Nitrogen-Containing Heterocyclic Quinones: A Class of Potential Selective Antitumor Agents

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Abstract: The development of prodrugs that are enzymatically activated into anticancer agents is a promising perspective in cancer therapy. Many nitrogen-containing quinoid heterocycles have been reported to show antitumor effect. The principal interest in these compounds lies on their potential to produce tumor-selective toxicity. Selectivity occurs by difference in oxygen tension between normal and tumor tissue and by levels of the required activating enzymes.

In this review a summary of the most interesting heterocyclic quinones is given together with their biological property. SAR studies concerning the importance of some structural features will be described.

Key Words: Quinones, anticancer agents, tumor-selective toxicity, SAR, prodrugs, heterocycles.

INTRODUCTION

The design of prodrugs that are converted into toxic species by bioactivating mechanisms is an important goal in cancer chemotherapy. This strategy may afford reduced unwanted side effects to patients when the activation of the prodrug is primarily a property of the tumor cells [1-5]. One approach to achieve selective toxicity is through enzymatic reduction of quinone moiety. The bioactivation of these prodrugs can be accomplished by one- or two-electron reductases and leads to toxic species which can act either in hypoxic regions of tumors or in tumors rich in the required activating enzymes [6-11].

A wide variety of nitrogen-containing heterocyclic quinones have been reported to show antitumor effect [12-14].

The mechanisms of their toxicity are various; they can act as bioreductive alkylating agents of biomolecules, as producers of reactive oxygen radicals by redox cycling, as DNA intercalating agents, as topoisomerase and phosphatase inhibitors and as apoptosis inducers. The first step of all these actions relies on the quinone bioreduction which generates electrophilic species toxic to cells. Many studies have been reported to correlate quinone structure with substrate specificity, DNA-damaging capability and toxicity [15-19]. However, the result of these attempts is somewhat controversial. Some key structural features seem to be important. For instance, the substituent on the quinone ring, by varying the quinone reduction ease, influences its single-strand DNA cleavage capability and therefore its activity [20]. Another structural requirement for the antitumor action is the presence of alkylating centers as well as the presence of good leaving groups [21-23]. Moreover, the number and position of nitrogen atoms substituted in the heterocyclic ring play a considerable role; generally, the increasing numbers of nitrogen atoms enhance the activity [24-26].

Another interesting aspect of this class of compounds lies on their potential to be used as "triggers" to release an effector which is the cytotoxic species. This effector (e.g. mustard) is capable of diffusion, resulting in a bystander effect [27-30].

Despite the large number of nitrogen quinonoid systems, few have reached clinical evaluation and additional studies are needed to understand their mechanism of action and to improve their efficacy.

This review describes the most significant nitrogen-containing quinones, the focus being directed to three aspects: chemical structure, SAR and antitumor effect. The great potential of these compounds might offer new opportunities for the development of selective anticancer agents and prompt the researchers to optimize their pharmacokinetics properties.

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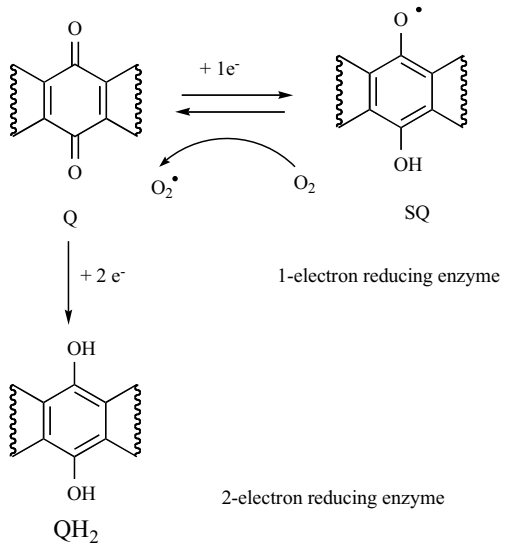
QUINONE BIOREDUCTION

The 1-electron reduction is catalyzed by cellular oxidoreductases, mainly by NADPH cytochrome c (P450) reductase, cytocrome b5 reductase (b5R), NADH dehydrogenase and ferredoxin NADP reductase and leads to formation of semiquinone anion radical. The non-enzymatic reoxidation of the semiquinone to the non-toxic quinone occurs *via* redox cycling in well-oxygenated cells, whereas in hypoxic tissues (e.g. solid tumors) is less efficient, resulting in a solid tumor-selective therapy. The redox cycling leads to generation of reactive oxygen species (ROS), but cellular defenses such as superoxide dismutase, catalase and glutathione peroxidase limit their toxicity. The 2-electron reduction is catalyzed mainly by NAD(P)H: Quinone oxidoreductase (NQO1), known as DT-diaphorase, and results in formation of hydroquinone. Hydroquinone can be conjugated with sulfate or glucuronic acid and excreted, or it can be the active form and lead to the formation of drug-DNA adducts (Scheme **1**).



Scheme 1. Quinone bioreduction.

Overexpression of this enzyme in a range of tumor types could result in a highly specific cytotoxicity for these tumors. Thus, selectivity occurs by difference in oxygen tension between normal and

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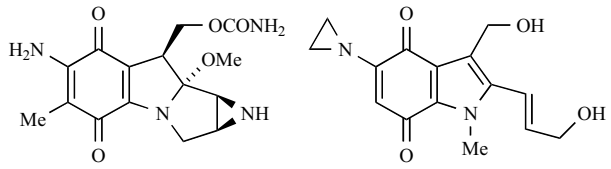
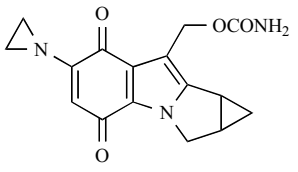
tumor tissue and by levels of enzymes catalyzing bioreductive activation.

