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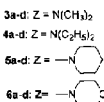
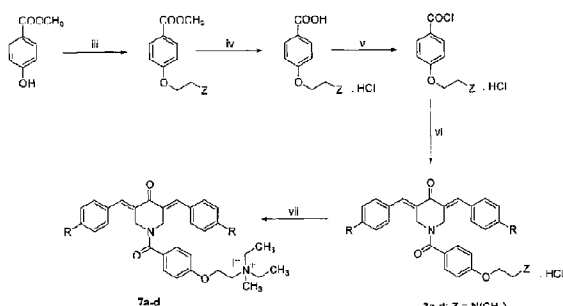
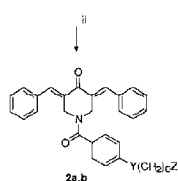
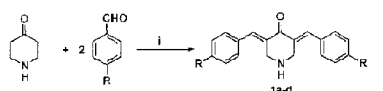
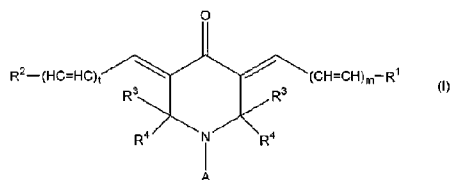
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[Continued on next page]

(54) Title: ANTINEOPLASTIC COMPOUNDS



(57) Abstract: The present invention relates to 4-piperidone derivatives represented by the following formula (I) and the acid addition salts thereof. The method of preparation and antineoplastic activity of the said compounds are disclosed. A number of the compounds possess submicromolar IC₅₀ and CC₅₀ values and have a selective toxicity for colon cancers and leukemic cells. In addition, many of the compounds are able to reverse multidrug resistance.



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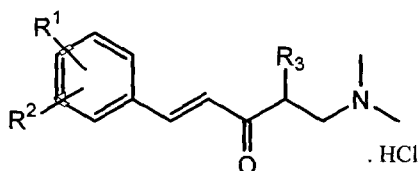
Antineoplastic Compounds

Field of Invention

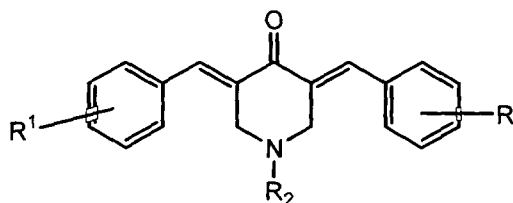
The present invention relates to 4-piperidone derivatives possessing antineoplastic activities and their use in the treatment of cancer.

Background of the Invention

A number of α,β -unsaturated ketones display cytotoxic and anticancer properties (Dimmock et al, Curr.Med. Chem. 1999, 6,1125-1149; Dimmock et al, J. Med Chem 1999, 42,1358-1366) and are believed to interact with cellular thiols with little or no affinity for hydroxyl and amino groups in nucleic acids (Mutus et al, Anal Biochem, 1989, 177, 237-243; Baluja et al Chem Ind. 1964, 2053-2054). This characteristic provides the potential to develop these compounds as cytotoxics, bereft of the genotoxic properties present in certain antineoplastic agents (Bevenuto et al, J. Pharm. Sci. 1993, 82,988-991). The Mannich base form of such compounds has been investigated. For instance, Mannich bases of some conjugated enones have been shown to lead to significant increase in both the rate of thiolation and alkylation (Dimmock et al, Can J. Chem 1980, 58,984-991) and cytotoxicity (Dimmock et al, J Pharm Sci 1975, 64, 241-249). However, compounds, such as the conjugated arylidene ketone of Formula (A) shown below, are toxic to mice.



(A)



(B)

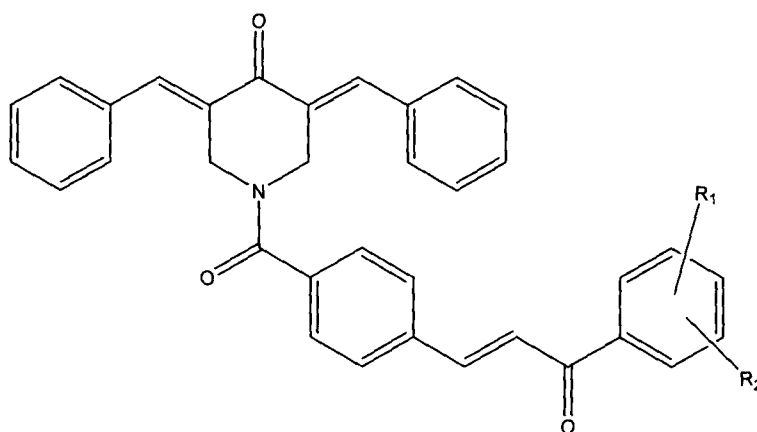
$R^1 = R^2 = \text{H, Cl, NO}_2, \text{N(CH}_3)_2, \text{alkoxy or alkyl};$

$R^3 = \text{H or alkyl}$

Related cyclic analogues of Formula (B), shown above, have been prepared to reduce the toxicity of compounds of Formula (A).

Compound B1 (Formula B with $R^1 = R^2 = \text{H}$) is a cyclic Mannich base of a dienone and possesses cytotoxic activity towards the murine lymphocytic leukemia P388/MRI cell line and does bind to a synthetic DNA, poly(d(AT)); more surprisingly, five consecutive daily doses of 240 mg/kg did not induce fatalities in mice (Dimmock et al., Drug Des Delivery 1990,6, 183-194).

Various N-substituted derivatives of compound B1 have been prepared and tested: N-3-carboxy-2-propenoyl derivatives of compound B1 were evaluated against P388/MRI cells (Dimmock et al, Drug Des.Deliv. 1990, 6,183-194). N-Acyl derivatives (Dimmock et al, Drug Des Discovery 1992, 10,291-299), N-amidic, and N-carbamate derivatives (Dimmock et al, Drug. Des. Discovery 1994, 12, 19-28) of compound B1 were screened against murine L1210 cells and a panel of human tumours. N-acryloyl amides (Dimmock et al, J. Med. Chem 2001,44,586-593), N-amidic derivatives (shown as Formula C below) (Dimmock et al. Eur J. Med.Chem., 2002, 37, 961-972), and N-maleamoyl derivatives (Dimmock et al, J. Enz.Inhib. Med. Chem., 2003, 18, 325-332) of compound B1 were evaluated against murine P388 and L1210, human Molt 4/C8 and CEM T-lymphocytes.



(C)

Compounds of Formula C have been shown to inhibit human N- myristoyltransferase and fyn kinase.

Nearly 50 per cent of human cancers are either completely resistant to chemotherapy or respond only transiently, after which they are no longer affected by commonly used anticancer drugs. This phenomenon is referred to as multidrug resistance (MDR) and is inherently expressed by some tumour types while others acquire MDR after exposure to chemotherapy treatment. The ability of a therapeutic to dampen or eliminate MDR in a cell is thus an important consideration.

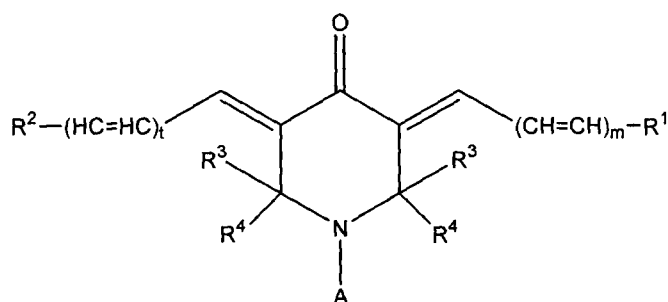
Summary of the Invention

In one aspect, the present invention provides novel antineoplastic compounds, structurally divergent from currently available anticancer drugs. This structural divergence may provide compounds devoid of cross resistance to contemporary medications including the antineoplastic alkylating agents. The synthesis of new compounds is

disclosed herein, together with experiments demonstrating their activity in cytotoxicity (IC_{50} and CC_{50}) assays against cancer cell lines. The compounds are also useful in reversing MDR (multidrug resistance).

