N-Oxides as Hypoxia Selective Cytotoxins

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Abstract: *N*-Oxide-containing compounds have been developed as prodrugs that are selectively bioactivated in the hypoxic cells in tumors. This selectivity is based on the net reduction of the *N*-oxide moiety in the absence of oxygen, in a one or two-electron process, by reductive enzymes. A wide range of *N*-oxides have been studied and some of them are currently in clinical use. This review covers the principal families of compounds under study and in clinical trials.

1. INTRODUCTION

1.1. Tumor Hypoxia

Because of their rapid growth, cancerous cells can become relatively isolated from the blood supply, and it becomes increasingly difficult for nutrients, especially oxygen, to diffuse to them, resulting in hypoxia. The hypoxic regions in tumors have been well characterized [1-9]. Cell greater than 120 – 150 micron distance from a blood vessel are starved of oxygen, die and ultimately constitute necrotic tumor regions. The tumor region between fully oxygenated and necrotic zone is composed of cells experiencing degrees of hypoxia varying from 20 mm Hg to radiobiological hypoxia (1 mm Hg) [10]. There is evidence that viable hypoxic cells may contribute up to 20 % of tumor mass [11]. Hypoxic cells are very resistant to radiation damage, and the same diffusional limitations for oxygen can apply to the reactive, 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 the normal growth cycle. 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 haem degradation. Flavoprotein containing dehydrogenases and oxidoreductases mediate the flow of electrons from reducing equivalents in the form of the nicotinamide dinucleotide coenzymes, NADH and NADPH, to dioxygen and other endogenous acceptor molecules. Various chemicals are also substrates for certain flavoprotein enzymes that ordinarily participate in intermediary metabolism [10].

On these basis is that bioreduction has been useful under hypoxic conditions, so that agents capable to undergo bioreduction (bioreductive drugs [12]) and produce further cytotoxic events have been developed as antitumoral drugs and are, currently, either in clinical use.

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1.2. Bioreductive Agents

Bioreductive antitumor agents are prodrugs. That is, they are inactive in their own right, but are able to undergo metabolism to species which damage biomolecules upon reduction process [13]. These agents are attractive antineoplastic drugs because their selectivity toward tumoral cells is the result of the degree of hypoxia and the level of reductive enzymes.

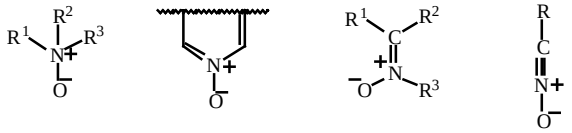
Several types of compounds that are toxic only under hypoxic conditions are known [14-16]. These agents belong to the classes of nitroderivatives [17-19], quinone derivatives [20], nitrogen mustard derivatives [21-23] and *N*-oxides derivatives [24]. In this article *N*-oxides derivatives as bioreductive compounds will be reviewed.

1.3. *N*-Oxides

1.3.1. Chemistry

The *N*-oxide functional group is the result of addition of an atomic oxygen to the lone pair electrons of the nitrogen atom. This moiety occurs in three important structures, (Fig. **1**): in *N*-oxide of tertiary amines (aliphatic and aromatic, **Ia** and **Ib** respectively), in *N*-oxides of imines or nitrones (**II**), and in *N*-oxide of nitriles (**III**) [25,26]. Only the first class of compounds have been described as bioreductive agents.

N-oxide of tertiary amines has been synthesized by different ways, such as by direct amine oxidation [25-28], cyclization process [29-33], addition to alkenes [34], thermolysis process [35], cyclocondensation [35-36].



Ia **Ib** **II** **III**

Fig. (1). Different functional groups containing *N*-oxide moiety. The resulting dative covalent nitrogen-oxygen bond is semi-polar and can be described formally as N^{+}-O^{-}.

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1.3.2. Biology of N-oxide as bioreductive pharmacophore

Two possible pathways describe the biological action, in hypoxic tumors, of the different *N*-oxide containing compounds. Several reports suggest that *N*-oxide reduction can be an enzymatic or non enzymatic mechanism, or both [10]. In cellular systems cytochrome P450 (NADH and/or NADPH dependent) and other haemoproteins have demonstrated been implicated in *N*-oxide reduction [37-44].

In one case (Fig. **2a**), when the *N*-oxide (**IV**) is bioreduced by an one-electron process, the cytotoxic metabolite (nitroxide radical, **V**) is generated [45]. In presence of oxygen a redox cycling process occurs, producing, probably, aerobic cytotoxicity [46,47]. The intermediate **V** is suggested to initiate radical-mediated oxidative DNA strand cleavage, unlike quinone and nitrocompounds, without covalent binding to DNA and protein [48,49].

In the second case (Fig. **2b**), *N*-oxide masks the cationic charge of tertiary amines that are protonated under physiological conditions. When the complete metabolic reduction of amine *N*-oxides (**VI**) is involved, donation of two electrons to the *N*-oxide, their respective amines (**VII**, a biomolecule-affinity agent) are produced. This process can be inhibited by oxygen. The hypoxic selectivity is considered to result from an increase in biomolecule (DNA, DNA and RNA polymerases and/or topoisomerases) binding affinity [10, 50-53]. DNA-interaction studies of anthraquinones derivatives, using molecular modeling, show that the electrostatic interaction between *N*-oxide and DNA-phosphates will be repulsive, whereas the δ(+) nature of amine in the protonated form allows for an attractive electrostatic interaction with phosphates [54].

2. *N*-OXIDES USED AS BIOREDUCTIVE COMPOUNDS

Several *N*-oxide containing compounds as intrinsically cytotoxic agents have been described [38,39,44,55-64]. But, nitromin (**39**, Fig. **11a**) was probably the first example of an *N*-oxide that could be selectively reduced in hypoxic conditions [10]. However, historically the benzotriazine N^1,N^4-dioxide was the first representative group of bioreductive compounds extensively studied and currently, fifteen years latter, the first drug to enter clinical trials purely as a bioreductive agent with toxicity to hypoxic cells belongs to this family of compounds [65].

