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STUDIES ON THE MOLECULAR PHARMACOLOGY OF GR63178A

A NOVEL PENTACYCLIC PYROLLOQUINONE ANTICANCER DRUG

JEFFREY CUMMINGS,* MARTIN A. GRAHAM,† BRIGID M. HOEY,‡ JOHN BUTLER,‡ ANDREW M. FRY,§ IAN D. HICKSON,§ GORDON LEONARD,‖ RAYMOND FRENCH and JOHN F. SMYTH

Imperial Cancer Research Fund, Medical Oncology Unit, Western General Hospital, Edinburgh EH4 2XU; † Cancer Research Campaign, Department of Medical Oncology, Beatson Laboratories, University of Glasgow, Glasgow G61 1BD; ‡ Cancer Research Campaign, Department of Biophysical Chemistry, Paterson Institute of Cancer Research, Christie Hospital and Holt Radium Institute, Manchester M20 9BX; § Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU; and ‖ Department of Chemistry, Edinburgh University, Edinburgh EH2, U.K.

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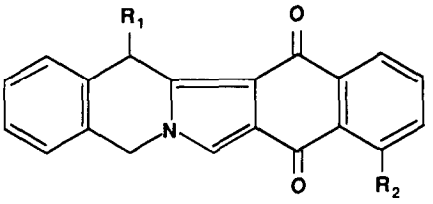
Abstract—GR63178A (NSC D611615) is the second pentacyclic pyrolloquinone to be evaluated clinically as an anticancer drug. Its mechanism of action is unknown but may be related either to its quinone group or planar ring system. In this report we have investigated the ability of GR63178A to bind non-covalently to DNA, inhibit topoisomerase II and undergo reduction to reactive free radical species. Using two DNA duplexes, a 12-mer oligonucleotide which is a preferred sequence for minor groove binders and a hexamer which is a preferred sequence for intercalators, no evidence of significant binding with GR63178A was found. Neither GR63178A nor GR54374X (its 9-hydroxy metabolite) inhibited purified human topoisomerase II in a decatenation assay. Free radical chemistry was studied by both pulse radiolysis and ESR spectroscopy as well as by *in vitro* drug incubations with NADPH-fortified rat liver microsomes and purified cytochrome P450 reductase. The one-electron reduction potential of GR63178A was -207 mV ± 10 which is much more positive than other quinone-containing anticancer drugs such as doxorubicin, mitomycin C and mitozantrone. GR63178A underwent enzyme-catalysed quinone reduction more readily than doxorubicin but produced significantly fewer reactive oxygen species. No evidence was detected of drug-induced, radical-mediated DNA damage *in vitro* using pBR322 plasmid DNA. Disproportionation of the GR63178A semi-quinone free radical proceeded with a rate constant of $1 \times 10^9\ M^{-1}\ sec^{-1}$ under anaerobic conditions, one order of magnitude faster than doxorubicin. The preferential disproportionation of the semi-quinone may explain our inability to detect a free radical signal by ESR. The hydroquinone of GR63178A was stable and exhibited strong visible absorption with a bathochromic shift of 120 nm over the parent drug. These unusual properties may be due to the hydroquinone undergoing a form of keto-enol tautomerization. Thus, GR63178A free radical formation does not appear to result in significant drug activation. In conclusion, GR63178A is unlikely to mediate its antitumour activity by DNA binding, topoisomerase II inhibition or free radical formation in direct contrast to similar anthracycline- and anthraquinone-based anticancer drugs.

GR63178A (NSC D611615, Fig. 1) is the first more water-soluble analogue of the pentacyclic pyrolloquinone mitoquidone (NSC 382057D, Fig. 1) to be evaluated clinically for anticancer activity. Both preclinical and clinical pharmacology studies have demonstrated that GR63178A exhibits unusual properties. Repeated drug administration for up to 21 days commencing 1 day after tumour implantation is necessary for full expression of activity against a panel of murine solid tumours and human xenografts [1]. Additionally, the drug is inactive against L1210 and P388 murine leukaemias which are normally more responsive to cytotoxic drugs, and it also appears to be inactive against a variety of tumour cell lines in culture [2]. Three different phase 1 protocols have been performed with GR63178A and these have demonstrated that the major side-effects associated with its administration are pain, often at the site of primary disease or metastases, headaches, nausea and vomiting rather than bone marrow suppression, which was absent [3].

At present the mechanism of action of GR63178A is unknown, but may be related to drug binding to DNA either through intercalation of its quasi-planar ring system or by association with the minor groove. Alternatively, activity may be due to bioreduction of the drug's quinone group with subsequent drug free radical formation, redox cycling and generation of a cascade of reactive oxygen species (ROS¶) resulting in DNA damage and/or lipid peroxidation. As part of a larger programme investigating the role of *in vivo* drug metabolism in the pharmacology of GR63178A, we have looked at

* Corresponding author. Tel: (031) 332 2471; FAX (031) 332 8494.