Thus, in one aspect, the present invention provides a 4-piperidone compound

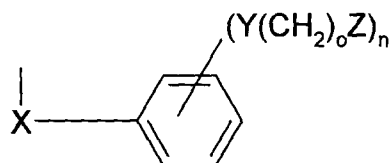
5 represented by structural formula (I):



(I)

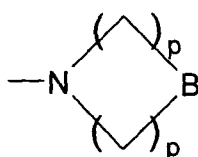
wherein R^1 and R^2 independently represent alkyl, alkoxy, aryl, or heteroaryl; R^3 and R^4 independently represent H, alkyl, hydroxyalkyl, or carboxylate ester; m and t independently represent an integer from 0 to 3; A represents H or A' , where A' represents

10



wherein n represent an integer from 0 to 4 and indicates the number of substituents on the benzene ring, o represent an integer from 0 to 4; X represents $(CH_2)_r$, CO , COO , SO , SO_2 , or O , where r represents an integer from 0 to 4; Y represents O , S , NH , $N(alkyl)$, $N(aryl)$, or $(CH_2)_s$, where s represents an integer from 0 to 4; Z represents H , $N(alkyl)_2$, $NH(alkyl)$, $N(aryl)_2$, $NH(aryl)$, $alkyl$, substituted $alkyl$, $+N(alkyl)_3$, or

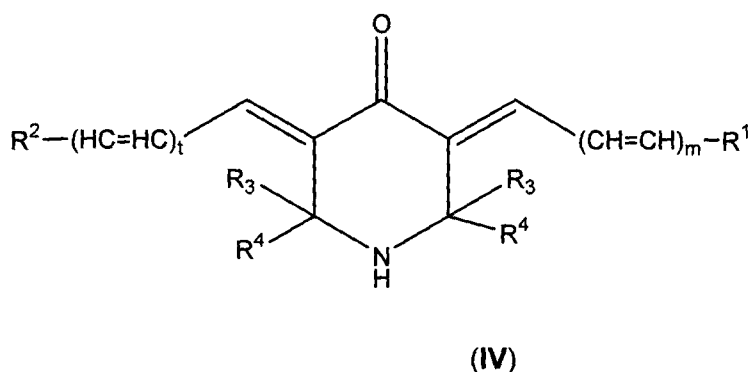
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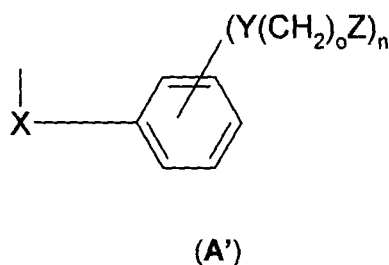
where B represents O , S , $(CH_2)_q$, NH , $N-alkyl$, or $N-aryl$ and p and q independently represent an integer from 0 to 4; or a pharmaceutically acceptable salt thereof, with the

proviso that the free base and the hydrochloride salt of compounds **1a**, **1b**, and **1c** are excluded.

In another aspect, the present invention provides a method of preparing the compounds of the invention, comprising the steps of: reacting a compound of formula (IV)



with a compound of formula T-A' (V), where T represents a halogen, and A' represents



10 in the presence of a base.

Formula (I) and Formula (IV) each contain two R³ substituents, which are the same or different, and are as defined herein. Formula (I) and Formula (IV) each contain two R⁴ substituents, which are the same or different, and are as defined herein.

15 In another aspect, the present invention provides uses of the compounds and pharmaceutical compositions thereof for treating cancer and for reversing multidrug resistance.

Brief Description of the Drawings

FIG. 1 is a schematic representation of the synthesis of some of the compounds of the invention.

Detailed Description of the Invention

Chemistry

Compounds of the invention include compounds of Formula (I).

Preferably, the alkyl or alkoxy groups are substituted or unsubstituted, branched or
 5 unbranched C1-C7, preferably C1-5. Preferred alkyl groups are methyl or ethyl. Preferred
 alkoxy groups are methoxy and ethoxy. Possible substituents are known in the art and
 include halogen, alkoxy, alkyl, phenyl, and/or nitro groups. Halogen may be fluorine,
 chlorine, bromine, or iodine, and preferably fluorine or chlorine.

Preferably R¹ and R² are the same or different substituted or unsubstituted, branched or
 10 unbranched alkyl, aryl, or heteroaryl groups, wherein the alkyl group is preferably as
 defined above. Aryl groups are preferably substituted or unsubstituted phenyl rings in
 which substitutions are preferably independently placed in the 2, 3, or 4 positions of the
 phenyl ring, or combined at 3,4; 2,4; 2,3; 2,5; 2,6; 3,5; 3,6; 4,6; 3,4,5; 2,4,6; or 2,3,4
 positions. Substituents may include halogen, alkoxy, alkyl, phenyl, nitro, and phosphonate
 15 groups. Phosphonate groups include -P(O)(OH)₂, -P(O)(OH)(O-alkyl), -P(O)(O-alkyl)₂, -
 P(O)(OH)(O-aryl), -P(O)(O-aryl)₂. Preferred substituents are 4-fluoro, 4-chloro, 4-
 methoxy, 4-nitro, 4-methyl, 3,4-dimethoxy, 3,4-dichloro, 3,4,5-trimethoxy and 2,4,6-
 trimethoxy. R¹ and R² may desirably be any one of phenyl, 4-chlorophenyl, 4-nitrophenyl,
 and/or 4-methylphenyl. The heteroaryl groups are preferably 2, 3, or 4- pyridyl, 2-thienyl
 20 or 2-furyl. Both m and t may independently be any integer from 0 to 3. In one aspect, m
 and t are both 0.

R³ or R⁴ may independently be H, alkyl, or carboxylate ester. Each R³ may be the same
 or different. Each R⁴ may be the same or different. The alkyl group is preferably as
 defined above. The carboxylate ester includes alkyl carboxylates and is preferably methyl
 25 or ethyl carboxylate. In one aspect, R³ and R⁴ are both H.

Compounds referred to herein include: when A is H, R³ = R⁴ = H, and m and t are 0 in
 Formula (I): R¹ = R² = Phenyl (**1a**); R¹ = R² = 4-Chlorophenyl (**1b**); and R¹ = R² = 4-
 Nitrophenyl (**1c**).