INDOLEQUINONES

Following the discovery of MMC (**1**), E09 (**2**) and related mitosene derivatives such as **3** there is great interest in the indolequinone based antitumor agents (Fig. **1**).



1 MMC **2 EO9**



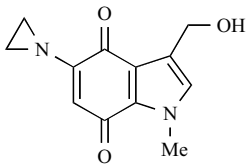
3

Fig. (1). Indolequinones.

It is well known that these compounds act as dual substrates for one- or two-electron reductases and show selectivity for DT-diaphorase-rich cells and hypoxia selectivity [31, 32]. Several structure-activity studies have been published. In general, these agents bear an aziridinyl moiety on the quinone ring and a leaving group and are converted by reductive metabolism to a bifunctional alkylating species able to cross-link DNA in the major groove. Reduction to the hydroquinone makes the aziridin nitrogen more basic resulting in protonation and nucleophilic attack [11, 21, 23]. The N-7 guanine is the usual nucleophile target (Scheme **2**). Reduction also activates the leaving group resulting in formation of an iminium species capable of alkylation DNA nucleophiles (Scheme **3**).

The best representative is the compound RB 95629 (**4**) (IC_{50} = 3 nM on the A549 breast tumor cell line under aerobic conditions) (Fig. **2**) [5, 10].

Many studies have been conducted to analyze the effects of different substituents on the quinone redox properties, but the results are often ambiguous. In many cases, no correlation between



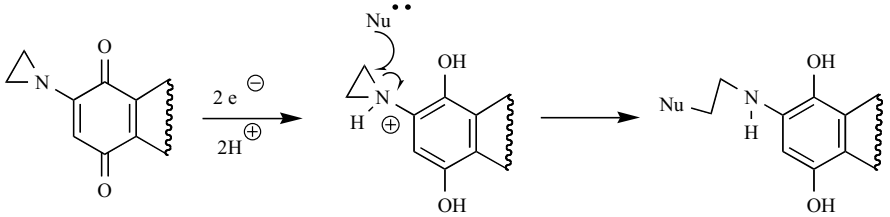
4 RB 95629

Fig. (2).

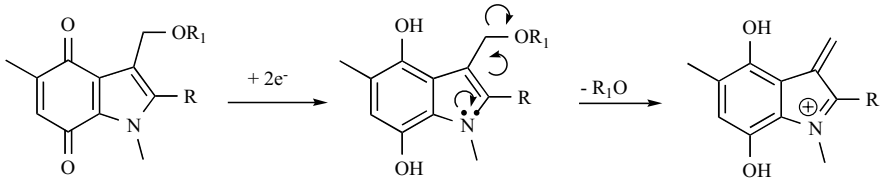
reduction potential and *in vivo* substrate specificity has been found [17, 18]. Probably, other factors, such as steric influence, oxygen tension and hydrogen bond formation capability may be important to determine the reduction efficiency of these compounds. Generally, the importance of aziridinyl moiety at position 5 is confirmed. Its shifting to position 6 or its substitution with the methylaziridine or with a methoxy group reduces potency, its omission causes loss of activity. A leaving group, such as hydroxymethyl or an acetate at the C-3 is essential. With regard to the 2-position, it was demonstrated that this substituent has a considerable effect on leaving group-mediated activation and that an increase in C-2 side chain length reduces cytotoxicity [17]. A summary of SAR studies is reported (Fig. **3**).

Moreover, the indole quinone structure has been used as trigger for the physical release of an effector which is the bioactive agent. The aim of this strategy is to achieve selectivity and to improve the biodistribution of the drug. The reduction of quinone generates an intermediate that spontaneously releases a cytotoxic mustard that can cross-link DNA. In addition, the evicted mustard is capable of diffusion, resulting in a "bystander" cell kill, so the effects of the prodrug beyond the cell in which it is activated, are amplified. An example is compound **5** (MUP98176) an indolequinone-mustard conjugate, where reduction is followed by direct C-N bond cleavage (IC_{50} = 0.07 μM under hypoxic conditions) [4, 5, 28] (Fig. **4**). The design of prodrugs that utilize the phosphoramidate release led to a series of 2- and 3- phosphorodiamidate indolequinones [29]. Some of them, such as **6** and **7** (LC_{99} = 0.11 and 0.07 μM on HT-29, respectively) appeared to be highly potent inhibitors of cell growth. Mechanisms for the activation of these prodrugs have been evaluated [30].

Both compounds undergo activation *via* two- electron reduction. Reduction to hydroquinone increases the electron density of the indole nitrogen, resulting in the generation of an iminium ion



Scheme 2. Activation of aziridinyl moiety.



Scheme 3. Elimination of leaving group and alkylation by the iminium species.