¶ Abbreviations: ROS, reactive oxygen species; DMSO, dimethyl sulphoxide; kDNA, DNA purified from the kinetoplast of *Crithidia fasiculata*; T_m, DNA melting temperature.



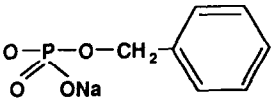
Name	R_1	R_2
Mitoquidone	H	H
GR 63178A	CH_3	
GR 54374X	CH_3	OH

Fig. 1. Structural formulae of GR63178A, its metabolite GR54374X and mitoquidone the parent drug in the class.

the ability of the parent drug and where possible its main metabolite GR54374X (due to very poor water solubility, Fig. 1) to bind to DNA and act as topoisomerase II poisons. In addition, we have studied the free radical chemistry of GR63178A, including its one electron reduction potential, rate constant for disproportionation of the semi-quinone free radical, ROS generation and radical-induced DNA damage (DNA nicking).

MATERIALS AND METHODS

Pure GR63178A (sodium salt) and GR54374X were a kind gift from the Infection and Oncology Department of the Medical Division of Glaxo Group Research Ltd (Greenford, U.K.); pure doxorubicin (hydrochloride salt) was from Farmitalia (Milan, Italy). NADPH, superoxide dismutase, adrenaline, dimethyl sulphoxide (DMSO) and dithiothreitol were all from the Sigma Chemical Co. (Poole, U.K.). pBR322 plasmid DNA, nuclease-free bovine serum albumin and ATP were from Boerhinger (Mannheim, Germany). kDNA purified from the kinetoplast of trypanosome *Crithidia fasciculata* was from TopoGEN Inc. (Colombus, OH, U.S.A.). The duplex DNA sequences d(CGCGAATTCGCG) and d(CGATCG) were prepared and purified in house, human topoisomerase II was purified from HeLa cells in house and purified rat liver cytochrome P450 reductase was a kind gift from Dr Roland Wolf, ICRF Laboratory of Molecular Pharmacology and Drug Metabolism, Edinburgh University. All buffers and standard laboratory reagents were of the highest grade available and were used as received.

Drug bioreduction and ROS generation. Drug bioreduction was determined by following the oxidation of NADPH spectrophotometrically at 340 nm. GR63178A (25–200 μM) was incubated at 37° in the presence of rat liver microsomes fortified with NADPH and utilization was monitored over 3 min [4]. ROS generation was measured indirectly using NADPH-fortified rat liver microsomes by the superoxide anion ($O_2^{\cdot-}$)-dependent reduction of adrenaline to adrenochrome, in the presence of either GR63178A (25–200 μM) or doxorubicin (100–200 μM).

Pulse radiolysis and ESR spectroscopy. Pulses of electrons (50 nsec) from the Paterson Institute linear accelerator [5] were used to irradiate GR63178A solutions which contained the drug (40–100 μM) in an argon-saturated buffer mixture of 0.1 M sodium formate, 0.01 M sodium phosphate, pH 7.0. Light transmitted through the solutions was passed through a Kratos monochromator into an EMI 9558Q photomultiplier (using bandwidths of 10 nm). Changes in optical transmission with time were then recorded using a Hewlett Packard HP9836s computer fitted with a Tektronix 7612D analogue to digital converter.

ESR spectroscopy was performed using a Varian E/9 X-Band spectrometer with 100 kHz modulation. Incubations included 50–200 μM GR63178A or doxorubicin, 1 mM NADPH and rat liver microsomes (6 mg protein) which had been purged with nitrogen. Scan rate was 20 G and a gain of up to 4×10^4 was employed.

DNA damage assay. Drug-induced DNA damage was measured by following the conversion of plasmid pBR322 DNA from form I (supercoiled) to form II (open circular, nicked relaxed) by gel electrophoresis after incubation of both GR63178A and doxorubicin with purified NADPH-cytochrome P450 reductase to activate the drugs. Assays were performed in a total volume of 200 μL which contained 500 ng pBR322; 100 U cytochrome P450 reductase; 50–200 μM doxorubicin or GR63178A; 0.2 mM NADPH in 100 μM Tris–HCL buffer, pH 7.4 and were incubated for 60 min at 37°. After incubation 100 μl of 5 M NaCl were added and the drugs were extracted with 4×200 μL water-saturated butan-1-ol. After drug extraction the DNA was ethanol precipitated (−20°, 1 hr) and was finally reconstituted in 10 μl of

10 mM Tris buffer pH 7.5, 1 mM EDTA to which was added 1 μl of a loading buffer consisting of 30% glycerol containing Bromophenol blue and xylene cyanole tracking dyes in H_2O. The DNA samples were run on a 1% agarose gel with a running buffer of 40 mM Tris, 20 mM sodium acetate, 20 mM NaCl, 2 mM EDTA, pH 8.15, the gel was stained for 30 min in ethidium bromide (2 μg/mL), and then destained for 30 min prior to being photographed (Polaroid type 55).