Compounds of the invention include, but are not limited to the following:

- 30 i) when A is H, R³ = R⁴ = H, and m and t are 0 in Formula (I):
 R¹ = R² = 4-Methylphenyl (**1d**)

(ii) when A is A' and Y (CH₂)_oZ is placed at the 4- position of the benzene ring and R³ = H, R⁴ = H, X = CO; m and t = 0; n = 1; Y=O; o = 2 in Formula (I):

R¹ = R² = Phenyl; Z = N (CH₃)₂ (**3a**)

R¹ = R² = 4-Chlorophenyl; Z= N (CH₃)₂ (**3b**)

5 R¹ = R² = 4-Nitrophenyl; Z= N (CH₃)₂ (**3c**)

R¹ = R² = 4-Methylphenyl; Z = N (CH₃)₂ (**3d**)

R¹ = R² = Phenyl; Z = N (C₂H₅)₂ (**4a**)

R¹ = R² = 4-Chlorophenyl; Z= N (C₂H₅)₂ (**4b**)

R¹ = R² = 4-Nitrophenyl; Z= N (C₂H₅)₂ (**4c**)

10 R¹ = R² = 4-Methylphenyl; Z = N (C₂H₅)₂ (**4d**)

R¹ = R² = Phenyl; Z = 1-piperidyl (**5a**)

R¹ = R² = 4-Chlorophenyl; Z = 1-piperidyl (**5b**)

R¹ = R² = 4-Nitrophenyl; Z= 1-piperidyl (**5c**)

R¹ = R² = 4-Methylphenyl; Z = 1-piperidyl (**5d**)

15 R¹ = R² = Phenyl; Z = 4-morpholinyl (**6a**)

R¹ = R² = 4-Chlorophenyl; Z = 4-morpholinyl (**6b**)

R¹ = R² = 4-Nitrophenyl; Z = 4-morpholinyl (**6c**)

R¹ = R² = 4-Methylphenyl; Z = 4-morpholinyl (**6d**)

R¹ = R² = Phenyl; Z = +N(CH₃)(C₂H₅)₂ I⁻ (**7a**)

20 R¹ = R² = 4-Chlorophenyl; Z = +N (CH₃)(C₂H₅)₂ I⁻ (**7b**)

R¹ = R² = 4-Nitrophenyl; Z= +N(CH₃)(C₂H₅)₂ I⁻ (**7c**)

R¹ = R² = 4-Methylphenyl; Z = +N(CH₃)(C₂H₅)₂ I⁻ (**7d**)

(iii) when A is H, R³ = R⁴ = H, and m and t are 0 in Formula (I):

25 R¹ = R² = 2-Thienyl (**8a**)

(iv) when A is A' and Y (CH₂)_oZ is placed at the 4- position of the benzene ring and R³ = H, R⁴ = H, X = CO; m and t = 0; n = 1; Y=O; o = 2 in Formula (I):

R¹ = R² = 2-Thienyl; Z = N (C₂H₅)₂ (**9a**)

30

(v) when A is A' and Y (CH₂)_oZ is H, and m and t are 0 in Formula (I):

R¹ = R² = Phenyl; R³ = R⁴ = H, X = CO; n = 1 (**2a**)

(vi) when A is A' and Y (CH₂)_oZ is placed at the 4- position of the benzene ring and R³ =

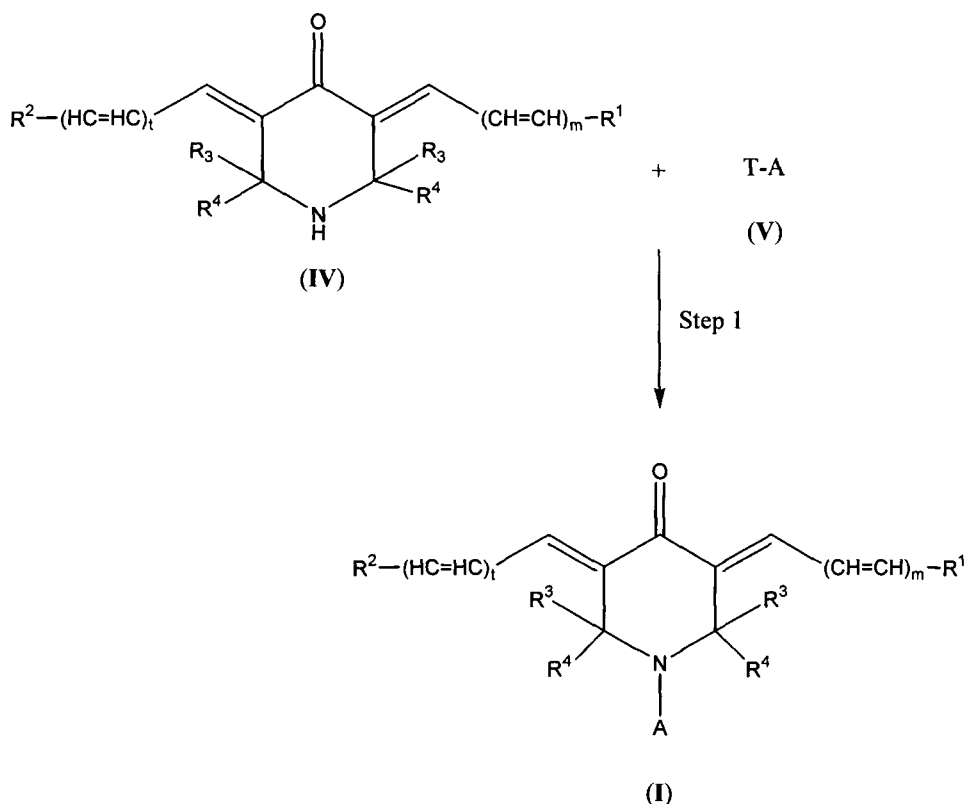
35 R⁴ = H, X = CO; n = 1; Y=O; o = 0; and m and t are 0 in Formula (I):

R¹ = R² = Phenyl; Z= CH₃ (**2b**)

The compounds of formula (I) include acid addition salts thereof. By "acid addition salts" it is meant any salt which may be formed for the purpose of isolation, purification, and storage, such as the oxalate salt, and pharmaceutically acceptable salts meant for administration of the compound to a host, such the hydrochloride, sulfate, acetate, and citrate.

Preparation

A compound of formula (I) can be prepared according to the following reaction step:



where A represents A', whose meaning is the same as discussed earlier. T represents halogen that has the same meaning as discussed earlier.

A compound of formula (I) can be obtained by reacting compound (IV) with (V) in the presence of a base in an inert solvent. The bases used may be inorganic bases such as potassium carbonate, sodium carbonate or secondary amines such as piperidine, pyrrolidine, morpholine or tertiary amines such as triethylamine, metal hydrides such as sodium hydride and the like may be used in an amount of 1-5 equivalents. The solvent can, for example, be a polar aprotic solvent (for example, tetrahydrofuran, acetonitrile) or a halogenated hydrocarbon (for example, chloroform, dichloromethane, 1, 2-

dichloroethane), which solvents may be used alone or in combination. The reaction is carried out at a temperature between -10°C and the boiling point of the solvent employed in the reaction, and is completed in 0.5 to 24 hrs.

The starting compound (IV) can be prepared according to the literature procedure (Dimmock et al, J. Med. Chem, 2001, 44, 586-593). The substituents on (IV) can be any of those mentioned for Formula (I). The starting compound (V) can be prepared according to the process described in the literature (Jones et al, J. Med. Chem. 1984, 27, 1057-1066).

The intermediates and the desired compounds in the processes described above can be isolated and purified by purification methods conventionally used in organic synthetic chemistry, for example, filtration, extraction, washing, drying, concentration, recrystallization and various types of chromatography. The intermediates may also be subjected to a subsequent reaction without isolation.

The acid addition salts of this invention are readily prepared by treating the base compound with a substantially equivalent amount of the chosen mineral or organic acid in an aqueous solvent medium or in a suitable organic solvent such as methanol or ethanol. Upon careful evaporation of the solvent, the desired solid salt is obtained.

The acids which were used to prepare the pharmaceutically acceptable acid addition salts of the base compounds of this invention are those which form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, such as chloride, bromide, iodide, nitrate, sulfate or bisulfate, phosphate or acid phosphate, acetate, lactate, citrate or acid citrate, tartrate or bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulfonate and pamoate [i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)] salts.

In the case where a quaternised salt of compound (I) is desired, compound (I) in the form of a free base is dissolved in a suitable solvent followed by addition of an alkyl halide to form a salt. The reaction is carried out at a temperature between 0 °C and the boiling temperature of the solvent employed in the reaction and is completed in 0.5 to 24 hrs.

Compound (I) and the acid addition salts thereof may be in the form of adducts with water or various solvents, which are also within the scope of the present invention.

