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1,5-Diaryl-3-oxo-1,4-pentadienes: A Case for Antineoplastics with Multiple Targets

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Abstract

A number of organic molecules which contain the 1,5-diaryl-3-oxo-1,4-pentadienyl group, referred to hereafter as the dienone moiety, have antineoplastic properties. Emphasis is made on the attachment of this structural moiety to several molecular scaffolds, namely piperidines, *N*-acylpiperidines, cycloalkanes and 3,4-dihydro-1*H*-napthalenes. Many of these compounds are potent cytotoxins having micromolar and nanomolar IC_{50} values towards a wide range of neoplastic and transformed cells. On occasions, greater toxicity towards neoplasms than normal cells has been demonstrated. A number of these compounds have *in vivo* anticancer properties and in general excellent tolerability in rodents is demonstrated. The way in which a number of physicochemical properties such as redox potentials, torsion angles, atomic charges and logP values govern cytotoxic potencies are presented. The importance of the shapes of different compounds as determined by molecular modeling in contributing to antineoplastic properties is outlined. Arguments are presented in favour of designing antineoplastics which have multiple sites of action in contrast to those bioactive molecules which have only one molecular target. A number of compounds which possess the dienone group have different modes of action some of which are chronicled in this review, such as inducing apoptosis, affecting respiration in mitochondria, inhibiting macro-molecular biosynthesis and both inhibiting and stimulating certain enzymes. Other important properties of these compounds are discussed including their anti-angiogenic, MDR-revertant and antioxidant properties. It is hoped that this eulogy of the importance of the dienone group will encourage researchers to consider incorporating this structural unit into candidate cytotoxins in the future.

Keywords

1,5-Diaryl-3-oxo-1,4-pentadienes; cytotoxicity; cycloalkanones; 4-piperidones; 3,4-dihydro-1*H*-naphthalen-2-ones; anticancer activity; antineoplastics; diaryldienones

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INTRODUCTION

The purpose of this review is to encourage consideration of the inclusion of the 1,5-diaryl-3-oxo-1,4-pentadienyl moiety into the design of novel antineoplastics. The reasons for this recommendation are based on the evidence that many compounds containing this structural unit display cytotoxic and anticancer properties, exercise their bioactivity in a number of different ways and display excellent tolerability in mammals.

A number of years ago, one of the authors prepared a lengthy review on the bioactivities of acyclic conjugated α,β-unsaturated ketones [1]. This monograph outlined some evidences that these compounds have a marked affinity for thiols whereas only minimal or non-existent interactions occur with amino or hydroxy groups. Later studies confirmed the thiol-selectivity of these compounds [2–3]. Since thiols are absent in nucleic acids, conjugated enones may avoid the problems of mutagenicity and carcinogenicity caused by certain contemporary anticancer drugs [4–5]. A subsequent review article from this laboratory on chalcones or 1,3-diaryl-2-propenones [6] provided further evidence of the potential usefulness of conjugated α,β-unsaturated ketones in cancer chemotherapy. Various series of compounds have been developed in which an aryl ring was placed adjacent to the β carbon atom in order to alter the polarity of the electrophilic centre which may correlate with cytotoxic potencies [7]. In addition, the steric and hydrophobic properties of molecules may be controlled by the nature of the aryl substituents.

However with the aim of preparing conjugated enones with selective or preferential toxicity to malignant cells rather than the corresponding normal cells, the theory of sequential cytotoxicity was proposed [8]. This hypothesis states that a chemical insult prior to a subsequent chemical attack on cellular constituents may produce greater toxicity to cancers than normal cells. The rationale for this theory is based on the observation that on a number of occasions, the lowering of cellular thiol concentrations before the administration of various anticancer agents enhances the cytotoxicity of these drugs towards tumours rather than normal cells [9–11]. In other words, greater sensitization of malignant cells may take place. In order to achieve successive interactions with cellular thiols, the 3-aryl-2-propenoyl group (Ar-CH=CH-CO-) present in various cytotoxins was expanded to a 1,5-diaryl-3-oxo-1,4-pentadienyl moiety (Ar-CH=CH-CO-CH=CH-Ar). Alkylation of thiols is predicted to occur as indicated in Scheme 1.

One of the principal reasons for the pursuit of 1,5-diaryl-3-oxo-1,4-pentadienes (hereafter referred to as diaryldienones) is that these compounds interact with a variety of cellular constituents. In the past, many efforts have been made to design compounds or monoclonal antibodies to interact with one primary molecular target [12], but the results have often been disappointing [13]. Hence an alternative strategy is presented in this review, namely describing compounds which have the potential for multiple sites of action.

The reasons for incorporating groups which are documented to have different molecular targets into a candidate cytotoxin include the following considerations. (1) The problem of drug resistance will probably arise rapidly if the ligand inhibits only one biochemical pathway. Hence the design of antineoplastic agents with multiple sites of action is more

likely to lead to effective anticancer drugs than a one ligand-one binding site approach. (2) When a normal cell becomes malignant, a number of dysregulated processes occur and an antineoplastic agent should possess pleotropic capabilities. (3) It is conceivable that one or more of the sites at which a multitargeted ligand interacts may assist its metabolism. For example, a diaryldienone may stimulate an isoform of glutathione S-transferase which will catalyze the conjugation of the unsaturated ketone with glutathione. Hence the elimination will ensure a lack of accumulation in the body and thus avoid the toxic symptoms caused by xenobiotics which are retained in body tissues. (4) A compound reacting at different locations in the body which contribute to the anticancer efficacy may be less toxic than an analog which has only one site of action. Thus consider a biochemical sequence W-X-Y-Z in a tumour cell while an analogous pathway is V-X-Y-Z in normal tissue. In addition, one may assume that the inhibition of Y into Z is considered to be the most damaging to the cells and above certain doses, cell death results. If the multitargeted ligand inhibits the target W but not V as well as inhibiting the conversion of Y into Z, then fewer compounds will be required than in the case of an analog which only inhibits the pathway Y into Z. Hence toxicity to normal cells will be less when the multitargeted cytotoxin is administered. (5) A multitargeted compound may reveal novel mechanisms of action. For example, in the case of the discovery that an antineoplastic agent acts an both W and inhibits the Y-Z sequence may reveal that these two loci of action are connected, i.e., the relationship W-X-Y-Z is demonstrated. (6) Various molecular targets may be expressed only in small groups of patients. Hence a multitargeted ligand may be effective towards a greater number of patients than using a compound which acts at one locus only. (7) Compounds which interact at a variety of sites in the body may demonstrate bioactivity in other clinical areas. For example, a cytotoxic sulfonamide may also display diuretic properties. In this case, the doses required for anticancer therapy and enhancement of diuresis should be similar.

However in order to obtain compounds with therapeutic potential, a balance needs to be achieved whereby the cytotoxin interacts with various molecular targets associated with carcinogenesis but spares those receptors which are required for the healthy functioning of the body. Unless this selective toxicity is achieved when interactions with multiple targets takes place, the differential between unwanted side effects and therapeutic gain will not be manifested.

Recently other authors have articulated the need to find anticancer agents which interact at multiple sites [14–16]. Thus this review will emphasize not only the antineoplastic properties of various diaryldienones but some of the different ways whereby antiproliferative properties are mediated. A further positive aspect of these compounds is that large doses of many of these diaryldienones can be tolerated in rodents which is in contrast to the marked toxicity of many contemporary anticancer drugs [17]. To the best knowledge of the authors, the antineoplastic properties of diaryldienones has not been reviewed previously with the exception of a brief monograph of 3,5-bis(benzylidene)-4-piperidones as novel anticancer agents [18].

This review aims to summarize different studies each of which illustrates the potential utility of diaryldienones in cancer chemotherapy. A major emphasis of this review is the demonstration that the diaryldienone motif is able to confer antineoplastic properties when

incorporated into different ligands. Hence the cytotoxicity of these compounds is emphasized while reference will also be made to their *in vivo* anticancer properties, modes of action and other bioactivities.

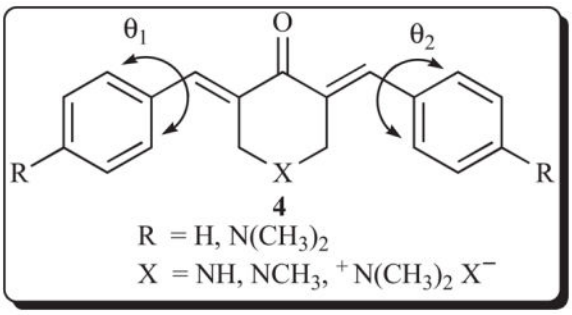
CYTOTOXICITY

Four classes of diaryldienones will be considered. In the first three groups of compounds, the 1,5-diaryl-3-oxo-1,4-pentadienyl group was mounted on piperidines, cycloalkyl and 3,4-dihydro-1*H*-naphthalenyl scaffolds while the fourth cluster of molecules are acyclic in nature.

1. 3,5-bis(Benzylidene)-4-piperidones

The design and cytotoxic properties of various 3,5-bis(benzylidene)-4-piperidones will be reviewed followed by an account of the cytotoxicity of various *N*-acyl products which were not described by Jha and Duffield [18].

The reasons for the initial development of these compounds as cytotoxins was based on a number of considerations. A previous investigation revealed that the second order rate constants between a biomimetic thiol and a cluster of Mannich bases of acyclic ketones **1** was approximately 240 times faster than the corresponding enones **2** when the same substituents are in the aryl rings [19]. However while some of the acyclic Mannich bases have cytotoxic and anticancer properties [20], respiratory depression was noted in low doses for many of these types of compounds when they were administered to mice [21]. The acyclic enones are flexible molecules and therefore able to assume a variety of different shapes, some of which may have antineoplastic properties while others are responsible for the unwanted toxicity. Hence while a conjugated arylidene keto group along with a basic centre beta to the carbonyl function was retained, molecular flexibility was reduced by the incorporation of most of the molecule into a rigid piperidine ring as illustrated in Fig. (1). In addition, the incorporation of a second alkylating group was undertaken with the aim of increasing cytotoxic potencies. These considerations led to the syntheses of **3a** (R^1 = H) which possesses an IC_{50} value of 16 pM towards murine P388D1 cells [22].



4

R = H, $N(CH_3)_2$

X = NH, NCH_3, $^+N(CH_3)_2$ X^-

Molecular modifications of this compound by altering the nature of the basic centre and placing a 4-dimethylamino group in the aryl rings gave rise to the analogs **4** with similar high cytotoxic potencies [22]. X-ray crystallography of many of these molecules revealed that both olefinic double bonds adopted the *E* stereochemistry and that the aryl rings are not

coplanar with the adjacent olefinic linkages. This latter phenomenon is due to nonbonded interactions between the equatorial protons at positions 2 and 6 of the piperidine ring and the ortho hydrogen atoms of the aryl rings. The torsion angles θ_1 and θ_2 differed in magnitude but no correlations between cytotoxic potencies and the θ_1 and θ_2 figures were observed.