2.1. Benzo[1,2-*e*]1,2,4-Triazine N^1,N^4-Dioxide

The benzo[1,2-*e*]1,2,4-triazine N^1,N^4-dioxide derivatives were described, for the first time in 1986, by Zeman *et al*. as bioreductive agents [66]. The compounds, developed and evaluated initially as radiosensitizers, could be prepared as shown in (Fig. **3a**) [67,68]. Recently, a convenient method to obtain 3-amino-6(7 or 8)-electron-withdrawing-substituentbenzo[1,2-*e*] 1,2,4-triazine N^1,N^4-dioxide was described [69] (Fig. **3b**).

The prototype compound of this family is 3-aminobenzo[1,2-*e*]1,2,4-triazine N^1,N^4-dioxide (**VIII**, R= H,

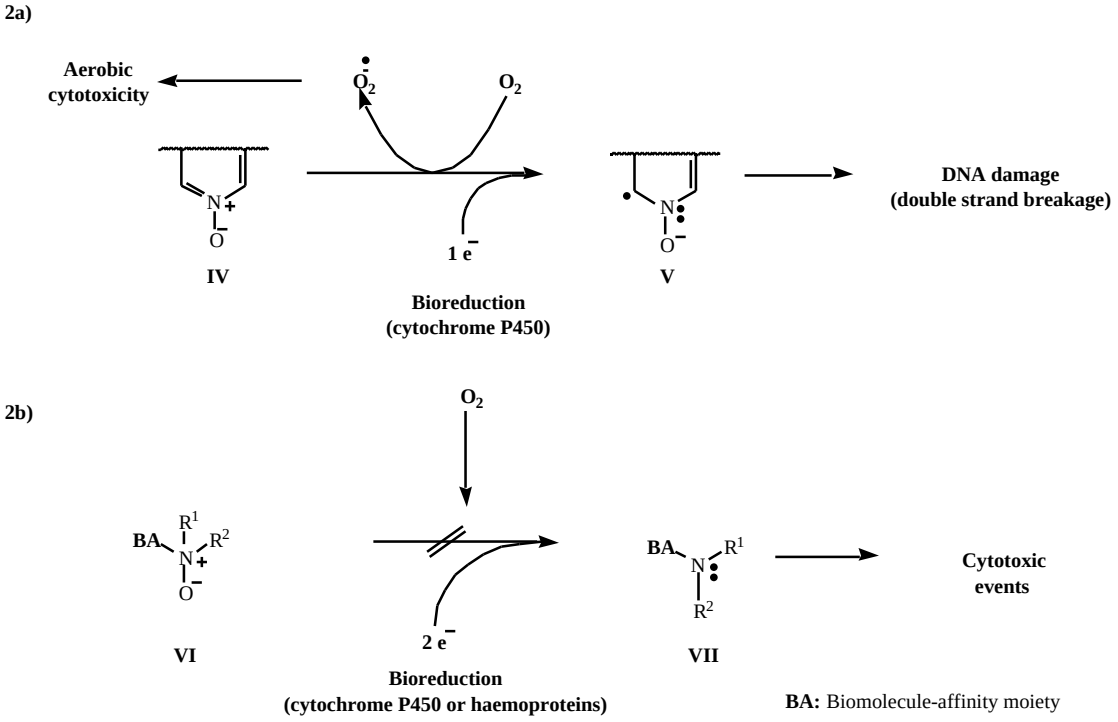


Fig. (2). Schematic mechanism of activation and action of *N*-oxide bioreductive compounds. **2a)** Activation by one-electron process. **2b)** Complete *N*-oxide reduction.