Drug binding to DNA. Drug binding was assessed as an increase in the melting temperature of duplex DNA measured by UV spectrophotometry. Two different sequences were studied: the 12-mer oligonucleotide d(CGCGAATTCGCG) and the hexamer d(CGATCG). Incubations were performed in 0.1 M NaCl, 10 mM sodium phosphate, 1 mM EDTA in 20% (v/v) DMSO at pH 7.0. In all cases the concentration of single-stranded DNA was 25 μM and drugs were added at a concentration to give a 1:2 molar ratio of duplex to drug.

Inhibition of topoisomerase II. Inhibition of topoisomerase II was measured using decatenation of kDNA essentially according to Davies *et al.* [6]. Briefly, GR63178A or GR54374X (2–100 μg/mL) was incubated with purified human topoisomerase II and 250–500 ng kDNA for 30 min at 30° in a total volume of 50 μL of buffer containing: 1 mM dithiothreitol, 1 mM ATP, 30 μg/mL bovine serum albumin, 0.5 mM EDTA, 120 mM KCl, 10 mM MgCl, 50 mM Tris HCl in 2% DMSO at pH 7.5. The reaction was stopped by adding 5 μL of loading buffer (50% sucrose, 50 mM EDTA, 0.1% Bromophenol blue and 0.01% SDS) and 20 μL was loaded onto a 1% agarose gel. After electrophoresis the gel was stained with ethidium bromide and photographed.

RESULTS

Bioreduction, redox cycling, ROS generation, free radical formation and radical-induced DNA damage

The ability of GR63178A to undergo bioreduction was determined by measuring an increase in microsomal NADPH oxidation (Fig. 2) over basal rates and was compared with that of doxorubicin. In separate studies (data not shown) HPLC analyses confirmed that in these incubations GR63178A was not consumed or metabolized indicating redox cycling. GR63178A stimulated NADPH oxidation in a concentration-dependent fashion to a peak of 7-fold at 150 μM from a basal rate of 12.8 ± 2.9 to 90.8 ± 5.8 nmol/min/mg. At 100 μM GR63178A produced a significantly larger stimulation of NADPH oxidation compared with doxorubicin (68.5 ± 1.4 vs 53.6 ± 2.9 nmol/min/mg $P < 0.05$, Student's *t*-test). In contrast, when superoxide anion formation was measured by reduction of adrenaline to adrenochrome, GR63178A was much less effective than doxorubicin: at 100 μM drug the rate of formation was 11.5 ± 0.5 nmol/min/mg for GR63178A as compared with 22.6 ± 1.6 nmol/min/mg for doxorubicin ($P < 0.01$, Student's *t*-test) (Fig. 3). At 200 μM GR63178A produced a 2.7-fold enhancement whereas doxorubicin produced a 5-fold enhancement. Superoxide dismutase (1 μg/mL) totally abolished both GR63178A and doxorubicin reduction of adrenaline.

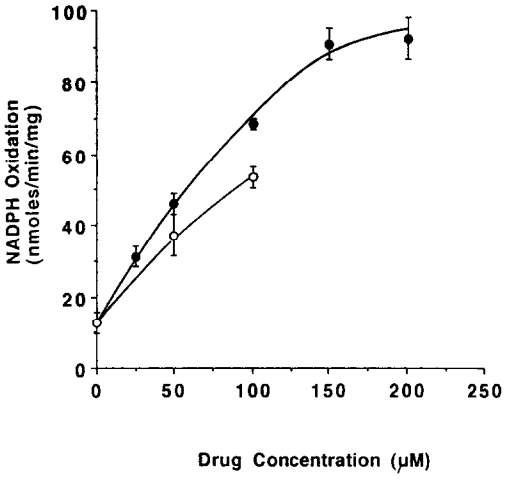


Fig. 2. Effect of GR63178A (●) and doxorubicin (○) on microsomal NADPH oxidation. Various drug concentrations (*X*-axis) were incubated with rat liver microsomes and the oxidation of NADPH was measured spectrophotometrically at 340 nm. NADPH utilization (*Y*-axis) is represented as nmoles oxidized per min per mg of microsomal protein. Values are means ± SD, N = 3–5.