Two experiments were undertaken with a representative compound **3a** [22]. First, this dienone lowered hepatic glutathione levels in mice confirming that it is a thiol alkylator. Second, administration of doses up to and including 240 mg/kg to mice on five successive days did not cause any mortalities. An examination was undertaken in order to find if these compounds exerted their potencies in additional ways. Two related compounds **4** (R = $N(CH_3)_2$; X = $+N(CH_3)_2\ ^-I$) and **4** (R = $N(CH_3)_2$; X = NCH_3) were added to three synthetic DNAs, namely poly[d(AT)], poly da·poly dT and poly [d(TG)]·poly[d(CA)]. The quaternary ammonium compound caused a rise in the melting temperature of all three DNAs but no changes were noted with the non-quaternary ammonium compound. The behaviour of the quaternary ammonium compound indicates AT selectivity and is consistent with binding in the minor groove of DNA. Further experimentation with three unsubstituted compounds (R =H) in which X is NH.HCl, CH_2 and $+N(CH_3)_2{}^-Br$ using poly[d(AT)] revealed that only binding occurred with the quaternary ammonium compound. A further study with related 3,5-bis(benzylidene)-1-methyl-4-piperidone methoiodides which are toxic to murine L1210 cells revealed that they too increased the melting temperature of poly[d(AT)] [23].

N-Acyl-3,5-bis(benzylidene)-4-piperidones

A number of *N*-acyl analogs of 3,5-bis(benzylidene)-4-piperidones have been prepared and the design of these compounds was based on the following considerations. First, the cytotoxicity of 3,5-bis(benzylidene)-4-piperidones may be considered to be due to the 1,5-diaryl-3-oxo-1,4-pentadienyl group interacting at a primary binding site. Hence the placement of various groups on the piperidyl nitrogen atom could enable interactions at an auxiliary binding site leading to potency increases. It is of course conceivable that groups on the nitrogen atom will impede alignment of the diaryldienone group at the primary binding site thereby lowering antineoplastic efficacy. These concepts are visualized in Fig. (2). Second, 3,5-bis(benzylidene)-4-piperidones are ionized under physiological conditions; the extent being determined by the pK_a of the piperidyl nitrogen atom as well as the pH of the biological milieu [24]. The ions are likely to experience difficulty in penetrating cell membranes and hence the formation of the *N*-acyl analogs will prevent the nitrogen atom being charged and the permeability into malignant cells may be enhanced. The resultant amide may be bioactive *per se* or be a prodrug liberating the 4-piperidones by amidic hydrolysis.

An initial study involved the preparation of *N*-acyl analogs of **5** in which an additional site for thiol alkylation is either present (**7**) or in a latentiated form (**9**) [25]. Examples of the compounds prepared are given in Scheme 2 and the number in parentheses refer to the potencies relative to the parent compound **5** which has an IC_{50} value of 2.5 μM towards murine L1210 leukemic cells. Clearly potency is elevated in both *N*-acylated products **7** and **9**. With a view to lowering the IC_{50} values still further, two electron-withdrawing chloro atoms were placed in the aryl rings in order to increase the electrophilicity of the olefinic

carbon atoms which gave rise to the potent cytotoxins **6**, **8** and **10**. The diaryldienones **5**-**10** along with a number of related analogs were evaluated against a panel of approximately 56 human tumour cell lines from different groups of tumours, namely leukemia, melanoma and colon, renal, small cell lung, non-small cell lung, central nervous system and ovarian cancers (referred to hereafter as the NCI screen). In particular, the average IC_{50} figure of **8** is 0.16 μM while the figure for melphalan is 26.3 μM revealing **8** to possess 164 times the potency of this reference drug. In particular, the average IC_{50} value of **8** towards five human leukemic cell lines is 36.3 nM indicating its potential as an antileukemic agent. Compound **9** and two analogs in which the dimethylamino hydrochloride group was replaced by trimethylammonium bromide and diethylamino hydrochloride substituents were incubated in a mixture of deuterated phosphate buffered saline and dimethylsulfoxide-d_6 for 48 hours and 37° C. ^{1}H NMR spectroscopy revealed the liberation of the corresponding *N*-acryloyl derivative **7** along with other decomposition products.

A subsequent study revealed that sodium borohydride reduction of the carbonyl group of **5** led to the corresponding alcohol **11** which has an IC_{50} value of 18.0 μM towards L1210 cells [26]. Thus reduction of the keto group lowered potency sevenfold suggesting that the integrity of the 1,5-diaryl-3-oxo-1,4-pentadienyl group should be maintained.

A more detailed study was undertaken subsequently in order to compare the relative cytotoxic potencies of the 3,5-bis(benzylidene)-4-piperidones **12** with the corresponding *N*-acryloyl derivatives **13** [27]. In addition, efforts were made to identify some of the physicochemical and biochemical parameters which contribute to the magnitude of the anti-neoplastic properties.

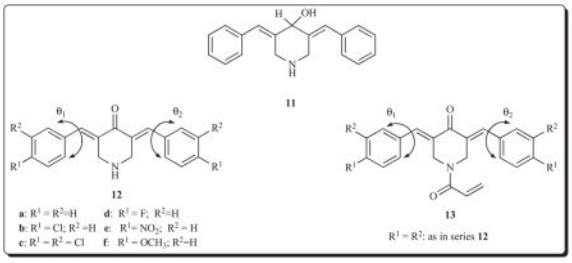
The average IC_{50} figures of the six compounds in series **12** towards human Molt 4/C8 and CEM T-lymphocytes as well as murine P388 and L1210 leukemic cells are 59.4, 33.6, 0.81 and 81.9 μM, respectively. In the case of the compounds in series **13** which have the same aryl substituents, the relative figures are 0.96, 1.13, 0.28 and 4.71 μM, respectively, i.e., the *N*-acryloyl derivatives **13** are, on average, 62, 30, 3 and 17 times more potent in each of these screens. Thus with the exception of the P388 cells, which are the most sensitive to these compounds, substantial increases in cytotoxic potencies were observed in creating an additional site for alkylation of cellular thiols.

The following relationships between physicochemical properties and cytotoxic potencies of the compounds in series **12** and **13** were observed. First, the Hammett σ values of the aryl substituents in series **13** correlated negatively with the IC_{50} figures in the P388 and L1210 bioassays. Second, the average torsion angles θ_1 and θ_2 in series **12** are −49.5 (± 0.05)° and 49.4 (± 0.11), respectively, while in the case of the *N*-acyl analogs **13**, the figures are −53.2 (± 0.17) and 61.0 (± 0.24), respectively. Third, correlations have been established between the redox potentials of various compounds and different bioactivities [28], including chalcones which contain the related 3-aryl-2-propenoyl group, and cytotoxicity [29]. When the same substituents are present in the aryl rings, the redox potentials are lower in series **13** indicating their greater reducing properties. These three observations have value in the design of further analogs with a view to increasing cytotoxic potencies. Thus the placement of one or more strongly electron-withdrawing groups in the aryl ring, the insertion of

substituents of different sizes in the ortho position of the aryl rings in order to increase the θ values and employing groups which have lower redox potentials should be considered.

The possible modes of action of representative compounds in series **12** and **13** were investigated. Both **12e** and **13e** inhibited DNA, RNA and protein biosyntheses in murine L1210 cells. The average IC_{50} values to accomplish these results for **12e** and **13e** are 239 μM and 3.90 μM, respectively. Thus 61-fold difference in potency is similar to the 78-fold difference in the IC_{50} values to L1210 cells. Both **12c** and **13c** inhibited RNA and protein biosyntheses as well as inducing apoptosis in human Jurkat T cells. In summary, the cytotoxicity of the compounds in series **12** and **13** likely includes interference with the syntheses of important biomacromolecules and by causing apoptosis.

A recent investigation also addressed the issue of placing different acyl groups on the piperidyl nitrogen of the diaryldienones **14** in order to glean further evidence of the structural requirements at the putative auxiliary binding sites [30]. Benzoylation of **14a** give the corresponding amide **15** which has a similar toxicity profile as the parent compound. In order to ascertain if groups on the amidic aryl ring of **15** which have a mixture of hydrophilic and hydrophobic properties increased cytotoxic potencies, **16a** was synthesized which possesses three times the potency of **14a** and **15** in the Molt 4/C8 bioassay. With a view to investigating the generality of whether the *N*-[4-(2-alkylaminoethoxy)phenylcarbonyl] group enhances the cytotoxicity of the 3,5-bis(phenylmethylene)-4-piperidones, the following structural alterations of **16a** were made. First, substituents were placed in the arylidene aryl ring which have substantially divergent σ and π values. Second, the dimethylamino group was replaced by other amines which differed in size and basicity. Such considerations led to the syntheses and cytotoxic evaluation of series **16-19** as indicated in Scheme 3.



The compounds in series **16-19** displayed potent cytotoxicity towards human Molt 4/C8 and CEM T-lymphocytes whereby three quarters of the IC_{50} values are less than the figures for melphalan (~ 2.9 μM). In particular, **16c**, **17c**, **18c** and **19c** where R^1 is a nitro group have submicromolar IC_{50} figures. The murine L1210 cells are less sensitive to these compounds but in 63% of the assays, the IC_{50} values are less than 10 μM. In general, the greatest cytotoxic potencies are found when the R^1 substituent is either nitro or methyl and the dimethylamino, diethylamino and 1-piperidyl groups are present in the *N*-acyl side chain. Comparisons were made between the IC_{50} values of the piperidines **14** and **16-19** when the same substituents are present in rings A and B. In 48% of the comparisons, the *N*-acylated products were more potent while in 35% of the cases, equal potencies were observed. The

conclusion drawn is that the *N*-[4-(2-aminoethoxy) phenylcarbonyl] side chain likely interacts at auxiliary binding sites thereby increasing potencies.

A number of the compounds in series **16**-**19** were evaluated against approximately 49 human tumour cell lines. The average IC_{50} values ranged from <0.52 (**19b**) to >11.5 (**18b**) μM. Compound **19b** was particularly cytotoxic towards the colon cancer HCC-2998 having an IC_{50} figure of less than 5 nM. A huge differential between the IC_{50} values were noted for most of these compounds, i.e., there was a selective toxicity towards certain tumour cell lines which may translate into a preferential lethality for neoplastic cells rather than the corresponding normal tissues. A TUNEL assay using human HepG2 liver cancer cells revealed that **19b** caused apoptosis. A subsequent study revealed that two representative molecules **16a** and **19d** stimulated respiration in rat liver mitochondria *vide infra* and none of these compounds caused mortalities when 300 mg/kg was administered to mice [31].

