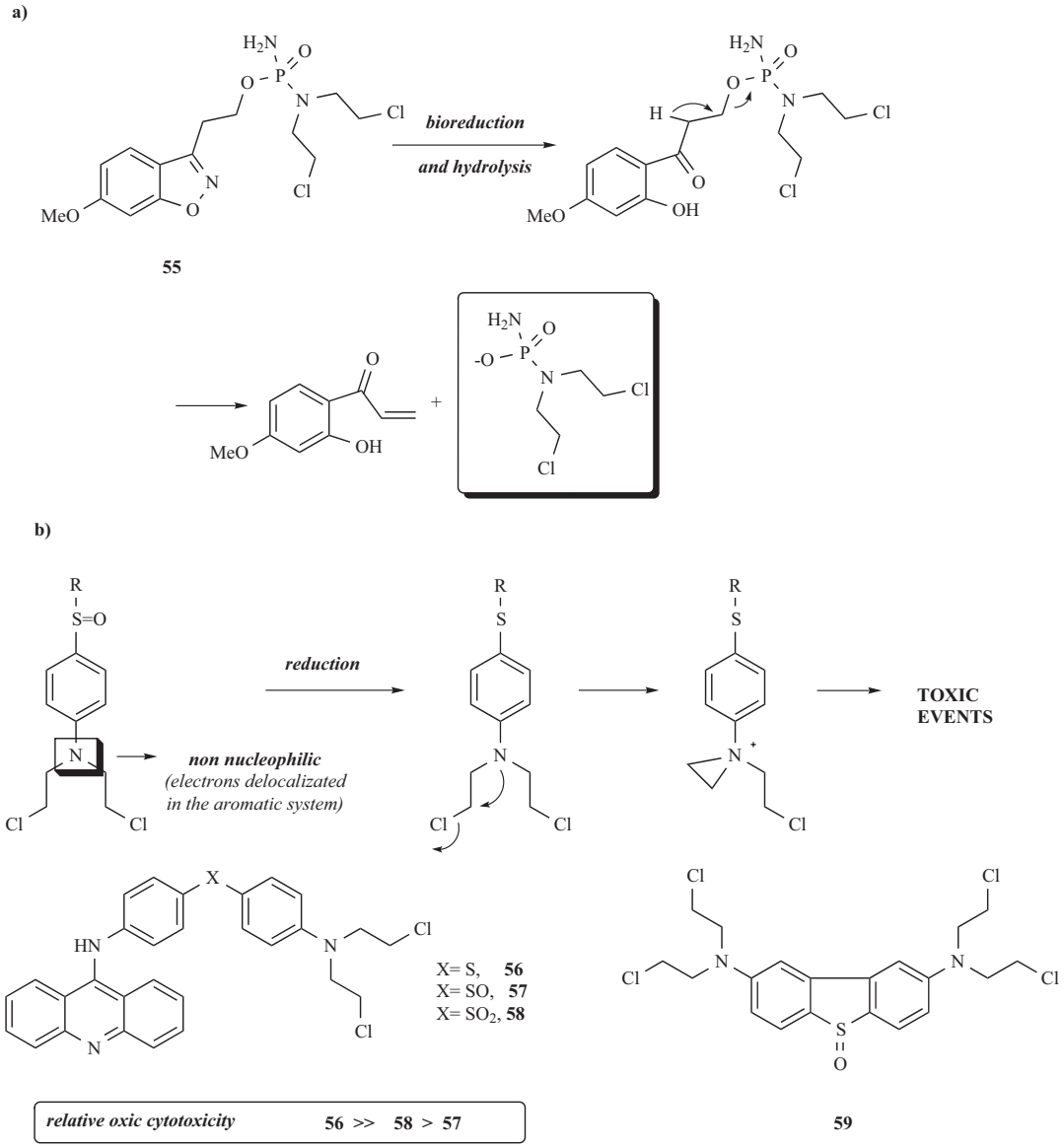


Fig. (21). Examples of novel moieties employed as redox-activated triggers.

targeting hypoxia-inducible factor 1 [110-112] or the use of recombinant anaerobic bacteria [113] should also be studied together with the production of new and more effective clinical candidates as antineoplastic drugs.

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