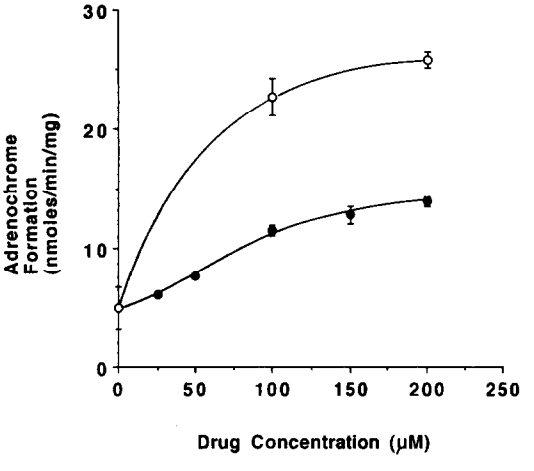


Fig. 3. Effect of GR63178A (●) and doxorubicin (○) on microsomal-catalysed superoxide anion formation. Various drug concentrations (*X*-axis) were incubated with rat liver microsomes and superoxide generation was measured by reduction of adrenaline to adrenochrome. Adrenochrome formation (*Y*-axis) is represented as nmoles formed per min per mg of microsomal protein. Values are means ± SD, N = 3–5.

The one-electron reduction potential for the formation of the semi-quinone free radical of GR63178A was determined to be −207 ± 10 mV and the equilibrium constant (K_1) for the semi-quinone radical in the presence of molecular oxygen (Eqn 1) to be 0.13 ± 0.02.

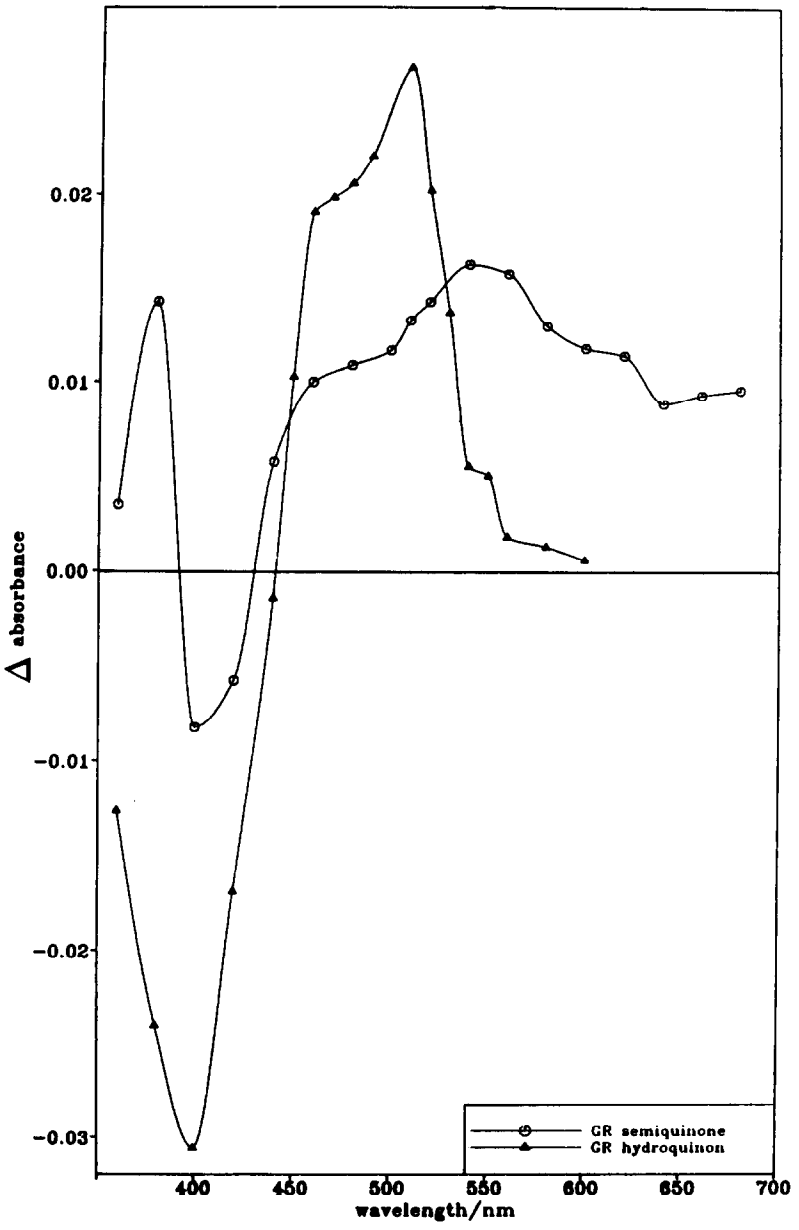


Fig 4. Difference between the spectra of native GR63178A and its one-electron reduced semi-quinone free radical form (○) and fully reduced hydroquinone form (▲). The spectra were recorded 28 μsec and 4.35 msec after the pulse. The reactive intermediates were produced by pulse radiolysis where GR63178A solutions received a dose of 10 Gy as described in Materials and Methods.