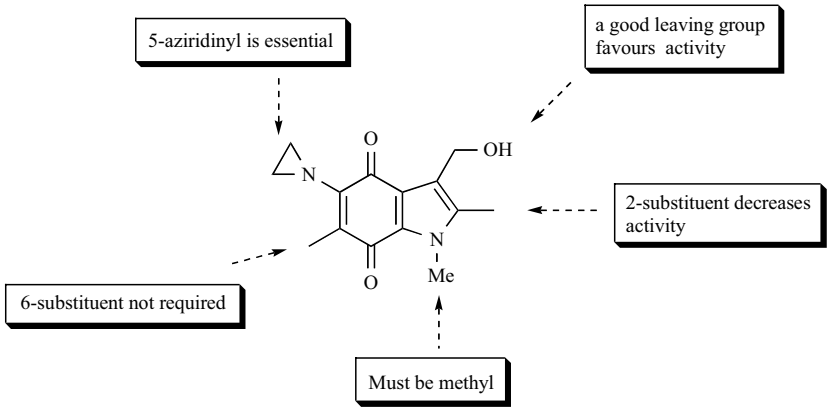
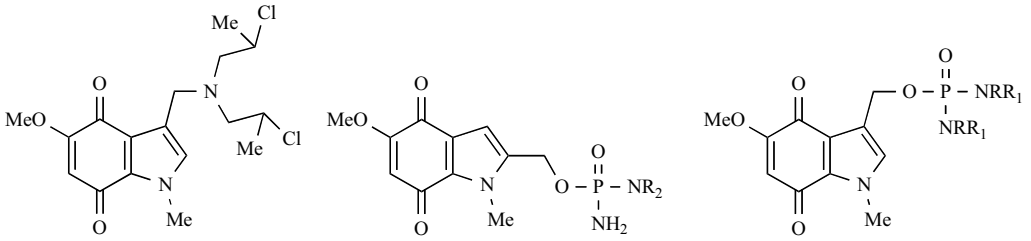


Fig. (3). SAR studies on indolequinones.



5 **MUP98176** **6** R= CH_2CH_2Cl **7** R=CH_3,R_1=CH_2CH_2Br

Fig. (4). Indolequinone used as trigger.

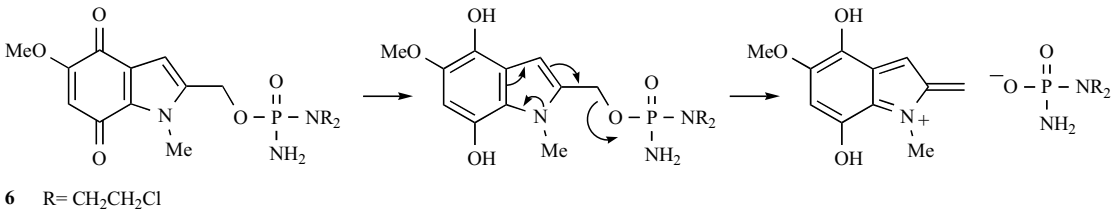
following expulsion of the phosphoramidate anion (Scheme **4**). The liberated phosphoramidate anion is capable of cross-linking DNA.

Alternative activation pathways are supposed for the 3-substituted indolequinones, such as **7**. The one-electron reductases also may contribute to their activation. Additionally, nucleophilic activation, occurring *via* direct displacement of the phosphoramidate anion from the indolequinone, might be another mechanism. The various routes of activations of the 3-derivatives decrease selectivity of these compounds. In contrast, the preferential activation following two-electron reduction of the 2- regioisomers makes these derivatives more selective antitumor agents.

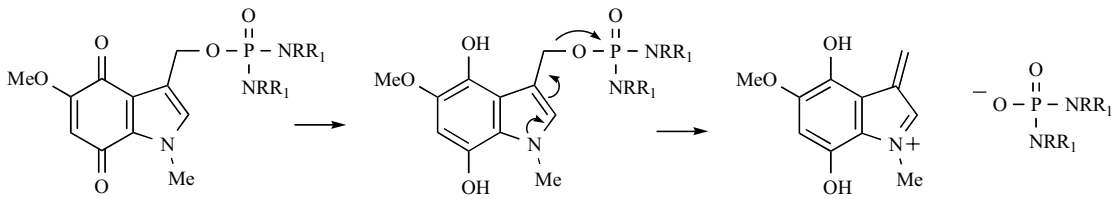
The addition of a fused cyclopentane ring to the indole system sent to derivatives with high specificity for the activating enzyme DT-diaphorase and high percent DNA alkylation [33, 34].

The cyclopent[b]indole **8** and its N-acetyl analogue **9** displayed significant cytotoxicity (Log LC_{50} = -4.99 and –5.06 on melanoma, respectively) and *in vivo* activity against syngeneic tumor implants (Fig. **5**).

The importance of a fused ring in reductive alkylation is probably related to quinone methide formation upon elimination of acetate from hydroquinone [35] (Scheme **5**). It has been hypothesized that the indole quinone methide recognizes the 3'-GT-5' sequence

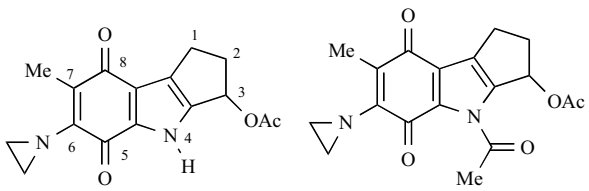


6 R= CH_2CH_2Cl



7 R= CH_3, R_1= CH_2CH_2Br

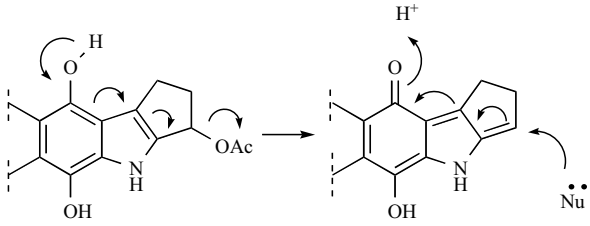
Scheme 4. Expulsion of phosphoramidate.



8 **9**

Fig. (5). Cyclopent[b]indoles.

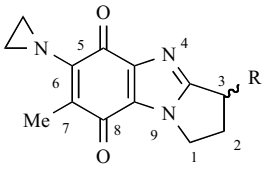
and alkylates the guanine N(7) and the thymine 6-carbonyl oxygen causing the hydrolytic removal of these bases. Moreover, the indole NH of compound **8** is supposed to have a hydrogen-binding role; while the carbonyl of the N-acethylated **9** might have the hydrogen bonding capability in the DNA major groove.