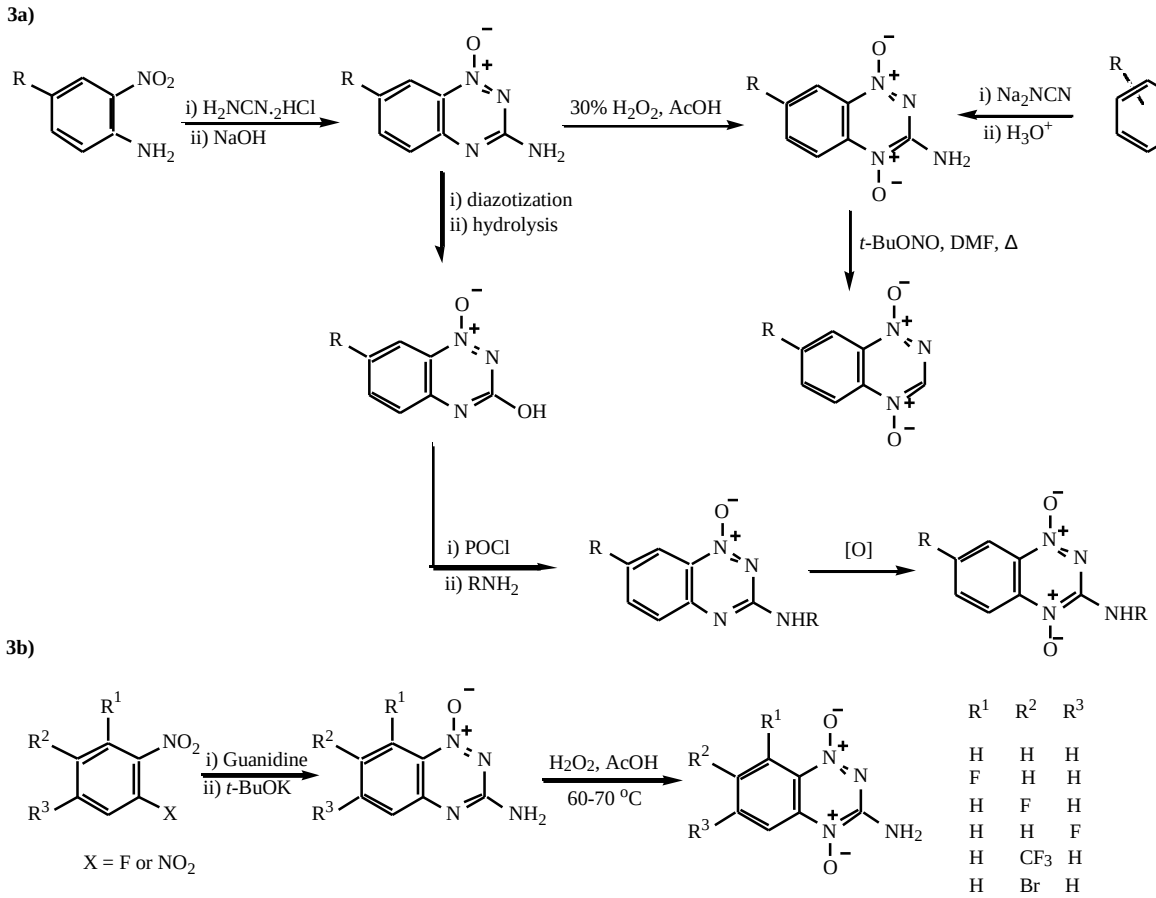


Fig. (3). 3a) Synthesis of benzo[1,2-*e*]1,2,4-triazine N^1,N^4-dioxide derivatives as described by Mason and Tennant and by Seng and Ley. **3b)** Synthesis of benzo[1,2-*e*]1,2,4-triazine N^1,N^4-dioxide derivatives as described by Suzuki and Kawakami.

(Fig. **4**), known as **SR 4233**, NSC 130181, WIN 59075, tirapazamine and Tirazone™). This drug possess differential hypoxic cytotoxicity for a great number of cell lines of hamster, mouse and human beings, with a hypoxic cytotoxicity ratio (HCR, relationship between concentration of drug in air and concentration of drug in hypoxia that produce the same level of cell killing) of approximately 25 to 200 for the different cellular lines [70-72]. Several groups have investigated the mechanism for the selective hypoxic toxicity of **SR 4233**, the first step (one-electron process) is the same that was shown in (Fig. **2a**). The product of two-electron reduction, SR 4317 (**IX**, R= H, Fig. **4**), is toxic neither to hypoxic, nor aerobic, cells [24]. The cellular damage described is the result of an H abstraction from biomolecules (principally DNA, Fig. **4**) by the nitroxide radical [73]. Also it has been proposed that the hydroxyl radical is involved in this DNA cleavage [74].

A large number of analogues of **SR 4233** were developed in order to improve the biological activity and/or decrease the systemic toxicity of this lead compound [75-77]. The principal modifications and the corresponding HCR values for Chinese hamster ovary (CHO) cells are shown in (Table **1**). Change the 3-amine moiety by 3-methoxy or 3-acetylamino substituents produces a lack of activity, but proton or 3-alkylamino substituents improve HCR. Although, derivatives **8**, **9**, and **11** (Table **1**) have comparable or higher HCR *in vitro* and similar animal toxicity, they

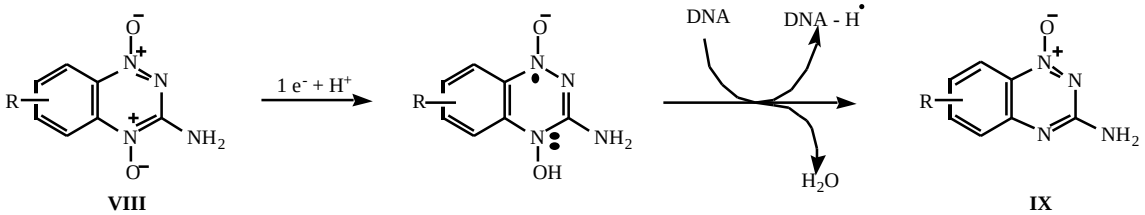
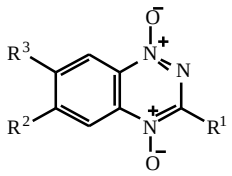


Fig. (4). Schematic mechanism of action of benzotriazine N^1,N^4-dioxide derivatives.

Table 1. More Characteristics Benzo[1,2-*e*]1,2,4-Triazine N^1,N^4-Dioxide Derivatives and Physicochemical and Biological Properties



Compound	R^1	R^2	R^3	$E_{1/2}$ [a] (V) at pH 7.4[b]	HCR (CHO cells)	Reference
1	OMe	H	H	- 0.206	1	[75]
2	NHCOMe	H	H	- 0.222	1	[75]
3	NH_2	CH_3	CH_3	- 0.391	12.5	[75]
4	NH_2	H	$OCH_2CH=CH_2$	- 0.338	17.5	[75]
5	$NH(CH_2)_2OH$	H	Cl	- 0.275	17.5	[76]
6	NH_2	Cl	H	- 0.285	35	[75]
7, SR 4233	NH_2	H	H	- 0.332	65.4	[75]
8	$NH(CH_2)_2NEt_2$	H	H	- 0.350	70.0	[76]
9	H	H	H	- 0.190	70.9	[77]
10	NH_2	H	NO_2	- 0.133	84	[76]
11	$NH(CH_2)_3NEt_2$	H	H	- 0.348	104.5	[77]
12	$NH(CH_2)_2NEt_2$.HCl	H	NO_2	- 0.140	211	[76]

Note: [a] $E_{1/2}$: Half-wave reduction potential. [b] Polagraphic studies.

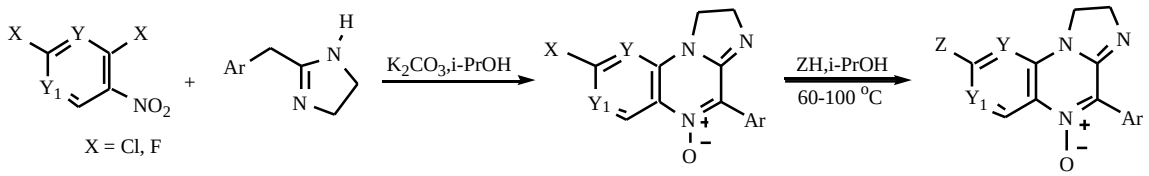
exhibit shorter elimination half-lives compared to **SR 4233** (7, 2.1, 12, and 15 min, respectively) [77]. On the other hand, the activity is enhanced by the presence of electron-withdrawing substituents in the 7-position. Also, 1,2,4-triazine N^1,N^4-dioxide derivatives were evaluated as bioreductive compounds [78], lack of the benzo system produces a total loss of cytotoxic activity (in oxia and hypoxia), probably due to the different electrochemical properties of these compounds.