$$Q + O_2^{\cdot-} \rightarrow Q^{\cdot-} + O_2. \quad (1)$$

GR63178A semi-quinone was observed to decay to a hydroquinone in a second order manner consistent with Eqn 2 with a rate constant of $1.0 \pm 0.1 \times 10^9\ M^{-1}\ sec^{-1}$.

$$Q^{\cdot-} + Q^{\cdot-} + 2H^+ \rightarrow Q + QH_2. \quad (2)$$

The difference spectra between GR63178A and its semi- and hydroquinone forms are in Fig 4. These show significant visible absorption for both the semi- and hydroquinone above 500 nm compared with the parent drug which has a visible absorption λ max of 370 nm. The hydroquinone was stable over several seconds. In a series of experiments with cytochrome P450 reductase to activate the drug, a stable species with visible absorption very similar to that of the hydroquinone of GR63178A generated in the pulse radiolysis experiments was measured spectrophotometrically. Addition of potassium ferricyanide to these incubations oxidized this product back to native GR63178A, confirming further the presence of a hydroquinone. Whilst a free radical signal was detected by ESR with doxorubicin under the con-

Table 1. The influence of GR63178A on the thermal melting (T_m) temperature of DNA

Sequence	T_m° (duplex only)	T_m°* (duplex + GR63178A)	T_m°† (duplex + 4'epiDoxorubicin)
1 = d(CGCGAATTCGCG)	61.2 ± 0.5	60.8 ± 0.4	—
2 = d(CGATCG)	25.5 ± 0.4	25.8 ± 0.3	34.5 ± 0.5

* No significant increase in T_m occurred.
† Significantly different at $P < 0.001$ (Student's t-test).
To determine whether GR63178A binds to DNA, the drug was incubated with two different duplexes according to Materials and Methods and the effect on T_m recorded. A known DNA intercalator (4'epiDoxorubicin) which is also related in structure to GR63178A was included for comparison [20]. Sequence 1 is preferred by minor groove binders and sequence 2 by DNA intercalators.
Values are means ± SD, N = 3–5.

ditons of our incubations (data not shown), no signal could be detected with GR63178A even up to 200 μM of drug.

Over a concentration range of 50–200 μM GR63178A did not induce a shift in electrophoretic mobility of pBR322 from form I (supercoiled) to form II (nicked relaxed) characteristic of DNA damage whereas doxorubicin under identical conditions did induce a shift (data not shown).

Binding to DNA and inhibition of topoisomerase II

The effect of GR63178A on melting temperature (T_m) of two different DNA oligonucleotides is shown in Table 1. The 12-mer oligonucleotide is a well-known sequence for the detection of drugs which bind to the minor groove of DNA and the hexamer a preferred sequence for intercalators [7]. In both cases no evidence was detected of a significant increase in T_m. Due to poor water solubility it was impossible to assess the effect of GR54374X. Neither GR63178A nor GR54374X inhibited topoisomerase II-catalysed decatenation of kDNA (see Fig. 5).

DISCUSSION

GR63178A is a novel structure currently being evaluated clinically as an anticancer drug and therefore determination of its mechanism of action is of importance with a view to rational design of second generation analogues. In this report, we have evaluated whether or not GR63178A exhibits properties in common with well-established anticancer drugs such as the anthracyclines, doxorubicin and daunorubicin, with which it shares structural similarities in the form of a quinone group and planar ring system. Several recent reports suggest that the mechanism of action of doxorubicin responsible for its anticancer activity is DNA intercalation followed by stabilization of a drug–DNA/topoisomerase II ternary complex, referred to as the cleavable complex [8, 9]. It is therefore of significance that GR63178A did not appear capable of binding to DNA and neither GR63178A nor GR54374X inhibited topoisomerase II.

