Quinone Methides and their Prodrugs: A Subtle Equilibrium Between Cancer Promotion, Prevention, and Cure

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Abstract: The importance of reactive drug metabolites in the pathogenesis of drug-induced toxicity has been investigated since the early 1950s, mainly to reveal the link between toxic metabolites and chemical carcinogenesis. This review mainly focuses on biologically active compounds, which generate reactive quinone methide (QM) intermediates either directly or after bioactivation. Several examples of anticancer drugs acting through the generation of QM electrophiles are given. The use of those drugs for chemotherapeutic purposes is also discussed. The key feature of those QM-generating drugs is their reactivity toward specific nucleophilic biological targets. Modulation of their reactivity represents a challenge for medicinal chemists because, depending on the reactivity of these QM intermediates, their interaction with critical proteins can alter the function of these key proteins and induce a wide variety of responses with functional consequences. Among the possible consequences, antiproliferative effects could be exploited for chemotherapeutic purposes. Information on how such QM-generating drugs can affect individual target proteins and their functional consequences are required to help the medicinal chemist in the design of more specific QM-generating molecules for chemotherapeutic use.

Keywords: Alkylating agent, antioxidant pool, cancer, DNA, prodrug, thiols.

INTRODUCTION

Although they have progressively become important chemical compounds, both in synthetic organic chemistry and in biology, drugs that contain QM moieties or drugs that are capable of generating QM intermediates require further in-depth investigation with respect to their implications in the pharmaceutical and medical sciences. This feature may relate to the fact that QMs are closely related to the structure of "simple" α,β-unsaturated ketones and, in particular, cyclic ones (Fig. **1**) [1,2]. However, if they share some reactive behavior with cyclic ketones, QM drugs and QM-prodrugs also show specific characteristics that render them more interesting for applications in biology [1].

QMs exist as two-position isomers: the p-QM (**1**) and the o-QM (**2**). Comparing the structures of QM and α,β-unsaturated ketones leads to three conclusions, as follows:

1. The position prone to nucleophilic attack in a QM, that is, the methide group, is generally less sterically hindered, unless it is disubstituted. In α,β-unsaturated ketones, the presence of chemical groups at the γ position forces atoms to come closer the β carbon, which, as a consequence, is more difficult to reach by the nucleophile (see the arrows in Fig. **2**).
2. The δ position of a QM is more electropositive than the β position in an α,β-unsaturated ketone. This is clearly demonstrated when the resonance structures are drawn, illustrating the larger electronic delocalization between the oxygen atom and the δ carbon atom (**3**). When the oxygen atom of a QM is protonated (**4**), the methide carbon is also rendered more electrophilic.
3. It is worth note that in a QM, the aromatization of the 6-membered cycle is crucial as this leads to a large gain in energy [1]. As a consequence, it is reasonably hypothesized that QMs are much more reactive than α,β-unsaturated ketones. Moreover, this reactivity can be directed towards the nucleophile on the δ carbon atom, as well as the electrophile on the oxygen atom.

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The consequence of these features is that the « native » form of a QM cannot be easily isolated. Indeed, during synthesis, isolation or purification processes, QMs can be easily destroyed by the reagents used. Only the most stable molecules can be characterized, that is, those molecules whose nucleophilic character has been decreased by electron-donating substituents (see Table **1** for examples).

It is also important to mention that some authors consider certain molecules as QMs, although their structure is quite different from the « classical » quinoid structure (classical QMs are generally considered as giving 1,6-nucleophilic additions) or they are produced by equilibria between several species. Within these categories are found the following:

1. Molecules having more than 4 carbon atoms between the oxygen and the electrophile (see Table **2**, compounds **29** – **32** for examples) and, consequently, giving 1,8- or 1,10-additions.
2. Molecules with nitrogen-containing rings that replace the traditional benzene ring (**25a**, **25b, 26a** and **26b**).
3. Molecules that have to tautomerize to obtain a QM state (for instance, see 3-methylbenzoquinone **37** and compound **38** in Fig. **3**) [3,4].

EXAMPLES OF QMS THAT ARE ANTICANCER COMPOUNDS

Anticancer Drugs the Chemical Structure of which Includes a QM Moiety (Table 1)

The anticancer activities of QMs and their derivatives have been long described on a large variety of chemical species, including terpenes, antitumoral antibiotics, simple QM, and quinone-imines [5].

Celastrol (**5**), a diterpenoid from thunder god vine (*Triperygium wilfordii* Hook), has been intensely investigated against a large range of diseases [6]. Its anticancer, anti-inflammatory, and immunosuppressing properties have attracted particular attention. Two targets that are structurally modified after exposure to celastrol have been identified. First, celastrol reacts with a CYS residue (CYS-149) in IKKβ and inactivates it.

IKKβ is involved in the phosphorylation and the degradation of IκBα, an enzyme responsible for the inhibition of the NF-κB pathway. Celastrol thus promotes the destruction of NF-κB, a factor that contributes to cancer development [6]. Second, celastrol prevents

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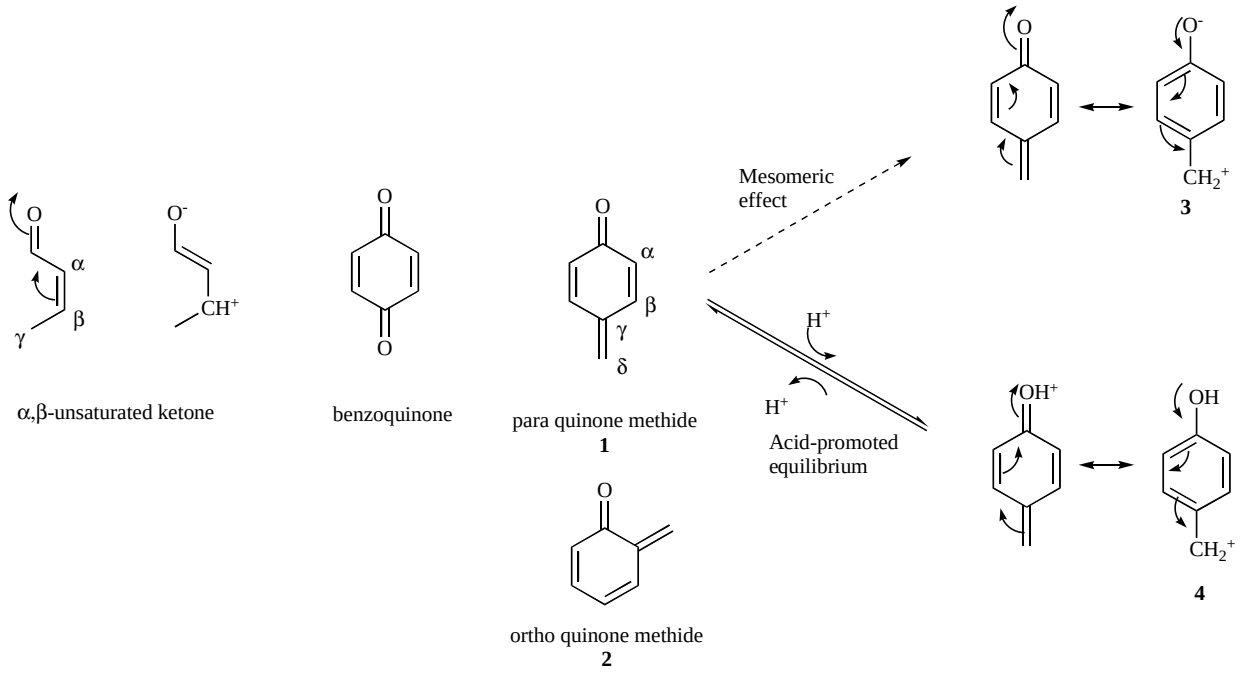


Fig. (1). Structures and resonance structures of α,β-unsaturated ketones and quinone methides.

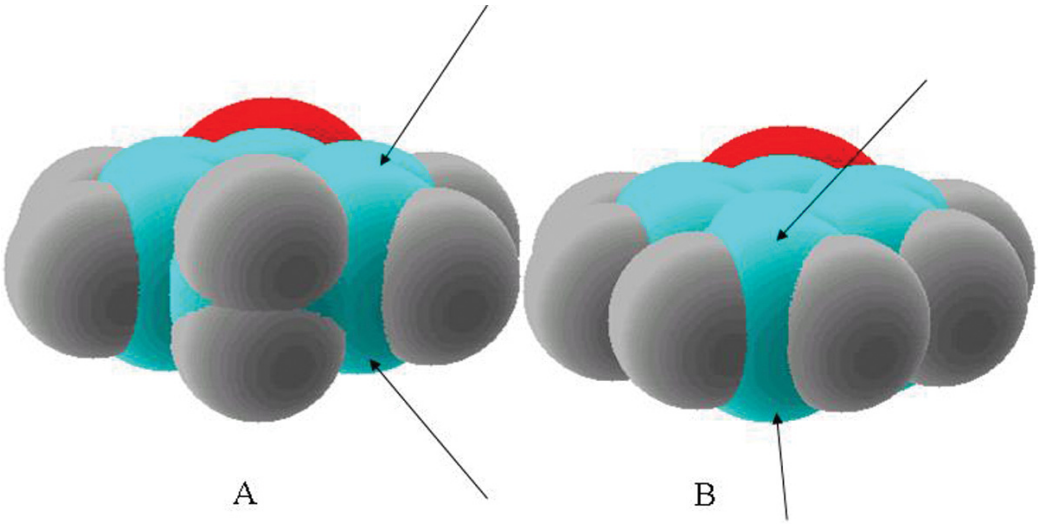


Fig. (2). Cyclic α,β-unsaturated ketone (A) and quinone methide QM (B). The arrows show the sites for nucleophilic attacks. The unsaturated ketone is slightly more sterically hindered than the QM at the positions prone to react.

Table 1. Anticancer Drugs the Chemical Structure of which Includes a QM Moiety

Compound	Structure
5	Celastrol

(Table 1). Contd…..