Biological Activity

The compounds of the invention may be used to treat cancer, particularly colon cancer and leukemia. In addition, the compounds demonstrate anti-multidrug resistance properties.

5 Compounds of the invention (compounds **1-7**) were evaluated for their cytotoxicity towards human transformed cells by treating them with human Molt 4/C8 and CEM T-lymphocytes. In addition, they were evaluated for their ability to inhibit the growth of murine L1210 cells, an ability of many clinically useful anticancer drugs. The results are shown as IC₅₀(micromolar) in Table 1. IC₅₀(micromolar) indicates the concentration of a
10 compound which is required to inhibit the growth of the cells by 50%. The results are compared to compound **10** and to melphalan. Melphalan is an anticancer drug that exerts its effect by alkylating cellular constituents. In almost all the cases, the compounds of the invention were more potent than melphalan in both the Molt 4/C8 and the CEM assays.

A number of compounds of the invention were further assessed against numerous human
15 malignant cell lines. An average of 49 cell lines from nine different neoplastic diseases, namely leukemia, melanoma, non-small cell lung, colon, central nervous system, ovarian, renal, prostate, and breast cancers were employed. Against most of the cell lines, compounds tested had average IC₅₀ values lower than those of melphalan, as shown in Table 2. For instance, **6b** and **6d** possess in excess of 48 and 38 times, respectively, the
20 potency of melphalan. In particular, the IC₅₀s were lower towards colon and leukemic neoplasms.

An important feature of novel antineoplastic agents is the display of greater toxicity for malignant cells rather than for the corresponding normal tissues. A differentiation in cytotoxicity toward the cell lines would reveal that the compound displayed selective
25 toxicity in contrast to being a general biocidal agent; such selectivity may translate into a preferential lethality for tumour cells rather than related non-malignant cells. Accordingly, a selective index (SI) figure for each compound was calculated as the ratio of the IC₅₀ values between the cell line which is the most sensitive and the cell line which is least sensitive to the compound (also shown in Table 2). The amides **3b**, **4a**, **6a**, **6b**, **6d** and
30 **7d** were more selective than melphalan, in particular, **4a**, **6b** and **6d**.

Mean IC₅₀ graphs were calculated as described previously (M.R. Grever, S.A. Schepartz, B.A. Chabner, Semin. Oncol. 19 (1992) 622-638). A review of the mean graphs revealed that in general the IC₅₀ values of the compounds were lower towards colon and leukemic

neoplasms than the other cell lines. The selective toxicity for these groups of cancers is revealed in Table 2 since the IC_{50} figures of many of the colon and leukemic cell lines were lower than the values for all cell lines. An effective drug against colon tumours is 5-fluorouracil. The data in Table 2 reveal that the IC_{50} figures for the compounds prepared in this study towards the six colon cancer cell lines indicated in Table 2 were lower than that of 5-fluorouracil in 67% of the cases. Melphalan is used in combination chemotherapy to treat chronic leukemias and in 80% of the comparisons made for the K-562, RPMI-8226, HL-60 (TB) and SR cell lines, the compounds in series 1, 3-7 had lower IC_{50} figures than melphalan. A general conclusion to be drawn from the evaluation of representative compounds against a panel of approximately 49 human tumour cell lines is that their potencies are substantially greater than certain clinically used drugs and that they have a particular toxicity towards colon cancer and leukemic cells.

In addition, the compounds were evaluated against human squamous cell carcinomas (HSC-2 and HSC-4) and human promyelocytic leukemia neoplasms (HL-60) and compared with normal cells, i.e. human gingival fibroblasts (HGF), human pulp cells (HPC) and human periodontal ligament fibroblasts (HPLF). The CC_{50} values towards these three cell lines are also indicated in Table 3. CC_{50} (micromolar) indicates the concentration of a compound which is required to kill 50% of the cells. These data are presented in Table 3. Most of the compounds have CC_{50} values less than 10 micromolar and the average CC_{50} values for 3c, 4c, 5c, and 6c are submicromolar. Furthermore, almost all of the compounds in series 1 and 3 to 7 have lower average CC_{50} values than the established anticancer drug melphalan.

Further evaluation of the compounds was done to determine whether they had the capacity to reverse multidrug resistance (MDR) and/or display selective toxicity to malignant cells. All of the compounds in series 1 and 3-7 were examined for their ability to reverse MDR using murine L-5178 lymphoma cells. The assay to determine MDR reversal employed murine L-5178 lymphoma cells transfected with the human MDR1 gene. The concentrations of rhodamine 123 were measured in treated and untreated transfected and parental cells and the ratios of the fluorescence intensities are referred to as fluorescence activity ratio (FAR) values. Since MDR is due, *inter alia*, to an increase in the efflux of a compound from cells, a FAR value of greater than 1 indicates that reversal of MDR has taken place. These data are also presented in Table 3. MDR-reversal was displayed by all of the compounds in series 3 to 6. Huge FAR values were obtained for many of the compounds using a concentration of 4 μ g/ml. In contrast to a concentration of

10 micrograms/ml of a reference drug verapamil, the FAR values of the compounds of the invention ranged from 8 to 32 times that of verapamil.

Compounds which reverse MDR and exert an antineoplastic effect may be classified as bi-modal. They are superior to those compounds which have only one of these effects.

- 5 The compounds of the invention can be used as either antineoplastics, MDR reversants, or for both effects.

Administration

The compositions of the present invention may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers. Thus, the active compounds of
10 the invention may be formulated for oral, buccal, intranasal, parenteral (e.g., intravenous, intramuscular or subcutaneous), topical or rectal administration or in a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically
15 acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known
20 in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats);
25 emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); and preservatives (e.g., methyl or propyl p-hydroxybenzoates or sorbic acid).

For buccal administration, the composition may take the form of tablets or lozenges formulated in a conventional manner.

- 30 The compounds of the invention can also be formulated for sustained delivery according to methods well known to those of ordinary skill in the art. Examples of such formulations can be found in U.S. Pat. Nos. 3,538,214, 4,060,598, 4,173,626, 3,119,742, and 3,492,397, which are herein incorporated by reference in their entirety.

The compounds of the invention may be formulated for parenteral administration by injection, including using conventional catheterization techniques or infusion.

Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in a powder form for reconstitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, the active compounds of the invention are conveniently delivered in the form of a solution, dry powder formulation or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, heptafluoroalkanes, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

The compounds of the invention including pharmaceutically acceptable salts and solvates thereof may be used on their own but will generally be administered in the form of a pharmaceutical composition in which the compound (active ingredient) is in association with a pharmaceutically acceptable adjuvant, diluent or carrier. Depending on the mode of administration, the pharmaceutical composition will preferably comprise from 0.05 to 99% w (percent by weight), more preferably from 0.10 to 70% w, of active ingredient, and, from 1 to 99.95% w, more preferably from 30 to 99.90% w, of a pharmaceutically acceptable adjuvant, diluent or carrier, all percentages by weight being based on the total composition.

Experimental

Synthesis

The synthetic chemical routes employed in producing the compounds of series 1-7 are portrayed in FIG. 1. The compounds in series 1 were prepared by acid-catalyzed condensation between various aryl aldehydes and 4-piperidone. The reaction of **1a** with different aroyl chlorides led to the isolation of **2a,b**. Alkylation of methyl 4-hydroxybenzoate with 2-dimethylaminoethyl hydrochloride gave rise to the corresponding ether which upon hydrolysis produced 4-(2-dimethylaminoethoxy)benzoic acid isolated as the hydrochloride salt. This compound was converted to the corresponding acid chloride which condensed with **1a-d** leading to the formation of **3a-d**, respectively. A similar synthetic pathway was employed using related N-(2-chloroethyl)amines hydrochlorides in place of 2-dimethylaminoethyl hydrochloride leading to the formation of **4a-d**, **5a-d** and **6a-d**. Reaction of the free bases derived from **4a-d** with methyl iodide gave rise to the corresponding quaternary ammonium salts **7a-d**. In addition, the preparation of 4-(2-diethylaminoethoxy)benzoic acid **10**, which is structurally related to the compounds in series 4, was synthesized.