Trying to gain insight into the mechanism of action, several physicochemical studies of **SR 4233** and analogues were performed [75-77,79-81]. Cyclic voltammetric studies, in organic solvents, show that the first reduction step for **SR 4233** is a reversible process, assigned to a monoelectron transference. The anion radical product is stable, although this intermediate shows a tendency to participate in a following chemical reaction, subsequent reduction steps are highly irreversible in nature. At pH 7.4 the half-wave reduction potentials ($E_{1/2}$) were determined by polarography (see Table **1**). Using pulse radiolysis the **SR 4233** one-electron reduction in aqueous solution was studied and reduction potential has been estimated as - 0.45 V *vs* NHE at pH 7 (see Table **2**). The pK_a of the one-electron reduced product was estimated from the pH dependence of the reduction potential (see Table **2**). The **SR 4233** free radical generated by microsomal reduction was characterized by electron spin resonance, resulting in the unpaired electron centered on N^1.

SR 4233 is currently in phase II and III trial, alone and combined with radiation [82-84], or combined with other chemotherapeutic drugs (cisplatin [85-87], cyclophosphamide [88], doxorubicin, etoposide, 5-fluorouracil, taxol, navelbine [89]), or with mild hyperthermia [90], or in cancer gene therapy [91], or with pharmaceutical delivery polymers [92].

2.2. Imidazopyridopyrazine *N*-Oxide and Imidazoquinoxaline *N*-Oxide

In 1993 a new class of cytotoxic compounds bioactivated in hypoxia was reported. The compounds are 1,2-dihydro-4-phenylimidazo[1,2-*a*]quinoxaline N^5-oxide derivatives, varying the 6- and 8-substituents (secondary and tertiary amines). Also, the 7- and 9-aza analogues have been developed [93]. The synthetic procedures and some of the derivatives and their corresponding activity are shown in (Fig. **5**). One deoxy derivative developed (RB92815), obtained by reduction with titanium trichloride, was 10-fold less active in hypoxia than the corresponding *N*-oxide analogue (**18**, Fig. **5**). Inclusion of a second bioreductive function, nitroimidazole moiety, in 8-position (**19**, Fig. **5**) showed a poor improvement of the activity. 1,2-Dihydro-8-(4-methylpiperazinyl)-4-phenylimidazo [1,2-*a*] pyrido [3,2-*e*]pyrazine N^5-oxide (**18**, known as **RB90740**) and 1,2-dihydro-8-[2-(dimethylamino)ethoxy]-4-phenylimidazo[1,2-*a*]pyrido[3,2-*e*]pyrazine (**21**, Fig. **5**), known as **RB93918**



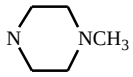
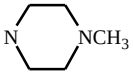
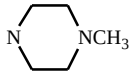
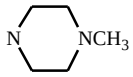
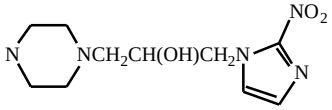
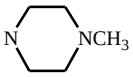
Compound	Y	Y_1	Ar	Z	HCR (V79-379A cells)	Reference
13	N	CH	*p*-OMe-Ph		1.4	[93]
14	CH	N	Ph	H	2.1	[93]
15	N	CH	Ph	H	8.6	[93]
16	CH	CH	Ph		10.2	[93]
17	N	CH	naphthyl		10.6	[93]
18, RB90740	N	CH	Ph		15.3	[93]
19	N	CH	Ph		16.7	[93]
20	N	CH	*p*-F-Ph		21.0	[93]
21, RB93918	N	CH	Ph	$O(CH_2)_2N(CH_3)_2$	65.0	[94]

Fig. (5). Synthetic procedures for the preparation of imidazopyridopyrazine *N*-oxide and imidazoquinoxaline *N*-oxide derivatives. Some structural modifications and displayed activity *in vitro* against V79-379A cells.

[94]) have turned out to be the best analogues of these family of compounds, having a good HCR *in vitro* (V79-379A cells) but *in vivo*, using RIF-1 tumor model, **SR4233** displays better activity [94].

In vitro **RB90740** causes DNA strand breaks only under extreme hypoxia, 0.02 % of oxygen. The lack of *in vivo* activity of this compound could be due to requirement for extreme hypoxia [95]. The biological system P450 reductase, cytochrome b5 reductase, and cytochrome b5 are implicate in the bioactivation of **RB90740**, but the relationship between the intracellular levels of P450 reductase and cytochrome b5 reductase and the hypoxic toxicity of the compounds is not clear [96].

Pharmacokinetic studies of **RB90740** in C3H mice indicate that the two-electron reduced derivative (deoxy analogue, RB92815) is present in some tumors, but it is not possible to confirm that the formation of this metabolite is the result of a two-electron detoxifying process or of a mono-electronation process (toxic free radical species formation) [97]. In air, this metabolic product (RB92815) was significantly more carcinogenic than the parent compound **RB90740** [98]. However, the same study indicates that this potential oncogenicity is no worse than that of γ-rays.