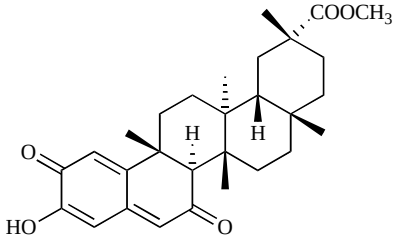
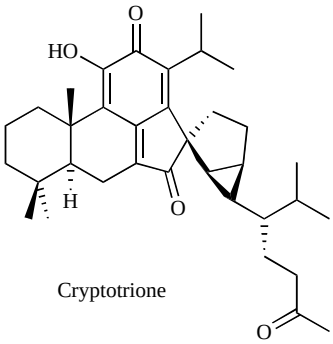
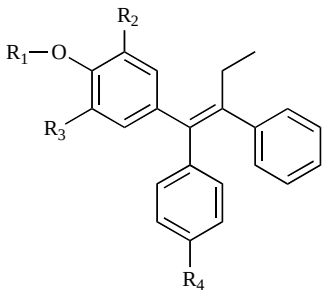
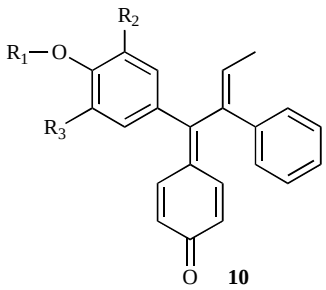
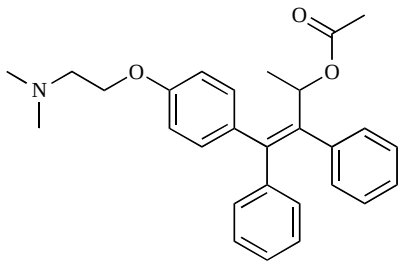
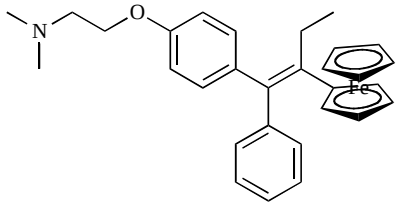
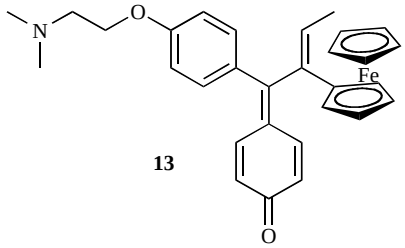
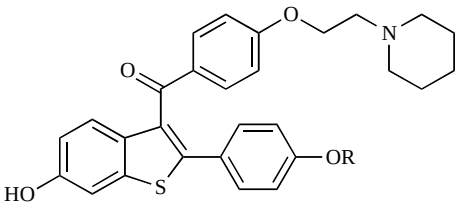
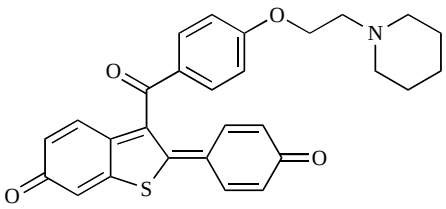
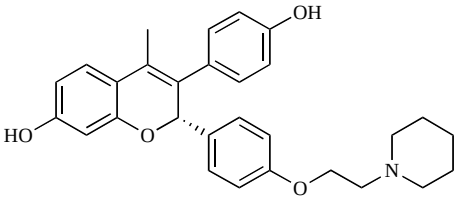
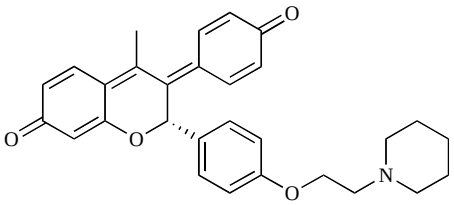
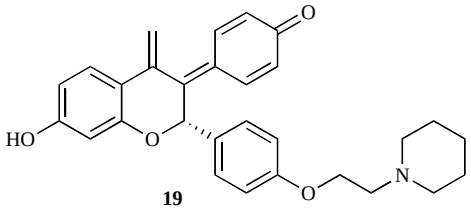
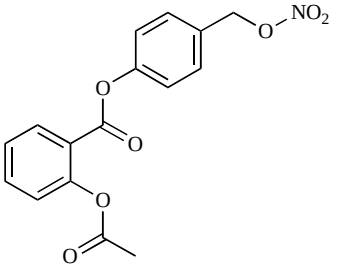
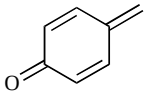
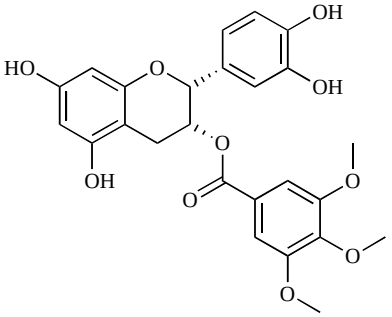
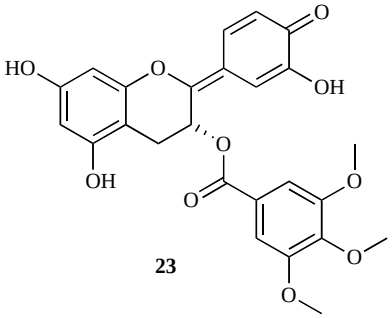
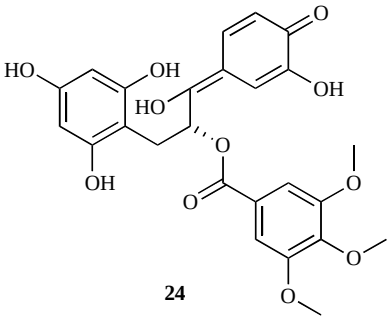
Compound	Structure
6	 Pristimerin
7	 Cryptotrione

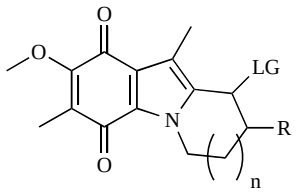
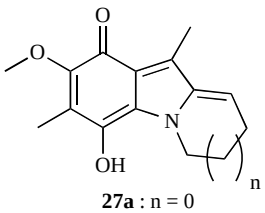
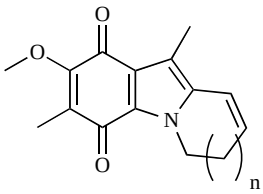
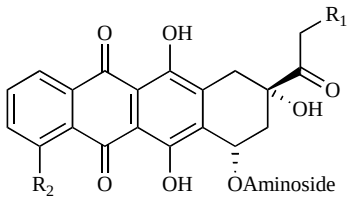
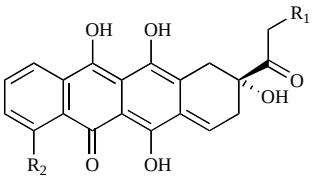
Table 2. Anticancer Drugs that Generate QM Intermediates after Enzymatic Transformation

Compound N°	Precursor	QM generated	Enzyme
8 = TAMO: R_1 = $CH_2CH_2N(CH_3)_2$, R_2 = R_3 = R_4 = H **9** = 4-OHTAMO : R_1 = $CH_2CH_2N(CH_3)_2$, R_2 = R_3 = H, R_4 = OH		 **10**	CYP450
11 = α-acetoxylTAMO		?[a]	
12		 **13**	CYP450

(Table 2). Contd…..

Compound N°	Precursor	QM generated	Enzyme
14 : R = CH_3 = RA **15** : R = H = Arzoxifene		 **16**	CYP3A4
17 = acolbifene		 **18**  **19**	
20 = NO-ASA		 **21**	Esterases
22 = TMECG		 **23**  **24**	Tyrosinase

(Table 2). Contd…..

Compound N°	Precursor	QM generated	Enzyme
Pyrroloindoles (n = 0): **25a** : R = H **25b** : R = LG Pyridoindoles (n = 1): **26a** : R = H **26b** : R = LG		 **27a** : n = 0 **27b** : n = 1  **28a** : n = 0 **28b** : n = 1	Reductases
29 and **30** : R_1 = OH, R_2 = OCH_3: doxorubicin and epirubicin (different aminosides) **31** : R_1 = H, R_2 = OCH_3: daunorubicin **32**: R_1 = H, R_2 = H : idarubicin		 Respective QM **33**, **34**, **35** and **36**	Oxidase + Reductase + NADH:oxidoreductase

[a]Authors give no information about possible metabolization and QM formation from **11**, although it is possible since this molecule is very closely related to TAMO metabolite **10**.

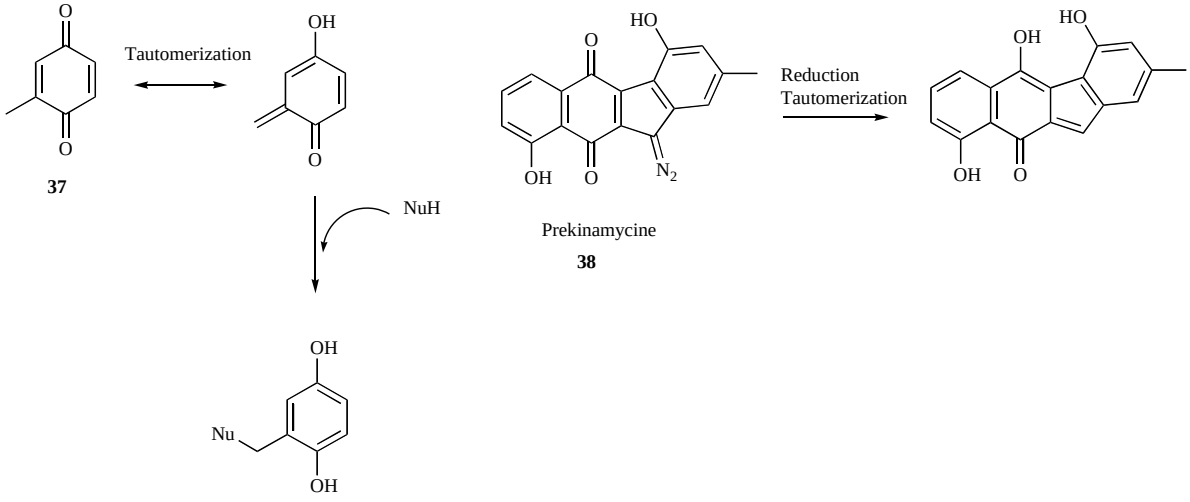


Fig. (3). Examples of compounds that are in chemical equilibrium with QM.