A major problem in treating cancers with drugs is that resistance often develops. Thus compounds are urgently required which will enable anticancer drugs to be retained in the malignant cells rather than be extruded rapidly by a drug efflux pump such as P-glycoprotein (P-gp). Recent studies have revealed that the mounting of the diaryldienone moiety on a *N*-acyl piperidinyl scaffold led to potent revertants of P-gp associated multidrug resistance. The biological assay employed in these studies used murine L-5178 lymphoma cells transfected with the human MDR1 gene [32]. The concentration of rhodamine 123 in treated and untreated transfected and parental cells are measured and the fluorescent activity ration (FAR) values calculated. A FAR value of greater than 1 indicates that reversal of MDR has occurred. This assay is referred to subsequently as the L-5178 P-gp reversal screen. Using concentrations of 4 μg/mL, the FAR figures of **16**-**19** ranged from 46–179 which are substantially greater than was noted for a reference drug verapamil which has a FAR value of 4.2 at this concentration [33]. The optimal basic group is the 1-piperidinyl ring present in series **18** while higher FAR values are associated with the compounds containing 4-methyl and 4-chloro substituents in the arylidene aryl rings. One may note that the precursor amines **14** are virtually bereft of MDR revertant properties using concentrations of both 4 and 40 μg/mL.

A brief summary is now presented of four series of compounds **20**-**23** which were principally designed to explore the tolerance of auxiliary binding sites to the acyl group attached to the nitrogen atom of various 3,5-bis(benzylidene)-4-piperidones. In general, evaluation of the cytotoxic properties of these compounds involved the use of human Molt 4/C8 and CEM T-lymphocytes and murine L1210 leukemic cells. Many of the compounds possessed submicromolar IC_{50} values. Furthermore in short-term toxicity studies, doses up to and including 300 mg/kg of these dienones did not produce mortalities.

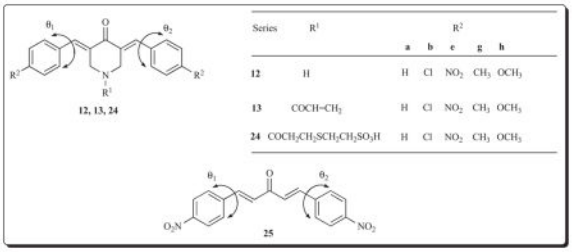
The first study involved the preparation of series **20** [34] in which the chalcone motif is present in the *N*-acyl group. It is conceivable that the compounds *per se* interact strongly at receptors or possibly they may undergo hydrolysis liberating the cytotoxic piperidinone and chalcones, many of which have cytotoxic properties [35,36]. In general, the compounds in series **20** (IC_{50} values of 1.7–94 μM) were less potent than **14a** (IC_{50} range is 1.7–8 μM) which may have been due to the bulk of ring A impeding alignment at a binding site.

However a second series of compounds **21** in which ring A had been excised has similar potencies as series **20**.

Series **22** was designed in which the olefinic double bond in the *N*-acyl group not only provided an additional site for thiol alkylation but also conferred rigidity to the molecules thereby controlling the location of terminal aryl ring B relative to the 3,5-bis(benzylidene)-4-oxopiperidinyl moiety [37]. In general, these compounds were more potent than the diaryldienones **13** (R^1=R^2=H) and **14a**. The IC_{50} values are submicromolar in over half of the compounds and less than 5 μM in all cases. All of these compounds are more potent than melphalan towards the T-lymphocytes. Changing the stereochemistry of the olefinic group in the *N*-acyl side chain from *Z* to *E* led to series **23** which, in general, was accompanied by a reduction in potency [38].

Attempts have been made to determine the modes of action of representative compounds in series **22** and **23**. First, the enzyme myristoyl-CoA:protein N-myristoyl transferase (HMT) catalyzes the attachment of the myristoyl group onto the N-terminal glycine residues of certain polypeptides [39]. In particular the cysteine-169 residue of human NMT (hNMT) is believed to be involved in the myristoylation process [40] and the activity of this enzyme is higher in some colorectal tumours than in the corresponding normal tissues [41]. hNMT may therefore be considered a molecular target in cancer chemotherapy. All of the compounds in series **22** were examined for their effect on human NMT (hNMT) using concentrations up to and including 40 mM. No inhibition or stimulation of the enzyme was observed [37]. On the other hand, two representative compounds in series **23** (R^1=3-Cl, R^2=4-Cl and R^1=4-CH_3, R^2=H) both inhibited the action of hNMT by 52% using concentrations of 100 and 50 μM, respectively [38]. A COMPARE program [42] of a number of compounds in series **23** revealed that the correlation coefficients were greatest in regard to tyrosine kinases which indicates a possible way that these diaryldienones exert their bioactivity.

Previously the impressive cytotoxic potencies of both various 3,5-bis(benzylidene)-4-piperidones **12** and the corresponding *N*-acryloyl derivatives **13** have been described. These bioevaluations utilized neoplastic and transformed cells. An important question to be resolved in considering the potential of these molecules for further development is whether selective toxicity to malignant cells is exhibited by these compounds. Furthermore in order to consider the possibility of increasing the selective toxicity of candidate cytotoxins for neoplasms, the decision was made to form adducts between series **13** and 2-mercaptoethanesulphonic acid leading to the formation of the acids **24**. This suggestion was made on the basis that coadministration of sodium mercaptoethanesulfonate (mesna) and various anticancer drugs led to reduction of the side effects in normal cells but did not alter the antineoplastic properties of the anticancer drugs [43–45]. Thus the compounds **12a,b,e,g,h**, **13a,b,e,g,h** and **24a,b,e,g,h** were prepared and evaluated against both malignant and tumourous cell lines [46].



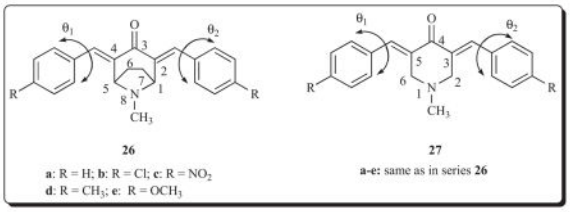
The compounds in series **12**, **13** and **24** were evaluated against the following human neoplastic cell lines, namely HSC-2 and HSC-4 squamous cell carcinomas as well as HL-60 promyelocytic leukemia cells. Three normal human cells were used viz HGF gingival fibroblasts, HPC pulp cells and HPLF peridontal ligament fibroblasts. In regard to the malignant cells, the average CC_{50} values (the concentrations of the compounds to reduce the number of viable cells by 50%) of **12a,b,e,g,h**, **13a,b,e,g,h** and **24a,b,e,g,h** are 29.4, 1.27 and 81.3 μM, respectively, revealing the marked potencies of the compounds in series **13**. In fact, the CC_{50} figures of the compounds in series **13** are submicromolar in 73% of the bioassays. In addition, since the average CC_{50} value of melphalan is 40.7 μM, the far greater potencies of the compounds in series **13** than this established drug are revealed.

Selectivity index (SI) figures were calculated which are the quotients of the average CC_{50} values of the normal and neoplastic cells. A SI figure of 10 was arbitrarily chosen as demonstrating noteworthy selectivity and was met by **12a,e,g** and **13a,b,e,g,h**. The SI figure for melphalan is >4.9. Thus the compounds in series **13** are clearly important lead molecules in terms of cytotoxic potencies and high SI values. The disappointing results of the compounds in series **24** was attributed to two phenomena. First, a stability study of a solution of a representative compound in series **24**, namely **24e**, revealed that no decomposition occurred after incubation at 37° C for 24h (the temperature and time of the cytotoxicity assays). In other words, mesna and **13e** were not liberated from **24e**. Second, the intact molecules **24** contain a polar sulfonic acid group which may impede the penetration of cell membranes and hence in future the corresponding ethyl esters should be made.

An experiment was undertaken to ascertain whether the compression of the 1,5-diaryl-3-oxo-1,4-pentadienyl group into rigid cyclic scaffolds influences cytotoxic potencies and SI values. Consequently the acyclic analog of **12e**, **13e** and **24e**, namely **25**, was prepared. The average CC_{50} values towards the three malignant cell lines of **12e**, **13e**, **24e** and **25** are 3.5, 0.26, 4.7 and 94 μM, respectively, and the SI figures are 13, 15, 7.7 and >4.3. These results indicate that in general the incorporation of part of the 1,5-bis-(4-nitrophenyl)-3-oxo-1,4-pentadienyl group into a piperidine ring makes an important contribution to the desired bioactivities in terms of potency and selectivity. In order to determine the reason for this phenomenon, models were built of these compounds and their shapes compared. While various interatomic distances are similar in **12e**, **13e**, **24e** and **25**, the torsion angles θ_1 and θ_2 as indicated in structures **12**, **13**, **24** and **25** are substantial in the case of **12e**, **13e** and **24e** (42–61°) but very small in **25** (0.1–0.3°) which may have contributed to the divergence in biological properties. The possibility of correlations between θ values and IC_{50} as well as SI figures should be explored in the future.

Substitutions on the C2 and C6 Piperidyl Carbon Atoms

A further structural modification of 3,5-bis(benzylidene)-4-piperidones involved the placement of a dimethylene bridge between the two ring carbon atoms attached to the piperidyl nitrogen atom. The dimethylene bridge could increase binding at receptors by van der Waals bonding; alternatively, it could cause steric impedance to alignment at a binding site thereby lowering cytotoxic potencies. As mentioned previously, there is a lack of coplanarity between the aryl rings and the adjacent olefinic groups in the 3,5-bis(benzylidene)-4-piperidones which is caused by non-bonded interactions between the equatorial proton attached at the C2 and C6 atoms and one of the ortho protons of the aryl ring [22]. The decision was made to replace the axial protons attached to the C2 and C6 carbon atoms in order that the torsion angles θ_1 and θ_2 present in compounds with and without a dimethylene chain will be similar and interpretation of the biodata will not be confused by more variables than necessary. Thus series **26** was prepared and also the corresponding analogs **27** which lack the dimethylene bridge [47].

