Recent Advances in Bioreductive Drug Targeting

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Abstract: Advances in the chemistry of bioreductive drug activation have led to the design of hypoxia–selective drug delivery systems. These prodrugs, comprising a bioreductive "trigger", "linker" and "effector" were first explored with nitrobenzyl quaternary ammonium mustards. Alternative nitroheterocycles were subsequently developed, together with new avenues of prodrug activation in ADEPT and GDEPT. Major advances have also been made in utilising indolequinone reductive chemistry based upon an appreciation of the kinetics of oxygen–sensitive reductive elimination.

Hypoxia and hypoxia/reperfusion injury are inherent and potentially exploitable features of most solid tumour types as well as other common conditions such as arthritic joints and inflammatory bowel disease. Where tumour therapy is concerned, hypoxic cells present a major therapeutic challenge because of their refractory properties with regard to radiation therapy and due to resistance conferred to many cytotoxic chemotherapeutic drugs. Tumour hypoxia is consequently a prognostic indicator for survival following radiotherapy [1]. Hypoxic cells also present a major therapeutic opportunity for the real targeting of already established drugs, both anti–tumour and anti–inflammatory, *via* bioreductive prodrug systems. The more recent developments in the organic and medicinal chemistry of these hypoxia–selective prodrugs is the focus for this review.

Bioreductive drugs have grown rapidly in interest and stature in the past ten to fifteen years, with the emphasis on compounds that are chemically reduced selectively and intracellularly to form active cytotoxic agents. The rationale for the development of this kind of therapeutic entity centres on the concept that compounds which are metabolically reduced to a cytotoxin should be more cytotoxic to hypoxic tumour cells than to normal oxygenated ones. Selectivity occurs because reduction involves free radicals, which react rapidly with oxygen to form superoxide radicals, which inhibits drug reduction in normal tissues [2]. The development of bioreductive drugs has been closely related to, and has indeed grown from the field of hypoxic cell radiosensitisers for use as adjuncts to radiotherapy [3,4]. The mechanism of radiosensitisation is through fast free radical mechanisms, and the abilities of compounds to act in this way is related to their redox properties [5-7]. This is also true of bioreductive drugs, and redox–related factors are key properties in the design of drugs selectively active in hypoxia, as has been recently and extensively reviewed by Wardman [8]. The selective hypoxic toxicity of drugs, in particular the nitroimidazoles, of interest as radiosensitisers [9,10] was first noticed by Sutherland in cellular spheroids close to the areas of central necrosis [11]. This was later shown to be hypoxia mediated and the development of drugs activated by bioreductive metabolism followed. The term "bioreductive drug" was first used by Sartorelli in relation to the metabolism of mitomycin C (MMC) [12], an archetypal drug which has since become the platform for many of the more recent advances in the field [13]. A number of classes of bioreductive compound are now known and some have reached advanced stages of development including clinical evaluation, exemplified by the *N*–oxide tirapazamine (SR 4233, **1,** Figure (1)) [14], the indolequinone EO9 (**2**) [15] and the 2–nitroimidazole RB6145 (**3**) [16]. These drugs act as substrates for one or more of the reductases present in most cells and can be targeted towards both solid tumours with defined hypoxic fractions and tumour tissues rich in the required activating enzymes. Thus, bioreductive drugs have become a significant weapon in the armoury of agents effective against these targets [13,17-24].

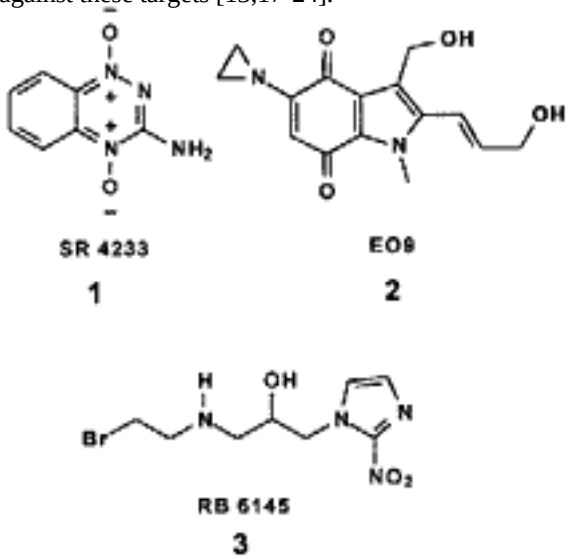

Fig. (1).

It is only comparatively recently that the release of a drug, mediated by selective reduction of a prodrug has been the focus of attention. The aim of this strategy has been to

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1389-5575/01 $20.00+.00

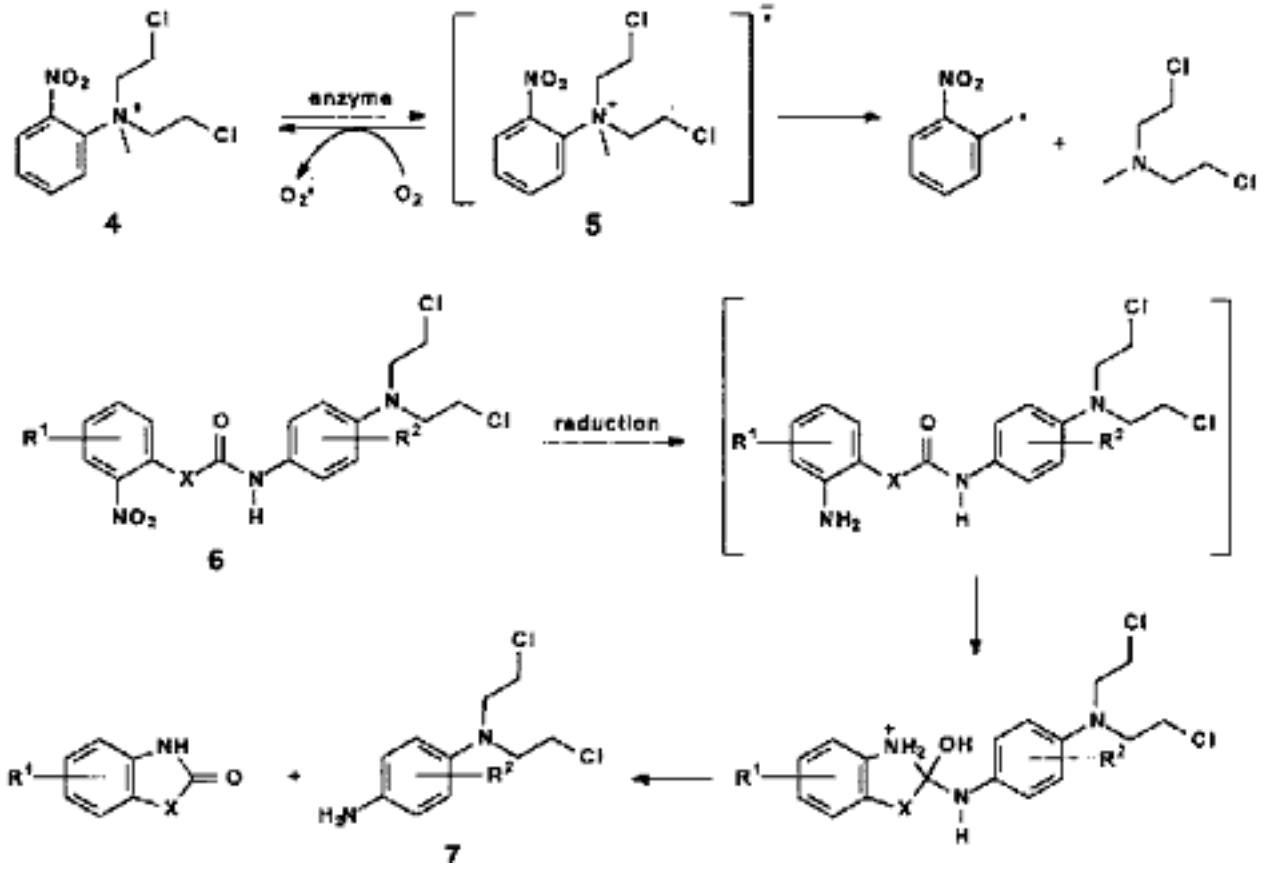
improve the biodistribution of such drugs, which have been described as being composed of Trigger, Linker and Effector units [13]. A bioreductive drug would ideally provide a bystander effect, whereby a diffusible cytotoxin (or other "effector") could be released upon bioactivation (reduction of a "trigger") so that a small proportion of hypoxic cells in a solid tumour could be maximally exploited. It was also recognised that this process could be an enzyme specific means of targeting[4,25]. To date a number of standard classes of bioreductively–activated "trigger–effector" compounds have been evaluated in both areas, with the initial studies focused upon nitroaromatic compounds, the first examples having been reported by Firestone *et al*. [26].