In FIG. 1, the substituents R in series 1, 3-7 are as follows : **a** : R = H; **b** : R = Cl; **c** : R = NO₂; **d** : R = CH₃. The nature of the Y(CH₂)₆Z moieties in series 2 are **a** : Y(CH₂)₆Z = H; **b** : Y(CH₂)₆Z = OCH₃. The following reagents were used in the synthetic routes viz i = HCl/CH₃COOH; ii = ClCOC₆H₄Y(CH₂)₆Z; iii = ClCH₂CH₂ZHCl/K₂CO₃; iv = NaOH/HCl; v = SOCl₂; vi = **1a-d**; vii = CH₃I/K₂CO₃.

Melting points are uncorrected. Elemental analyses (C, H, N) were undertaken on **1d** and **2-7** by the Microanalytical Laboratory, Department of Chemistry, University of Alberta and were within 0.4% of the calculated values. Compounds **3b-d**, **4a**, **5a,d**, **6a,c,d** and **7a,d** were isolated as the hemihydrates, **7b** as the monohydrate and **5c** was obtained with 1.5 moles of water of crystallization. The ¹H NMR spectra (500 MHz) of all of the compounds in series 1-10 and the ¹³C NMR spectra (125 MHz) of representative compounds were determined in deuterated solvents using a Bruker AM 500 FT NMR machine.

Synthesis of 1a-d: Compounds **1a-d** were prepared by a literature procedure (Journal of Medicinal Chemistry **44**, 586 (2001). Generally, the appropriate aryl aldehyde (36.71 mmol) was added to a suspension of 4-piperidone hydrochloride monohydrate (13.03 mmol) in acetic acid (35 mL). Dry hydrogen chloride was passed through this mixture for 0.5 hours during which time a clear solution was obtained. After stirring at room

temperature for 24 hours, the precipitate was collected and added to a mixture of a saturated aqueous potassium carbonate solution (25% w/v, 25 mL) in acetone (25 mL); the resultant mixture was stirred for 0.5 hours. The free base was collected, washed with water (50 mL) and dried. The compounds were recrystallized. **1d** was recrystallized from chloroform-ethanol to give the desired product.

3,5-bis(4-Methylphenylmethylene)-4-piperidone (1d). M.p. 180-181°C. Yield: 79%. ¹H NMR (CDCl₃): 2.40(s, 6H), 4.16(s, 4H), 7.24(d, 2H, J = 7.75 Hz), 7.31(d, 2H, J = 7.85 Hz), 7.80 (s, 2H). Found C, 82.87; H, 6.94; N, 4.60 %. Anal. (C₂₆H₂₁NO₂) requires C, 83.13; H, 6.98; N 4.62 %.

General procedure for the synthesis of 2a, b: A solution of the aroyl chloride (0.015 mol) in 1,2-dichloroethane (50 ml) was added over 0.5 h to a solution of **1a** (0.01 mol) and triethylamine (0.03 mol) in 1,2-dichloroethane (30 ml) at ~ 5-6°C. After stirring at room temperature for 6 h, the solvent was removed *in vacuo*. An aqueous solution of potassium carbonate (10% w/v, 50 ml) was added to the residue and the resultant slurry was stirred for 4 h at room temperature. The precipitate was collected, washed with water and dried. The products were purified by crystallization from chloroform-methanol.

1-Benzoyl-3,5-bis(phenylmethylene)-4-piperidone (2a). M.p. 165-166°. Yield: 85%. ¹H NMR (CDCl₃): 4.72 (bs, 2H), 5.04 (bs, 2H), 7.06 (t, 2H), 7.22 (d, 4H), 7.38(bs, 9H), 7.92 (s, 2H). Found C, 82.02; H, 5.49; N, 3.56 %. Anal. (C₂₆H₂₁NO₂) requires C, 82.30; H, 5.58; N 3.69 %.

1-(4-Methoxybenzoyl)-3,5-bis(phenylmethylene)-4-piperidone (2b). M.p. 177-178°. Yield: 72 %. ¹H NMR (CDCl₃): 3.70 (s, 3H), 4.87 (bs, 4H), 6.51 (d, 2H, J = 10.62 Hz), 7.17 (d, 2H, J = 10.69 Hz), 7.40 (bs, 10H), 10.07 (s, 2H). Found C, 79.19; H, 5.72; N, 3.25%. Anal. (C₂₇H₂₃NO₃) requires C, 79.19; H, 5.66; N 3.42 %.

General procedure for the synthesis of series 3-6: The general method employed for the preparation of the compounds in series **3-6** is exemplified by the synthesis of **4a**. A mixture of **10** *vide infra* (4.10 g, 0.015 mole), thionyl chloride (23.10 g, 2 mol) and dimethylformamide (0.01 ml) was heated under reflux for 3 h. Evaporation *in vacuo* led to the isolation of the corresponding acid chloride which was dissolved in 1,2-dichloroethane (50 ml) and added over a period of 0.5 h to a solution of **1a** (2.75 g, 0.01 mol) and triethylamine (3.03 g, 0.03 mol) in 1,2-dichloroethane (30 ml) at ~ 5-6° C. After stirring at room temperature for 6 h, the solvent was removed *in vacuo*. The solid obtained was suspended in a solution of potassium carbonate (10% w/v, 50 ml) and stirred at room

temperature for 4 h. The precipitate was collected, washed with water and dried to give 1-[4-(2-diethylaminoethoxy)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone, i.e., the free base corresponding to **4a**. The hydrochloride salt was prepared by dissolving the free base in a mixture of isopropanol and chloroform (3:2; 50 ml) and after the addition of activated charcoal (0.5 g), the mixture was stirred at room temperature for 1 h. The solution was acidified with dry hydrogen chloride gas and the mixture stirred at room temperature for 1 h. The solvent was removed in vacuo to yield a viscous oil to which was added acetone (50 ml) and the mixture was heated under reflux for 0.5 h. On cooling to room temperature, the precipitate was collected and recrystallized from acetone to give **4a**. The analogs **4b-d** were prepared using the same methodology except isopropanol was used as the recrystallization solvent for **4b** and **4c**. Series **3**, **5** and **6** were obtained in a similar manner from methyl 4-hydroxybenzoate and the appropriate 2-chloro-N-substituted-ethylamine hydrochloride. The solvent used in recrystallizing **3a-d**, **5a,b** and **6a-d** was isopropanol. Diethyl ether-methanol was used to purify **5c**, while **5d** was recrystallized from acetone.

[4-(2-Dimethylaminoethoxy)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone hydrochloride (3a). M.p. 244-245°. Yield: 76%. ¹H NMR (DMSO-d₆): 2.83 (s, 6H), 3.46 (t, 2H), 4.22 (t, 2H), 4.80 (bs, 4H), 6.55 (d, 2H, J = 10.55 Hz), 7.08 (d, 2H, J = 10.65 Hz), 7.37 (bs, 10H), 7.76 (s, 2H), 11.08 (bs, 1H). Found C, 71.80; H, 5.90; N, 5.50 %. Anal. (C₃₀H₃₁ClN₂O₃) requires C, 71.63; H, 6.21; N 5.57 %.