Scheme 5. Quinone Methide.

PYRROLOBENZIMIDAZOLE QUINONES

Skibo *et al*. described a large number of 6-aziridinyl-pyrrolo[1,2-*a*]benzimidazole quinones (PBIs) [36-39]. Some of them, **10**-**14**, are reported in (Fig. **6**).



PBIs

10 R = OAc
11 R = Carbamate
12 R = NH2
13 R = (S)-NH-L-Phe
14 R= (S)-NH-L-Phe-L-Phe

Fig. (6). PBIs.

These compounds are reduced by DT-diaphorase and show strong antitumor activity by virtue of their ability to cleave DNA upon reductive alkylation. The mechanism of PBIs cytotoxicity involves DNA cleavage as a result of binding to the major groove followed by phosphate backbone alkylation. The authors suggested that these reductive alkylating agents target the oxygen anion of the phosphate backbone, because of the electron-deficient character of the pyrrolo benzimidazole system. The activated aziridine alkylates the phosphate oxygen to afford a hydrolytically labile phosphotriester. Hydrolysis of the resulting phosphotriester results in DNA cleavage [21] (Scheme **6**).

All the features of this system have been extensively studied to gain information about the structure-activity relationship. The main conclusions are:

1) the position of the aziridine ring substituent is essential for activity; its shifting from position C(6) to C(7) decreases the cytotoxic potency.
2) the influence of the 7-methyl substituent is confirmed; its omission or its replacement by a methoxy group or by the aziridinyl moiety reduces the activity. The reason of the beneficial impact of the 7-methyl substituent in these compounds is probably due to steric and electronic effects; this moiety influences the rotational conformation of the aziridinyl group and favours the nucleophile-trapping and hence the antitumor activity.
3) the most important determinant of activity appears to be the 3-substituent: the presence of a nitrogen, either as an amine, carbamido, or amide, results in increased potency. Probably, the 3-substituent basicity enhances the degree of DNA alkylation by hydrogen bonding in the major groove. To evaluate the importance of the configuration of the 3-stereocenter, the R(+) and the S(-) stereoisomers have been studied. The R(+) enantiomer seemed to be more active than the S(-) enantiomer against some cancer panels. A summary of SAR studies is reported in (Fig. **7**).

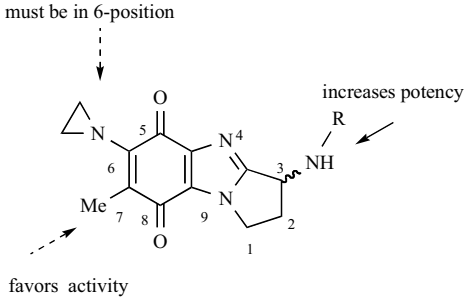
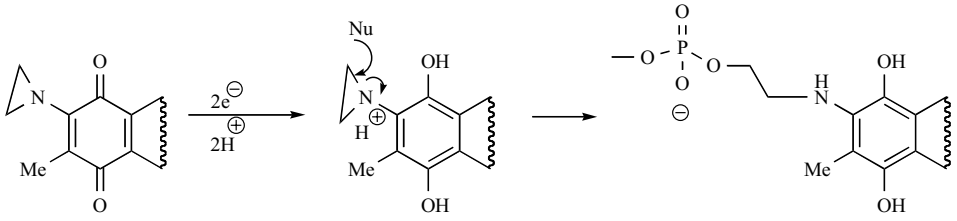


Fig. (7). SAR studies on PBI derivatives.

Among the best representatives, compound **12**, namely, 3-amino-6-aziridinyl-2,3-dihydro-7-methyl-1H-pyrrolo-[1,2-α] benzimidazole-5,8-dione combined all the favourable requirements and showed high cytotoxicity and antitumor activity (LC_{50} <10 $^{-8}$ in human cancer cell lines) [37]. After reduction, the hydroquinone hydroxyl, the 4-nitrogen and the 3-amino group of this compound are hydrogen bonded to the DNA major groove.

The presence of different substituents such as heterocycles, aminoacids and peptides linked to the 3-amino center of the PBI,



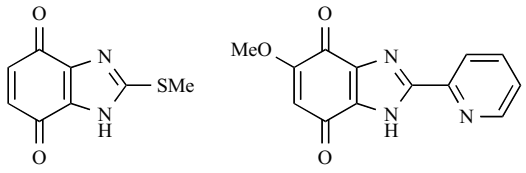
Scheme 6. Phosphate Alkylation.

was made to evaluate the possibility of additional interactions in the major groove [40].

Some 3- substituents increased the specificity of DNA hydrolytic cleavage, but not the cytotoxic action. It is evident that, in many cases, a high DNA alkylation capability is not correlated with good activity. The linking of one phenylalanine resulted in compound **13** endowed with high cytostatic and cytotoxic action and high cancer selectivity. A further optimization was obtained when another phenylalanine was added. Compound **14** showed highly selective activity against melanoma. This compound is a weak DNA alkylator and the authors suggested that the activity could be related to active uptake mediated by the L-amino acid transporter.

BENZIMIDAZOLE-4,7-DIONES

The great potential of this class of compounds prompted our group to study a series of benzimidazole-4,7-diones bearing at the 2-position the thiomethyl group or the pyridinyl moiety [41]. The derivatives **15** and **16** (Fig. **8**) were more active or equiactive, respectively, than MMC against human lymphoblastic leukemia cells.