NITROBENZYL COMPOUNDS

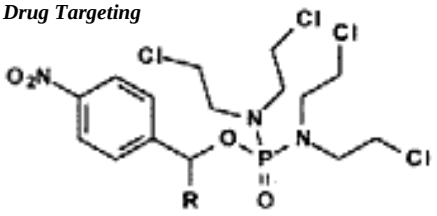
Nitrobenzyl quaternary ammonium salts are known to undergo fragmentation on reduction[27,28]. Hence, nitrobenzyl prodrugs containing latent cytotoxic species were designed to exploit this characteristic[29]. In the absence of oxygen, enzyme induced one–electron reduction of *N*,*N*–bis(2–chloroethyl)–*N*–methyl–*N*–(2–nitrobenzyl)ammonium chloride ((**4**) Scheme (1)) generated a reactive nitrogen mustard, mechlorethamine, and a nitrobenzyl radical *via* a putative radical anion intermediate (**5**). The parent prodrugs possessed a number of attractive properties. The reduction potential for compound (**4**) of –320 mV, determined by cyclic voltammetry, fell within the –300 to –450 mV range considered desirable for hypoxia–selective cytotoxins. Water solubility was good, and the mustard moiety was deactivated by the positive charge on the nitrogen atom. The 3–nitro and 4–nitro regioisomers were also investigated as part of the study. The 3–nitro analogue was non–toxic in cultures of Chinese hamster ovary cells under both aerobic and hypoxic conditions. Both the 2– and 4–nitro isomers demonstrated hypoxia–selective toxicity (200– and 8–9–fold respectively) and, as a result, led to the further investigation of other nitrobenzyl related triggers. Experimental data also provided evidence for the reductive activation of these compounds rather than thiol activation, as was initially proposed. In actual fact, the 2–nitro conjugate was remarkably stable to diethyl dithiocarbamate in buffered solution for 2 days at 37°C.

Alternative means of releasing "effector" mustards from bioreducible prodrugs have been explored. For example, in efforts to separate the optimisation of substituent effects influencing nitro–group reduction and mustard reactivity, which have opposing electronic requirements, Atwell *et al*. reported 2–nitroaryl amides (**6**) (Scheme (1)) which cyclise to "extrude" the active mustard (**7**) [30]. This chemistry could clearly be adapted for the bioreductive delivery of other pharmacologically active amines.

Exploration of the use of phosphoramidite deactivated mustards in combination with a nitrobenzyl trigger (Compounds (**8**)–(**12**) Figure (2)) [31], yielded, as expected,



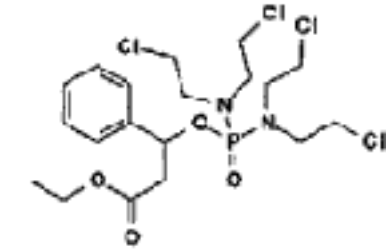
Scheme 1.



8 R = H
9 R = CH_3
10 R = $CH_2CO_2CH_2CH_3$
11 R = $CH_2CO_2CH_2CH_2N(CH_3)_2$
12 R = $CH_2CO_2CH_2CH_2CH_2N(CH_3)_2$

Fig. (2).

greater cell kill when HT–29 cells were treated under hypoxic conditions. The greatest differential in cytoxicity belonged to the α–methyl substituted analogue (**9**) with an aerobic/hypoxic toxicity ratio of >90. The inactivity of compounds (**13**) and (**14**) (Figure (3)), afforded further proof of the necessity of having a nitro group and an electrophilic substituent, in this case bound to nitrogen to form a latent mustard, for cytotoxity. Further experimental data, from aerobic bone marrow progenitor cells, suggested that cellular prodrug activating mechanisms in addition to bioreduction were in operation. In some recent related work Shyam *et al*. report on the development of prodrugs of the 1,2–bis(sulfonyl)–1–(2–chloroethyl)hydrazine family of cytotoxic agents using similar reductive activation chemistry [32].



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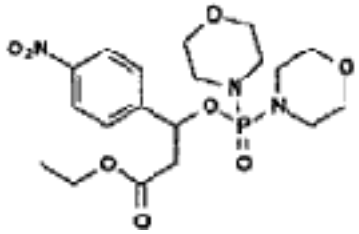
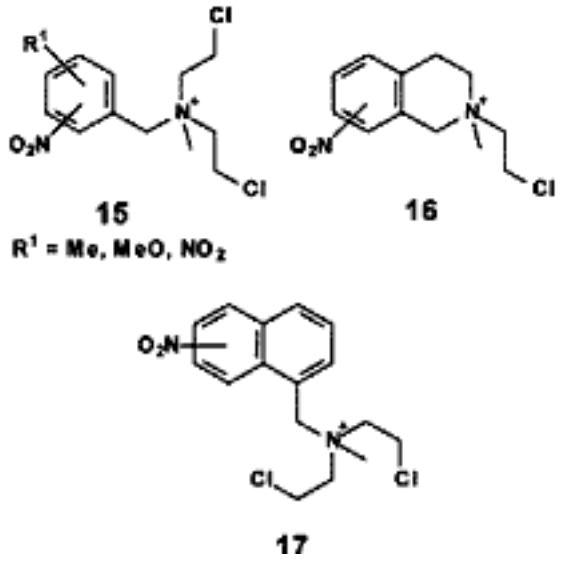


Fig. (3). **14**

In 1996, Tercel *et al*.[33] reported the evaluation of a series of mono– and disubstituted nitrobenzyl compounds possessing both single and fused ring systems. The new substituted benzyl (**15**), tetrahydroisoquinolium (**16**) and naphthyl (**17**) analogues (Figure (4)), generally possessed favourable physicochemical properties but, on the whole, were coupled with indiscriminate toxicity. The need to 'fine–tune' prodrug activation thus making them more selective assumed a greater urgency in bioreductive drug discovery. New avenues of prodrug activation were soon explored in response to rapid advances made in molecular biology, including ADEPT (antibody–directed enzyme prodrug therapy) and GDEPT (gene–directed enzyme prodrug therapy).



15 **16**

R^1 = Me, MeO, NO_2

17

Fig. (4).