To study the mechanism of action of these analogues and compare it with the corresponding to **SR4233** several electrochemical studies were performed [99-102]. In this way reduction potential, rate constant for the drug radical reacting with oxygen, and radical pK_a (influence of pH or H-donor in the reduction) were determined (Table **2**). Like the reduction potential, the compounds result less electron-affinic than **SR4233**. This is reflected in the high reactivity of the radicals of these analogues with oxygen, which could prevent radioresistant hypoxic cells from being killed by the cytotoxin, except in extreme hypoxia, thus accounting for the poor *in vivo* activity of drugs of this type despite potent tumor cell killing under anoxia *in vitro.*

Table 2. Physicochemical Properties for Some Imidazopyridopyrazine *N*-Oxide Derivatives and SR 4233

Compound	Reduction potential (V) vs NHE[a] at pH 7	Rate constant reacting with oxygen at pH 7.4 (dm^3 mol^{-1} s^{-1})	Radical p*Ka*	Reference
15	- 0.70 ± 0.01[b]	16 x 10^7	7.4 ± 0.1	[101]
RB90740	- 0.79 ± 0.03[c]	32 x 10^7	6.2 ± 0.1	[101]
19	-	3.1 x 10^7, 2.8 x 10^6	4.3, 5.6 (nitro), 7.6 (*N*-oxide)	[102]
RB93918	- 0.79 ± 0.01[b] (- 0.81 ± 0.03[c])	18 x 10^7	5.5 ± 0.1	[101]
SR4233	- 0.45 ± 0.01[b,c]	0.8 x 10^7	5.6 ± 0.2	[80,100]

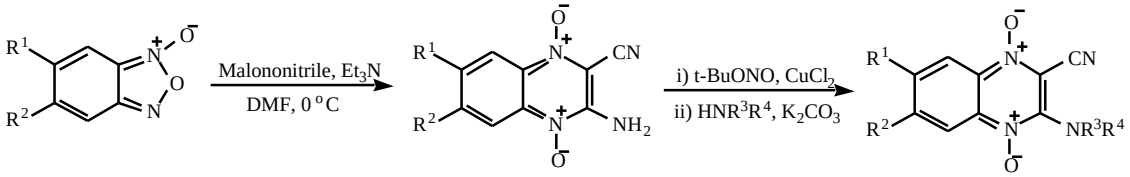
Note: [a] NHE: Normal hydrogen electrode. [b] Using benzyl viologen as redox indicator. [c] Using 1-methylnicotinamide as redox indicator.

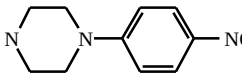
2.3. Quinoxaline *N,N'*-Dioxide

In 1994 the capacity of the quinoxaline *N,N'*-dioxide derivatives to act as bioreductive drugs in hypoxia was described [103]. On other hand, the deoxy derivatives have resulted inactive neither in oxia nor hypoxia. In order to study the relationship between activity and structure a great number of derivatives were prepared, following the Beirut reaction (Fig. **6**), varying 2,3,6,7, and/or 8 subtituents [104-107]. The best *in vitro* biological results were obtained with the 3-amino-2-carbonitrile derivatives. In order to improve the delivery properties 3-alkylamino derivatives were developed, resulting in compound **29** (Fig. **6**) the best *in vitro* derivative but with an *in vivo* half-live too short to be used in therapy [108-110].

Electrochemical properties (voltammetry studies) of the quinoxaline *N,N'*-dioxide derivatives showed that as the electron-withdrawing nature of the 6-(7)-substituent increases, the reduction potential becomes more positive and the compounds are more readily reduced. This is in accordance with the developed hypoxia cytotoxicity [105] (Fig. **6**).

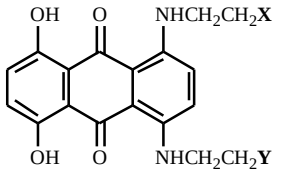
Recent report have demonstrated that the cyano-substituted quinoxaline *N,N'*-dioxide may exhibit DNA-



Compound	R^1	R^2	NR^3R^4	HCR (V79 cells)	E_{pc}[a], V vs SCE[b,c]	Reference
22	H	*p*-NO_2-Ph	NH_2	15	nr[d]	[108]
23	Cl	OCH_3		> 50	nr	[107]
24	CF_3	H	NH_2	75	- 0.65	[105]
25	Cl	Cl	NH_2	80	- 0.62	[105]
26	H	H	NH_2	80	- 0.88	[105]
27	Cl	H	$NH(CH_2)_2NMe_2$.HCl	100	nr	[108]
28	Cl	H	NH_2	150	- 0.74	[105]
29	Cl	H	$NH(CH_2)_3NMe_2$.HCl	250	nr	[108]
30	H	H	$NH(CH_2)_3NMe_2$.HCl	300	nr	[108]
31	CF_3	H	$NH(CH_2)_3NMe_2$.HCl.H_2O	340	nr	[108]
SR 4233	-	-	-	75	- 0.90	[105]

Notes- [a] E_{pc}: Reduction potential. [b] SCE: Saturated calomel electrode. [c] Using cyclic voltammetry. [d] nr: no reported.

Fig. (6). Schematic synthesis of active quinoxaline *N,N'*-dioxide derivatives. Selected analogues and their biological and electrochemical properties.



Compound	X	Y
32, Mitoxantrone	$NHCH_2CH_2OH$	$NHCH_2CH_2OH$
33	$N^+(O^-)(CH_3)_2$	$N^+(O^-)(CH_3)_2$
34	$N^+(O^-)(CH_3)_2$	$NHCH_2CH_2OH$

Fig. (7). General and particular structure of alkylaminoanthraquinone *N*-oxide and parent compound (**32**) used as hypoxic selective cytotoxins.

phototoxicity [111], so this could be related to the aerobic cytotoxicity of these kinds of compounds.

2.4. Aminoalkylaminoanthraquinone *N*-Oxide

Aminoalkylaminoanthraquinone *N*-oxide are prodrugs designed to harness tumor-cell selectivity for the activation process. The aminoalkylaminoanthraquinone *N*-oxide are essentially inactive DNA-binding agents in oxygenated conditions, but are capable of neoplastic cell killing selectivity through bioreductive conversion, via two electrons, in hypoxic tumors, as shown in (Fig. **2b**).

The parent compound of these kinds of drugs was mitoxantrone (**32**, Fig. **7**), a DNA binding-topoisomerase II poison [112]. Mitoxantrone cannot be derivatised to an *N*-oxide, being a secondary amine, thus other structural approaches were performed [113,114]. (Fig. **7**) shows the two most important derivatives developed (**33**, known as **AQ4N** or **AQ4NO**, and **34**, known as **AQ6N**).