15 **16**

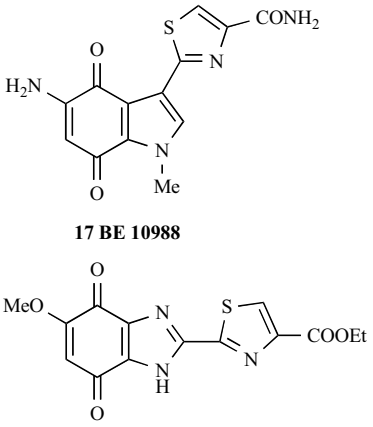
Fig. (8). Benzimidazole-4,7-diones.

Both compounds exhibited high activity on human non-Hodgkin lymphoma cells. It is evident that the substituent on the quinone ring has a marked effect on activity. In the 2-thiomethyl series the substituents have a negative effect, while in the 2-pyridinyl series the presence of an electron-donating group, such as the methoxy, leads to a very good activity. In a further study [42], it was observed that two different electron-donating groups, such as the methoxy and the methyl at C-5(6), are able to influence to a different extent the antiproliferative action: the methoxy group leads to a significant activity on erythroleukemia cells, the methyl function shows a high activity on colon carcinoma. Moreover, the position of the nitrogen atom in the pyridine moiety plays an important role. Shifting the nitrogen from 2- to 3- and 4- positions progressively decreases the antiproliferative action, independent of the presence or the absence of substituents on the quinone ring. Probably, the distance of the pyridine nitrogen from the benzimidazole ring is critical for the interactions between the compounds and the biological target(s) accountable for the antiproliferative effects.

The thiazolylindolequinone BE 10988 (**17**), (Fig. **9**) inspired the synthesis of thiazolyl benzimidazolequinones [43]. The best representative of the series was **18** which showed a very good activity on erythroleukemia cells (IC_{50} = 1.16 μM on K562). The activity is associated both with the presence of a methoxy group on the quinone ring and with the presence of the carboxylate moiety at the thiazole 4-position.

QUINONES AS INTERCALATORS

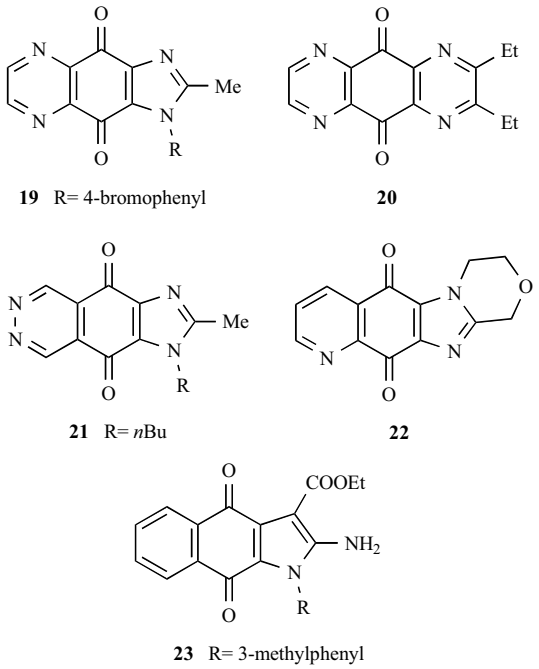
One of the cytostatic action mechanism of coplanar polycyclic compounds is their intercalation with human DNA. The intercalation causes enzymatic blockade and reading errors during the replication process. According to Moore and Pindur, the optimal intercalation needs three or four coplanar rings with a length of 3-4 Å and a width of 6-8 Å. It must also have a p-conjugated quinone containing a nitrogen atom, because this enables hydrogen bonding with DNA [44, 45]. A number of coplanar tricyclic and tetracyclic quinones with three or four nitrogen atoms have been studied. Compound **19**, namely 2-methyl-4,9-dihydro-1-(4-bromophenyl)-1H-imidazo-[4,5-*g*]quinoxaline-4,9-dione, and compound **20**, namely 2,3-diethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione [46] exhibited potent cytotoxic activity against human gastric adenocarcinoma cells (IC_{50} = 1.30 and 7.61 μM, respectively) (Fig. **10**). Both compounds bearing bulky side chains are supposed to interact with



17 BE 10988

18

Fig. (9). BE analogue.



19 R= 4-bromophenyl **20**

21 R= *n*Bu **22**

23 R= 3-methylphenyl

Fig. (10). Quinones as DNA intercalators.

DNA and form stable intercalation complexes. The 1-*n*-butyl-2-methyl-1H-imidazo[4,5-*g*]phthalazine-4,9-dione **21** [47] showed *in vitro* high cytotoxicity against several human tumor cell lines (IC_{50} = 0.001 μM on SNU-638). Addition of the fused oxazino ring to the imidazoquinolinedione structure led to **22** endowed with potent action on human colon tumor cell lines *in vitro* (IC_{50} = 0.0019 and 0.026 μg/mL on A549 and HCT 15, respectively) [26]. A further improvement of antitumor action was observed in compound "**23**, namely, 2-amino-3-ethoxycarbonyl-N-(3-methyl-phenyl)-benzo[*f*] indole-4,9-dione, [48] which showed *in vitro* antitumor activity

comparable or superior to doxorubicin against the human ovarian tumor cells (ED_{50} = 0.04 μg/mL) and the human CNS cells.

QUINONES AS TOPOISOMERASE INHIBITORS

Many heterocyclic quinones act as topoisomerase inhibitors *via* DNA-intercalation. Topoisomerases are DNA-modifying enzymes essential to the control of DNA topology. They are involved in all cellular processes (replication, transcription, chromatin condensation and recombination) in which the topology of the DNA molecule has to be changed without changing its chemical structure. They catalyze the passage of individual DNA strands (topo I) or double helics (topo II) through one another. The activity of the topoisomerases is important in cell division and therefore many types of cancer cells possess elevated levels of these enzymes [49].