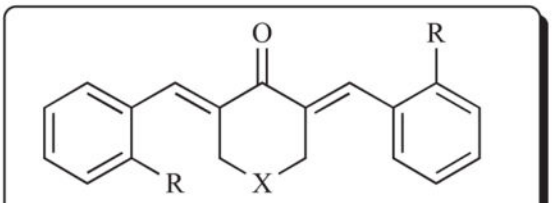


Apart from the anomolous behaviour of **26b** and **26c**, the torsion angles θ_1 and θ_2 are in the range of 43–47° for all of the compounds in series **26** and **27**. Thus substitution on the axial C2 and C6 atoms of **27a–e** leading to the tropinones **26** does not appear to affect the magnitude of the torsion angles in general. The biodata generated for **26** and **27** using the Molt 4/C8, CEM and L1210 bioassays reveal clearly that the insertion of a dimethylene bridge between the C2 and C6 atoms of series **27** leading to **26a–e** causes a reduction in cytotoxic potencies. This conclusion was drawn from the fact that the compounds in series **27** are more potent than the tropinones **26** which have the same R^1 values in the aryl rings except that **26e** is more cytotoxic than **27e** in the L1210 assay. The most potent compound in each series is the un-substituted analog, namely **26a** and **27a**, which have average IC_{50} values of 9.77 and 4.69 μM, respectively. These results may indicate that an E_4 operating parameter is in effect [48], i.e., that para substitution exerts an unfavourable steric effect at binding sites. The compounds were examined against HSC-2, HSC-3, HSC-4 and HL-60 neoplasms as well as HGF, HPC and HPLF normal cells. SI figures of over 10 were obtained for **26a** (>15), **27b** (>19) and **27c** (>11) which serve as lead molecules for future development.

An attempt was made to determine some of the ways that representative compounds exert their bioactivity and to provide an explanation for the differences in potencies between various compounds. A number of the acyclic Mannich bases derived from conjugated arylidene ketones affect respiration in isolated mitochondria [49, 50]. Since 4-piperidone can be considered to be a cyclic Mannich base, two representative compounds with widely differing potencies were evaluated for their effects on respiration in rat liver mitochondria.

The average IC_{50} values of **26a** and **26d** in the Molt 4/C8, CEM and L1210 bioassays are 9.77 and > 500 μM, respectively. Both compounds stimulate respiration in mitochondria but the latent period is much shorter with **26a**. Furthermore, **26a** produced rapid swelling of the mitochondria whereas **26d** does so but more slowly. Thus interference with mitochondrial function appears to be one way in which cytotoxicity is caused and influences the magnitude of the cytotoxic potencies.

Some further aspects of the potential of 3,5-bis(benzylidene)-4-piperidones for utilization in cancer chemotherapy may be illustrated with compound **28a**. This cytotoxic molecule may be linked to other structural units in order to increase selective toxicity to neoplasms, is effective *in vivo* towards certain tumours and has anti-angiogenic properties. A complex of compound **28a**-linker-tripeptide-methylketone-factor VIIa has been prepared [51, 52]. Factor VIIa interacts with tissue factor which is expressed on the surface of tumours and also on the endothelial cells of the tumour vasculature. Hence the complex should hone in preferentially on malignant cells, undergo endocytosis and then release the cytotoxin. This complex inhibits the growth of both human MDA-MB-231 breast cancer cells and human RPMI-7951 melanoma cells to a greater extent than when **28a** was used alone. This compound **28a**, which demonstrated promising activity in the NCI *in vitro* screen, was administered subcutaneously to nude mice inoculated with human MDA-MB-231 breast cancer cells [53]. The average tumour weight was reduced by 30% and 55%, respectively, using doses of 20 and 100 mg/kg, respectively. The 100 mg/kg dose did not cause any liver, kidney or spleen toxicity and the animals had normal weight gain. Anti-angiogenic compounds have a suppressive effect on proliferating endothelial cells and selectively inhibit the blood supply to tumours. Compounds **28a** and **28b** inhibit endothelial cell growth *in vitro* and further evaluation revealed that **28a** reduced cord formation and cell migration in human umbilical vein endothelial cells (HUVEC) at a concentration of approximately 1 μM [53]. Thus such compounds, even if bereft of antineoplastic properties, could be administered with anti-cancer drugs. In addition, development of enones possessing both cytotoxic and anti-angiogenic properties may well prove to be useful agents to combat carcinogenesis as is the case, for example, with certain combretastatins [54,55].



28

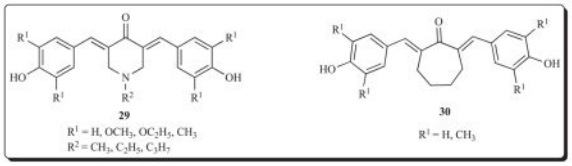
a: R = F; X = NH CH_3COOH

b: R = OH; X = NCH_3

A number of diaryldienones are antioxidants and the importance of this property is deemed to be as follows. The body has a number of natural defences to harmful chemically reactive species such as various enzymes, e.g., superoxide dismutase, glutathione peroxidase and

catalyse. In addition, reduced glutathione plays a part in the strategies to eliminate various chemically reactive species. However when these defences are overcome, then a variety of toxic reactions can occur such as the initiation of carcinogenesis. In order to counteract these problems, antioxidants may be used to scavenge harmful reactive oxygen and nitrogen species [56], and hence may be referred to as chemoprotectant agents. In the case of phenolic compounds, the free radical scavenging capacity is associated with their ability to donate hydrogen atoms [57].

A variety of diaryldienones which contain a 4-hydroxy substituent in the aryl rings have demonstrated antioxidant properties. The series of compounds **29** and **30** were prepared as antioxidants and their relative ability to scavenge free radicals was determined towards diphenylpicrylhydrazyl [58]. The pseudo first order rate constants (K $min^{-1} \times 10^{-2}$) were determined from which the following correlations between structure and antioxidant properties were noted. First, in series **29**, the placement of one or two methoxy, ethoxy or methyl substituents ortho to the 4-hydroxy group increased the radical scavenging properties compared to the parent compound (R^1 groups are hydrogen atoms). Increasing the size of the *N*-alkyl group in series **29** from methyl to ethyl to propyl led to compounds displaying a corresponding increased reaction rate to the diphenylpicrylhydrazyl free radical. This observation is surprising as the diaryldienone moiety is separated from the *N*-alkyl group by three sigma bonds. Second, replacement of the 4-piperidone ring by a cycloheptyl scaffold produced series **30** with similar K $min^{-1} \times 10^{-2}$ values. In addition, the ability to scavenge the oxygen free radical produced by the addition of phorbol-12-myristate-13-acetate to peripheral multinuclear neutrophil cells revealed that all of the compounds inhibited free radical formation with the highest activity being present in series **29**.



2. bis(Benzylidene)cycloalkanones

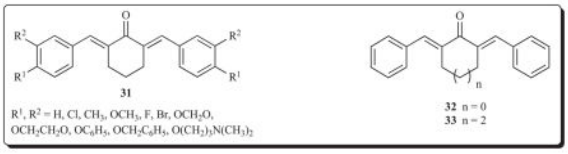
The previous section outlined in some detail the cytotoxicity of a number of 3,5-bis(benzylidene)-4-piperidones and indicated some of the ways by which bioactivity is mediated. The 1,5-diaryl-3-oxo-1,4-pentadienyl group has also been mounted on alicyclic scaffolds which are now presented. In general, the compounds **31**-**38** were evaluated against human Molt 4/C8 and CEM T-lymphocytes and the IC_{50} values are generally in the range of 0.1–10 μM, while somewhat higher figures of 0.5–15 μM are found in the murine L1210 screen. In addition, doses up to and including 300 mg/kg could be administered to mice without causing lethal effects. In this section emphasis is placed on important SAR correlations, probing for the mechanisms of action of representative compounds, the identification of lead molecules and guidelines for future development.

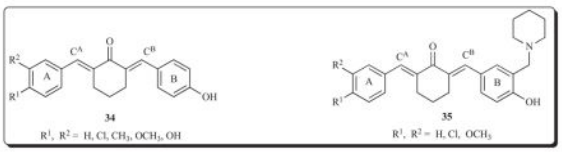
A series of 12 bis(benzylidene)cyclohexanones **31** were assessed against murine L1210 cells and the IC_{50} values ranged from 0.2 to >50 μM [59]. A Topliss analysis [48] based on the biodata of five compounds revealed a $+\sigma$ dependency. This result indicates that extension of

this project should include the placement of strongly electron-withdrawing groups in the aryl rings. The most potent compound [**31**, $R^1 = O(CH_2)_3N(CH_3)_2$, R^2 = H] possesses an IC_{50} value of 0.2 μM revealing its potency to be twice that of the reference drug melphalan. In addition, the average IC_{50} figure of this compound towards a number of human tumour cell lines from different neoplastic conditions (the NCI screen) is 1.55 μM which is 17 times lower than the average IC_{50} value of melphalan. This compound is clearly a lead molecule.

In order to obtain an appreciation of the importance of the relative locations of the olefinic carbon atoms, two analogs of **31** ($R^1 = R^2$ = H), namely **32** and **33** were prepared. An overlap of the three compounds as indicated in Fig. (3) reveals that the nature of the alicyclic scaffold controls the relative positions of the olefinic protons where interactions with thiols is considered to occur. The IC_{50} figures towards L1210 cells of **31** ($R^1 = R^2$ = H), **32** and **33** are 4.4, 7.5 and 16 μM, respectively, suggesting that retention of the six-membered ring is preferred.

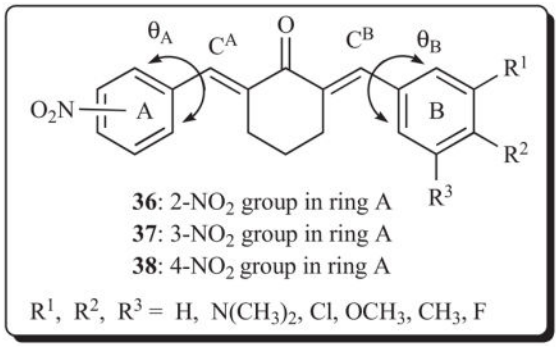
Two further series of 2,6-bis(benzylidene)cyclohexanones **34** and **35** were prepared in which the group in rings A and B were different in most cases [60]. This strategy led to variations in the atomic charges on the olefinic carbon atoms C^A and C^B which should facilitate the concept of sequential cytotoxicity taking place. A number of tumours are more acidic than the corresponding normal cells [61]. The placement of a 1-piperidinylmethyl group in ring B in series **35** should lead to a higher percentage of charged species in malignant cells than the corresponding normal tissues which would deplete the electron density on the C^B atom and could make it more reactive towards thiols. In both series of compounds, linear plots between the atomic charges on the C^A atoms and the Hammett σ values were observed ($p < 0.05$). The IC_{50} values of the majority of the compounds are in the low micromolar range (3–6 μM) and have comparable potencies to melphalan.