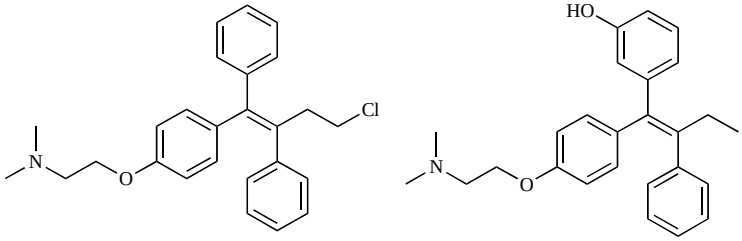
the interaction between HSP90 and Cdc37 through binding to three CYS residues on HSP90 [7]. This complex activates numerous protein kinases that are described as oncogenic [8]. Celastrol also inhibits the chymotrypsin-like activity of rabbit proteasome 20S [8]. From a larger perspective, celastrol has been showed to activate a series of genes involved in the expression of anti-oxidant proteins and heat-shock proteins, such as HSP90 and Hsf1. These effects are reversed by high concentrations of thiols, thus, showing that the electrophilic character of the QM moiety is responsible for the cellular response towards the molecule [9].

Pristimerin (**6**), a very close structural analog of celastrol, displays the same biological profile, but its molecular mechanism of action has not been studied as in-depth as that of celastrol [10].

Cryptotrione (**7**), a naturally occurring QM from *Cryptomeria japonica* (L.f.) Don, is a weakly cytotoxic compound, but the involvement of the QM in the anticancer properties has not yet been clarified [11].

Anticancer Drugs that Generate QM Intermediates after Enzymatic Transformation (Table 2)

Selective estrogen receptor modulators (SERMs) have been extensively studied for their QM-generation capacity, according to the fact that they possess a phenol group, from which they can generate QM intermediates after their metabolization by CYP450 enzymes. One of the first SERMs to be used in breast cancer therapy was



39 **40**

Fig. (4). Structure of toremifene (**39**) and droloxifene (**40**).

tamoxifen (TAMO, **8**). Although it is a powerful drug in hormone-dependant breast cancer, some doubt has been cast over its safety following the discovery of a higher incidence of endometrial cancers in women and liver cancer in rats: studies of the carcinogenetic effects of TAMO have included some active metabolites, such as 4-OHTAMO (**9**), α-acetylTAMO and the QM derivative of 4-OHTAMO (**11** and **10,** respectively) [12]. In fact, only 4-OHTAMO (**10)** was able to create mutations in DNA sequences at an appreciable level; adducts with QM were 10-fold less present [12,13].

Research performed by the Jaouen group has shown that replacing the C ring of 4-OHTAMO (**9**) with a ferrocenyl moiety (see molecule **12**) increased its *in vitro* anticancer activity in human breast cancer cells [14]. Because QMs (especially **13**) are produced from **12**, studies of the metabolism of these new species have been undertaken. These authors demonstrated that, owing to the lower reduction potential of ferrocenyl-TAMO, the QM of the latter is formed more easily than with **8**; they also isolated and characterized compound **13** after oxidation using both chemical reagents and rat liver microsomes [15]. Furthermore, the shift of the phenol moiety on ring A from a p- to a m-position was found to lower the *in vitro* anticancer activity [16]. Meta-positioned phenols are unable to give QMs, a fact in favor of the idea that QM formation participates in the anticancer effects of ferrocenyl-derivatives of TAMO. Therefore, a parallel can be drawn between TAMO and its structurally related ferrocenyl-containing compounds.

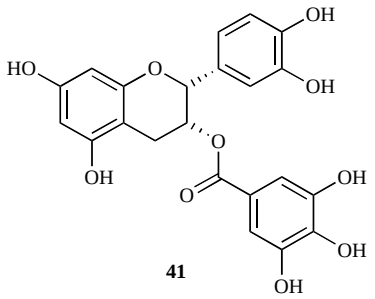
As with its congener, TAMO, the anticancer drug raloxifene (RA, **14**) has been extensively studied for its effect on protein and DNA. Raloxifene is metabolized by CYP3A4 in several hydroxylated compounds, including 3-hydroxy-RA and 7-hydroxy-RA [17,18]. Adducts of RA with reduced glutathione (GSH) have also been detected [19]. Furthermore, a study of the precise mechanism of oxidation using ^{18}O labeled reagents has led to the conclusion that a DQM (**16**) was the intermediate species for the production of 7-hydroxy-RA and the glutathione adduct. Due to the very short half-life (< 1 second) of RA DQM, it has been postulated that this molecule could not react with nucleophiles just after its formation inside CYP3A4 [19]. However, further investigation has proven that RA DQM is able to bind to amino-acid residues outside the binding domain of CYP3A4 and, as a consequence, is able to react with proteins surrounding the cytochrome proteins [13]. Interestingly, RA and the structurally related arzoxifene (**15**) are also oxidized by peroxidases in the uterus, a tissue where these compounds act as drugs [20].

Acolbifene (**17**) is oxidized into QMs (**18**) and diquinone methides (DQMs) (**19**), which are able to react with the usual thiolated compounds but also with DNA. Its toxicity is, however, not currently known [21]. The same metabolic profile has been observed with toremifene (**39,** see Fig. **4**) and droloxifene (**40**), for which adducts between their respective QMs with GSH and deoxynucleosides have been detected *in vitro* [22].

Research has been recently undertaken on NO-releasing anti-inflammatory molecules initially intended to afford NSAIDs with less side effects [23]. These compounds have been named NO-ASAs, such as compound **20**. These molecules were also tested on colon cancer cell lines because some NSAIDs are known to exert chemopreventive effects against colon cancer [23]. These compounds, especially NO-donating aspirin, displayed *in vitro* anticancer activity in the micromolar range [23]. Further investigations on the mechanism of action pointed to the possible involvement of a QM (**21**), which is, in fact, the simplest one in terms of chemical structure; it is produced by the esterase-induced hydrolysis of the ester linker, followed by the subsequent spontaneous degradation of the intermediate through an elimination reaction [24]. Further inquiry revealed that the chemistry and biology of NO-ASA were much more subtle than previously thought. Indeed, QMs from NO-ASA have a very short lifetime in H_2O (~ 200 ms) and are able to trap 25% of the total intracellular GSH after 10 min [25]. The depletion of GSH by the QM derived from NO-ASA has been suggested to be a strong apoptosis-inducing phenomenon, especially through the activation of caspase-3 [25]. Several other mechanisms of action have been evoked to explain the biological effects of NO-ASA. A first mechanism proposes that QM-derived NO-ASAs are able to react with bionucleophiles, including DNA, to generate cytotoxic and genotoxic compounds and, consequently, trigger DNA fragmentation [26]. A second mechanism argues the fact that these QMs can act upon antioxidant responsive elements (AREs) that, in turn, activate the transcription of genes for antioxidant cytoprotective enzymes, such as NAD(P)H-dependant quinone oxireductase 1 (NQO1) [27]. Thus, there is a balance between these two mechanisms of action that lead, respectively, to cytotoxic versus cytoprotective effects [27]. These mechanisms are controlled, at least partly, by the protein, Keap1, wherein the alkylation of CYS residues by Michael reagents leads to the dissociation of its complex with Nrf2 and eventual ARE activation [28]. NO-ASA-related SAR analysis has revealed that a structure containing a leaving group bound to a methylene moiety on an o- or p-substituted phenol ester is essential for obtaining the above-mentioned effects [26], whereas m-substituted phenols are unable to provide similar effects [26]. Additional studies have revealed that stronger anticancer effects can be obtained by replacing the acetylsalicylate ester by an acetate or a phenylacetate ester [26]. Due to steric reasons, these latter are more rapidly hydrolyzed for the release of the QM precursor. Finally, it also appears that the nitrate group can be exchanged with other leaving groups, such as chloride. Thus, the starting pharmacophore of NO-ASA has been refined to afford a model where all of the nonessential elements (acetylsalicylic and nitrate moieties) could be simplified [26].

Epicatechin gallate (ECG, **41**, see Fig. **5**) is a compound endowed with good anticancer properties due, among other mechanisms, to the inhibition of DHFR. However, it has limited bioavailability and poor pharmacokinetics. A lipophilic derivative of ECG, TMECG (**22**), has been designed to improve its pharmacological characteristics [29]. This transformation led to a stronger antitumor compound but only on melanoma cells. The selectivity for these cells has been ascribed to the presence of the oxidizing enzyme, tyrosinase (TYR), which is responsible for the synthesis of melanin.

TMECG is oxidized into an o-quinone, which tautomerizes (**23**) and is hydrolyzed into a QM (**24**). This species, closely related to the DHFR inhibitor trimethoprim does not behave as other QMs. Indeed, it is considered to be very stable because it is able to inhibit irreversibly DHFR without forming a covalent adduct with the enzyme, unlike most of the other known irreversible inhibitors [29].



41

Fig. (5). Structure of epicatechin gallate (**41**).

Quinones derived from pyrrolo[1,2-*a*]indoles (**25a** and **25b**), and pyrido[1,2-*a*]indoles (**26a** and **26b**) are compounds related to QM, but the structure of which is polycyclic and contains a nitrogen atom [30]. These QM precursors are activated by an *in vivo* quinone reduction, followed by the elimination of a leaving group to produce several QMs (**27a**, **27b**, **28a** and **28b**). The similarity is clear between those compounds, which possess one leaving group and undergo 1,6-addition, and the "true QM" precursors while other molecules bearing two leaving groups give a 1,8-addition. Among this series, only the pyrroloindoles have antitumoral activity, being deactivated by a steric hindrance at the attacked-ring position. However, the antitumor potency of the QMs derived from **25b** and **26b** remains low, due to their reported lack of bis-alkylation. pKa values of the carbonyl moiety are very important in these types of molecules. Indeed, QM must have a pKa ranging from 6 to 8 to form a carbocation that is able to trap nucleophiles [30].