Doxorubicin and daunorubicin were first reported to generate free radicals in 1975 [10] and since then the role of free radicals in the mechanism of action of both drugs has remained controversial [11]. The one-electron reduction potential of GR63178A is much more positive (−207 mV) than that of other well-known quinone-containing antitumour drugs including doxorubicin itself (−347 mV), mitomycin C (−310 mV) and mitozantrone (−527 mV) [12] and its value is almost optimal for NADPH-cytochrome P450 reductase [13]. A more positive reduction potential has two important ramifications. First GR63178A should undergo more facile bioreduction, and this has been confirmed in this present study. Second the semi-quinone drug free radical will be relatively more stable in the presence of molecular oxygen ($E_7^1[O_2/O_2^-] = -155$ mV). Again, this has been confirmed both by reduced ability to redox cycle and generate ROS and by reduced capability to damage DNA. However, GR63178A semi-quinone free radical disproportionates into the hydroquinone with a rate constant one order of magnitude faster than that of doxorubicin [12].

Under anaerobic conditions, hydroquinones of doxorubicin and mitomycin C are highly unstable and rearrange chemically with the elimination of a leaving group (daunosamine in the case of doxorubicin; methanol in the case of mitomycin C) into a quinone methide which is sufficiently reactive, at least in the case of mitomycin C, to alkylate DNA [14, 15]. The hydroquinone of GR63178A, on the other hand, appears to be much more stable with a half-life in the order of seconds. Unusually for a hydroquinone GR63178A has visible absorption. Even more unusual is the fact that the hydroquinone of GR63178A has a visible λ max which exhibits a large bathochromic shift over the parent drug (from 370 to 510 nm), suggesting an increase in conjugation despite reduction of the double-bonded quinone group. A possible explanation is that the hydroquinone undergoes a form of keto-enol tautomerism resulting in an increase in conjugation extending over the whole pentacyclic ring system (see Fig. 6). Such a rearrangement may be responsible for the apparent stability of the hydroquinone. The preferential disproportionation of the GR63178A semi-quinone into the hydroquinone and the relative stability of the hydroquinone may explain why we were unable to detect a free radical signal by ESR. Thus, with GR63178A free radical formation does not appear to result in appreciable activation of the drug. Although we were unable to derive data for