A continuation of the concept of exacerbating the differential in the atomic charges on the olefinic carbon atoms has been made recently [62]. In these compounds, the strongly electron-withdrawing nitro group was placed in the ortho, meta or para locations in ring A while a variety of substituents having different σ values was placed in ring B. Such considerations give rise to series **36-38**. The differences in the charge densities on the olefinic carbon atoms C^A and C^B are approximately 0.02–0.07 esu. Interestingly the atomic charges are lower on the C^B atom, indicating that the polarization of the π electrons in the 1,5-diaryl-3-oxo-1,4-pentadienyl group is towards ring A. The compounds were evaluated in

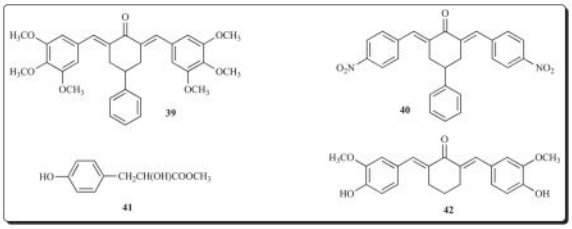
the Molt 4/C8, CEM and L1210 bioassays and the IC_{50} values are generally in the 1–10 μM range.



The following correlations between structures and cytotoxic potencies were noted. First, positive correlations ($p < 0.05$) were found when linear plots were constructed between the electron densities at carbon atom C^B and the IC_{50} values in all three screens for series **36** and **38** and in the L1210 assay in series **37**. Thus potency increases (IC_{50} values diminish) as the charge densities are reduced at the site of the initial alkylation at C^B. Hence in the future, substituents with large σ values should be placed in ring B. Second, the same groups are present in ring B in series **36**-**38**. A comparison of the IC_{50} values in the Molt 4/C8, CEM and L1210 bioassays revealed that the order of potencies is **36** > **37** > **38**. The rate and extent of thiolation at the C^A and C^B atoms may be influenced by the magnitude of the torsion angles θ_A and θ_B. The θ_B angles in all three series of compounds are virtually constant viz 51.4±0.5°, whereas the θ_A figures in series **36**, **37** and **38** are 76.8°, 51.3° and 51.1°, respectively. Hence the creation of new series of analogs in which larger θ_A values are manifested should be prepared such as the formation of congeners with large Taft E_S constants. Third, the average log P figures of series **36**, **37** and **38** are 4.86, 5.06 and 5.08, respectively, and hence the lower lipophilicity in series **36** may have contributed to its superior potencies.

Since a number of thiol alkylators react with mercapto groups in mitochondria [63], three representative compounds **36d**, **37d** and **38d** were chosen, each of which has the 3,4,5-trimethoxy substitution pattern in ring B. The average IC_{50} figures of **36d**, **37d** and **38d** in the three bioassays are 0.88, 43.8 and 6.00 μM, respectively. Using a concentration of 10μM, the two most potent compounds, viz **36d** and **38d**, stimulated the rate of respiration in rat liver mitochondria while **37d** had no effect at 10 μM nor when the concentration of this compound was raised to 100 μM. While the variation in cytotoxicity is likely multifactorial, the different effects on mitochondrial function may contribute to the differences in the observed potencies.

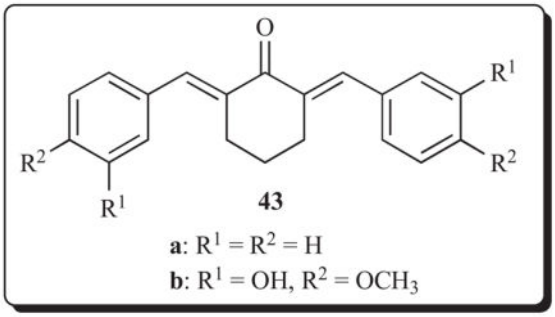
A further aspect of the contribution that some of these compounds may make to cancer chemotherapy is the ability to reverse multidrug resistance. For example, using a concentration of 4 μg/mL, **38d** and a related analog **38** ($R^1 = R^2 = OCH_3$; $R^3 = H$) have FAR values of 16 and 20, respectively, in the L-5178 P-gp reversal screen [64].



Various 2,6-bis(benzylidene)-4-phenylcyclohexanones have IC_{50} values in the low micromolar range when evaluated against murine B16 melanoma and L1210 leukemia cell lines and in the case of **39** and **40**, the IC_{50} figures in the B16 and L1210 assays are 0.51 and 0.35 μM, respectively, and are clearly lead molecules [65]. There is a structural similarity of some of these compounds, particularly those possessing several methoxy aryl substituents, to agents which inhibit tubulin polymerization. Two representative compounds, namely **39** and **40**, were examined for this property. However using concentrations at least 25 times greater than the IC_{50} values did not disrupt microtubules. Hence the way in which these compounds exert their cytotoxic properties is by mechanisms other than inhibition of tubulin polymerization.

Another important example of a 2,6-bis(benzylidene) cyclohexanone is RSV-101. Compound **41,** known as methyl p-hydroxyphenyllactate, is a cell growth regulating agent which occurs naturally [66]. It binds to nuclear type II sites in non-malignant cells thereby ensuring a relatively low level of cell proliferation. However **41** is metabolized in tumour cells which leads to high levels of unoccupied nuclear type II sites and hence a loss of regulatory control. The enone RSV-101 (**42**) binds with high affinity to nuclear type II sites in neoplastic cells which inhibits malignant cell proliferation [67, 68].

The 2,6-bis(benzylidene)cyclohexanones **43** have anti-angiogenesis properties causing a 90–94% inhibition of SVR endothelial cells using a concentration of 3 mg/mL [69].



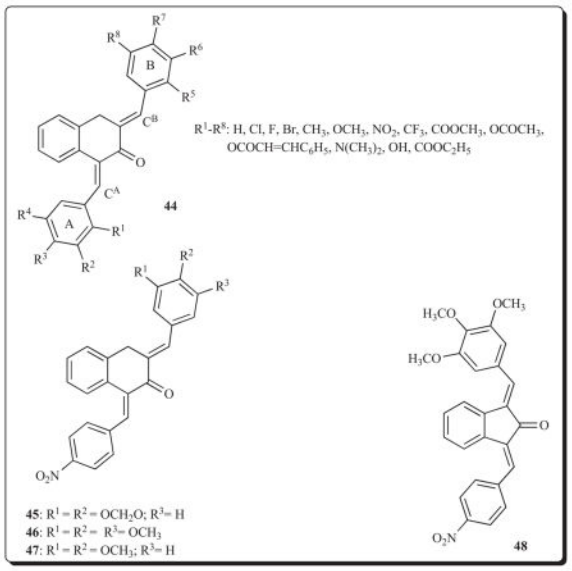
3. 3,4-Dihydro-1*H*-naphthalen-2-ones

A further investigation designed to create molecules which permit successive reactions with cellular thiols to occur involved the preparation of a series of 1,3-bis(benzylidene)-3,4-dihyro-1*H*-naphthalen-2-ones **44** [70]. In these compounds the olefinic carbon atoms C^A and C^B are in different topographical environments. Thus when both rings A and B are unsubstituted, the Mulligen charges on C^A and C^B are −0.105 and −0.095, respectively. All

of the diaryldienones **44** were examined in the Molt 4/C8, CEM, L1210 and P388 bioassays and selected compounds in the NCI screen. The IC_{50} figures are generally in the low micromolar range. Three representative compounds, namely the unsubstituted, 2-nitro and 4-nitro congeners, inhibited DNA, RNA and protein biosynthesis while the unsubstituted and 2-nitro analogs did not bind to calf thymus DNA. Thus, in part at least, the cytotoxicity of these compounds is by interference with macromolecular biosynthesis.

A subsequent investigation with three representative compounds was undertaken in an attempt to find additional ways by which cytotoxicity is mediated [71]. The three compounds chosen, namely **45-47** have IC_{50} values in the 1.0–1.6 μM range towards Molt 4/C8 and CEM cells. First, a TUNEL assay revealed that **45** caused apoptosis in human HepG2 liver cancer cells at a concentration of 1 μM, suggesting that apoptosis is an important way whereby cytotoxicity is mediated. Second, there is higher activity and expression of hNMT in some colon cancers than the surrounding tissues *vide supra*. At the maximum concentration utilized, namely 1 mM, **46** inhibited hNMT by 23%. Third, a number of arylidene compounds which have several methoxy groups on the aryl ring inhibit tubulin polymerization [72]. However **47** inhibited this biochemical process by 16% only using a concentration of 50 μM. In summary, an important mechanism of action of these novel cytotoxins is by induction of apoptosis while the inhibition of hNMT and tubulin polymerization appear at most to be marginal contributors to the bioactivity observed.

A further consideration is whether this cluster of diaryldienones possesses selective toxicity to malignant cells. Accordingly **45**-**47** and several analogs were evaluated against HSC-2, HSC-3, HSG and HL-60 neoplastic cell lines as well as non-malignant HGF, HPC and HPLF cells [64]. These compounds display high potencies towards the tumorous cells, e.g., the average CC_{50} value of **46** is 1.75 μM. On the other hand, the CC_{50} figures for several of these compounds towards the non-malignant cells is in excess of 400 μM. The selective index (SI) figures range from 12 to 229 (in the case of **46**) which affords further evidence of the importance of developing these compounds. Of interest is the observation that the nor analog of **46**, namely **48**, was far less potent towards the malignant cells (average CC_{50} value of >335 μM) and had a SI figure of 0.6. This observation confirms the importance of the relative locations of different functional groups in these compounds with the bioactivity observed. This conclusion was also reached by comparing the cytotoxic potencies of **49** with its vinylog **50** [73]. In the Molt 4/C8, CEM and L1210 assays, **50** is on average nine times less potent than **49** (average IC_{50} value is 4.68 μM) and further experimentation is required in order to determine whether the optimal spacer between the aryl rings is an 3-oxo-1,4-pentadienyl group.