It should be mentioned that anticancer agents of the anthracycline family, such as doxorubicin (**29**), epirubicin (**30**), daunorubicin (**31**) and idarubicin (**32**), are sometimes considered QM precursors. Indeed, they are metabolized by the reduction of the quinone ring and by hydrolysis of the glycosidic bond. The structures obtained are proposed to be QMs (see molecules **33** – **36**), but they are not, strictly speaking, QM, as they give 1,10-additions [31]. The skin toxicity observed in patients treated with daunorubicin (**31**) is also considered to be produced by its QM metabolite [32].

Lastly, it must be noted that the mechanism involving QM formation has failed to explain the cytotoxicity of some molecules. The case of callipeltin B (**42**, see Fig. **6**) and its parent, desmethoxycallipeltin B (**43**), can be given as an example. Indeed, the removal of the OMe moiety, which was seen as a putative leaving group leading to the QM, gave a compound with the same cytotoxicity (somewhat) as the former molecules [33].

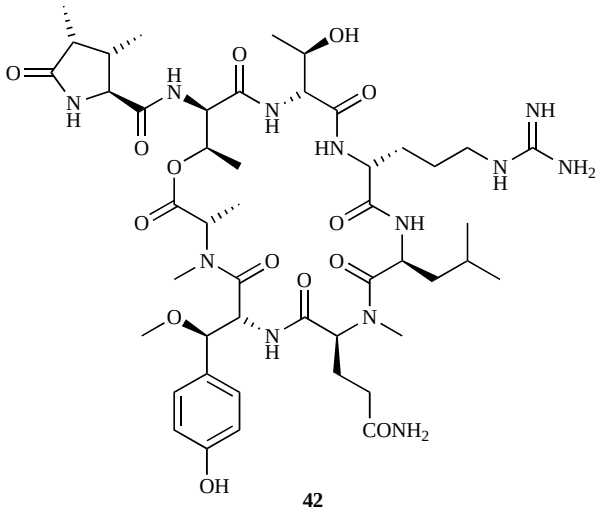
If we refer to those compounds illustrated in Tables **1** and **2**, no clinical applications in oncology has yet been performed with these compounds as actual QMs. For example, celastrol (**5**) has only been assessed against autoimmune arthritis in animal models (mice) [34]. Pristimerin (**6**) and cryptotrione (**7**) have not been investigated in any biological models to state a more precise indication than just the putative « anticancer ». Tamoxifen (**8**), its hydroxylated metabolite hydroxytamoxifen (**9**), raloxifene (**14**), arzoxifene (**15**), and acolbifene (**17**) are used as anticancer agents against hormono-dependent breast cancer for decades. However, their anticancer action is mediated through their action on estrogen receptors (modulation) instead of alkylating abilities [35]. TMECG (**22**) is considered as a possible anti-melanoma drug owing to its activation by tyrosinase, an enzyme present in melanoma cancer cells [29]. However, no clinical trial is under way. Pyrroloindoles (**25a** and **25b**) and pyridoindoles (**26a** and **26b**) have been assessed for their antitumor activity on cellular models.

EXAMPLES OF CHEMICALLY-DERIVED QMs

Molecules that Generate QM Intermediates after Enzymatic Transformation (Table 3)

Butylhydroxytoluene (BHT, **44**) as a source of QMs has been extensively studied, probably because BHT is widely used as a preservative in chemicals and pharmaceuticals. Owing to its two *t*-butyl groups, this compound is very soluble in lipophilic media and, especially, in cell membranes and oils (Table **3**). It was considered as a good free radical and oxidizing-compound scavenger until it was discovered that its structure was profoundly modified when exposed to these species, generating diverse QMs or non-QM structures [36,37]. BHT is a substrate for oxidizing enzymes, particularly the CYP2B isoform of CYP present in the liver and lungs, and it is transformed into several metabolites, among which hydroxy-BHT (BHTOH, **45**), BHT-QM (**46**) and BHTOH-QM (**47** and **48**) have been the most studied [38] (Table **3**).

Some structure-activity relationship studies have been conducted to elucidate the reactivity of BHT-derived QMs. Mizutani



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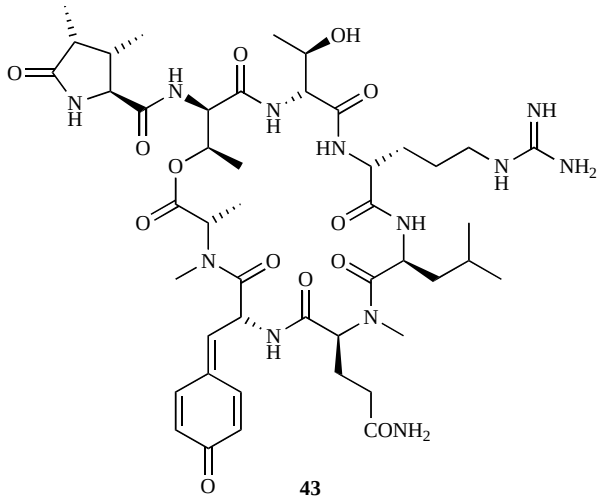

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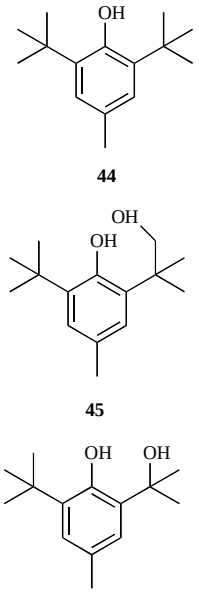
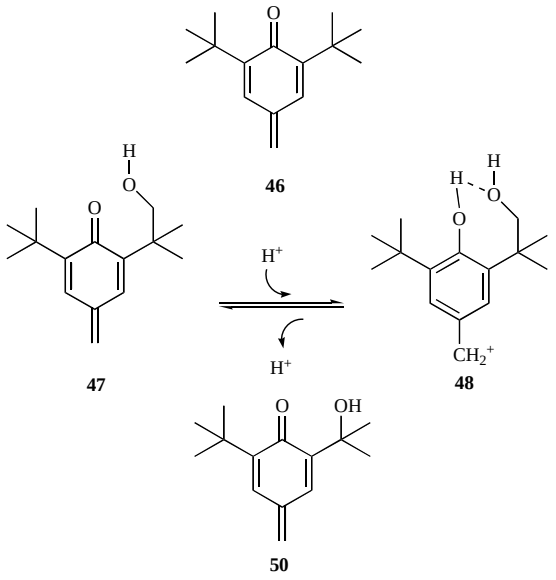
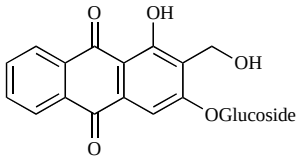
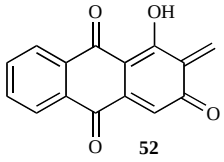
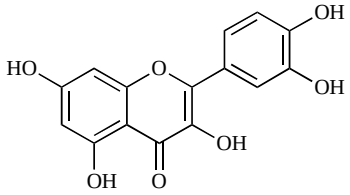
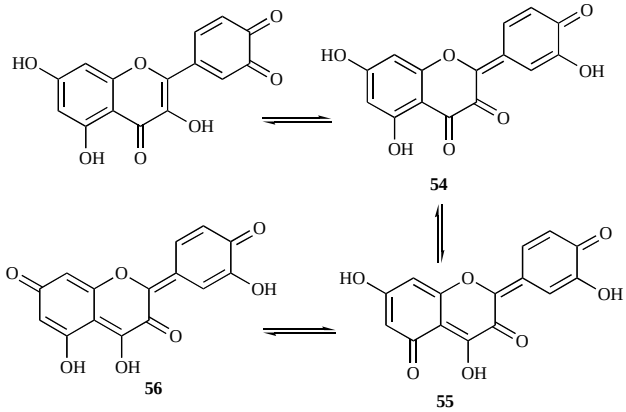
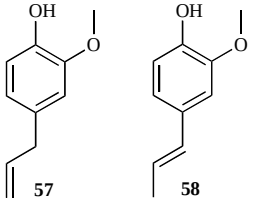
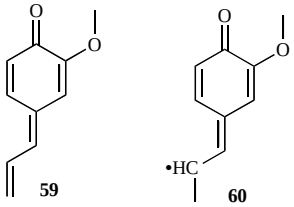
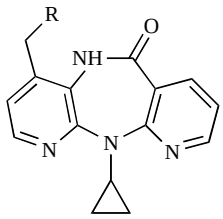
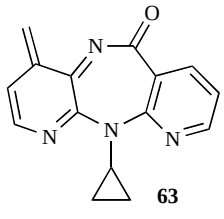
Fig. (6). Structures of callipeltin B (**42**) and desmethoxycallipeltin B (**43**).

Table 3. Non-Anticancer Molecules that Generate QM Intermediates after Enzymatic Transformation

Compound N°	Precursor	QM generated	Enzyme
44 : BHT **45** : BHTOH **49** : BPPOH			CYP450
51: lucidin-3-*O*-primeveroside			Glycosidase Sulfotransferase
53: quercetin			CYP450
57 : eugenol **58** : isoeugenol			CYP450 Peroxidases
61 : nevirapine (R = -H) **62** : hydroxynevirapine (R = -OH)			CYP450

et al. [39] have determined the molecular features for QM production from BHT derivatives. They observed that a phenol and a methyl group (an ethyl group is suitable under some conditions) on the p-position, with at least one t-butyl, but not t-amyl moiety, in the o-position, were essential for the enzymatic transformation. The *t*-butyl is considered to play a very important role in BHT-QM, because it decreases its reactivity towards nucleophiles by preventing the interaction of H_2O with the oxygen atom of the quinone. Such an interaction polarizes the C=O bond and, thus, renders the methide group still more electropositive [40] Bolton *et al*. have shown that BHTOH-QM is a more reactive species than BHT-QM and, as a consequence, reacts not only with thiols but also with the amino groups of proteins. [41,42] The origin of this higher reaction rate has been ascribed to the potential H-bridge between the OH and the oxygen of the carbonyl group, which leads to a more stable, cationic resonance form (**48**). Furthermore, Bolton *et al*. have also synthesized a precursor of a more reactive QM of BHT (BPPOH, **49**) to study its reactivity. The QM **50** was still more reactive than the QM **47**, owing to the more favorable H-bridge present in the structure (Table **3**). They concluded that most reactive QMs lose their ability to react with thiol- or nitrogen-containing nucleophiles, because they are first hydrolyzed by H_2O [41].