ADEPT exploits enzyme activation of a non–toxic prodrug using a tumour specific enzyme–antibody conjugate. In some cases, the enzyme utilised is nitroreductase (NTR) which can be isolated from *Escherichia coli* B, and in conjunction with NADH or NADPH will reduce certain aromatic nitro– groups to the corresponding hydroxylamine. An important factor of the ADEPT drug delivery concept is that prodrugs should not be activated by endogenous enzymes. This approach was encapsulated by Mauger [34] who attached various drugs (effectors) to a nitrobenzyl trigger *via* a carbamate linker (Figure (5)). Effectors such as actinomycin D (AMD), doxorubicin and mitomycin C (MMC) were chosen so that, upon derivatisation, they would become far less toxic than the respective parent. Previous studies showed that *N*–acylation of these compounds significantly reduced their biological activity. Exposure of these self–immolative prodrugs to NTR did indeed generate active species from the AMD (**18**) and MMC (**19**) conjugates. However, the prodrug of doxorubicin (**20**), a substrate for NTR, did not fragment according to HPLC analysis, thus in this case the intermediate hydroxylamine was not self immolative.

The nitrobenzyl trigger and carbamate linker was further applied in GDEPT investigations by Hay and co–workers [35]. In this case, GDEPT was based on transfecting cells with an NTR gene which in turn would express the requisite enzyme within tumour cell lines. The enzyme thus activates suitable non–toxic prodrugs into cytotoxic species. Enediynes were chosen as effectors due to their superlative potency (generally in the low pM region). It was postulated that reduction of a nitro– moiety, with subsequent prodrug fragmentation, would allow the enediyne core to adopt a conformation beneficial for Bergman cyclisation (Scheme

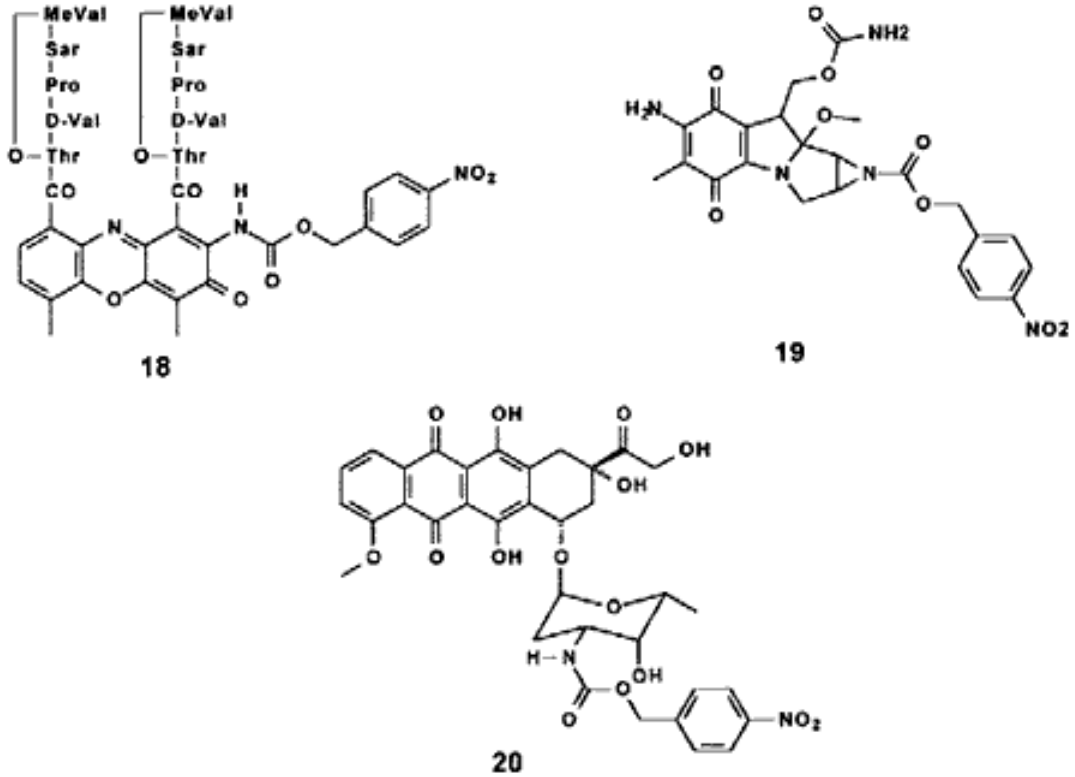
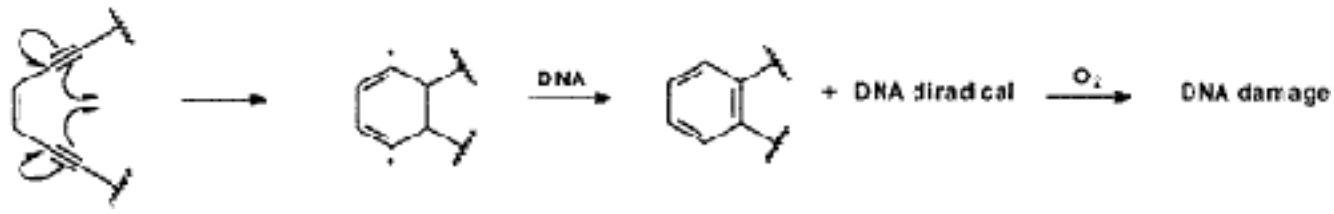
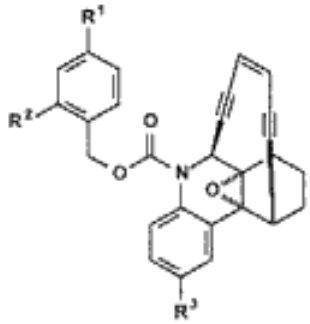


Fig. (5).

(2)), a process which ultimately causes double strand DNA breaks *via* a diradical initiated cascade reaction. As in previous investigations (*vide supra*), prodrugs of both 4–(**21**) and 2–nitro (**22**) regioisomers (Figure (6)) were evaluated alongside an analogue containing a pendant hydroxyethyl chain (**23**). Intracellular NTR activated the 4–nitro to a greater extent than the 2–nitro isomer in transfected cell lines (SC3.2, WC14.10, T79–A3). The tethered analogue provided the greatest activity differentials across the board. Unfortunately for this class of prodrugs, the enediyne core requires oxygen for maximum potency, particularly against efficient self repairing cells. Ultimately, this requirement will effectively lower the efficacy of these compounds in hypoxic tumour regions.



Scheme 2.



21 $R^1 = NO_2$, $R^2 = H$, $R^3 = H$

22 $R^1 = H$, $R^2 = NO_2$, $R^3 = H$

23 $R^1 = NO_2$, $R^2 = H$, $R^3 = OCH_2CH_2OH$

Fig. (6).