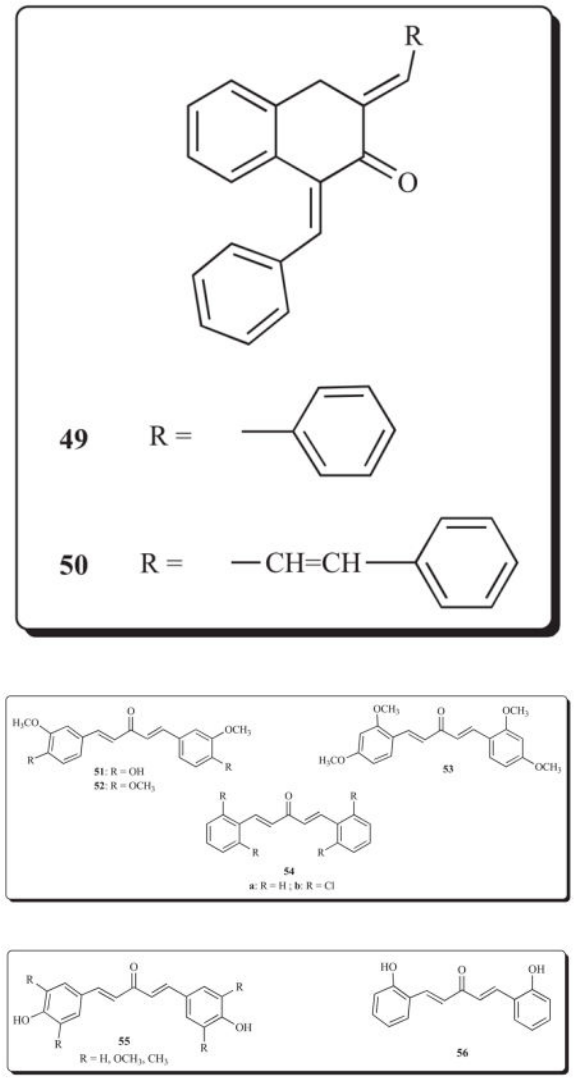


The importance of developing 3,4-dihydro-1*H*-naphthalen-2-ones is that a number of these molecules demonstrate significant potencies in the L-5178 P-gp reversal screen. In particular **46** and **47** have FAR values of 134 and 44, respectively, using a concentration of 4 μg/mL [64].

4. Acyclic 1,5-diaryl-3-oxo-1,4-pentadienes

Recently the acyclic derivatives **51**-**53** were evaluated using two human prostate cancer cell lines, namely PC-3 cells which are androgen-independent and LNCaP cells which are androgen-dependent [74]. Both **51** and **52** display excellent growth-inhibiting properties with IC_{50} values in the 1.4–3.8 μM range towards both cell lines. On the other hand, when the two methoxy groups are located in the 2 and 4 positions of the aryl rings as in **53**, the potencies towards PC-3 and LNCaP cells compared to **52** are lowered by approximately sixfold. In this study involving a number of structurally related compounds with larger unsaturated conjugated spacer groups between the aryl rings than the five carbon chain in **51**-**53**, the importance of 3,4-disubstitution on the aryl rings in conferring marked potencies was noted.

Another feature of certain acyclic diaryldienones is their anti-angiogenic properties. Thus the unsaturated ketones **54a** and **54b** inhibited the growth of SVR endothelial cells by 97% and 87%, respectively, using a concentration of 3 mg/mL [69].



Series **55** were prepared as candidate antioxidants and shown to possess radical-scavenging properties as determined towards diphenylpicrylhydrazyl [58]. Other studies with compounds related to **55** displayed good antioxidant properties and maximum potencies are displayed by compounds possessing two small substituents ortho to the aryl hydroxy group [75]. However another investigation revealed that the presence of an aryl hydroxy group does not automatically confer antioxidant properties [76]. It is of interest to note that **56** not only inhibited superoxide formation and lipid peroxidation but displayed marked cytotoxicity towards Dalton's lymphoma ascites cells and Ehrlich ascites cells [77].

5. Concluding Remarks on Cytotoxicity

The account so far has revealed that marked potencies are displayed by a variety of compounds in which the 1,5-diaryl-3-oxo-1,4-pentadienyl groups is present in a number of piperidines, cycloalkanes, arylalicyclics and acyclic molecules. In the future, the diaryldienone moiety should be mounted on further scaffolds with the goal of optimizing the biological responses. In this review is described the marked potencies of some

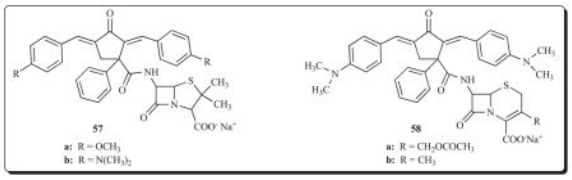
diaryldienones which is accompanied by such favourable properties as greater toxicity to neoplasms than non-malignant cells. Various physicochemical parameters of some of the molecules described herein such as atomic charges and torsion angles influence the magnitude of the biological responses. The modes of action include the induction of apoptosis, stimulation of respiration in mitochondria and possibly a minimal effect on human myristoyl-CoA:protein N-myristoyltransferase (hNMT). In virtually all cases the series of compounds are well tolerated by mice.

Mention may be made of three further studies which aim to unify some of these observations. First, the 3D QSAR methodologies of comparative molecular field analysis (CoMFA) [78,79] and comparative molecular similarity indices analyses (CoMSIA) [80] were applied to the cytotoxic potencies of various 1,3-bis(benzylidene)-3,4-dihydro-1*H*-naphthalen-2-ones, 2,6-bis(benzylidene)cyclohexanones and 3,5-bis(benzylidene)-4-piperidones in the Molt 4/C8, CEM and L1210 screens [81]. From these determinations, the compounds interact at binding sites which have the following characteristics, namely two electropositive pockets to accommodate the electron-withdrawing group in the para position of the aryl rings, the aryl rings align at an electro-negative pocket and no groups should be present in the ortho and meta locations, a sterically forbidden site at which one of the olefinic groups interacts (i.e., no substitution on one of the methine carbon atoms) and a bulky hydrophobic pocket into which the group beta to the carbonyl function fits (e.g., the *N*-acyl group in the case of the 4-piperidones).

Second, a recent review indicated that many bioactive compounds contain an allylic group with an oxygen atom at one end of this substituent [82]. The oxygen atom can be located in a hydroxy, ester, ether, ketal or carbonyl substituents. Furthermore various compounds which lack this functionality may be converted into one of these groups by metabolism and in particular by the cytochrome P-450 enzymes. The allylic bioactive molecules may exert their influence in a variety of ways including controlling the phosphorylation of proteins, reducing the electron flow in mitochondria and lowering the concentration of glutathione in cells.

Third, as mentioned in the introductory remarks of this article, compounds interacting with multiple targets may exert more than one type of bioactivity. An example of the success of this strategy is as follows. An investigation was launched with a view to producing novel compounds with both antitumour and antimicrobial properties. In the molecules **57** and **58**, the penicillin and cephalosporin side chains contain the antineoplastic diaryldienone group [83]. The percentage total growth inhibition in the murine S-180 screen for **57a**, **57b**, **58a** and **58b** are 36, 68, 41 and 58, respectively, and 38, 69, 0 and 54, respectively, in the murine S-37 bioassay. The percentage increases in the survival times of mice inoculated with Ehrlich's ascitic carcinoma are 25, 100, 0 and 0, respectively. Clearly **57b** in particular is a promising lead molecule. These compounds are well tolerated in mice insofar as the LD_{50} values ranged from 1250–3000 mg/kg compared to 2.6 mg/kg for the reference compound carminomycin. In addition, these molecules suppressed the growth of *Staphylococcus aureus* and *Staphylococcus albus* in the concentration range of 0.39–1.56 μg/mL. Two further positive attributes of these compounds are their greater resistance to both β-lactamase and acidic conditions than benzylpenicillin. This investigation illustrates the importance of the

1,5-diaryl-3-oxo-1,4-pentadienyl group in producing compounds capable of combating multiple pathological conditions.



MODES OF ACTION

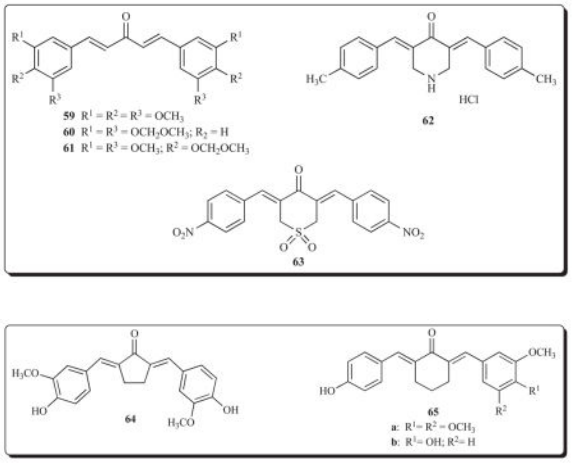
Some of the ways in which certain diaryldienones exert their antineoplastic properties have been mentioned previously such as inducing apoptosis, inhibiting macromolecular biosyntheses and stimulating respiration in mitochondria. Some further comments revealing the array of different molecular targets of these compounds will now be presented.

The diaryldienones **59**-**61** possess an IC_{50} value of 0.25 μM towards human HCT116 colon cancer cells and the way whereby cytotoxicity is mediated has been investigated [84]. Cell cycle analysis revealed that treatment of HCT116 cells by **59**-**61** impaired the synthesis of DNA. In addition, both **60** and **61** caused an increase in the sub-G_1 population of the cells which indicates that apoptosis has occurred. Apoptosis can proceed *via* two major pathways. First, Fas (CD95/APO-1)-associated death domain (FADD) binds to procaspase 8 leading to the formation of caspase-8. Second, cytochrome c is released from the mitochondria leading to a complex with Apaf-1 which binds to procaspase-9 and activates various caspases including caspase-9. When **60** and **61** were added to HCT116 cells treated with an inhibitor of caspase-3 and caspase-8, namely Z-DEVD-fmk, the sub-G_1 population returned to normal values. Thus two modes of action of these compounds are by inhibiting DNA synthesis and inducing apoptosis. Furthermore **60** and **61** induced down regulation of a number of oncoproteins, including ErbB-2, c-Myc, cy-clin D1 and Ki-ras.

Both **62** and **63** cause apoptosis by a Bcl-2-dependent but apoptosome-independent pathway of caspase activation in E1A/C9 cells [85]. This pathway is not followed by many anticancer agents [86]. Both **62** and **63** inhibit ubiquitin isopeptidases as do other cross-conjugated α,β;-unsaturated dienones with two sterically accessible electrophilic β-carbon atoms [87]. Hence ubiquitin-dependent protein degradation is impeded.