3,5-bis(4-Chlorophenylmethylene)-1-[4-(2-dimethylaminoethoxy)phenyl-carbonyl]-4-piperidone hydrochloride hemihydrate (3b). M.p. 250-251°. Yield: 71%. ¹H NMR (DMSO-d₆): 2.92 (s, 6H), 3.47 (t, 2H), 4.22 (t, 2H), 4.81 (bs, 4H), 6.58 (d, 2H, J = 7.61 Hz), 7.14 (d, 2H, J = 7.65 Hz), 7.39 (m, 8H), 7.82 (d, 2H, J = 10.75 Hz), 12.84 (bs, 1H). Found C, 62.45; H, 5.15; N, 4.86 %. Anal. (C₃₀H₂₉Cl₃N₂O₃·0.5 H₂O) requires C, 62.02; H, 5.03; N 4.82 %.

1-[4-(2-Dimethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)-4-piperidone hydrochloride hemihydrate (3c). M.p. 215-217°. Yield: 62%. ¹H NMR (DMSO-d₆): 2.78 (s, 6H), 3.42 (t, 2H), 4.21 (t, 2H), 4.85 (bs, 4H), 6.70 (d, 2H, J = 10.28 Hz), 7.15 (d, 2H, J = 10.25 Hz), 7.72 (m, 4H), 7.82 (s, 2H), 10.27 (bs, 4H), 11.08 (bs, 1H). Found C, 59.45; H, 4.93; N, 9.08 %. Anal. (C₃₀H₂₉ClN₄O₇·0.5 H₂O) requires C, 59.85; H, 4.85; N 9.30 %.

1-[4-(2-Dimethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-methylphenyl-methylene)-4-piperidone hydrochloride hemihydrate (**3d**). M.p. 224-226°. Yield: 64%. ¹H NMR (CDCl₃): 2.40 (s, 6H), 2.90 (t, 2H), 3.43 (t, 2H), 4.42 (t, 2H), 4.87 (bs, 4H), 6.58 (d, 2H, J = 10.10 Hz), 7.23 (m, 10H), 7.88 (s, 2H), 13.08 (br s, 1H). Found C, 71.38; H, 6.75; N, 5.21 %.

5 Anal. (C₃₂H₃₅ClN₂O₃·0.5 H₂O) requires C, 71.16; H, 6.53; N 5.18 %.

1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone hydrochloride hemihydrate (**4a**). M.p. 184-185°. Yield: 72%. ¹H NMR (CDCl₃): 1.45 (t, 6H, 2 x CH₃), 3.22 (m, 4H, 2 x NCH₂CH₃), 3.40 (t, 2H, OCH₂CH₂N), 4.41 (t, 2H, OCH₂CH₂N), 4.85 (bs, 4H, 2 x piperidyl NCH₂), 6.50 (d, 2H, aryl H, J = 10.1 Hz), 7.17 (d, 2H, aryl H, J = 9.05 Hz), 7.44 (bs, 10H, aryl H), 7.92 (s, 2H, =CH), 12.70 (s, 1H, NH). ¹³C NMR(CDCl₃): 187.41(C=O), 170.14(-C=O-N-), 158.77, 134.88, 132.21, 130.77, 130.08, 129.70, 129.25, 127.99, 114.19, 63.05(OCH₂), 50.95(OCH₂-CH₂-N), 47.39(NCH₂), 10.89(CH₃). Found C, 71.22; H, 6.41; N, 4.79 %. Anal. (C₃₂H₃₅ClN₂O₃·0.5 H₂O) requires C, 71.16; H, 6.71; N 5.18 %.

10 3,5-bis(4-Chlorophenylmethylene)-1-[4-(2-diethylaminoethoxy)phenylcarbonyl]-4-piperidone hydrochloride (**4b**). M.p. 210-211°. Yield: 68 %. ¹H NMR (CDCl₃): 1.45 (t, 6H), 3.22 (bs, 4H), 3.41 (t, 2H), 4.45 (t, 2H), 4.81 (bs, 4H), 6.57 (d, 2H, J = 10.34 Hz), 7.17 (d, 2H, J = 10.30 Hz), 7.31(m, 4H), 7.41(d, 2H, J = 6.54 Hz), 7.84(s, 2H), 12.70(bs, 1H). Found C, 64.28; H, 5.26; N, 4.98 %. Anal. (C₃₂H₃₃Cl₃N₂O₃) requires C, 64.06; H, 5.54; N 4.67 %.

1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)-4-piperidone hydrochloride (**4c**). M.p. 197-198°. Yield: 61%. ¹H NMR (CDCl₃): 1.46 (t, 6H), 3.19 (bs, 2H), 3.26 (bs, 2H), 4.45 (t, 2H), 4.45 (t, 2H), 4.82 (bs, 4H), 6.60 (d, 2H, J = 10.25 Hz), 7.14 (d, 2H, J = 10.25 Hz), 7.51 (bs, 4H), 7.89 (s, 2H), 10.26 (d, 2H, J = 6.35 Hz), 12.57(bs, 1H). Found C, 62.06; H, 5.38; N, 9.19 %. Anal. (C₃₂H₃₃ClN₄O₇) requires C, 61.88; H, 5.36; N 9.02 %.

1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-methylphenylmethylene)-4-piperidone hydrochloride (**4d**). M.p. 204-205°. Yield: 62%. ¹H NMR (CDCl₃): 1.45 (t, 6H), 2.40(s, 6H), 3.22 (m, 4H), 3.40 (t, 2H), 4.46 (t, 2H), 4.87 (bs, 4H), 6.57 (d, 2H, J = 10.53 Hz), 7.23 (d, 2H, J = 10.37 Hz), 7.32 (m, 4H), 7.88(s, 2H), 12.62 (bs, 1H). Found C, 73.34; H, 6.86; N, 5.36 %. Anal. (C₃₄H₃₉ClN₂O₃) requires C, 73.04; H, 7.03; N 5.01 %.

1-[4-{2-(1-Piperidinyloxy)}phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone hydrochloride hemihydrate (**5a**). M.p. 214-215°. Yield: 52 %. ¹H NMR (CDCl₃): 1.43 (t,

1H), 1.88 (t, 3H), 2.28 (q, 2H), 2.76 (t, 2H), 3.34 (bs, 2H), 3.60 (t, 2H), 4.44 (bs, 2H), 4.86 (bs, 4H), 6.50 (d, 2H, J = 7.75 Hz), 7.16 (d, 2H, J = 7.2 Hz), 7.38 (bs, 10H), 7.90 (s, 2H), 12.61 (bs, 1H). Found C, 71.48; H, 6.38; N, 5.04 %. Anal. (C₃₃H₃₅ClN₂O₃·0.5 H₂O) requires C, 71.79; H, 6.38; N 5.07 %.

- 5 *3,5-bis(4-Chlorophenylmethylene)-1-[4-{2-(1-piperidinyloxy)}phenylcarbonyl]-4-piperidone hydrochloride (5b)*. M.p. 257-258°. Yield: 75 %. ¹H NMR (CDCl₃): 1.44 (q, 1H), 1.92 (t, 3H), 2.32 (q, 2H), 2.80 (q, 2H), 3.38 (bs, 2H), 3.63 (d, 2H, J = 10.23 Hz), 4.54 (bs, 2H), 4.80 (bs, 4H), 6.57 (d, 2H, J = 7.84 Hz), 7.17 (d, 2H, J = 7.99 Hz), 7.34 (m, 8H), 7.90 (s, 2H), 12.68 (bs, 1H). Found C, 64.35; H, 5.39; N, 4.45 %. Anal. (C₃₃H₃₃Cl₃N₂O₃) requires C, 64.77; H, 5.44; N 4.58 %.