The principal member of these kinds of compounds, 1,4-bis[2-(dimethylamino)ethylamino]-5,8-dihydroxyanthracene-9,10-dione bis-*N*-oxide (**AQ4N**, Fig. **7**), has been prepared from the anthraquinones **X** or **XI** (Fig. **8a**). Throughout intermediate **XII**, which reacts with excess of the corresponding amine and oxygen to produce **XIII** that is then oxidized to **AQ4N** [115,116]. Because this method appeared to have limitations (low intermediate purity, low overall yield and extensive purification procedures) alternative synthetic routes have been reported, included a viable route for large-scale high-purity preparation of **AQ4N** [117,118]. This provides **AQ4N** in an overall yield of 20 % from a cheap starting material and requires only one chromatographic purification (Fig. **8b**).

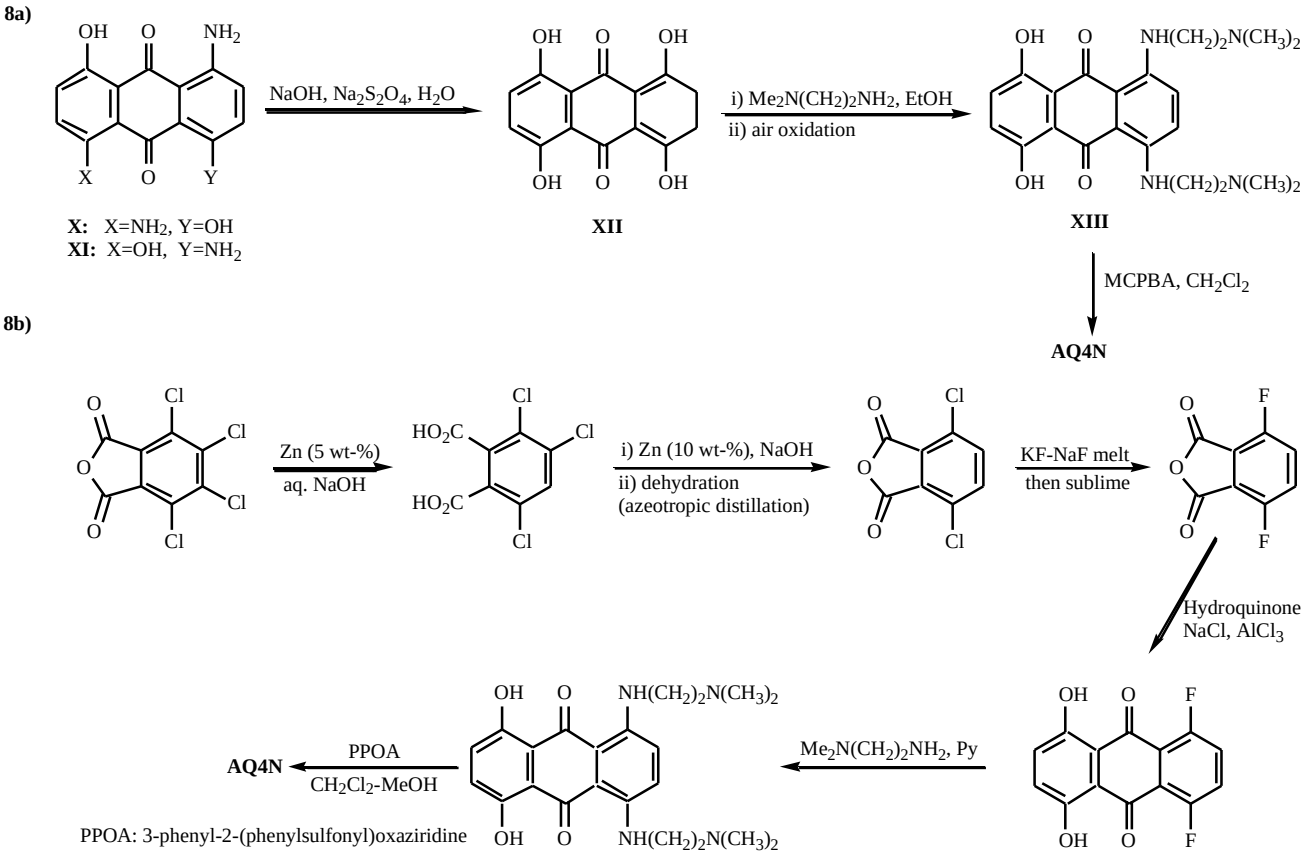


Fig. (8). 8a) Traditional synthesis of **AQ4N**. **8b)** Large-scale preparation of **AQ4N**.

Several studies confirm the mechanism of action proposed for these compounds. *In vitro* studies using human cytochrome P450 (CYP) 3A enzymes, enzymes highly express in a broad spectrum of human cancers, show that **AQ4N** is bioactivate for these systems in anaerobic conditions [40]. An important interspecies difference between the metabolism of **AQ4N** was described, the enzymatic system responsible of the bioreduction in rat is CYP 2B and 2E [42]. In aerobic conditions studies, using a cell line that overproduces DNA topoisomerase IIα, it was observed that the cytotoxicity produced by AQ4, AQ6, **AQ6N** and mitoxantrone in this system was enhanced whereas **AQ4N** was essentially inactive [52], and DNA-binding ranked in the order of mitoxantrone>AQ6>AQ4> **AQ6N**>>**AQ4N** [51]. The DNA-affinity ranking and the retention of topoisomerase-targeting capacity by **AQ6N** shows that its side chain without *N*-oxide is the responsible of oxic cytotoxicity. Other study indicates that **AQ4N** and AQ4 protect DNA against radiation [119].

In vivo hypoxic activity of **AQ4N** was demonstrated when its biological properties were enhanced in a co-administered schedule with 5,6-dimethyl xanthenone-4-acetic acid, a tumor blood flow inhibitor [50]. When combined with radiation or chemotherapy (cyclophosphamide, **SR4233**, cisplatin and thiotepa), **AQ4N** substantially increased the effectiveness of these modalities in a range of *in vivo* model systems [50, 87, 119-122]. **AQ4N** does not cause retinal damage, a side effect described for other bioreductive compounds (CI-1010 and **SR4233**) [123].

2.5. Aminoalkylaminoacridine *N*-Oxide

In the same manner of aminoalkylaminoanthraquinone *N*-oxide, these compounds are essentially inactive masked DNA-binding agents in oxygenated conditions that are bioactivated in hypoxic conditions.