Another site of action of a diaryldienone **64** is topoisomerase 1. This unsaturated ketone, which inhibits the growth of a number of human tumour cell lines in the low micromolar range, interferes with two functions of topoisomerase 1, namely DNA relaxation and DNA single strand breaks [88]. It does not intercalate with DNA but binds to AT-rich sites in the minor groove of this nucleic acid. Cell cycle analysis revealed that treatment of KB cells with **64** caused accumulation in the G2/M phase. Camptothecin is an anticancer drug which interferes with the function of topoisomerase-1 but no cross-resistance was noted with two camptothecin-resistant cell lines. This compound also inhibits tubulin polymerization and causes apoptosis in KB cells whereby activation of caspase-3 was observed. A further study with **64** indicated that this molecule activated p53 but not Bcl-2 in KB cells [89].

Furthermore it did not cause perturbed mitochondrial membrane potential in contrast to **69**, *vide infra*, which emphasized how minor structural changes affect the modes of action of the 1,5-diaryl-3-oxo-1,4-pentadienes.



Thioredoxin reductase catalyzes NADPH-dependent reduction of the disulfide group in thioredoxin. Hence this enzyme is important in controlling the redox potential and proliferation in cells. A number of tumours have elevated concentrations of thioredoxin reductase [90] and thus molecules which inhibit this enzyme may lead to products which are useful in cancer chemotherapy.

Following the observation that curcumin inhibits rat thioredoxin reductase [91], a number of analogs of this naturally occurring compound have been evaluated, including **65a** and **65b** [92]. The IC_{50} values of **61a** and **61b** towards thioredoxin reductase are 24.9 and 5.1 μM, respectively, and this mechanism of action may contribute to the antineoplastic properties of some or all conjugated dienones.

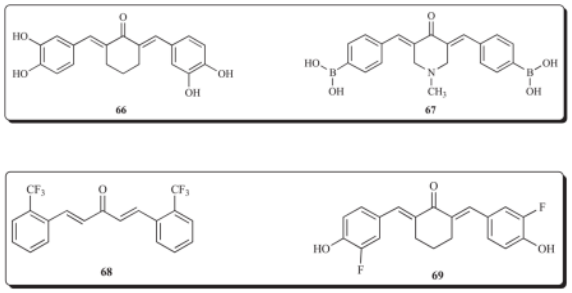
Recently a quest was initiated with the goal of finding compounds which stimulate an enzyme associated with cellular proliferation for the following reasons. The theory of sequential cytotoxicity is based on the differences in the vulnerability between neoplastic and non-malignant cells to chemical insults *vide supra*. The possibility exists that this differential in sensitivity could be exacerbated by the rate of proliferation in cancer cells being increased to a greater extent than occurs with normal tissue. Hence the first stage to explore this hypothesis is to find a cluster of compounds which stimulates an enzyme which controls cell proliferation, such as a protein kinase (PK). Fyn kinase is a PK and is overexpressed in certain tumours [93]. The 4-piperidone **5** stimulates fyn kinase, albeit weakly, namely approximately 15% using the concentration range of 25–100 μM [94]. However conversion of **5** to various *N*-acyl analogues led to compounds with increased enzyme-stimulating properties, e.g., **15** caused a 56% increase in fyn kinase activity at a concentration of 0.1 nM [94]. In addition, this compound is a cytotoxin having an average IC_{50} value of 5.11 μM in the Molt 4/C8, CEM and L1210 screens [30]. Hence the structural features of **15** could be included in the design of more complex molecules which initially release **15** or related compounds. Hence a greater increase in sensitivity to a subsequent

liberation of a cytotoxin in malignant cells rather than the corresponding normal cells may result.

Another way in which diaryldienones exert their antineoplastic properties is by interactions with nuclear type II sites. Ligands which react with this receptor inhibit cellular growth and proliferation since the type II sites located in the nuclear matrix are believed to assist DNA synthesis and replication [95,96]. Compounds **42** and **66** bind to type II sites in rat uterine cells with very high affinity [68]. A correlation was noted between the extent of binding and the percentage inhibition of the growth of human MCF-7 breast cancer cells. In addition, **66** reduced the growth of T-364 L mammary tumours in mice revealing it possessing anticancer properties *in vivo*.

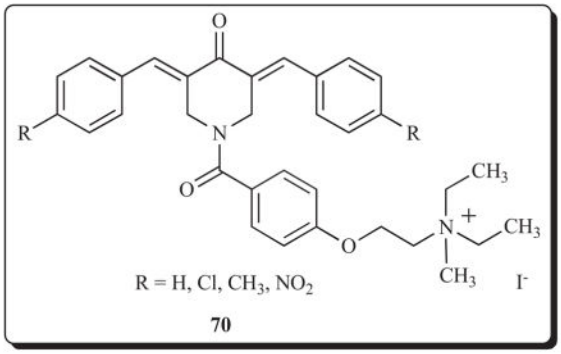
The proteasome is an enzyme complex involved in the degradation of proteins which is accomplished by ubiquitin conjugation. The diaryldienone **67** inhibits the activity of the 20S proteasome *in vitro* with an IC_{50} value of approximately 1 μM [97]. Thus the cytotoxicity of **67** towards cancer cells may be *via* inhibition of the proteasome leading to the accumulation of p53 and ubiquitinated proteins.

Another site of action of certain cyclic and acyclic diaryldienones is NFkappaB (NFκB) expression. This transcription factor is activated in a number of tumours and is important in metastasis [98]. NFkB protects certain malignant cells from apoptosis and hence compounds which inhibit its expression may be valuable in cancer chemotherapy. An acyclic and cyclic series of compounds have been prepared as exemplified by **42, 56** and **68** [99]. These diaryldienones were evaluated for their capacity to inhibit TNFα-induced activation of NFκB in cells stably infected with NFkB-dependent reporter construct using the Panomics Reporter Stable Cell Line. Compounds **42**, **56** and **68** have IC_{50} values of approximately 4.5 μM.



An important cellular target of compounds containing the 1,5-diaryl-3-oxo-1,4-pentadienyl group is the mitochondria. In particular these compounds have the capacity to increase the rates of respiration in these organelles. Compound **69** increases respiration rates in mitochondria and causes a collapse of the membrane potential [100]. This effect was reversed by 6-ketocholestanol which is a recoupling agent suggesting that **69** is an uncoupler. This compound inhibits the production of superoxide and oxidizes certain thiol groups which lead to swelling of mitochondria and the release of cytochrome c.

The possibility that different effects on mitochondria contribute to variation in cytotoxic potencies and murine toxicity was addressed recently [101]. Reference has already been made to the excellent cytotoxicity to series **17** [30] and that these compounds are well tolerated in rodents [31]. Quaternization of the diaryldienones **17** led to **70** which have reduced potencies [30] and most of the compounds are lethal when 300 mg/kg is administered to mice [31]. Using a concentration range of 10–100 μM, all of the compounds in series **17** and **70** stimulate respiration in rat liver mitochondria revealing one of the ways in which these compounds exert their bioactivity. However in virtually all cases when the same substituent was present in the arylidene aryl rings, respiration was greater in the quaternary ammonium salts. Furthermore using a concentration of 50 μM, all of the members of **70** caused swelling of mitochondria in contrast to a representative member of series **17** (R=H). While the greater effect on mitochondria by **70** does not explain their diminished cytotoxic properties compared to **17**, the pronounced murine toxicity noted in the quaternary ammonium salts may be due to a greater biochemical effect on mitochondria.



70

CONCLUSIONS

A number of diaryldienones have IC_{50} values towards neoplastic and transformed cells in the submicromolar range (0.1–0.9 μM) while single and double digit nanomolar values are observed in some cases. The diaryldienones have the capacity to alkylate cellular thiols sequentially which may permit greater toxicity to malignant cells rather than the corresponding normal cells. In this regard, the clear demonstration of the preferential toxicity to neoplastic cells has been observed. Arguments have been presented in favour of compounds which have multiple sites of action and this review has revealed a number of different ways in which diaryldienones exert their modes of action. The observation that antiangiogenic, MDR-revertant and antioxidant properties coexist with antineoplastic activity affords further support for the development of these compounds as candidate anticancer agents.

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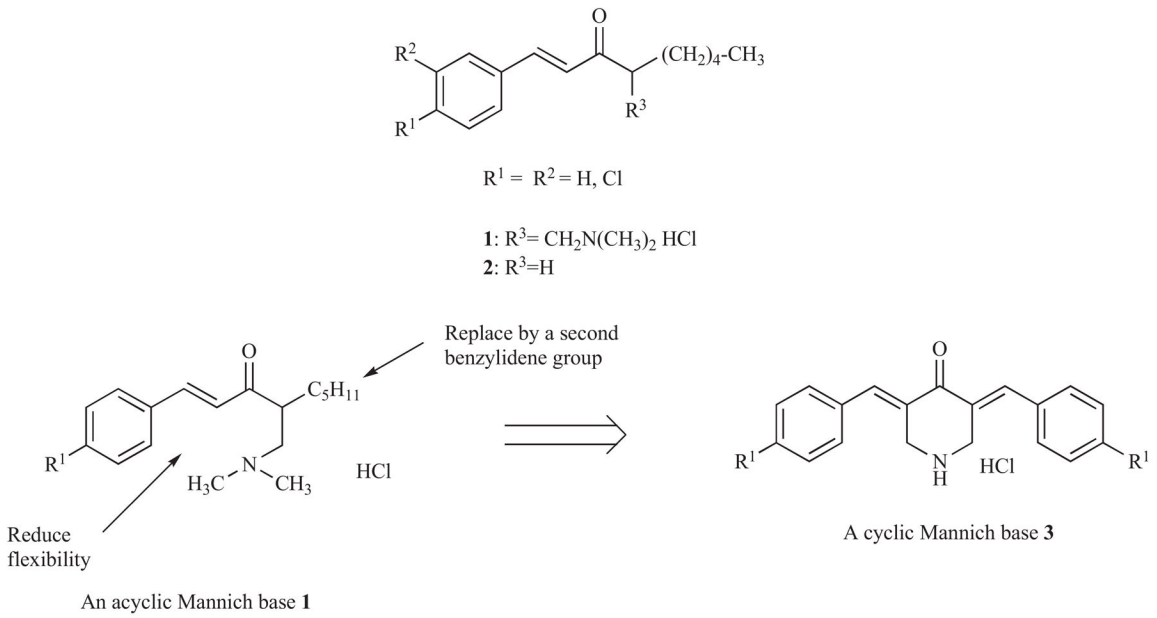


Fig. 1.

The design of 3,5-bis(benzylidene)-4-piperidones as candidate antineoplastic agents.