NITROIMIDAZOLES

In an effort to expand and improve nitroaromatic trigger utility, various other heterocyclic cores have been studied, as indeed occurred during the height of radiosensitiser development [36-40]. Nitroimidazoles were a fairly obvious choice of electron–affinic heterocyclic moiety due to their established bioreductive properties [41-45] and existing utility in medicine [46]. Hay and co–workers reported some studies on the attempted "extrusion" of mustard–type compounds from reduced nitroimidazoles [47]. However, Everett *et al*.[48] were the first to report on reductive elimination of a leaving group (bromide (**24**), salicylate (**25**), aspirin (**26**)) from a reduced 2–nitroimidazole (Figure (7)). The 2–nitroimidazoles were reduced by a one electron donor, $CO_2^{\bullet-}$, which was generated by γ–radiolysis of a buffered

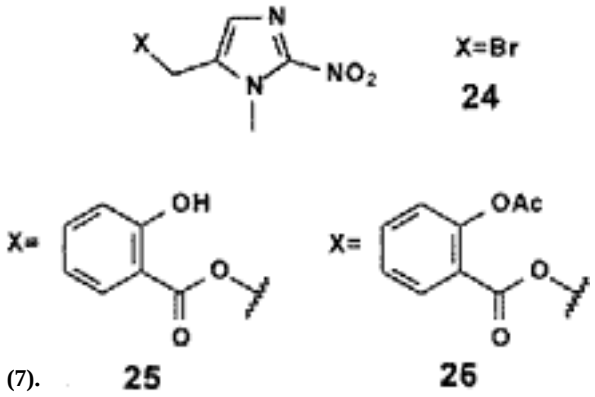
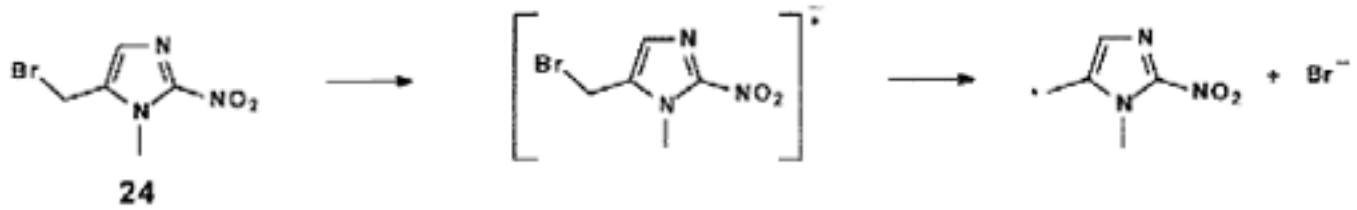


Fig. (7).

aqueous solution. Reaction kinetics revealed that bromide was eliminated from a one–electron reduced species, i.e. the radical anion (Scheme (3)). In contrast, the reduction stoichiometry inferred that the salicylate and aspirin trigger appended to a poly(ADP–ribose) polymerase (PARP) inhibitor, 5–bromoisoquinol–1–one, as effector ((**28**) Scheme (5)). PARP inhibitors act as radiosensitisers by interfering with repair of radiation damaged DNA. Reductions were carried out using: sodium borohydride / palladium / methanol, zinc / ammonium chloride and stannous chloride as mimics for hypoxia–induced bioreduction. Both the 'palladium' and 'zinc' conditions yielded the drug along with some over–reduced (debrominated) material. However, analysis of the stannous chloride reaction indicated the absence of free drug even though the nitro– group was reduced. Presumably the stannous chloride acts as a Lewis acid by complexing with amino / hydroxyamino intermediates, thus removing the driving force for drug release.

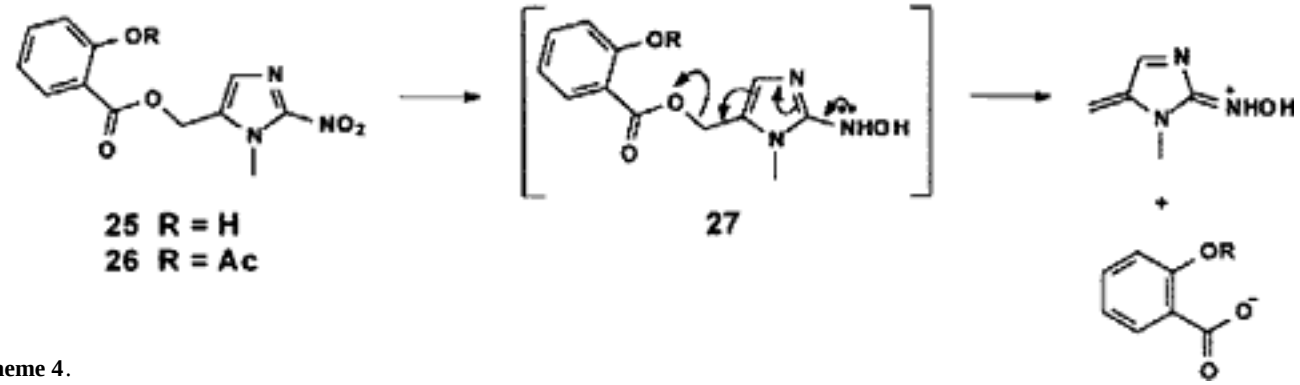
Hay *et al*. embellished their previous ADEPT and GDEPT work (*vide supra*) by targeting amino–*seco*–CBI–TMI, an effective DNA minor groove alkylating agent, in



Scheme 3.

conjugates required a four–electron reduction and subsequent elimination from a hydroxylamine intermediate (**27**) (Scheme (4)). Thus, it was recognised at an early stage that leaving group properties influence this type of chemistry and in particular the rates of bioreductive elimination.

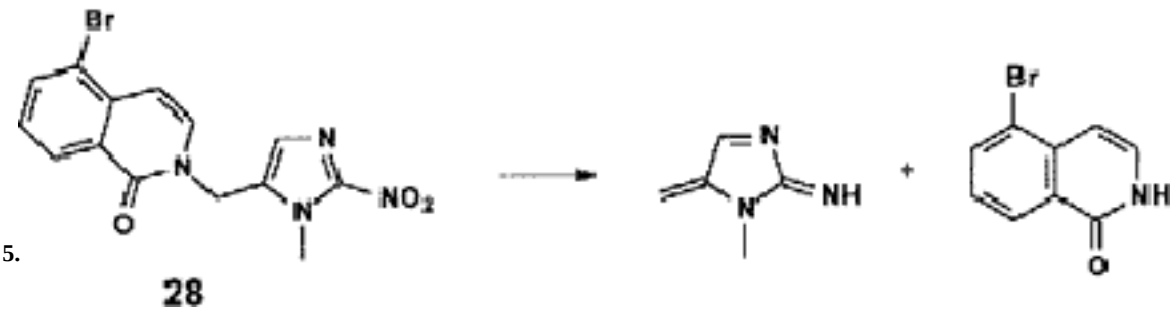
association with the 1–substituted–2–nitroimidazole trigger ((**29**) Figure (8)) [50]. This prodrug was tested for cytotoxicity in human ovarian carcinoma cell lines (SKOV3) using exogenous NTR with NADPH to mimic potential ADEPT conditions. Results revealed minimal toxicity when



Scheme 4.

Parveen and co–workers [49] have described related studies with nitroimidazoles. They used a 2–nitroimidazole

compared with the free amine, the masked amine alone or in the presence of co–factor (NADPH) without NTR. However,



Scheme 5.

an 11–fold increase in prodrug activation was duly recorded with the extracellular NTR / NADPH combination. GDEPT experiments were carried out with the suitably transfected SC3.2 cell line expressing the NTR enzyme, and produced a 21–fold activity increase (IC_{50} of 3.5 nM) over the normal type SKOV3 cell line (IC_{50} of 75 nM). Interestingly, the results outlined above were conducted under oxic (20% O_2 in the gas phase) conditions. The prodrugs were then tested using the SKOV3 cell line under hypoxic (<0.01% O_2) conditions, and demonstrated preferential cytotoxicity in this environment (15 to 40–fold increase). It could therefore be concluded that the nitroimidazole conjugate was a substrate for one electron reductases *in vitro*.