The tumor growth promotion of BHT metabolites has been ascribed to their ability to bind to nucleophiles, including DNA and proteins. Particular attention has been drawn to the QM derivatives of BHT as the culprits of the degeneration of normal cells to cancer cells. However, Nagai *et al*. [43,44] have shown that BHT-QM, unlike the other metabolites of BHT (such as aldehydes), was not able to cleave DNA, although it was very unstable and able to react with proteins. The main target of BHT-QM in this situation seemed not to be DNA, but proteins, even if the BHT-QM was able to bind nucleic acids [45]. Covalent adducts of BHT-QM with thiolated proteins and also with GSH have been detected in the lung and liver cells of mice, and the transcriptional activity of stress genes has been mainly observed in cells containing thiolated proteins [46]. BHT-QM inhibits the GSH-conjugating enzyme, GSTP1-1, by binding to CYS residues [47]; it also reacts with many other proteins involved in tumor progression [48]. These new molecular entities are not able *per se* to induce tumorigenesis, but depletion in the levels of antioxidant molecules could be the factor that triggers tumor development in the organs. As a matter of fact, the addition of BSO (an inhibitor of GSH synthesis) increased tumor growth, while the addition of thiol-containing antioxidants, such as CYS, had a strong protective effect against carcinogenesis. Rather than protein modification, the effective cause of tumor progression in these cases was the depletion of the antioxidant pool. In this context, it was demonstrated that cancer cells were less sensitive to lower concentrations of antioxidants, when compared to normal cells. Usually, normal cells have the ability to slow the cell divisions of cancer cells and to contain the tumor growth. When exposed to an oxidizing stress, their survival abilities are limited, and they are out-competed by cancer cells [49]. Moreover, BHT has many other additional effects. It decreases calpain-II activity, induces ODC expression (a widely recognized marker of tumor progression) and activates the MAPK family member, ERK, in keratinocytes, thus promoting tumor formation [41,50,51]. BHT also binds and inhibits peroxiredoxin 6, Cu-Zn SOD and CR, which are all antioxidant enzymes [49,52,53].

The complete analysis of BHT metabolism has also revealed that the peroxides generated along with QMs, or as intermediates of QM formation, have deleterious effects, among which, the promotion of skin tumors can be given as a striking example [54].

A food additive in Japan, madder (*Rubia tinctorum* L.), contains several compounds, including lucidin-3-*O*-primeveroside (**51**). This molecule has drawn attention, as it has been stated that it was carcinogenic in rat liver and kidney. Compound **51** is not toxic by itself, but it is metabolized in the liver by cleavage of the glycosidic bond, followed by the sulfonation of the OH in position 3. The resulting sulfate undergoes an elimination of HSO_4^- to produce QM **52**. This latter molecule has been shown to react with DNA, thus, prompting carcinogenic effects [55] (see Table **3**).

The natural products, flavonoids and, especially, quercetin (**53**), are known for their cancer-preventive properties [56]. Despite these beneficial effects, they also exert toxic effects through transformation into various QMs. The case of quercetin has been extensively investigated because these molecules exert pharmacologic, as well as antioxidant, properties. Regarding the latter point, quercetin and, in general, compounds having an OH group, a double bond and a ketone in position 3, 2 and 4, respectively, can be oxidized chemically or enzymatically, leading to different tautomeric forms. Three QMs (**54**, **55** and **56**) can be obtained that are able to alkylate DNA, GSH and proteins, such as GSTP1-1, thus producing direct and indirect mutagenic effects (Table **3**). The resulting carcinogenic effects were rather variable and depended upon the model used, thus, leading to confusing results that were impossible to extrapolate to humans. This conclusion has also been supported by the transient nature of the DNA and GSH adducts with quercetin, which were unstable and prone to decompose into the starting reagents [57].

Eugenol (**57**), widely used as a disinfectant and anesthetic in dentistry, can be transformed into a QM after oxidation by oxidizing enzymes, such as peroxidases [58]. These species can be trapped by GSH and other thiol-containing proteins. Moreover, studies on the anti-/pro-oxidant effects, cytotoxicities and GSH trapping of eugenol and isoeugenol (**58**) have demonstrated that isoeugenol was more cytotoxic than its parent compound. Isoeugenol induced more reactive oxygen species (ROS) and reacted more with GSH [59]. These discrepancies have been explained by the fact that the above-mentioned cellular events are mediated by free radicals for isoeugenol and by QM for eugenol. This showed that, while being cytotoxic, QM is less harmful for the body than ROS, which are less-selective reactive species and, as a consequence, destroy many relevant biomolecules [58]. Finally, studies on cultured liver cells have shed light on other eugenol-QM effects, such as the inhibition of gap junction-mediated intercellular communication or a slight perturbation in the mitochondrial and plasma membrane potentials [60].

The antiviral drug, nevirapine (NEV, **61**), is widely used as an anti-HIV agent. The use of NEV is frequently associated with skin rashes as a side effect. Recently, a link has also been invoked between the extensive use of NEV and cancer development [61]. Because both of these clinical manifestations are often ascribed to, respectively, protein (allergic reactions) and DNA (mutagenic effects) modification by chemicals that bind covalently to these species, it has been postulated that a metabolite of NEV could produce these effects. A QM (**63**) that is derived from the hydroxymethyl metabolite of NEV had been identified previously [62]. Although it is not the only electrophilic compound generated, it could participate in the generation of the NEV adducts that were detected in the liver tissue of treated patients [63].

AN UPDATE ABOUT QMs AND CANCER

The strictly spoken QM drug (i.e. **5**, **6** and **7**) are not currently involved in clinical trial. About QM prodrugs, the following data are available. Ferrocenyl derivative **12** has only been tested in *in vitro* models. NO-ASA (**20**) has been assessed on colon cancer tumor xenograft in mouse [64] and has been showed to act as a sensitizing agent to cisplatin in human ovarian xenograft tumor by decreasing the thiol pool in cancer cells [65]. A NO-ASA derivative has been undergoing clinical trial in 2003, but this molecule do not generate QM since it is a m-substituted phenol [66]. The tetracyclins doxorubicin (**29**), epirubicin (**30**), daunorubicin (**31**), and idarubicin (**32**), along with mitomycin (**64**) are currently used as che-

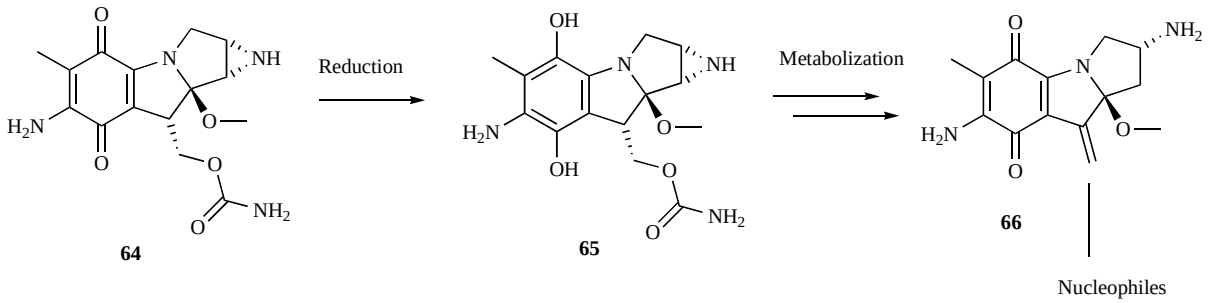


Fig. (7). Structure of mitomycin (**64**), its reduction product **65** and derived QM **66**.

motherapeutic agents in clinical pratice worldwide. The phenol phosphate ester derivatives **67** and **68** have never reached clinical trials.

Alkylating agents remain among the most important drugs used to fight cancer. Platinum complexes, nitrogen mustard derivatives, methanesulfonic esters and other atypical molecules, such as mitomycin (**64,** Fig. **7**), are known to be very potent anticancer drugs [67]. They react with DNA (mainly the N7 of guanine) but also with other nucleophiles, among which, thiol-containing compounds are the most important. As strong electrophiles, QMs are, of course, prone to the same reactions, as it has been proven for example with celastrol (**5**) [68,69]. QMs have also been compared to free radicals (FRs) that act as chemical modifiers of the intracellular contents. FRs are more reactive and are able to induce chemical and structural modifications in many cellular components, such as DNA, proteins and lipids. QMs react in qualitatively and quantitatively different ways than FRs: they are less reactive and more selective towards the kind of nucleophile they will bind to [69]. In contrast, they have the same features as alkylating agents.

Another interesting point is that the QMs produced by enzymatic reactions could render the chemotherapeutic agents specific for a cancer cell line, by following the enzymatic (and, as a consequence, the proteomic) profile of the cell. The widespread enzymes, such as esterases, which are required for the hydrolysis of an ester bond and, so, generate the QM precursors (as it is the case for NO-ASA **20**), could be a good start. However, to our knowledge, no example of cancer-cell selectivity based on esterases and QM formation has been reported in the literature. Moreover, NO-ASA derivatives have shown either tissue or tumor specificity [23]. In spite of this, three striking examples can be found. The first is given by the drug mitomycin (**64**, Fig. **7**), which acts preferentially on hypoxic cancer cells. These cells contain rather specific reductase enzymes, such as NADPH:cytochrome C (P-450) reductase and DT-diaphorase [70,71], which activate the drug into a QM, thus leading to a specific cytotoxicity. Another interesting strategy has been designed with molecules that were not originally indicated as anticancer agents. These simple QM-generating entities are (4-fluoromethyl)phenyl phosphate (**67**) and (4-acetoxymethyl)phenyl phosphate (**68**), which are both substrates and inhibitors of prostatic acid phosphatase (Fig. **8**) [72]. This enzyme seems to play important roles in regulation and metabolism in prostate cancer cells and, especially, in the regulation of androgen-receptor activity. The QMs produced *in situ* are able to inhibit this enzyme, but the results of this inhibition, in terms of anticancer activity, have not yet been assessed. The last molecule worth mentioning is TMECG (**22**), which is metabolized into an anticancer DHFR inhibitor by TYR, an enzyme located in cells that synthesize melanin, such as melanoma cells [29]. In this case, TMECG could be used as a more specific inhibitor of melanoma-cell growth.