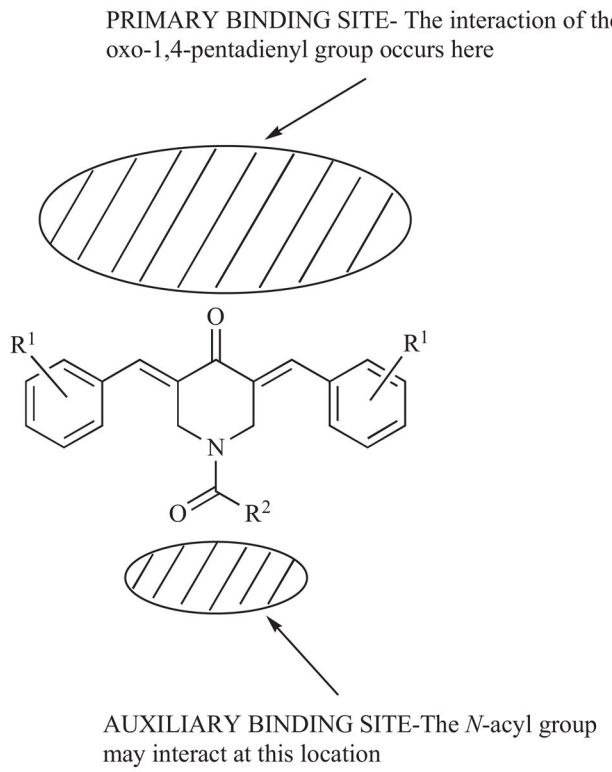


Fig. 2.

The postulated interactions of *N*-acyl-3,5-bis(benzylidene)-4-piperidones at binding sites.

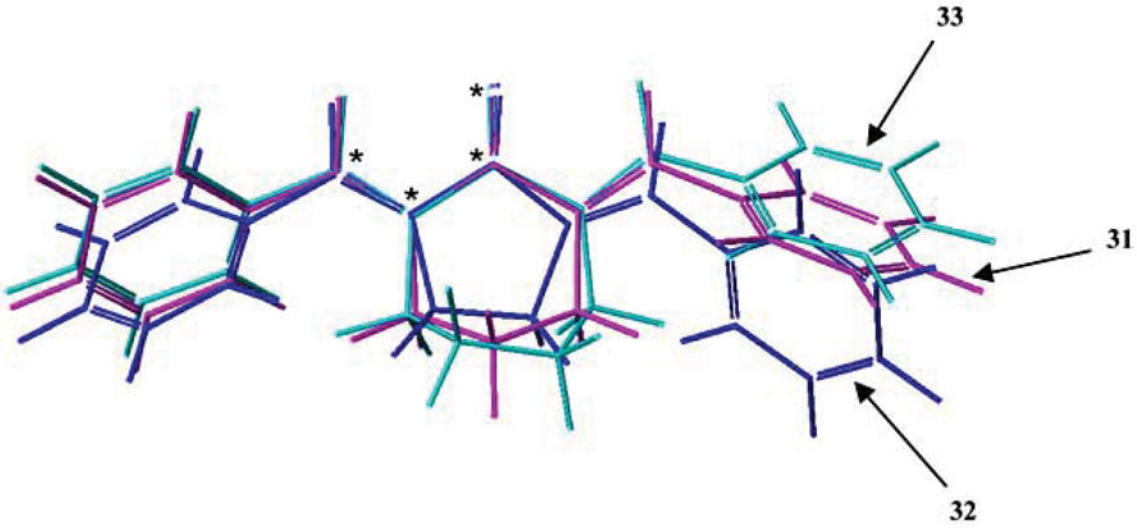
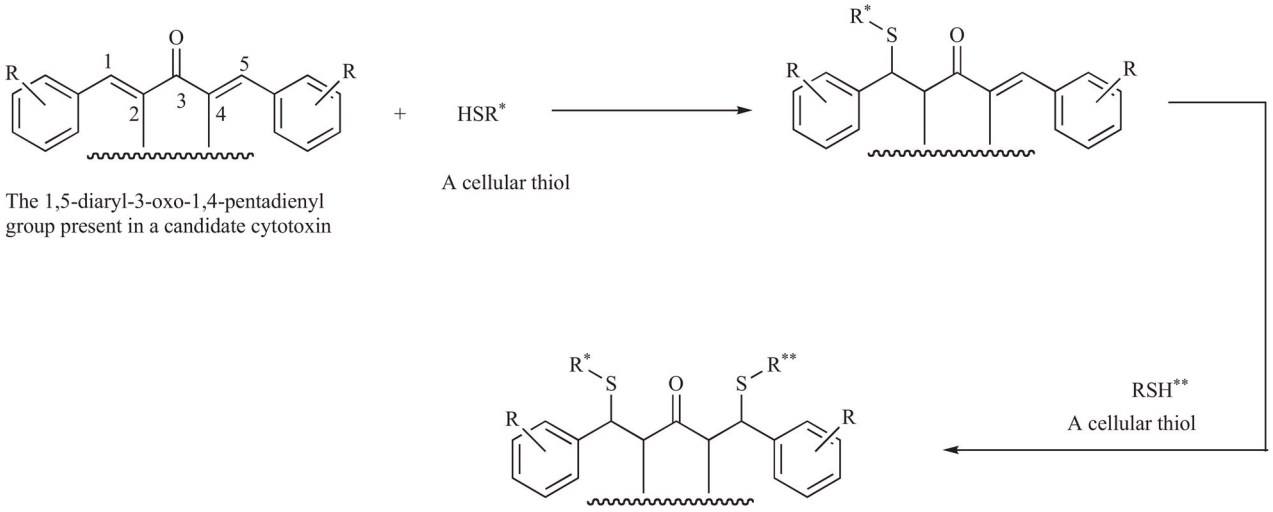


Fig. 3.

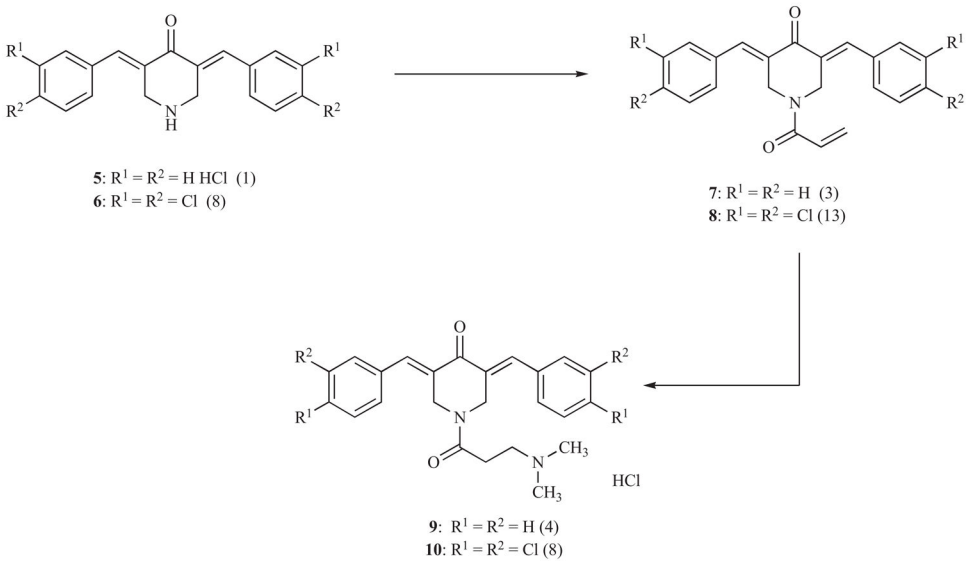
Overlap of models of **31** ($R^1 = R^2 = H$), **32** and **33**. The four atoms which are superimposed are indicated with an asterisk.



The 1,5-diaryl-3-oxo-1,4-pentadienyl group present in a candidate cytotoxin

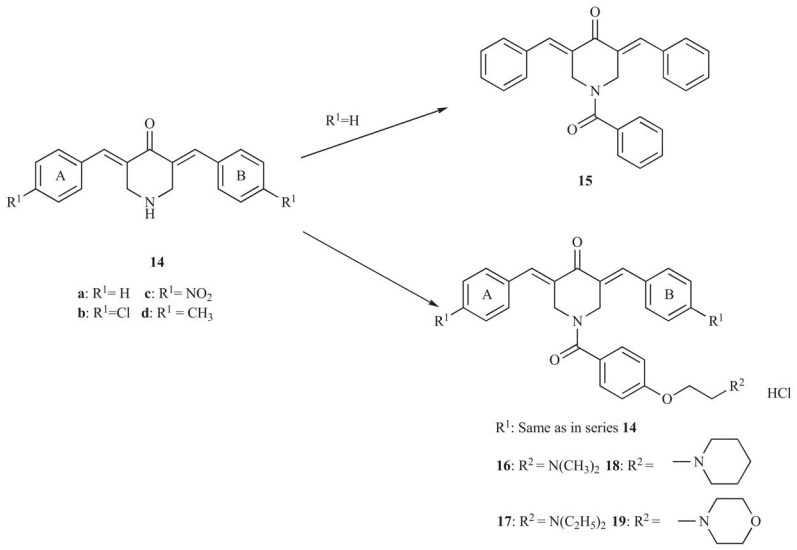
Scheme 1.

The sequential alkylations of cellular thiols by compounds containing the 1,5-diaryl-3-oxo-1,4-pentadienyl group.



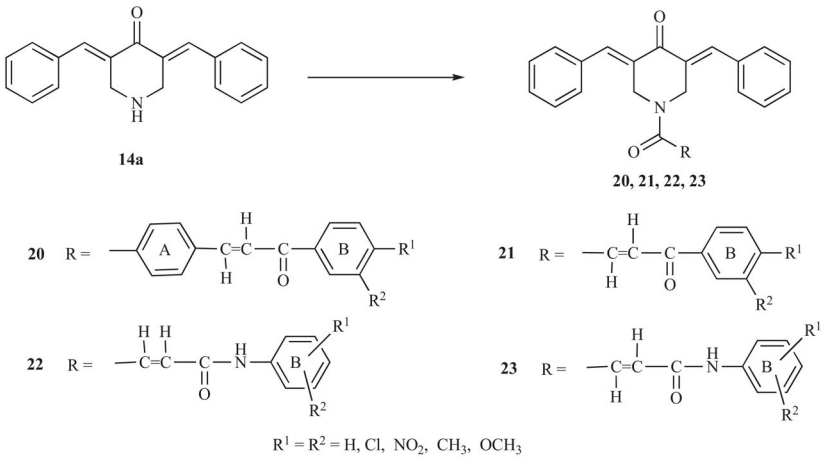
Scheme 2.

Conversion of the 3,5-bis(benzylidene)-4-piperidones **5** and **6** into the *N*-acylated products **7**-**10**.



Scheme 3.

Formation of the *N*-acyl products **15**-**19** from series **14**.



Scheme 4.

Synthesis of **20-23** from **14a**.