- 10 *3,5-bis(4-Nitrophenylmethylene)-1-[4-{2-(1-piperidinyloxy)}phenylcarbonyl]-4-piperidone hydrochloride 1.5 hydrate (5c)*. M.p. 224-226°. Yield: 64%. ¹H NMR (DMSO-d₆): 1.42 (t, 1H), 1.81 (t, 3H), 2.04 (q, 2H), 2.86 (q, 2H), 3.36 (d, 2H), 3.48 (m, 2H), 4.32 (bs, 2H), 4.75 (bs, 4H), 6.56 (d, 2H, J = 10.25 Hz), 7.07 (d, 2H, J = 10.10 Hz), 7.60 (m, 4H), 7.78 (s, 2H), 10.16 (bs, 4H), 11.53 (s, 1H). Found C, 71.38; H, 6.75; N, 5.21 %. Anal. (C₃₂H₃₅ClN₂O₃·1.5 H₂O) requires C, 71.16; H, 6.53; N 5.18 %.

- 15 *3,5-bis(4-Methylphenylmethylene)-1-[4-{1-piperidinyloxy}phenylcarbonyl]-4-piperidone hydrochloride hemihydrate (5d)*. M.p. 105° (dec). Yield: 65%. ¹H NMR (CDCl₃): 1.42 (q, 1H), 1.89 (t, 3H), 2.22 (q, 2H), 2.80 (bs, 2H), 3.40 (bs, 2H), 3.65 (d, 2H, J = 9.56 Hz), 4.48 (bs, 2H), 4.88 (bs, 4H), 5.44 (bs, 2H), 6.56 (d, 2H, J = 7.91 Hz), 7.23 (m, 10H), 7.99 (s, 2H), 11.80 (bs, 1H). Found C, 71.38; H, 6.75; N, 5.21 %. Anal. (C₃₂H₃₅ClN₂O₃·0.5 H₂O) requires C, 71.16; H, 6.53; N 5.18 %.

- 20 *1-[4-{2-(4-Morpholinyl)ethoxy}phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone hydrochloride hemihydrate (6a)*. M.p. 208-209°. Yield: 73 %. ¹H NMR (CDCl₃): 3.05 (q, 2H), 3.40 (bs, 2H), 3.53 (d, 2H, J = 12.02 Hz), 4.02 (dd, 2H), 4.30 (t, 2H), 4.47 (bs, 2H), 4.86 (bs, 4H), 6.51 (d, 2H, J = 10.56 Hz), 7.19 (d, 2H, J = 10.50 Hz), 7.44 (bs, 10H), 7.92 (s, 2H), 13.69 (bs, 1H). Found C, 69.54; H, 5.95; N, 4.96 %. Anal. (C₃₂H₃₃ClN₂O₄·0.5 H₂O) requires C, 69.36; H, 6.00; N 5.05 %.

- 25 *3,5-bis(4-Chlorophenylmethylene)-1-[4-{2-(4-morpholinyl)ethoxy}phenylcarbonyl]-4-piperidone hydrochloride (6b)*. M.p. 240-241°. Yield: 74%. ¹H NMR (CDCl₃): 3.06 (bs, 2H), 3.40 (bs, 2H), 3.55 (t, 2H), 4.04 (d, 2H, J = 11.97 Hz), 4.29 (t, 2H), 4.30 (t, 2H), 4.50 (bs, 2H), 4.81 (bs, 4H), 6.59 (d, 2H, J = 7.30 Hz), 7.14 (d, 2H, J = 7.47 Hz), 7.51 (m, 4H),

7.90 (s, 2H), 10.26 (bs, 4H), 13.59 (s, 1H). Found C, 62.39; H, 4.94; N, 4.47 %. Anal. ($C_{32}H_{31}Cl_3N_2O_4$) requires C, 62.60; H, 5.09; N 4.56 %.

1-[4-{2-(4-Morpholinyl)ethoxy}phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)-4-piperidone hydrochloride hemihydrate (**6c**). M.p. 224-226°. Yield: 64%. ^1H NMR ($CDCl_3$):
 5 3.05 (q, 2H), 3.40 (bs, 2H), 3.53 (d, 2H, $J = 12.02$ Hz), 4.02 (dd, 2H), 4.30 (t, 2H), 4.47 (bs, 2H), 4.86 (bs, 4H), 6.51 (d, 2H, $J = 10.56$ Hz), 7.19 (d, 2H, $J = 10.50$ Hz), 7.44 (bs, 10H), 7.92 (s, 2H), 13.59 (bs, 1H). Found C, 59.52; H, 4.72; N, 10.51 %. Anal. ($C_{32}H_{31}ClN_4O_8 \cdot 0.5 H_2O$) requires C, 59.67; H, 4.85; N 10.69 %.

3,5-bis(4-Methylphenylmethylene)-1-[4-{2-(4-morpholinyl)ethoxy}phenylcarbonyl]-4-piperidone hydrochloride hemihydrate (**6d**). M.p. 235-236°. Yield: 66%. ^1H NMR ($CDCl_3$):
 10 2.40 (s, 3H), 3.03 (m, 2H), 3.40(bs, 2H), 3.54(d, 2H, $J = 11.98$ Hz), 4.02 (dd, 2H), 4.29 (t, 2H), 4.50 (bs, 2H), 4.87 (s, 4H), 6.58 (d, 2H, $J = 10.63$ Hz), 7.24 (m, 10H), 7.88 (s, 2H), 13.68 (bs, 1H). Found C, 70.39; H, 6.69; N, 4.79 %. Anal. ($C_{34}H_{37}ClN_2O_4 \cdot 0.5 H_2O$) requires C, 70.15; H, 6.57; N 4.81 %.

15 **General procedure for the synthesis of 7a-d:** Activated charcoal (0.5 g) was added to a solution of the free base of **4a** *vide supra* (4.95 g, 0.01 mol) in acetone (25 ml) and the mixture was stirred at room temperature for 1 h. The suspension was filtered via celite and the celite bed was washed with acetone (10 ml). Methyl iodide (2.13 g, 0.015 mol) was added to the combined filtrates and the mixture was stirred at room temperature for
 20 4-5 h. The resultant precipitate was collected, dried and recrystallized from diethyl ether/methanol to give the desired products.

1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone methiodide (**7a**). M.p. 161-162°. Yield: 85 %. ^1H NMR ($CDCl_3$): 1.45 (t, 6H, 2 x CH_3), 3.34 (s, 3H, N- CH_3), 3.70 (m, 4H, 2 x NCH_2CH_3), 4.17 (bs, 2H, OCH_2CH_2N), 4.39 (bs, 2H, OCH_2CH_2N), 4.86 (bs, 4H, piperidyl H), 6.56 (d, 2H, aryl H, $J = 10.58$ Hz), 7.19 (d, 2H, aryl H, $J = 10.03$ Hz), 7.39 (bs, 12H, aryl H), 7.91 (s, 2H, olefinic H). Found C, 61.11; H, 5.64; N, 4.23 %. Anal. ($C_{33}H_{37}IN_2O_3 \cdot 0.5 H_2O$) requires C, 61.39; H, 5.77; N 4.33 %.

3,5-bis(4-Chlorophenylmethylene)-1-[4-(2-diethylaminoethoxy)phenylcarbonyl]-4-piperidone methiodide (**7b**). M.p. 220-223°. Yield: 69 %. ^1H NMR ($DMSO-d_6$): 1.32 (t, 6H), 3.07 (d, 3H, $J = 7.99$ Hz), 3.45 (t, 4H), 3.74 (t, 2H), 4.30 (bs, 1H), 4.39 (bs, 1H), 4.61 (bs, 4H), 6.60 (d, 1H, $J = 10.22$ Hz), 7.11(d, 1H, $J = 10.35$ Hz), 7.23 (d, 1H, $J = 10.36$ Hz), 7.71 (bs, 1H), 7.89 (s, 2H). Found C, 54.44; H, 4.79; N, 3.75 %. Anal. ($C_{33}H_{35}Cl_2IN_2O_3 \cdot 0.5 H_2O$) requires C, 54.78; H, 4.87; N 3.92 %.