Finally, as it was explained above, the electrophilicity of a QM is not the only way to exert cytotoxic activity. The recent idea that cancer cells have a higher content of antioxidants has led several research teams to attempt to fight cancer by destroying the antioxidant pool in the tumor tissue. Indeed, because these cells have an intensive metabolism and generally divide more quickly than normal cells, they also produce more toxic compounds, such as FRs [73]. To protect themselves against these FRs, they utilize antioxidant compounds, such as GSH, metallothionein and other reducing molecules. Apart from the classical chemotherapeutic agents, it has recently been shown that cancer cells could be killed efficiently by combining these "old" drugs with pro-oxidant compounds that are able to deplete the cells of these defensive compounds [74].

In term of QM-related toxicity, to the best of our knowledge, there is no preclinical or clinical study undertaken directly on QM but several investigations have been made on molecules that give QM after metabolization. As a consequence, some toxic manifestations could be ascribed to QM.

Estrogen receptor modulator such as TAMO (**8**), RA (**14**), arzoxifene (**15**), and acolbifene (**17**) have been extensively studied in

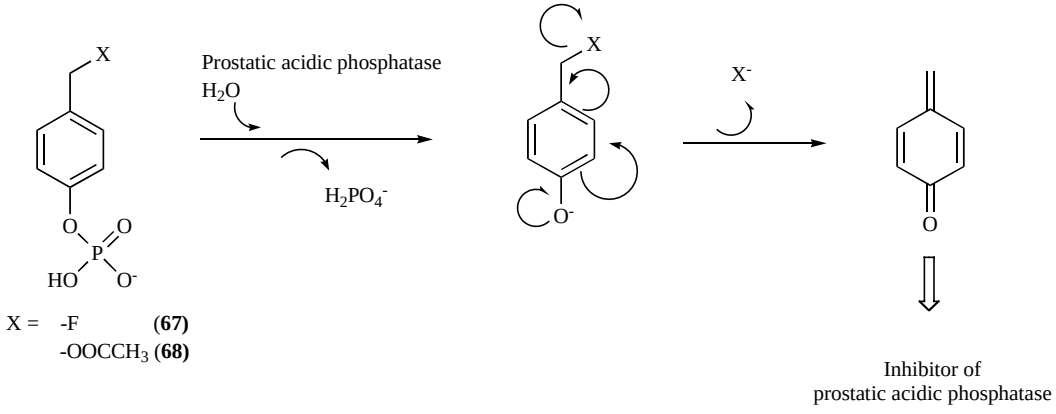


Fig. (8). Generation of prostatic acid phosphatase inhibitors through enzymatic cleavage of the phosphate bond.

human models during the clinical tests needed for their approval. These drugs are metabolized by CYP450 enzymes into several species and, among them, QMs. Toxicity of these drugs has been assessed and, among many effects spotted, endometrial cancer cases have been observed. The prevelance of this side effect was higher for TAMO than for the other drugs and, especially, RA [75]. These cancers can have many origins but the involvement of QMs produced by metabolization has not been clearly demonstrated. One can expect that, owing to the electrophilic character of QM, mutagenic adducts with DNA could be formed and cause the development of cancers of the genital system, as it is the case with TAMO and RA for instance. Albeit adducts of their respective QM and DNA have been observed, the involvement of the former ones in carcinogenic effects of the drugs has not been established but only suspected [18, 21].

With TAMO, clinical tests showed that the risk of developing an endometrial cancer increases with the exposition to the drug. However, a direct relationship between TAMO use and the risk to develop an endometrial cancer remains difficult to establish, as demonstrated by meta-analyses [76]. On the other hand, TAMO [77] and RA [78] have been shown to trigger liver damage with tissue necrosis but for which a relationship with QM has not been made.

In the anthracyclin anticancer drug family, the skin toxicity of daunorubicin (**31**) has been attributed to the QM metabolite of the drug formed by reduction. Within this group of compounds, the observation that a correlation exists between the severity of the lesions and the reactivity of the QM generated support an involvement of the later in this toxicity [32].

The toxicities of BHT (**44**), BHTOH (**45**) and BPPOH (**49**) have been assessed on lungs in animals [41] and are well established in this preclinical model [79].

Nevirapine (**61**) is an approved anti-HIV drugs and, as a consequence, has been assessed in clinical trials. The skin toxicity and allergic reactions observed with nevirapine are rather ascribed to its metabolite, 12-hydroxynevirapine (**62**), which can be further transformed into a QM (**63**). However, the relationship between a QM derived from nevirapine and these side effects is just hypothesized [80].

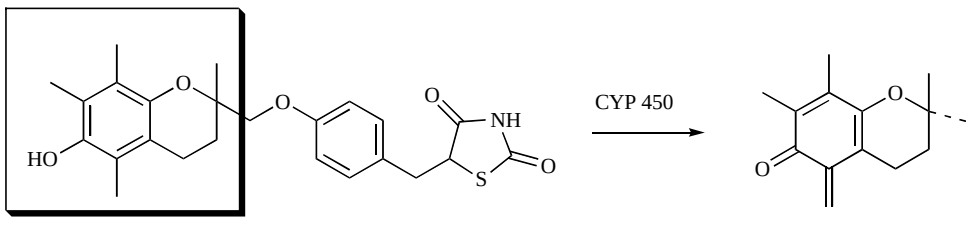
Hepatotoxicity likely related to the formation of several chemical species in liver, among which QM **70**, has also been described for the antidiabetic drug troglitazone (**69**) which is subjected to metabolization in this organ (see Fig. **9**) [81]. This has led to its withdrawal from the U.S. market.

MODULATION OF QM REACTIVITY TOWARDS NUCLEOPHILES

If one succeeds in modulating the chemical reactivity of the QM intermediate, then the possibility to selectively alkylate biological nucleophiles does exist. The modulation of the reactivity of the QM depends on the electronic and steric properties of the substituents linked to the QM or its precursor that gives rise to the QM. Substituents close to the oxygen atom of the QM, usually an o-position, may increase the reactivity by enhancing the polarization of the C=O bond, as is the case, for instance, with an electron-attracting group or a moiety that is able to form H-bridges with the oxygen [41,42]. Regarding the steric hindrance, Bolton and colleagues have shown, using eugenol (**57**) derivatives, that substituents on the methide group decreased the half-life in H_2O from 6-fold for the mono-substituted methide to almost 100-fold for the disubstituted methide [82]. For bigger nucleophiles (i.e., thiolated proteins or DNA), the effects of bulky groups should be, of course, much more dramatic. Finally, another drop in the reactivity was obtained with allylic substituents, for which reactions with nucleophiles break the resonance between the cyclic quinoid system and the double bond. Those substituents influence not only the rate of formation of this QM, but also its stability (half-life) and its electrophilicity, which directly influence its reactivity [69]. These structural considerations indicate that all QM species do not have the same reactivity towards biological nucleophiles, either in terms of potency or selectivity. The reactivity of QMs is dependent on their stability, and these can alkylate proteins at different sites, for example, at CYS residues or amino groups, particularly the Nα of the N-terminal residue, the Nε of LYSs or the amino groups of guanines. This has been shown with BHT-QM (**46**), the decreasing reactivity order of which is the following: thiols > terminal protein amino groups > LYS = HIS [83]. Such reactivity between QM and biological nucleophiles can be viewed as a reaction between soft and hard species. Biological nucleophiles can be *soft* nucleophiles (large atoms with a diffuse, partial or formal charge, such as RS^- and CN^-) or *hard* nucleophiles (small atoms with a dense formal or partial charge, such as H_2O, R-NH_2 and $RCOO^-$), from proteins, DNA, or any other accessible nucleophiles [84]. The QM electrophils, depending on their chemical substituents, can also be considered as hard or soft electrophils.

The possibility to modulate the electrophilic character of the generated QM intermediates should represent a possible way to specifically target biological nucleophiles; however, it should be kept in mind that strong nucleophiles (protein thiols) may react with weak electrophiles, and weak nucleophiles (DNA) react preferentially with strong QM electrophiles. To illustrate the different rates of reaction of QM following the type of nucleophile, it has been shown that the QM derived from TAMO (**10**) has a half-life of 4 hrs in H_2O (hard nucleophile) and 4 min. in H_2O in the presence of GSH (soft nucleophile) [85]. Consequently, some QMs with moderate electrophilic character may indifferently alkylate various nucleophiles, depending on the accessibility of those nucleophiles and, moreover, trigger different biological effects owing to the nucleophiles involved (for examples, see Fig. **10**). These effects rely on several signaling elements, such as ARE and NQO1 [27].

In other words, because hundreds of protein targets have been identified in mammalian systems, and the fact that these represent as many targets of damage for the electrophils of QMs, one can expect numerous possible incidences on the critical components of signaling pathways and metabolic networks and their damages at a systems level. In this perspective, it is tempting to classify drugs



69 **70**

Fig. (9). Troglitazone (**69**) and its QM derived metabolite **70**.