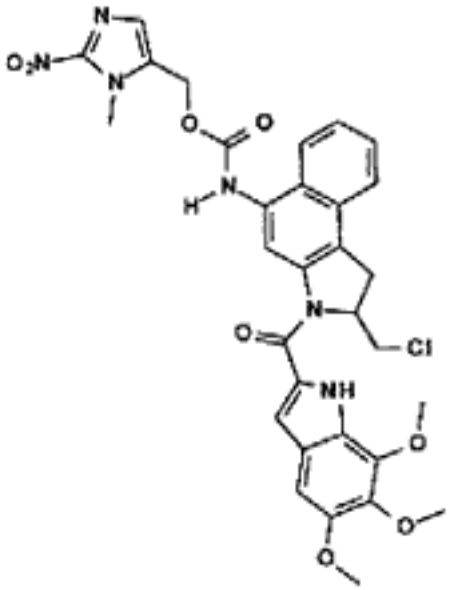


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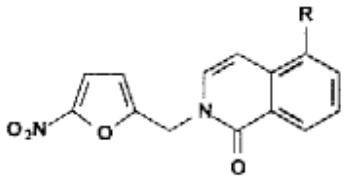
Fig. (8).

OTHER NITROHETEROCYCLIC COMPOUNDS

As previously discussed [49], PARP inhibitors were being used in bioreductive drug delivery experiments using the nitroimidazole trigger. However, these studies were superceded by the use of a 2–nitrofuran moiety ((**30**) Figure (9)) [51]. This heterocycle possesses a relatively high reduction potential (suggested to be in the region of *ca*.–325 mV, but with many compounds reported with much higher values) [36,52] which, it was surmised, would be beneficial for tumour specific reduction by endogenous enzymes such as cytochrome P50 reductase. The trigger was also attached to an alkylboranylamine ((**31**) Figure (9)) *via* a carbamate linker for investigation into boron neutron capture therapy (BNCT). Irradiation of the ^{10}B isotope with slow ('thermal') neutrons induces an (n, α) reaction, which yields ^{7}Li and an α–particle possessing kinetic energy equivalent to 2.31 MeV. Biological damage can be inflicted by the moving α–particle within one cell diameter of its origin, thus affecting only those cells containing boron. Preliminary studies revealed a 60% inhibition of the PARP enzyme by the prodrug versus >95% inhibition by the isoquinoline drug in L929 murine areolar cells. Biological activity of the BNCT prodrugs was not assessed. Biomimetic chemical reduction of these compounds, using sodium borohydride / palladium / propan–2–ol, furnished the carboranyl propylamine effector which was subsequently trapped as a benzyl carbamate (CBZ group) in 26% yield.



30 R = H, Me

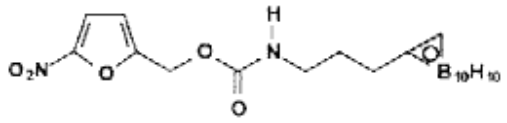


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Fig. (9).

Alternative substituted heterocycles were hitherto unexplored until the work of Borch *et al*. [53] who outlined the synthesis and *in vitro* evaluation of some bioreductively–activated nitroheteroaryl phosphoramidite mustards. Alluding to their previous work (*vide supra*), it was known that halopropyl mustard side–chains, pendant on a phosphoramidite conjugate, would be inactive under oxic conditions but become cytotoxic under anoxia. A wide range of nitroheterocyclic moiety were investigated as potential triggers ((**32**) Figure (10)). Cytotoxicity of target compounds was appraised using clonogenic assays against a number of different cell lines (B16 murine melanoma, wild–type and 4–hydroperoxycyclophosphamide resistant MCF–7 human breast cancer cells, and selected compounds against HT–29 human colon carcinoma) under both aerobic and hypoxic conditions. The 4–acetamido, 4–cyano, and 4–dimethylamino analogues of the 5–nitrothienyl triggers elegantly illustrated the difference that electronic and / or steric effects may have on activity. When tested against the HT–29 cell line, a decrease in hypoxic efficacy was demonstrated by all three analogues. Furthermore, this effect was small for the cyano– analogue but significant (50–fold reduction) for the dimethylamino– compound. This was considered to be because of nitrogen lone pair conjugation into the aromatic nucleus, which in turn fixes the *N*–methyl groups in the plane of the ring. Results gleaned from aerobic experiments revealed increased toxicity (1.5–2–fold) for electron withdrawing groups, and a 130–fold decrease for electron donating groups reflecting changes in the reduction potentials of these compounds. The less electron–affinic dimethylamino– analogue is presumably more easily re–oxidised because of its more negative one–electron reduction potential. The greatest hypoxic selectivity was shown by the 5–nitroimidazole conjugate, being virtually non–toxic under oxic conditions whilst demonstrating moderate toxicity against hypoxic HT–29 cells. It was also demonstrated that expression of DT–diaphorase, cytochrome b_5 reductase, and cytochrome P450 reductase in the NCI human tumour cell lines were not wholly responsible for aerobic bioactivation.

Finally, expulsion of the phosphoramidite was examined as a consequence of nitro– reduction, induced in this case by sodium dithionite. Release of the phosphoramidite anion was complete within 4 minutes (as determined by ^{31}P NMR) and the released anion had vanished with a half–lifeof approximately 23 minutes. In the absence of reducing agent, the prodrug was stable under the reaction conditions for over 12 h.

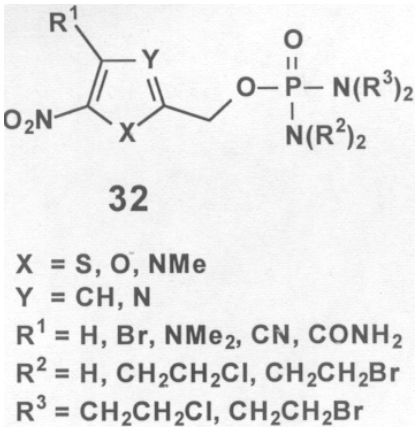
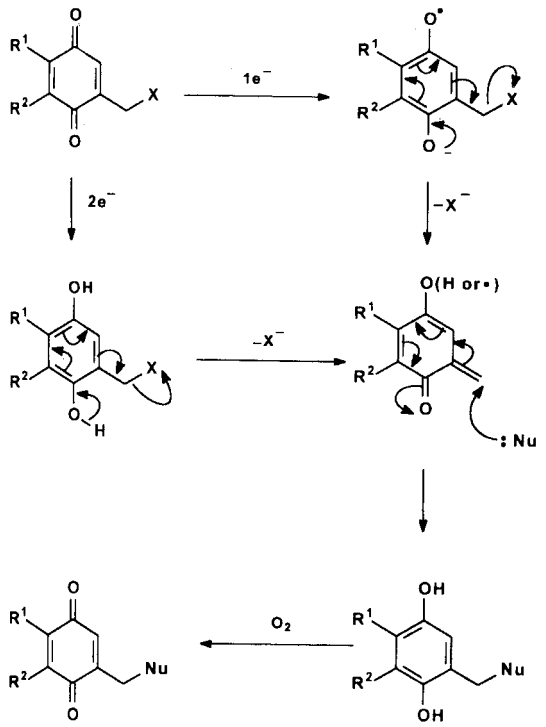


Fig. (10).