Interaction with DNA and with topoisomerase II is supposed to be one of the major mechanisms of action of some azaanthraquinones (Fig. **11**). This class of compounds has been developed in the attempt to overcome the cardiotoxicity and cross-resistance problems caused by anthracenediones [50]. They are structurally related to mitoxantrone, but containing one or more nitrogen atoms, and would present a major affinity for DNA, due to the presence of sites suitable for hydrogen bonding or ionic interactions. Moreover, the electron-withdrawing character of the heterocyclic rings should favour the formation of DNA-damaging anion radicals [51]. Among the 2-azaanthraquinones, pixantrone, BBR 2778, namely 6,9-bis[(2-aminoethyl)-amino]benzo[*g*]isoquinoline-5,10-dione dimaleate (**24**), emerged as the most promising antitumor agent [52]. BBR 2778 was curative against L1210 murine leukemia and YC-8 murine lymphoma in nanomolar range and showed an activity comparable to that of mitoxantrone and doxorubicin on solid tumors [53-55]. It is in phase III trials for the treatment of non-Hodgkin's lymphoma. The most interesting aspect of this compound is its lack of cardiac side effects. BBR 2778 exerts antitumor action through intercalative interaction with DNA. Probably, this intercalation is stabilized by interactions between the protonated primary amine on the side chain of BBR 2778 and the negatively charged phosphate group of DNA. Like other topo II inhibitors, this compound stabilizes the DNA-protein complex resulting in the stimulation of topo II-mediated DNA cleavage. Moreover, BBR 2778 showed *in vitro* pro-apoptotic effects [54]. The presence of a terminal tertiary amine on both side chains resulted in BBR 2378 (**25**), endowed with a cytotoxic action similar to that of BBR 2778. In contrast to BBR 2778, BBR 2378 was highly active against multidrug resistance cells (IC_{50} =0.07 and 0.14 μg/mL in MDR sensitive S180 and resistant S180/A10 cells, respectively) [55]. 1,8-diazaanthraquinones such as **26** and **27**, bearing a 3-pyrrolidinomethyl and 3-piperidinomethyl moiety, respectively, were more potent than doxorubicin against some cancer cell lines (IC_{50} = 1.60 and 0.30 μM on KB-3-1) [56]. Moreover, these compounds showed cytotoxic action against the doxorubicin-resistant cell lines, implying their therapeutic potential to doxorubicin-resistant tumors. Studies on their mechanism of action revealed that they are not DNA intercalators, but they inhibit topo II-mediated DNA relaxation *in vitro*. Among the 1,5-diazaanthraquinones, compounds **28** and **29** exhibited marked activity against some types of solid tumours (IC_{50} = 0.04 and 0.03 μM, respectively, on A-549 and MEL-28) [57]. The 6-acetamidopyrrolo[1,2*a*]benzimidazole quinone derivatives, such as **30** and **31** have been found to be catalytic inhibitors of topo II [58] (Fig. **12**).

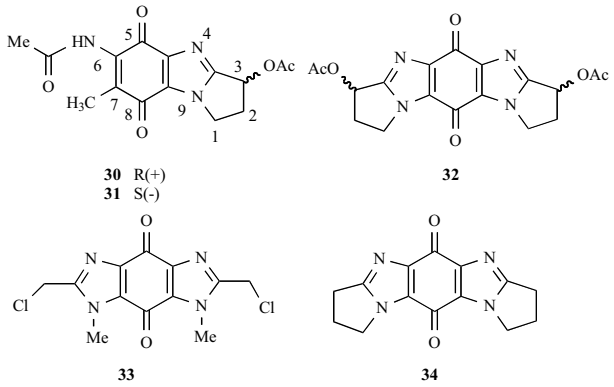


Fig. (12). 6-acetamidopyrrolo[1,2-*a*]benzimidazolequinones dipyrroloimidazobenzimidazole- and benzodiimidazolequinones.

These compounds are inactivated upon DT-diaphorase reduction and only the quinone form inhibits the first step of topoisomerase II-mediated relaxation of supercoiled DNA by intercalation. In this system the configuration of the 3-position and the bulk of the 7-substituent play an important role in the cytotoxicity because influence the interaction with DT-diaphorase and topoisomerase II.

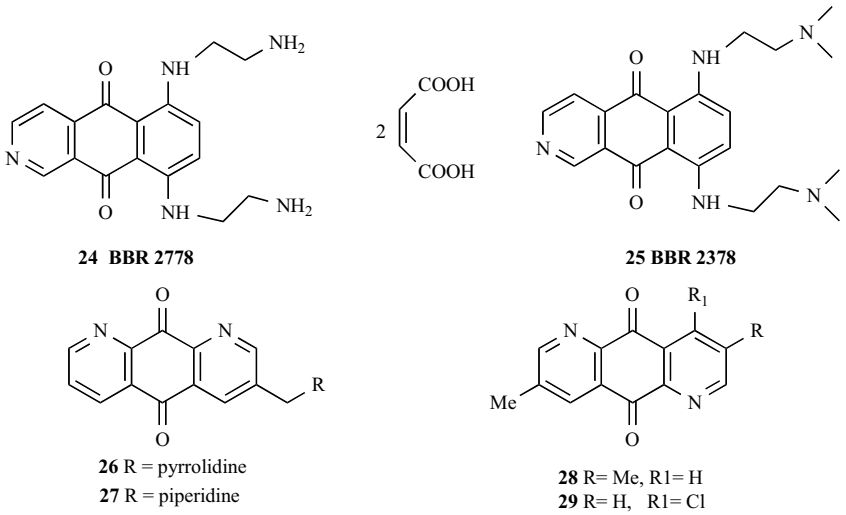
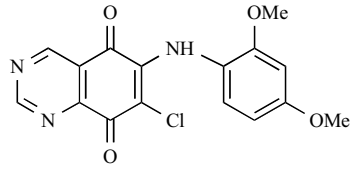


Fig. (11). Azaanthraquinones.