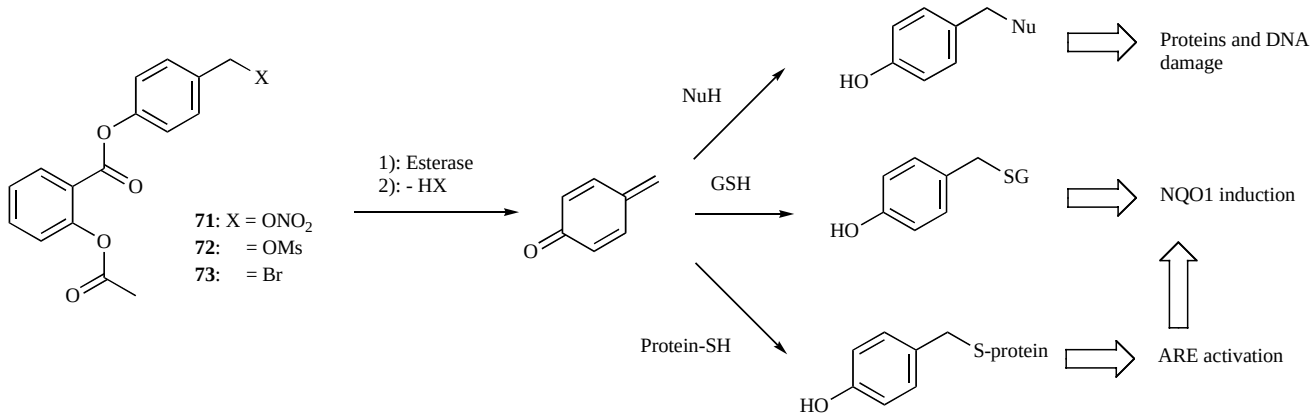


Fig. (10). Example of reactions undergone by several derivatives of acetylsalicylic acid that generate QM (Nu = diverse nucleophiles such as amino groups of proteins, DNA, etc.).

that can generate QM intermediates according to their capacity to form covalent adducts with various biological nucleophiles, but it should be taken into account that this classification is only based on the isolated adducts that have been reported in the literature, and it should be taken with caution.

MODULATION OF QM REACTIVITY TOWARDS NUCLEOPHILES

Drugs with QM Intermediates that React Preferentially with DNA

Pande and colleagues have developed an experimental model to study the reactivity of o-QMs (**2**) generated *in situ* in the presence of synthetic DNA [86]. They have shown that, just as other alkylating agents, such as cisplatin, this particular QM binds with a great selectivity to particular bases. Of the isolated bases and single-stranded DNA, cytidine was alkylated at the highest rate, followed by guanidine (~ 10-fold lower) and adenine (~ 30-fold lower). Thymine showed no detectable alkylation. On duplex DNA, guanosine was more prone to react, followed by cytosine and adenine (with 10- and 20-fold lower rates, respectively), while thymidine was virtually unreactive. The prevailing sites of reaction were the exocyclic amino groups.

A compound named CC-1065 (**74**, Fig. **11**) (along with more recent duocarmycin derivatives) is among the most potent antitumor agents discovered to date [86-91]. Strictly speaking, CC-1065 is not a QM, but the cyclopropane moiety present on its structure is, from a reactivity point of view, a bioisosteric form of the methide found in QMs. It binds preferentially to double-stranded B-DNA within the minor groove, with a sequence preference for 5'-d(A/GNTTA)-3' and 5'-d(AAAAA)-3', and it alkylates the N3 position of the 3'-adenine with its left-hand CPI segment [92-93].

The heterocyclic derivatives, 3-oxo-3*H*-pyrazolo*[1,5-a]*indoles **(**Fig. **12**), are also compounds that generate reactive alkylating QMs that mainly interact with DNA (they are structurally related to **25a**, **25b**, **27a**, **27b**, **28a**, and **28b**). These compounds, analogs of QMs, showed some cytotoxicity against some cancer cell lines, but were ineffective in an *in vivo* test against murine leukemia L1210 [94].

Drugs that Release QMs that React Preferentially with Protein Thiols and/or Amino Groups from CYS, HIS and LYS Proteins

Among NO-containing compounds, O-NO drugs (compound **20** in Table **2** and **71**, **72** and **73** in Fig. **10**) are known to induce GSH depletion through the release of a QM intermediate, in response to which, the cell initiates a variety of signaling pathways, including caspase 3 activation, which leads to cytotoxic and cytostatic effects [26].

Cathechol estrogens (naturally occurring compounds equilin, **75,** and estrone, **80**, in Fig. **13**) and their terpenic derivatives (**5**, **6**, and **7**), whose decomposition products include p-QM intermediates, are recognized as enhancing drugs for breast cancer risks [95-97]. These QM intermediates react preferentially with GSH, leading to GSH depletion, which can cause death of both tumorigenic cells, as well as normal cells, depending on the cell lines.

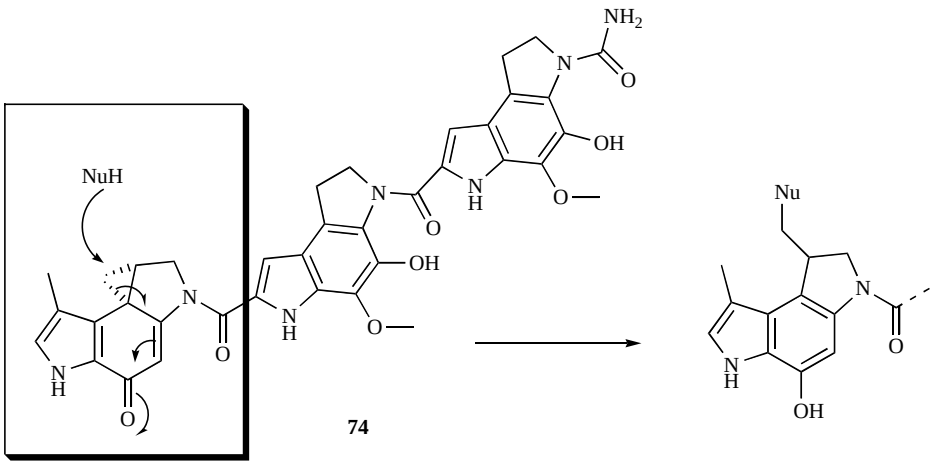


Fig. (11). QM-analogue (+)-CC-1065 (**74**) and its addition product with nucleophiles (NuH).

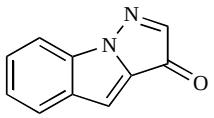


Fig. (12). General structure of pyrazolo[1,5-a]indole derivatives.

The QMs derived from naphthoquinones drugs (**29**-**32** and **51**) promote oxidative stress by a nucleophilic attack on GSH, as do also other quinoid compounds, such as menadione and plumbagine [98]. This has also been demonstrated with "simpler" derivatives of naphthoquinones, produced by photochemical reactions (**83** and **86**, see Fig. **14**) [99]. These species, although not closely related to standard QMs, as they undergo a [1-8] addition, can be generated in situ by irradiation between 310 and 360 nm. The QMs originating from binaphtols were 100-fold more reactive that the naphthyl QMs, and they were also produced from the water-soluble leaving group, trimethylammonium iodide; the QMs obtained were also able to alkylate DNA. This approach could lead to a more specific photodynamic treatment of localized cancers by direct irradiation into the tumors.

The antioxidant agent, BHT, and its derivatives **(44, 45** and **49)** are known to give several adducts with amino or thiols groups of specific proteins [48].

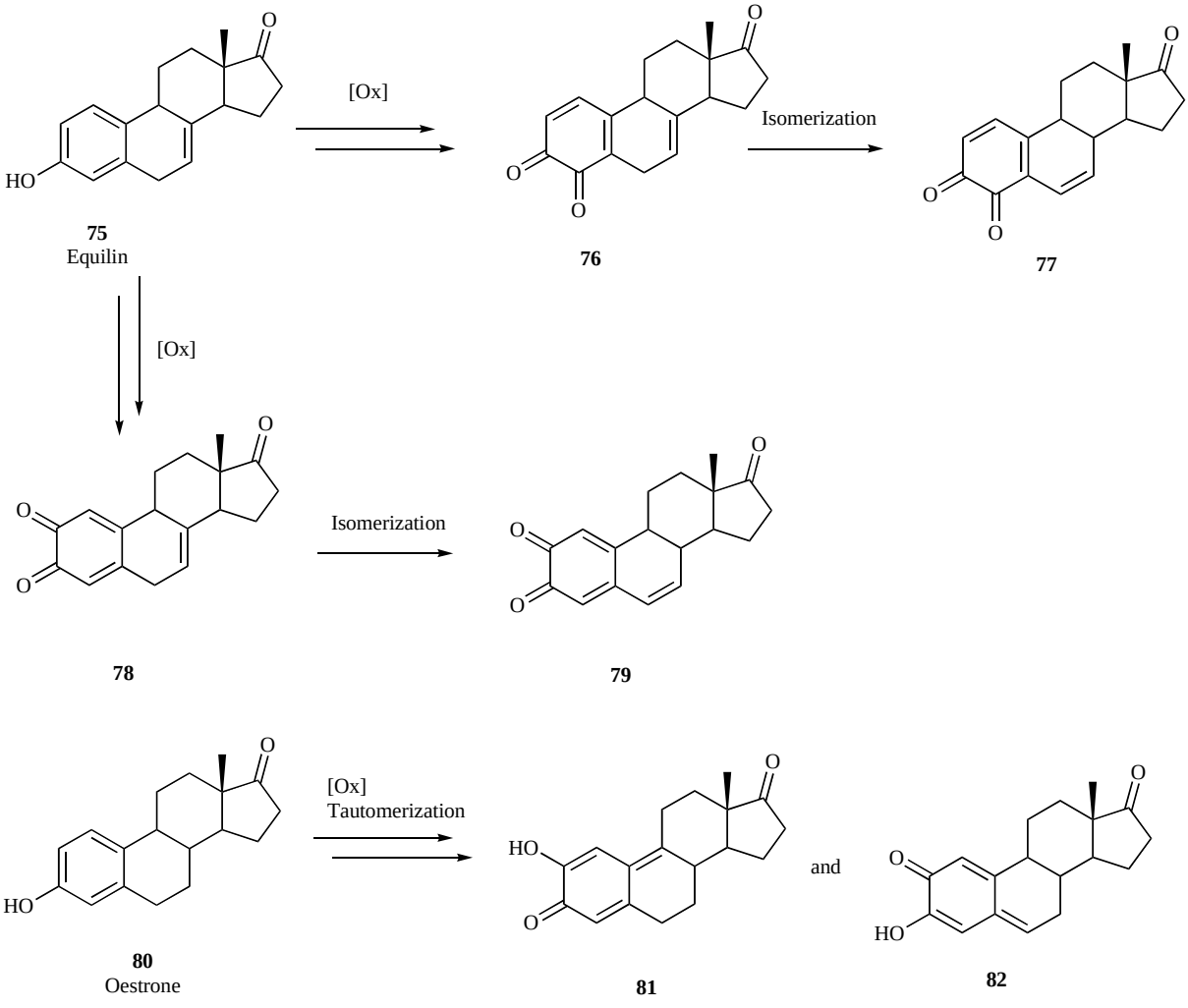
The bis N,N-[(8-hydroxyquinoline)methyl]-substituted benzylamines, such as JLK1486 (**89**, Fig. **15**), are potent antitumor drugs. Their antiproliferative activities are strongly correlated with their ability to generate relatively stable and moderately reactive QM intermediates, which react with thiols but not with DNA, probably due to their low electrophilicity and, consequently, low reactivity with weak nucleophilicity [100].