QUINONOID COMPOUNDS

Quinonoid compounds have long been known to have the potential to act as bioreductively–activated alkylating agents [54]. The archetypal compound is MMC ((**33**) Figure (11)), an antibiotic isolated from *Streptomyces* species, which cross–links DNA following enzymatic reduction of the quinone moiety and spontaneous elimination of the tertiary methoxy and C–10 carbamate groups [55-57]. MMC is then capable of binding to the N–2 of guanine in DNA *via* the C–1 site or *via* both the C–1 and C–10 reactive sites [58,61]. Similar bioreductive activating processes are implicated in the activation of quinones bearing appropriately placed leaving groups, although without the necessity for the initial activating and ring–opening steps in the MMC activation cascade. The pioneering work in the field centred on the proposition that simple benzo– and naphthoquinones (e.g. (**35**) and (**36**)) with cognate structural features might function in an equivalent manner and that enzymatic reduction of these quinones to semiquinones and hydroquinones would result in spontaneous degradation to a common reactive intermediate (a "quinone methide") with the potential to bind covalently to nucleophiles (e.g. DNA, Scheme (6)) [54,62,63].

Benzoquinones, ((**34**) Figure (11)) [12,62], 2– and 6–methylnaphthoquinone derivatives ((**35**) and (**36**), Figure (11)) [63,64] and anthraquinones (e.g. (**37**) and related



Scheme 6.

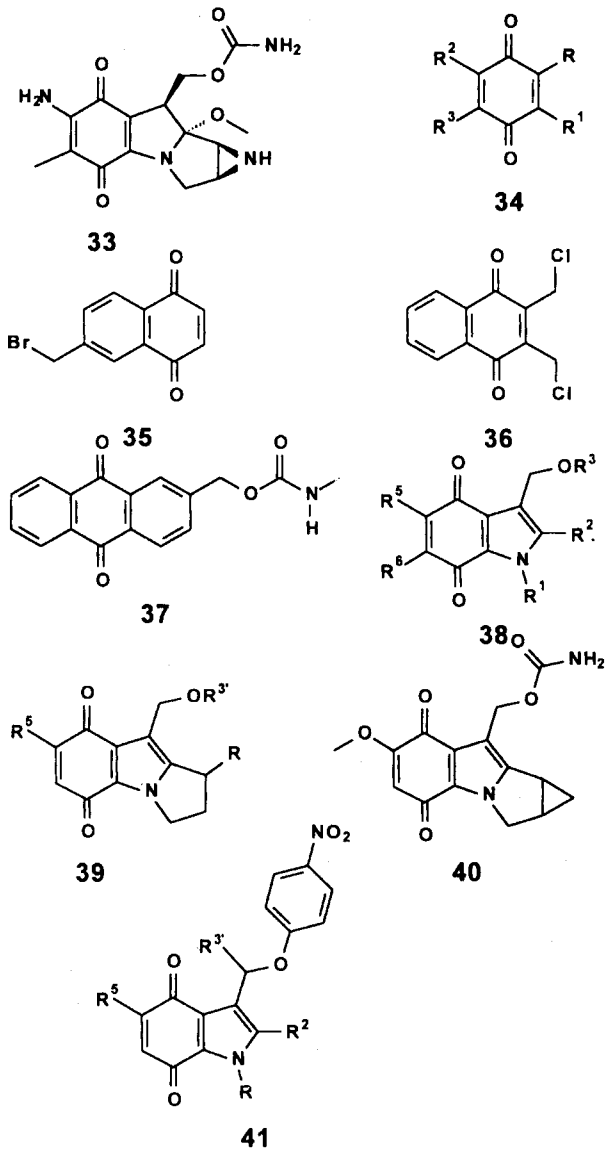
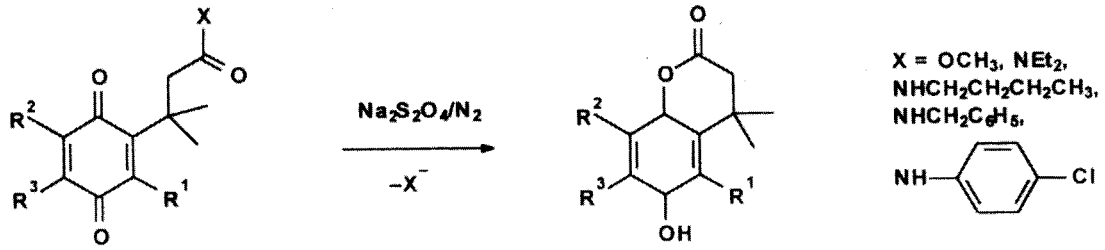


Fig. (11).



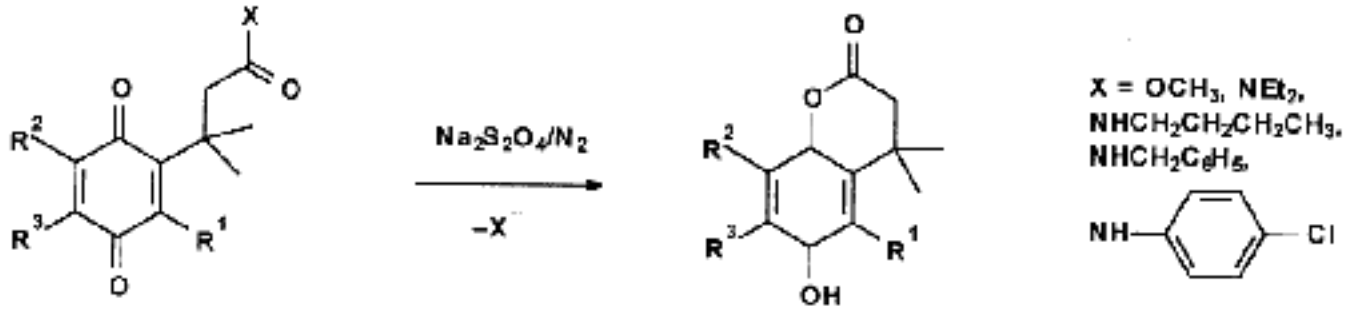
Scheme 7.

compounds) [54] possess selective toxicity towards hypoxic cells *in vitro* and various derivatives possess antitumour activity. Early studies addressed the mode of action of the haloalkyl benzo–and naphthoquinones ((**34**)–(**36**)), however, the potential of these compounds to act as bioreductive prodrug delivery systems was not recognised at that time and only recently has this chemistry been exploited. Particular emphasis in this area has been placed on the indolequinones ((**38**)–(**41**) Figure (11)), in which there has been much interest, although other systems have been explored. For example reductive lactonisation of strategically methylated quinone propionic acid esters and amides (Scheme (7)) [65,66] has been recognised as a potential bioreductive drug delivery system. Although no examples of prodrug compounds of this type have been subsequently reported, this chemistry has been developed towards useful redox sensitive protecting groups [67].