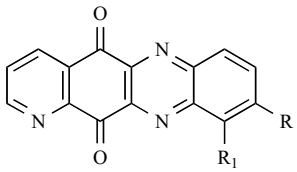
The S(-) enantiomer is more cytotoxic than the R(+) enantiomer and racemate because it has a lower specificity for reductive inactivation by DT-diaphorase and a more potent inhibition of topo II. The increased steric bulk at the 7-position (butyl instead of methyl) enhances the cytotoxicity, by decreasing the DT-diaphorase reductase activity. However, the bulk of the 7-substituent decreases the ability of these compounds to inhibit topoisomerase II, probably by interfering with DNA intercalation [59]. The addition of fused pyrrolo and fused imidazo rings to pyrrolo[1,2-*a*] benzimidazolequinone was made with the aim of increasing the bulk about the quinone moiety, so as to prevent DT-diaphorase reductive inactivation. Some dipyrroloimidazobenzimidazole quinones such as **32** and benzodiimidazole quinones such as **33** were found to have broad spectrum cytotoxicity, but no specificity. Compound **34** showed a high specificity against melanoma cell lines. Both systems are catalytic inhibitors of topo II-mediated relaxation of supercoiled DNA by intercalation [60].

Other nitrogen-containing heterocyclic quinones showed *in vitro* potent antitumor activity. DNA intercalation and topo I and topo II inhibition could be responsible for their cytotoxic effect. Among them, a series of 6-arylamino-7-chloro-quinazoline-5,8-diones, [25] and some pyridophenazinediones [61] showed potent activity against human stomach cancer cells. The possible mechanism of this action has been suggested as a DNA topo I and topo II inhibition.

The best representatives are compounds **35** (IC_{50} = 0.61 μM on SNU-638), **36** (IC_{50} = 0.06 μM on SK-OV-3) **37** (IC_{50} = 0.06 μM on XF-498) (Fig. **13**).



35



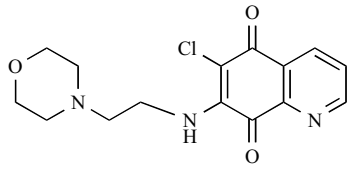
36 R= $OCH(CH_3)$; R_1 = H
37 R= R_1 = Me

Fig. (13). Quinoxaline-5,8-dione Pyridophenazine-6,11-diones.

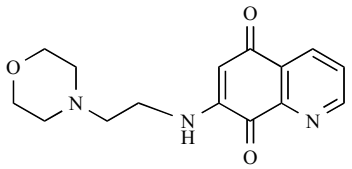
QUINONES AS CDC25 PHOSPHATASES INHIBITORS

The Cdc25 dual specificity phosphatases play an important role in coordinating cellular signaling processes and cell proliferation. These enzymes are involved in the activation of the cyclin-dependent kinases, the central regulators of the cell division cycle, at the major cell cycle phase transitions [62-66]. Three homologs exist in humans: Cdc25A, Cdc25B and Cdc25C. Cdc25A is responsible for the initiation of DNA synthesis in the S-phase of the cell cycle, whereas Cdc25B and Cdc25C are regulators of the G_2/M transition [67, 68]. Additional support for the importance of Cdc25 phosphatase activity has come from studies correlating Cdc25 inhibition by small molecules with growth arrest or cell cycle blocks [69]. Because of their overexpression in a wide variety of cancers, the Cdc25s are attractive targets for the development of chemotherapeutic agents [70-74].

Several quinoid agents have been identified as Cdc25 inhibitors; among them some quinoline-5,8-diones have been widely investigated [75]. The most potent were DA3003-1 (**38**) or NSC 663284 or 6-chloro-7-(2-morpholin-4-ylethylamino) quinoline-5,8-dione and the dehalogenated congener JUN1111 (**39**) 7-(2-morpholin-4-ylethylamino) quinoline-5,8-dione which selectively inhibited Cdc25 phosphatases *in vitro* in an irreversible manner and arrested cells in the G_1 and G_2/M phases of the cell cycle [76, 77] (Fig. **14**).



38 DA3003-1



39 JUN1111

Fig. (14). Quinoline-5,8-diones as Cdc25 Phosphatases inhibitors.

To gain information about the structure-activity relationship of these derivatives, various structural changes were evaluated. Replacement of the quinolinedione by isoquinoline-, phtalazine-, quinazoline-diones reduces the inhibitory effect. The presence of the 2-morpholin-4-ylethylamino moiety at the 7-position is crucial; its substitution with different groups or its shifting to 6-position decreases the activity.

The chlorine substituent at the 6-position is not required for inhibition. In a first study with DA3003-1, this moiety was considered essential for activity, because the dehalogenation of the inhibitor occurred with formation of a covalent adduct between compound and a serine residue adjacent to the catalytic cysteine in Cdc25A.

Further investigations revealed that covalent adduct formation is not the major mechanism of Cdc25 inhibition by these quinones. Inhibition could be due, at least in part, to reactive oxygen species (ROS) production and irreversible oxidation of the catalytic cysteine of Cdc25B [78, 79]. A summary of SAR studies is reported in (Fig. **15**).