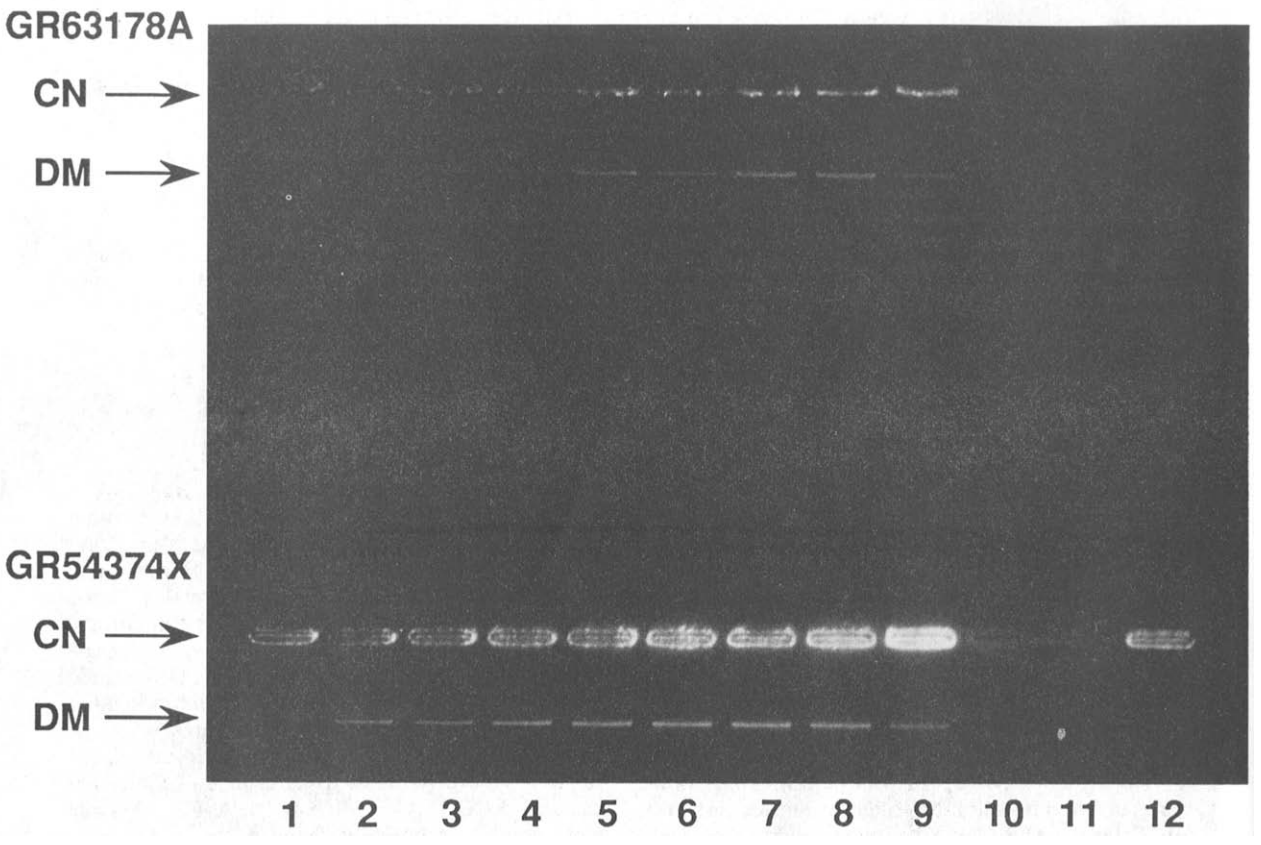


Fig. 5. Effect of GR63178A (upper portion of gel) and GR54374X (lower portion of gel) on decatenation activity of human topoisomerase II (topo II) purified from HeLa cells. Each lane contains 1% DMSO for drug solubilization and 500 ng of kDNA; incubations were performed at 30° for 60 min. CN, catenated DNA networks; DM, decatenated DNA minicircles. For both GR63178A and GR54374X: lane 1, kDNA alone; lane 2, kDNA + topo II showing normal enzyme activity; lanes 3–9, kDNA, topo II + 0, 2, 5, 10, 20, 50, 100 μg/mL drug or metabolite, respectively; lane 10, blank; lane 11, topo II + drug or metabolite at 5 μg/mL, no kDNA; lane 12, topo II + drug or metabolite at 100 μg/mL, no kDNA. At no drug or metabolite concentration was there a reduction in the intensity of the DM band, thus indicating that no significant inhibition of the enzyme had occurred. The increase in intensity of the CN band was due to the presence of increasing concentrations of drug or metabolite in the wells of the gel.

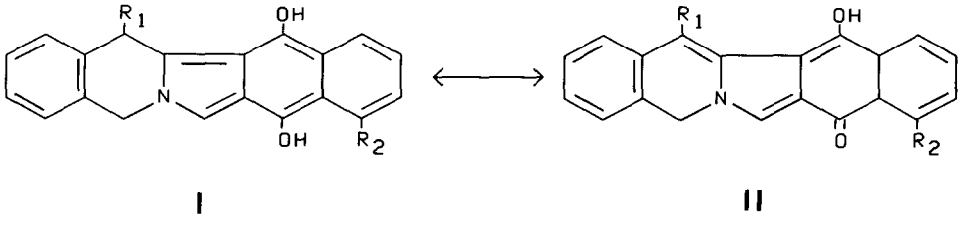


Fig. 6. Proposed scheme for the tautomerization of GR63178A hydroquinone (structure I) into the keto form (structure II) with increased conjugation of double bonds extending over the complete ring system. R_1 is a methyl group and R_2 is the benzyl phosphate ester group (see Fig. 1).

GR54374X due to its very poor water solubility, similar free radical chemistry is envisaged.

Both GR63178A and GR54374X appear not, therefore, to be working through conventional mechanisms of action and this is strengthened by the fact that both are non-cytotoxic against a panel of cell lines. This includes the colon carcinoma line HT 29 which nevertheless responds to the drug as a

xenograft [2]. The question remains as to how GR63178A produces its antitumour activity in animal models. One possibility is that the drug is biotransformed into cytotoxic metabolites. Apart from GR54374X at least five further metabolites have been detected in patient plasma and urine, indicating that the drug does undergo extensive metabolism [16]. However, in a murine tumour model where GR63178A produced a significant growth delay, we failed to detect any species other than the parent drug and GR54374X present in the tumour (unpublished observations). Additionally, there was no indication of an *in vivo* drug delivery problem for GR63178A itself, as the tumour appeared to reach equilibrium with blood-borne drug quickly (30 min) and levels were maintained over several hours. An alternative explanation is that GR63178A or GR54374X is acting as a biological response modifier and is working through non-cytotoxic mechanisms such as immunomodulation or specific alteration in tumour biology such as vascular collapse. Flavone acetic acid, which shares several properties in common with GR63178A [17], is now believed to work through a tumour vascular mechanism probably mediated via the immune system [18]. A recent report has shown that GR63178A also causes vascular collapse and tumour necrosis but to a lesser extent than flavone acetic acid [19].

In summary, although GR63178A shares chemical features in common with well-established classes of cytotoxic anticancer drugs, such as a quinone group and planar ring system, it is unlikely that it owes its *in vivo* antitumour activity to DNA binding, and inhibition of topoisomerase II, or generation of free radicals and ROS.