The most representative drug of this family is 9-[3-(dimethylamino)propylamino]-1-nitroacridine N^3-oxide (**35**, **(**Fig. **9a**); known as nitracrine *N*-oxide or **NC-NO**) [124]. This compound was the first reported example of such a "bis-bioreductive" agent, with two independent oxygen-sensitive redox centers (nitro and *N*-oxide moieties) (Fig. **9a**). Reduction of the nitro group appears to generate reactive intermediates responsible for DNA alkylation [125,126], while metabolism of the *N*-oxide increases intercalative DNA binding. **NC-NO** has no topoisomerase II poisoning activity [50,127].

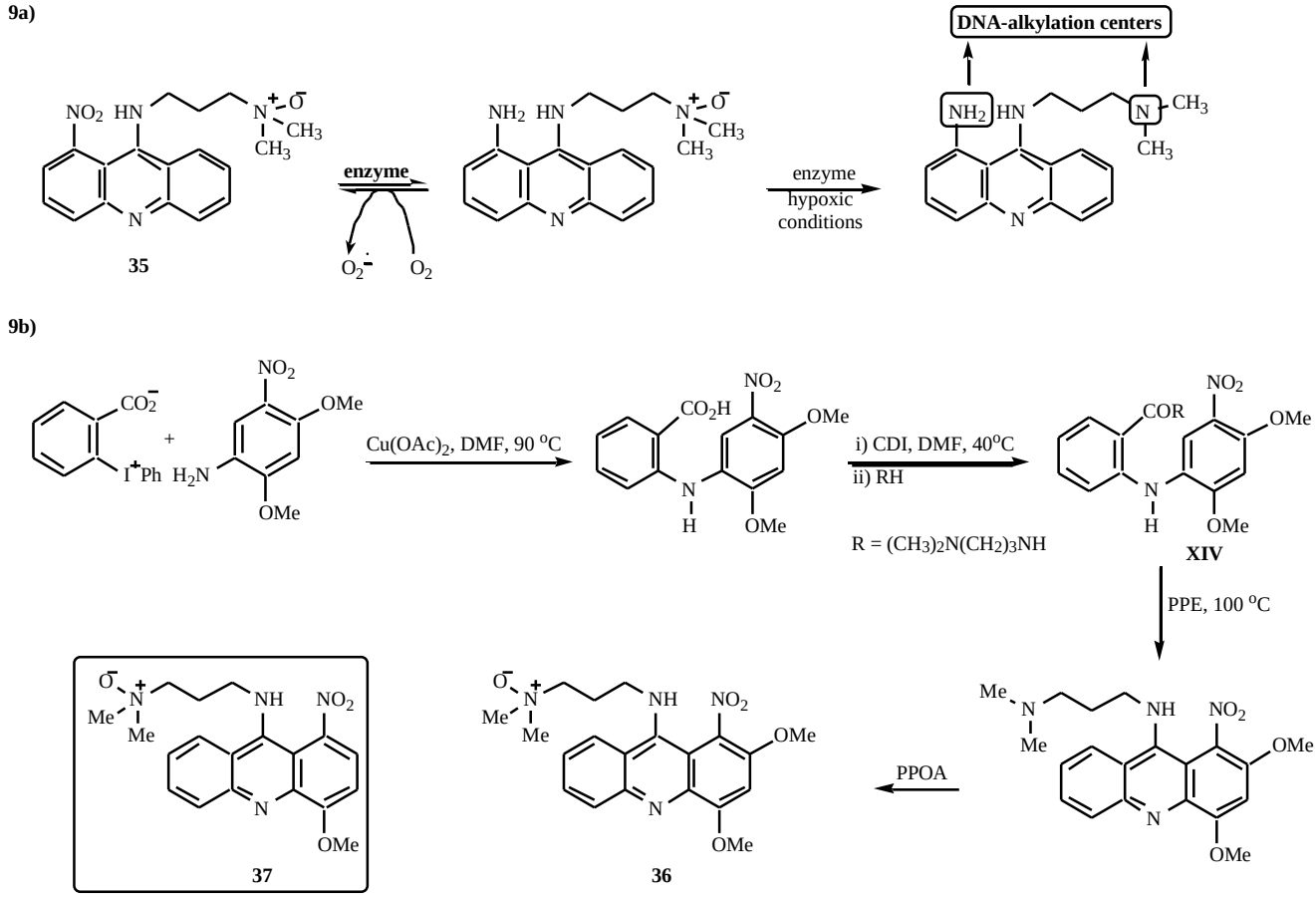


Fig. (9). 9a) NC-NO (**35**) and reductive biological pathways. **9b)** Synthesis of methoxy analogues of **NC-NO**. Structures of derivatives **36** and **37**.

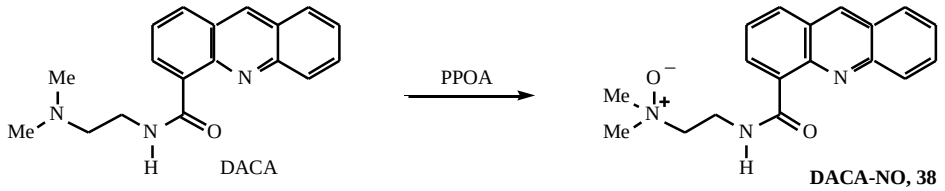


Fig. (10). DACA-NO, preparation from DACA.