Both compounds displayed significant growth inhibition against some human cancer cell lines (**38** IC_{50} = 0.5 μM, **39** IC_{50} = 0.1 μM on human breast cancer MDA-MB-435).

The thiazole ring fused with the quinone moiety resulted in BN82685 (**40**) (Fig. **16**) [80]. This compound was found to specifically inhibit Cdc25 *in vitro* and in cultured cells within the nanomolar range.

Furthermore, BN82685 inhibited the growth of the human tumor cell lines with an IC_{50} in the submicromolar range (IC_{50} = 90, 118, 134 nmol/L on DU-145, Mia PaCa-2 and A2058, respectively). This inhibitory effect is irreversible on both the purified Cdc25 enzyme *in vitro* and on tumor cell proliferation. BN82685 was also active against human tumor *in vivo*. Replacement of the thiazole with the oxazole ring led to **41-44** [81]. All these compounds show strong inhibition of Cdc25 phosphatases and good antiproliferative action against pancreatic carcinoma and androgen-independent prostate carcinoma with IC_{50} values ranging from 0.15 to 0.44 μM (Fig. **16**).

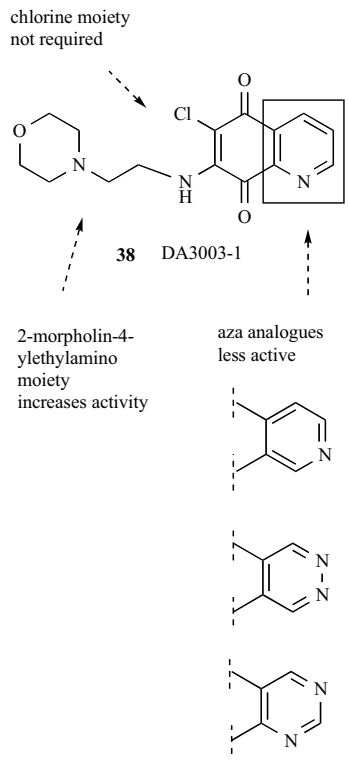
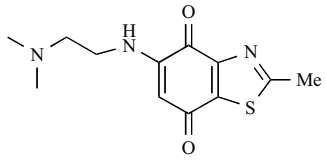
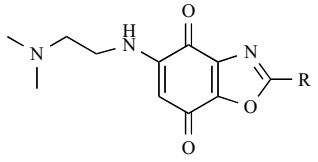


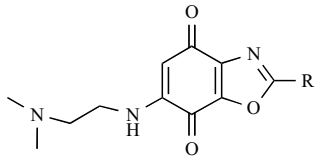
Fig. (15). SAR studies on DA3003-1.



40 BN82685



41 R= Et
42 R= Ph



43 R = Et
44 R = Ph

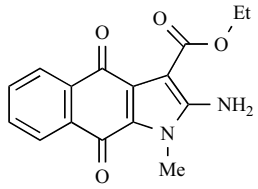
Fig. (16). Quinones as Cdc25 Phosphatase Inhibitors.

QUINONES AS APOPTOSIS INDUCERS

A promising aspect of this class of compounds is their potential to act as apoptosis inducers [82].

Apoptosis, also called programmed cell death, is a physiological mechanism by which unneeded harmful, or damaged cells commit suicide [83, 86]. During apoptosis, various characteristic biological and morphological changes in cells occur, including DNA fragmentations, membrane blebbing, cell shrinkage and apoptotic bodies constitution [87, 88]. The regulation of cell cycle progress and expression of checkpoint-related proteins are also highly involved in apoptotic cell death or proliferation. The tumor suppressor gene p53 is a multifunctional protein responsible for maintaining genomic integrity and it is the most frequently mutated gene in human cancers. p53 is stabilized in response to cellular stress leading to growth arrest or apoptosis [89, 90]. Since apoptosis is a modality by which tumor cells can be eliminated, the identification of compounds able to induce apoptosis in different tumor cell types, is an important goal in cancer therapy. In addition, it has been observed that resistant tumor cells evade the action of anticancer agents by increasing their apoptotic threshold [91-93].

Several benzo-and naftoquinones were found to induce DNA cross-linking followed by induction of apoptosis [94-96]. In some cases, the mechanism of apoptotic pathway is involved in the regulation effect of p53 protein on cell cycle arrest at the G_2/M phase. Unfortunately, at this time, few examples of nitrogen-containing heterocyclic quinones as apoptotic inducers are reported. Among them a series of benz[*f*]indole-4,9-diones showed a potent growth inhibition of a panel of human cancer cell lines [97, 98]. Their mechanism of action might be related to the induction of apoptosis by means of cell cycle arrest at G_2/M phase. The best representative is SME-6 (**45**), namely 2-amino-3- ethoxycarbonyl-N- methyl-benz[f]indole-4,9-dione with IC_{50} values ranging from 0.3 to 1.5 μM on A549, Co12 and SNU-638 (Fig. **17**).



45

Fig. (17). Quinone as apoptosis inducer.

CONCLUSIONS

The versatility of nitrogen-containing heterocyclic quinones and their potential to be selectively toxic to tumor cells offer a great opportunity to anticancer therapy. Despite the extensive work on the design of this class of compounds, the results are disappointing. Quinones that appeared very promising *in vitro* or in preclinic studies, have been ineffective in the clinic. The reasons of this failure are various. Firstly, the mechanisms of action of these compouds are not fully elucidated; the large variety of structures and functional groups makes their contribution to cytotoxicity uncertain. The enzymes responsible for the activation are multiple and it is difficult to know which of them plays a predominant role. Moreover, studies with tumor cell lines cannot duplicate the *in vivo* situations. Further research is needed to understand the mechanisms of tumor-specificity and to design more selective and effective agents.

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