REFERENCES

1. Fenton RJ, Kumar KA, O'Sullivan M, Neate MS, Spilling CR and Knox P, *In vivo* anti-tumour activity of the mitoquidone analogue GR63178A. In: *Proceedings of the Sixth NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, 7–10 March, 1989*, p. 89.
2. Eccles DM, Cummings J, Stewart ME, Nicolson M, Evans S, Cornbleet MA, Leonard RCF and Smyth JF, Phase I and pharmacology studies of GR63178A, a water soluble analogue of mitoquidone. In: *Proceedings of the Sixth NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, 7–10 March, 1989*, p. 90.
3. Eccles DM, Cummings J, Stewart ME, Nicolson M, Cornbleet MA, Leonard RCF and Smyth JF, A phase I and pharmacological study of GR63178A a water soluble analogue of Mitoquidone. *Cancer Chemother Pharmacol* **29**: 375–378, 1992.
4. Graham MA, Newell DR, Butler J, Hoey B and Patterson LH, The effect of the anthrapyrazole antitumour agent C1941 on rat liver microsome and cytochrome P-450 reductase mediated free radical processes. Inhibition of doxorubicin activation *in vitro*. *Biochem Pharmacol* **36**: 3345–3351, 1987.
5. Butler J, Hodgson BW, Hoey BM, Land EJ, Lea JS, Lindley EJ, Rushton FAP and Swallow AJ, Experimental studies of some moderately fast processes initiated by radiation. *Radiat Phys Chem* **34**: 633–646, 1989.
6. Davies SM, Robson CN, Davies SL and Hickson ID, Nuclear topoisomerase II levels correlate with the sensitivity of mammalian cells to intercalating agents and epipodophyllotoxins. *J Biol Chem* **263**: 17724–17729, 1988.
7. Kennard O and Hunter WN, Single crystal X-ray diffraction studies of oligonucleotides and oligonucleotide drug complexes. *Angew Chem Int Ed Engl* **30**: 1254–1277, 1991.
8. Tewey KM, Rowe TC, Yang L, Halligan BD and Liu LF Adriamycin induced DNA damage by mammalian DNA topoisomerase II. *Science* **226**: 466–468, 1984.
9. Cummings J, Anderson L, Willmott N and Smyth JF, The molecular pharmacology of doxorubicin *in vivo*. *Eur J Cancer* **27**: 532–535, 1991.
10. Handa K and Sato S, Generation of free radicals of quinone group containing anti-cancer chemicals in NADPH-microsome systems as evidenced by initiation of sulfite oxidation. *Gann* **66**: 43–47, 1975.
11. Keizer HG, Pinedo HM, Schuurhuis GJ and Joenje H, Doxorubicin (adriamycin): a critical review of free radical mechanisms of cytotoxicity. *Pharmacol Ther* **47**: 219–231, 1990.
12. Butler J and Hoey BM, Are reduced quinones necessarily involved in the antitumour activity of quinone drugs. *Br J Cancer* **55** (Suppl III): 53–59, 1987.
13. Powis G and Appel PL, Relationship of the single electron reduction potential of quinones to their reduction by flavoproteins. *Biochem Pharmacol* **29**: 2567–2572, 1980.
14. Kleyer DL and Koch TH, Electrophilic trapping of the tautometer of 7-deoxydaunomycinone. A possible mechanism of covalent binding of daunomycin to DNA. *J Am Chem Soc* **105**: 5154–5155, 1983.
15. Peterson DM and Fisher J, Autocatalytic quinone methide formation from mitomycin C. *Biochemistry* **25**: 4077–4084, 1986.
16. Cummings J, French RC, MacLellan A and Smyth JF, Chromatographic characterisation of six human metabolities of the new anticancer drug GR63178A. *Cancer Chemother Pharmacol* **28**: 27–32, 1991.
17. Cummings J and Smyth JF, Flavone 8-acetic acid: our current understanding of its mechanism of action in solid tumours. *Cancer Chemother Pharmacol* **24**: 269–272, 1989.
18. Bibby MC, Phillips RM, Double JA and Pratesi G, Anti-tumour activity of flavone acetic acid (NSC 347512) in mice—influence of immune status. *Br J Cancer* **64**: 57–62, 1991.
19. Hill SA, Williams KB and Denekamp J, A comparison of vascular-mediated tumour cell death by the necrotising agents GR63178A and flavone acetic acid. *Int J Radiobiol*, in press.
20. Leonard G, Brown TA and Hunter WN, Anthracycline binding to DNA: high resolution structure of d(TGTACA) complexed with 4'epiDoxorubicin. *Eur J Biochem* **204**: 69–74, 1992.