Drugs with QM Intermediates that can React with Thiols and Nucleosides

The QM metabolites of TAMO (**8**) and some related drugs (**9**, **12**, **14**, **15** and **17**) are known to react with both GSH and DNA through binding to the exocyclic N7 amino group of adenine [101-105]. However, some doubt remains regarding this, because some of these metabolites have been found to be extraordinarily stable, so that no adduct could be observed *in vitro* with DNA [105].

GENERATING SPECIFIC QM COMPOUNDS AGAINST CANCER CELLS: IS IT POSSIBLE?

The recent integration of novel affinity chemistries for electrophilic probes, shotgun proteomics methods and systems-modeling tools has led to the identification of hundreds of protein targets of electrophiles in mammalian systems. The technology now exists to map the targets of damage to critical components of signaling pathways and metabolic networks and to understand mechanisms of damage at a systems level. The chemical knowledge to conceive drugs that incorporate QM moieties or drugs that could produce QM intermediates after biological activation, has been, at least in



75 Equilin **76** **77** **78** **79** **80** Oestrone **81** **82**

Fig. (13). Estrogens and their quinone and QM metabolites.

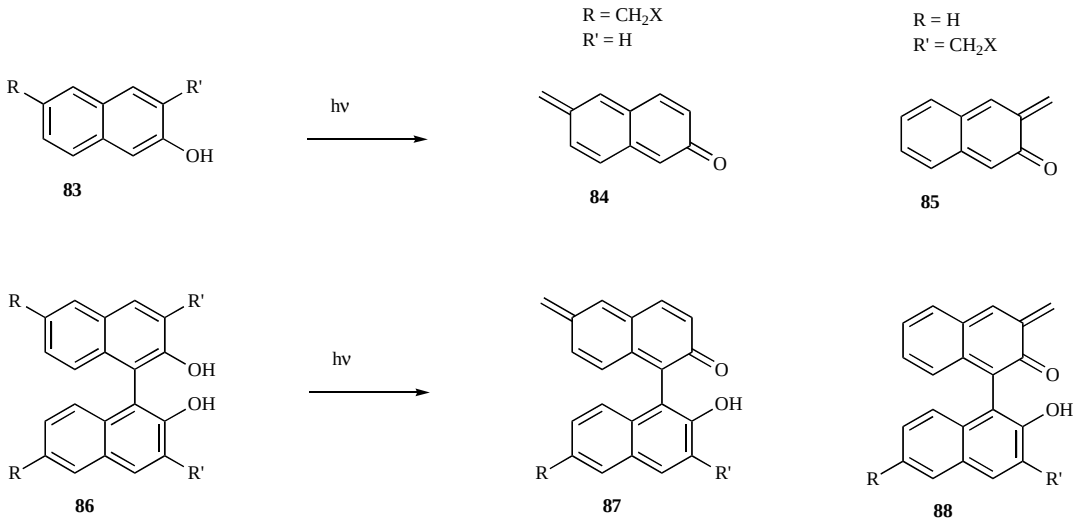


Fig. (14). QMs generated by photochemical reactions (X = -OH. NMe_2 or $N^+Me_3I^-$).

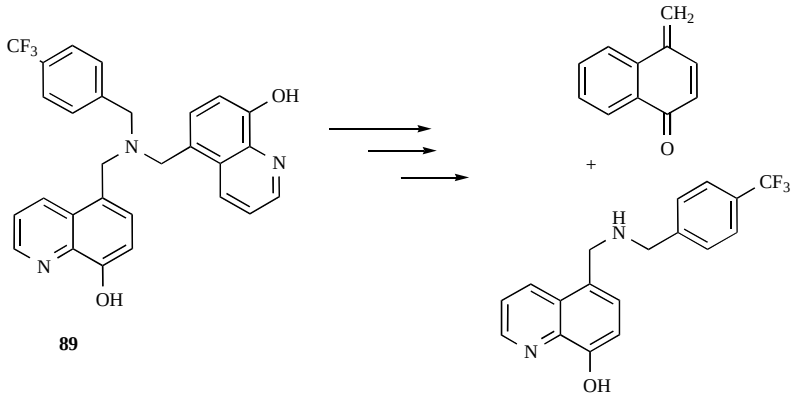


Fig. (15). Structures of JLK1486 (**89**) and the QM generated.

part, overcome by synthetic chemistry. Through subtle structural modifications, such as the extension or reduction of QM conjugation, the stability and reactivity of such drugs can be modulated, and, therefore, one can expect that those QMs may react with different selectivities. Nevertheless, the main obstacle, which remains to be overcome if one wants to use QMs as an efficient weapon against cancer cells, is the ADMET properties of such drugs. This ADMET obstacle is general for all drugs, whatever their pharmaceutical application, but probably due to their high chemical reactivity and general short stability, these QM drugs are particularly sensitive molecules to ADMET parameters. Among these ADMET parameters, toxicity is probably the most important, as a major challenge in toxicology is to understand how reactive intermediates trigger signals that lead to cell death, stress responses or adaptation to stress. Finally, the *chemist* can design chemically reactive metabolites, including QMs derived from small organic molecules or small molecules including QM moieties, the *drug metabolist* can determine the propensity of a novel entity to undergo bioactivation in model systems from expressed enzymes, genetically engineered cells, or whole animals, and *drug safety experts* can determine the time course of the toxicity, the nature of the toxicity and the direction that the toxicity takes in a particular patient. The taskforce that results from the unification of these three specialists could provide the possibility to use QMs as powerful clinically practical drugs.

CONCLUSIONS

There is overwhelming evidence that chemically reactive metabolites that are derived from simple, small molecules and therapeutic drugs are often the causes of a wide range of undesirable effects and hepatic injuries. Nevertheless, the reactive intermediates generated by drugs or simple molecules directly or after bioactivation could be also exploited for therapeutic purposes, predominantly in the field of cancer chemotherapy. In this review, we have pointed out the specific reactivity of QM intermediates, known as electrophilic intermediates, generated from small molecules that react with biological nucleophiles to give covalent bonds with proteins. Those protein alkylation reactions induce cellular biological pathway events that, in some cases, lead to antiproliferative cellular effects, and consequently, those compounds could be promising anticancer drugs. The medicinal chemists can address this latter issue by seeking to modulate the reactivity of QM intermediates through different means, firstly, through the introduction of specific substituents on molecules, incorporating a QM moiety into their structure. Such substitutions modulate the electrophilicity of the QM and, consequently, its reactivity and selectivity toward the different biological nucleophiles (e.g., amines, alcohols, thiols) generally encountered on proteins, DNA or any other biological target. Examples of drugs classified as alkylating anticancer drugs that incorporate a QM moiety in their structure have also been described to alkylate DNA guanines. Secondly, molecules can also be designed to undergo QM intermediates only after bioactivation. In this case, the reactivity of the generated QM can be also modulated by the introduction of specific substituents on the parent drug.

The challenge for the medicinal chemist is to acquire a more fundamental understanding of the impact of the binding of QM intermediates on specific proteins or DNA and of the pathway ef-

fects or injury phenotypes that can be exploited in order to overcome the proliferation of cancer cells. Emerging evidence from studies with electrophile-affinity probes clearly shows that different binding patterns lead to distinct biological responses. The crucial question remains to identify which protein or DNA binding site (or which biological target) is the most relevant in the reaction with the QM to induce the desired response or whether there is an antiproliferative effect on tumor cells. To answer these questions, it is mandatory to analyze the specific adducts formed between the QM intermediates and the biological nucleophiles and to analyze protein damages that are involved in the observed antiproliferative activity of such drugs. At the same time, the possible role of such QM intermediates should be defined, and how those intermediates interfere with signaling and the sequence of molecular events that impair cell defense should be elucidated. Only when such a mechanistic framework is well established can we envision QM-generating molecules as promising chemotherapeutic drugs.

ABBREVIATIONS

ADMET = Adsorption, Distribution, Metabolism, Excretion, Toxicity

ARE = antioxidant responsive element

BHT = Butylhydroxytoluene

BSO = buthionine sulfoximine

CR = Carbonyl reductase

CYP = cytochrome P450

DHFR = dihydrofolate reductase

DQM = diquinone methide

ECG = epicatechin gallate

ERK = Extracellular signal-regulated kinases

FR = free radical

GSH = Glutathione

GSTP1-1 = glutathione S-transferase P1-1

Hsf1 = heat-shock transcription factor 1

HSP90 = heat-shock protein 90

IκBα = nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha

IKKβ = Inhibitor of nuclear factor kappa B kinase beta subunit

KEAP1 = Kelch-like ECH-associated protein 1

LG = leaving group

MAPK = Mitogen-activated protein kinase

NEV = Nevirapine

Nrf2 = NF-E2-related factor 2

NSAID = non-steroidal anti-inflammatory drugs

NF-κB = nuclear factor κB

NQO1 = NAD(P)H-dependant quinone oxireductase 1

ODC = ornithine decarboxylase

QM = quinone methide

RA = Raloxifene

ROS = reactive oxygen species

SAR = structure-activity relationship

SERM = Selective estrogen receptor modulators

SOD = superoxide dismutase

TAMO = Tamoxifen

TMECG = trimethylepicatechin gallate

TYR = tyrosinase

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