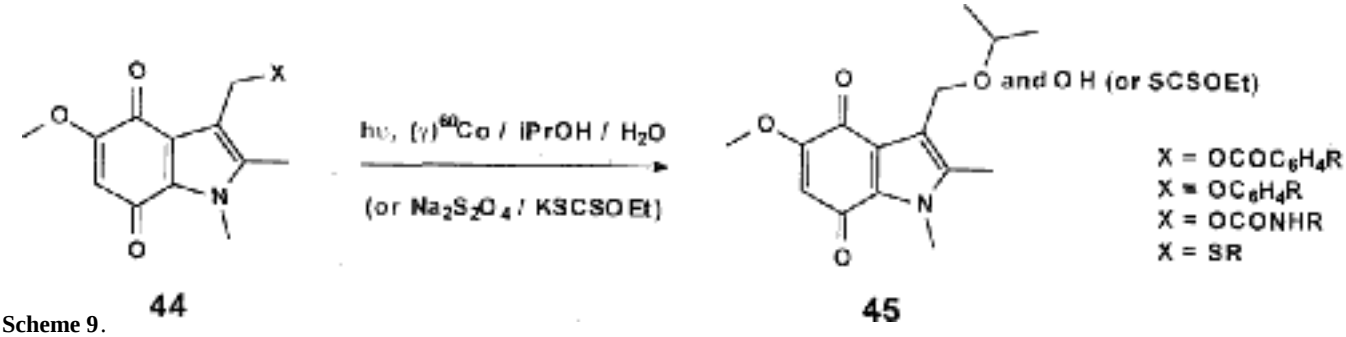
The indolequinone group of bioreductively–activated cytotoxic drugs have shown distinct antitumour potential [15,68,74]. In particular, the diol EO9 (**2**) [15,74] and related mitosenes (**38**), (**39**) [77,71,75,76] have shown improved properties over MMC [55,77-79] and the large number of analogues reported in the past 20 years [80-84]. The mitosene class of agent have an established requirement for reductive activation and the formation of electrophilic species toxic to cells, with the C–10 (or equivalent) position becoming the focus of attention in later studies on the alkylation process. This was achieved through the design and synthesis of the cyclopropamitosenes (**40**) (Figure (11)) in which the electrophilicity at C–1 is reduced through the presence of a cyclopropane ring in place of the naturally occurring aziridine [68,73,85]. It was confirmed that the carbamate group could be eliminated from C–10 of the cyclopropamitosene (**42**) upon chemical reduction with sodium dithionite, and the resulting intermediate (**43**) (Scheme (8)) trapped by added nucleophiles such as potassium ethyl xanthate or 4–toluidine [73]. No displacement of the carbamate by the nucleophile occurred in the absence of the reducing agent.

2–Alkyl derivatives more closely related to EO9 have since been synthesised and evaluated, including the 2–cyclopropylindolequinones ((**38**) R^2 = cyclopropyl) [72]. The most effective compounds, both in terms of hypoxic potency and hypoxia–selectivity *in vitro* and in model tumour systems *in vivo* included the 5–methoxy–3–carbamoyloxy(methyl) derivatives[72]. The carbamate group is also present in naturally occurring mitomycins and this moiety and its position soon became the focus of attention. Variation in the 5–substituent of indolequinones and the equivalent positions of some mitosenes had already been studied extensively [15,68,72,80,82] but only a limited variation in C–3 carbinyl (C–10 in mitosenes) leaving group (principally carbamates and acetoxy derivatives) had been examined [15,68,70,72,74,76,80]. The ability of 3–indolyl carbinyl substituents to undergo elimination, well known in indole chemistry [86] was soon found to be a crucial property of these compounds. Such reactivity is only observed upon reductive–activation of the indolequinone, through the participation of the 1–nitrogen lone pair electrons which are deactivated in the quinone parent prodrug where they are partially delocalized into the quinone carbonyl at C–4. The resulting iminium species is then a potential electrophilic DNA–alkylating or other cellular–damaging species. Compounds with more efficient leaving groups such as carbamate and actetoxy derivatives (e.g. (**39**) R = $OCOCH_3$, $R^{3'}$ = $COCH_3$, $CONH_2$) have generally shown greater potency and DNA cross–linking abilities [70,71] but more quantitative studies using radiolytic reduction of indolequinones bearing diverse leaving groups ((**44**) X = OAr, OCOR, $OCONH_2$, SR) followed [87]. These studies have found that the useful range of leaving groups is diverse (Scheme (9)) and the potential for the exploitation of this chemistry as a bioreductive delivery system has been recognised [87-91].

In view of the particular propensity of 3–indolyl carbinyl substituents to undergo this elimination process upon reductive–activation, these compounds have now been studied extensively to examine this potential to release a



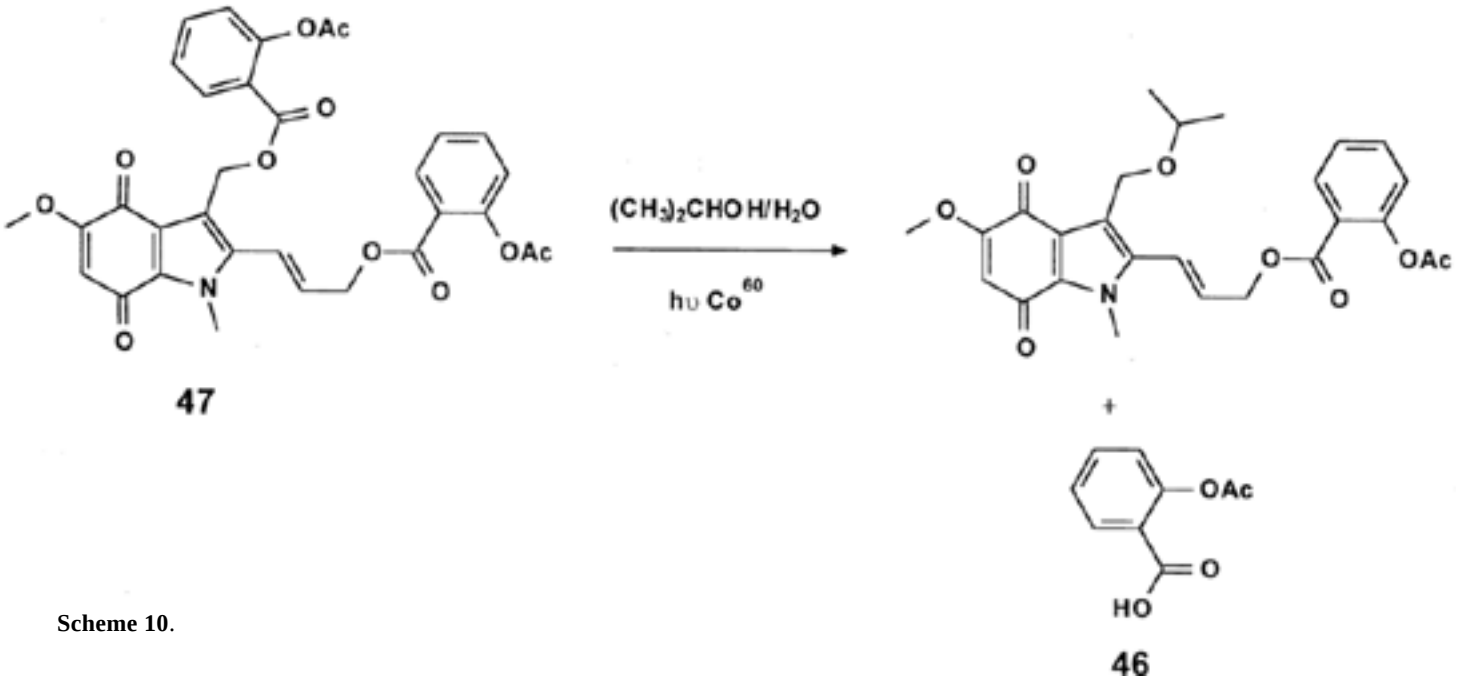
Scheme 8.