NC-NO is prepared using the classical synthesis of 9-(aminoalkyl)acridines with a posterior selective *N*-oxidation of the tertiary aliphatic nitrogen using 3-phenyl-2-(phenylsulfonyl)oxaziridine (PPOA) [27,128]. *In vitro* studies, using intact spheroids, indicate that **NC-NO** has high rates of metabolism, however *in vivo* studies show that **NC-NO** is recovers unchanged in bile and urine [129]. The high drug metabolism is due, probably, to **NC-NO** posses a nitro group with a high reduction potential (– 0.283 V by pulse radiolysis at pH 7) that allow rapid biotransformation to the corresponding amine. In this way, a great number of 2-, 4-, 2,4-, and 4,5- electron-donating substituted acridines were prepared and physicochemical and biological characterized [17]. Complex mixture of reactions in the ring closure-acridine formation procedure when the dimethoxy substituents are present, take to used the alternative route, *via* cyclodehydration of intermediate **XIV** with polyphosphate ester (PPE), as shown in (Fig. **9b**) for derivative **36**. This study show that the inclusion of electron-donating groups produce a significant metabolic stabilization in hypoxic cell cultures, the 4-OMe derivative (**37**, Fig. **9b**) is more selective (> 1000-fold) than **NC-NO** but less potent. This derivative is better diffusible in a multicellular environment than **NC-NO**. Another attempt to obtain compounds less metabolized was reported, changing the environment of *N*-oxide moieties [130]. The strategies were to obtain more weakly basic and different steric bulk around *N*-oxide derivatives. The results show that either such modifications do not exert significant effects in the *in vivo* activity.

A recent study described the biological characterization of **NC-NO** and related compounds, such as nitracrine aromatic *N*-oxide (NC-aO), non-nitro analogue (DAPA) and DAPA aliphatic and aromatic *N*-oxides derivatives (DAPA-tO and DAPA-aO respectively) [53]. HCR ranked in the order of **NC-NO**>>NC>NC-aO>DAPA, DAPA-tO, DAPA-aO for CHO AA8 cells, that clearly show the importance of the aliphatic *N*-oxide and the presence of the NO_2-moiety for an adequate bio-activity.

Another attempt to obtain adequate bioreductive drug from aminoalkylacridine *N*-oxide was described using *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA), a well known DNA intercalator currently in phase I clinical trial [131,132]. The corresponding *N*-oxide (**38**, Fig. **10**), known as **DACA-NO**) is prepared by direct oxidation of DACA using PPOA as oxidative agent. The hypoxia-selective cytotoxicity of **DACA-NO** is adequate to consider it as a good bioreductive compound, although the **AQ4N** *in vivo* activity is superior [50].

2.6. Other *N*-Oxides

Classical antineoplastic compounds were transformed into the corresponding *N*-oxide when this was possible [133]. The more characteristic group of classical antitumoral drugs converted and studied as *N*-oxide is formed by the nitrogen mustards, for example mechlorethamine and chlorambucil [134,135]. The *N*-oxide derivative of the first one is known as nitromin (**39**, Fig. **11a**) and has been reported to present a hypoxia selectivity due to bioreductive release to the mustard of 4-fold [136]. Chlorambucil *N*-oxide (**40**, Fig. **11a**) shows in AA8 cells little hypoxic selectivity, of only 1.7-fold [135].

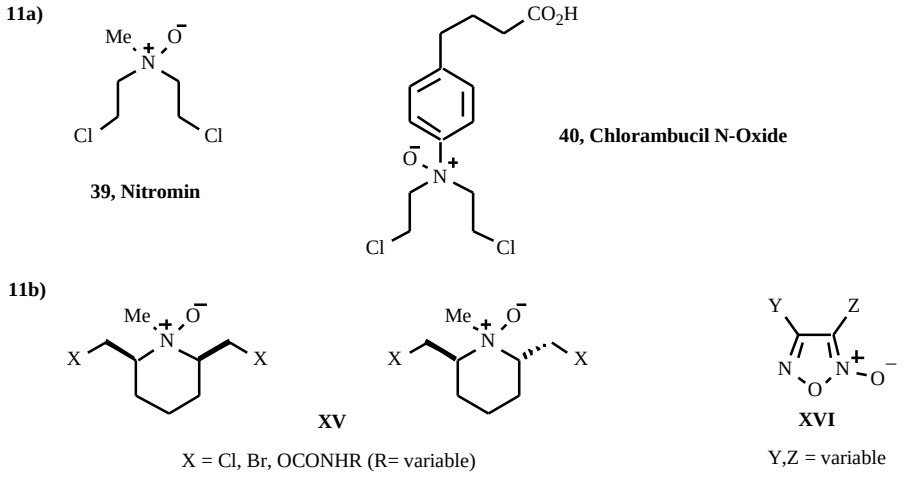


Fig. (11). Nitrogen mustard *N*-oxide derivatives (**39**, **40**, and general structure **XV**) and general structure of 1,2,5-oxadiazole *N*-oxide (**XVI**).

Also, 2,6-disubstituted-*N*-methylpiperidine *N*-oxide derivatives such as conformationally restricted nitrogen mustards were prepared and tested on human colon carcinoma cell lines under oxic and hypoxic conditions (**XV**, Fig. **11b**) [137]. These *N*-oxide prodrugs did not display cytotoxicity under any conditions.

On the other hand, 1,2,5-oxadiazole *N*-oxide derivatives as misonidazole-related compounds were synthesized and their cytotoxicity tested in both conditions (**XVI**, Fig. **11b**). Some compounds were potent cytotoxins but not selective to hypoxia conditions [138-140].

3. CONCLUDING REMARKS

The information amassed from the development of *N*-oxides as bioreductive drugs in hypoxia, with respect to selective cytotoxicity, biochemical pathways and mechanism of action have resulted in their clinical use as antineoplastic compounds. However, new *N*-oxide containing compounds, derived from well known parent drug or structurally news, could be obtained in order to produce a more efficient clinical-antineoplastic drug.

ACKNOWLEDGEMENT

The authors wish to thank the Comisión Honoraria de Lucha contra el Cáncer (Uruguay) for financial support, and Dra. Ana Martínez-Gil for the critical discussion of this review.

ABBREVIATIONS

HCR	=	Relationship between concentration of drug in air and concentration of drug in hypoxia that produce the same level of cell killing
CHO	=	Chinese Hamster Ovary
$E_{1/2}$	=	Half-wave reduction potential
PPOA	=	3-Phenyl-2-(phenylsulfonyl)oxaziridine
PPE	=	Polyphosphate ester
NHE	=	Normal hydrogen electrode
E_{pc}	=	Reduction potential
SCE	=	Saturated calomel electrode

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