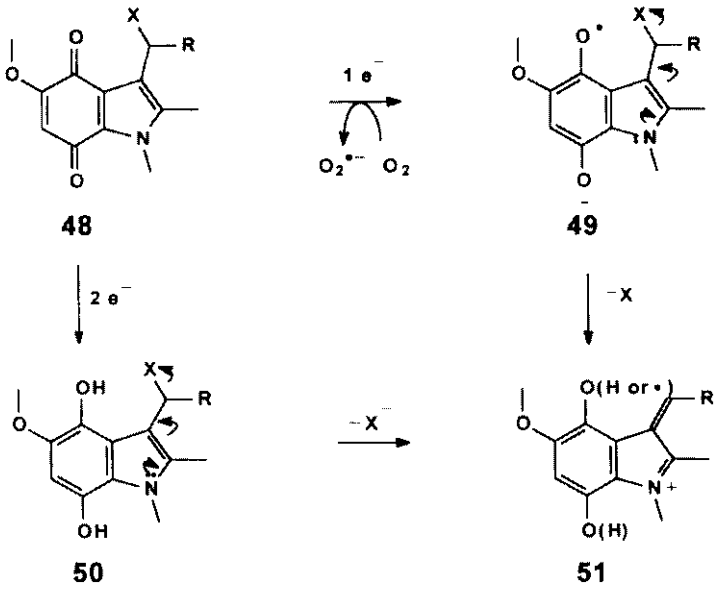
Scheme 9.

variety of drugs in a reductive environment. The ultimate aim was the design of drugs to give a secondary effect in addition to the cytotoxic iminium derivative formed on reduction and elimination. The potential for bioreductive drug targeting in this way has been demonstrated, for example the reductive elimination of aspirin (**46**) from indolequinone (**47**) and related compounds has been reported (Scheme (10)) [90]. However, it was known that the oxygen sensitivity of reduced quinone radicals might limit the usefulness of such chemical systems [2,92] and the kinetic studies that have followed have addressed many of the questions and provided the answers required to realise the potential of the approach [91,93].

As depicted in Scheme (11) there are two possible reductive pathways to drug activation involving either one–electron reduction (*via* reductase enzymes such as P450) of indolequinones of type (**48**) to the intermediate semiquinone radical ($Q^{\bullet-}$, (**49**)) and/or two–electron reduction to the hydroquinone (QH_2 , (**50**)). Both pathways result in elimination of leaving group (free drug or "effector") X and trapping of the intermediate (**51**) by cellular nucleophiles or experimental solvents. The two–electron process occurs following reduction by DT–diaphorase (NQO1) [94], for example, where QH_2 is formed directly *via* hydride transfer, bypassing $Q^{\bullet-}$ radical formation [95]. The hydroquinone has been shown to autoxidise slowly in oxygenated solution at rates that cannot compete with the reductive elimination of leaving groups [93]. Thus the release of leaving groups from the hydroquinone was not significantly oxygen–sensitive and hence not tumour selective on the basis of oxygenation status. Conversely, the elimination of carboxylate and phenolic (indol–3–yl)methyl substituents from the semiquinone was inhibited by oxygen too efficiently unless the semiquinone reacts with targets on a time–scale of milliseconds. Modification of redox properties was explored with the aim of changing this oxygen sensitivity [93], however it has become clear that only structural modification of the indolequinone, likely to achieve a significant increase in rate of elimination, was likely to affect this oxygen sensitivity. Thus, more recently the synthesis has been reported of a series of indolequinones with varying alkyl and aryl substituents ((**48**) R = H, CH_3, Ph, thienyl,) on the exocyclic (indol–3–yl)carbinyl group bearing the 4–nitrophenol moiety as a model leaving group (X) [91]. A comparison of the rate of release of the 4–nitrophenol drug model, in competition with oxygen enabled the influence of substitution of the indole core on this elimination rate to be ascertained, with the ultimate aim of achieving hypoxia–selective elimination with such drugs at a variety of chosen oxygen tensions and thus real tumour targeting. It was found that all the compounds underwent intramolecular fragmentation and highly efficient elimination of 4–nitrophenol from the (indol–3–yl)methyl position when reduced but only $Q^{\bullet-}$ radicals of the 3–carbinyl substituted derivatives did so with sufficiently short half–lives to compete with electron transfer to oxygen. The hydroquinones were not sufficiently oxygen sensitive to

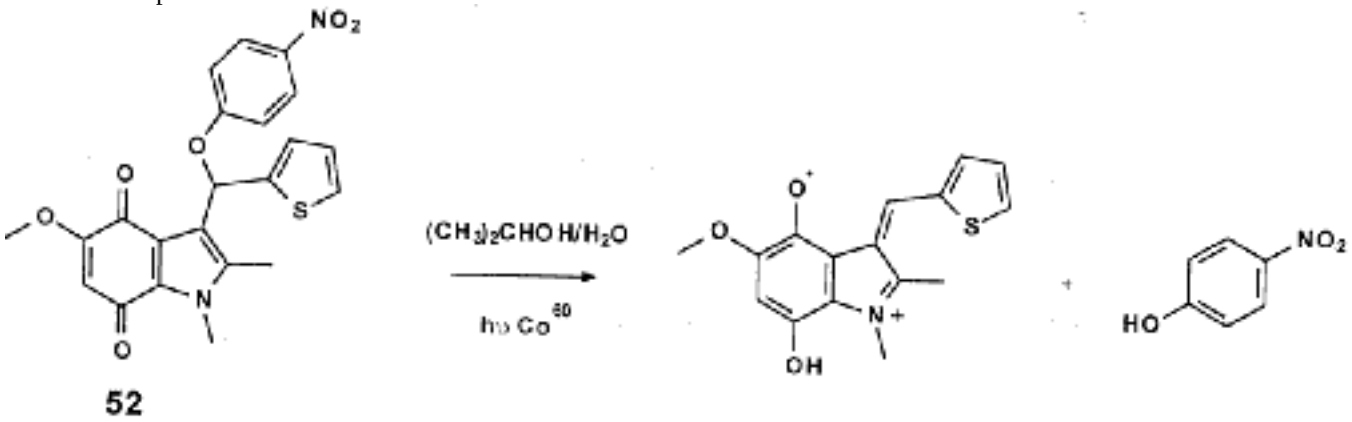


Scheme 10.



Scheme 11.

prevent the elimination of 4–nitrophenol under normoxic conditions. Hence the kinetics of the elimination process was clearly the critical factor in the design of more efficient bioreductive targeting systems. For the indolequinones to undergo fragmentation selectively in hypoxic tumour cells a balance needs to be achieved between the reactivities of both the semiquinone radical ($Q^{\bullet -}$) and hydroquinone (QH_2) with oxygen and their corresponding rates of reductive elimination. The most recent studies suggested that hypoxia–selective drug liberation may only be achieved through 3–carbinyl substitution, and ideal rates were achieved with trigger compounds such as the thienyl substituted indolequinone (**52**) and related alkyl derivatives (Scheme (12)) [91]. Compounds related to these new derivatives are therefore expected to provide the basis for the design and synthesis of genuine hypoxia–selective drug delivery systems which are able to target oxygen tensions ranging from anoxia to more moderate hypoxia. Similar studies will be required for nitroaromatic–based bioreductive triggers, which although less oxygen sensitive, are less efficient [48], and will require optimum rates of drug elimination over a range of levels of hypoxia, either tumour or other disease–specific.



Scheme 12.

ACKNOWLEDGEMENTS

This work is supported by The Gray Laboratory Cancer Research Trust (MAN) and Angiogene Pharmaceuticals (PT). The authors are grateful to Professor P. Wardman for helpful comments.

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