1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)-4-piperidone methiodide (**7c**). M.p. 193-195° (dec). Yield: 74 %. ¹H NMR (DMSO-d₆): 1.24(t, 6H), 3.00 (s, 3H), 3.38(q, 4H), 3.64(t, 2H), 4.27(t, 2H), 4.40 (bs, 4H), 6.70(d, 2H, J = 10.41 Hz), 7.16(d, 2H, J = 10.39 Hz), 7.74(bs, 4H), 7.83(s, 2H), 10.27(bs, 4H). Found C, 54.41; H, 4.71; N, 7.60 %. Anal. (C₃₃H₃₅IN₄O₇) requires C, 54.55; H, 4.86; N 7.71 %.

1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-methylphenylmethylene)-4-piperidone methiodide (**7d**). M.p. 212-213°. Yield: 72 %. ¹H NMR (DMSO-d₆): 1.24 (t, 6H), 2.34 (s, 6H), 3.01(s, 3H), 3.39 (q, 4H), 3.68 (t, 2H), 4.33 (t, 2H), 4.87 (bs, 4H), 6.75 (d, 2H, J = 10.34 Hz), 7.22 (d, 2H, J = 10.30 Hz), 7.28 (m, 8H), 7.72 (s, 2H). Found C, 62.65; H, 6.25; N, 4.10 %. Anal. (C₃₅H₄₁IN₂O₃·0.5 H₂O) requires C, 62.40; H, 6.13; N 4.15 %.

Synthesis of 8a: 3,5-bis(thiophen-2-ylmethylene)-piperidin-4-one hydrochloride (**8a**): **8a** was prepared in the same manner as **1d**. The reaction of 4-piperidone hydrochloride with 2-thiophene-carboxaldehyde afforded **8a**, m.p. 297(dec) in 72% yield. δ (DMSO-d₆): 4.45 (brs, 4H, 2×NCH₂), 7.32 (t, 2H, 2×C₃-H), 7.70 (s, 2H, 2×=CH-ylidene protons), 7.94-8.05 (d, 4H, 2×C₂-H & 2×C₄-H). Found C 55.36 %, H 4.30 %, N 4.19 %. C₁₅H₁₄ClNOS₂ requires C 55.63 %, H 4.36%, N 4.32%.

Synthesis of 9a: 1-[4-(2-Diethylamino-ethoxy)phenylcarbonyl]-3,5-bis(thiophene-2-ylmethylen)-piperidin-4-one hydrochloride monohydrate (**9a**): Following the above procedure described for compound **4a**, the reaction of 3,5-bis(thiophen-2-ylmethylene)-piperidin-4-one with V. HCl (X= CO, T = OH, Y = O, o = 2, Z = N(C₂H₅)₂) (0.015 mol) afforded **9a**, m.p. 193-194° in 82% yield. δ (CDCl₃): 1.44-1.47 (t, 6H, 2×CH₃), 3.17-3.31 (m, 4H, 2×NCH₂), 3.56 (br s, 2H, NCH₂), 4.53 (t, 2H, OCH₂), 4.93 (br s, 4H, 2×NCH₂), 6.75-6.77 (d, 2H, C₂-H & C₆-H), 7.18-7.19 (t, 2H, 2×C₃'-H), 7.38-7.40 (d, 4H, C₃-H, C₅-H & 2×C₄'-H), 7.66-7.67 (d, 2H, C₂'-H), 12.67 (s, 1H, NH). Found C 59.90 %, H 5.76 %, N 4.81%. C₂₈H₃₁ClN₂O₃S₂·H₂O requires C 59.92 %, H 5.56 %, N 4.99 %.

Synthesis of 10: A mixture of methyl 4-hydroxybenzoate (15.21 g, 0.1 mol), anhydrous potassium carbonate (34.55 g, 0.25 mol), 2-chloro-N,N-diethylamine hydrochloride (25.81 g, 0.15 mol), potassium iodide (0.166 g, 0.001 mol) and acetone (75 ml) was heated under reflux for 10-9 h. On cooling, the solid was removed by filtration and the solvent was evaporated. The residue was dissolved in toluene (75 ml) and the solution was washed with sodium hydroxide solution (2% w/v, 30 ml) and deionized water (2 x 30 ml). Removal of the solvent afforded methyl 4-(2-diethylaminoethoxy)benzoate as an oil

which was dissolved in ethanol (50 ml) and added to a solution of sodium hydroxide (10.0 g, 0.2 mol) in water (50 ml). The mixture was heated under reflux for 2 h. The ethanol was removed *in vacuo* and the aqueous solution was acidified with hydrochloric acid (12N) at 5-6° C. The solid was collected, triturated with water (previously cooled to 5-6° C), filtered and dried at 55-60° C *in vacuo* and recrystallized from water to give **10**.

4-(2-Diethylaminoethoxy)benzoic acid hydrochloride (10). M.p. 170-172° [lit.[22], m.p. 171-174°]. Yield: 73%. ¹H NMR (D₂O): 1.17-1.20 (t, 6H, 2 x CH₃), 3.13-3.24 (m, 4H, 2 x CH₂), 3.47-3.48 (t, 2H, OCH₂CH₂N), 4.26-4.28 (t, 2H, OCH₂CH₂N), 6.89-6.91 (d, 2H, aryl H), 7.80-7.82 (d, 2H, aryl H).

Biological Activity

Cytotoxic evaluations against Molt 4/C8, CEM and L1210 cells: All of the compounds in series **1-7** were evaluated against human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 cells. In addition, compound **10** was included in these assays. These data are presented in Table 1. The cytotoxic potencies of various compounds towards a panel of various human tumour cell lines are given in Table 2.

The compounds in series **1-7** and **10** and melphalan were evaluated against Molt 4/C8, CEM and L1210 cells by a literature procedure (J. Balzarini, E. De Clercq, M.P. Mertes, D. Shugar, P.F. Torrence, *Biochem. Pharmacol.* 31 (1982) 3673-3682). In these assays, at least three different concentrations of compounds were incubated with the neoplastic cells at 37° for 48 h after which time inhibition of growth was determined.

Selected 4-piperidones were examined for antineoplastic properties towards a panel of 49 (38-59) human tumour cell lines using a literature method (M.R. Boyd, K.D. Paull, *Drug. Dev. Res.* 34 (1995) 91-109). In addition, both 5-fluorouracil and melphalan were evaluated towards 57 cell lines. In this assay, compounds were incubated for 48 h using five different concentrations at series tenfold dilutions of 10^{-10.3}M to 10^{-4.3}M except for **1a**, **4a** (10⁻⁸M to 10⁻⁴), 5-fluorouracil (10^{-6.6}M to 10^{-2.6}M) and melphalan (10^{-7.6}M to 10^{-3.6}M). The mean graph midpoint (MG MID) figures for **1a**, **3b**, **3d**, **5d**, **7d** and melphalan were IC₅₀ values. MG MID is the average of the potencies of the compound to approximately 50 neoplastic cell lines. In the following cases, the number of cell lines compared to the total number of cell lines for which an IC₅₀ figure were not obtained were as follows [maximum or minimum concentrations (molar) in parentheses], namely **4a** : 2/53 (< 10⁻¹⁰), **5b** : 2/50 (> 10^{-4.3}), **6a** : 3/48 (> 10^{-4.3}), **6b** : 11/38 (< 10^{-10.3}), **6d** : 3/44 (< 10^{-10.3}), **7a** : 36/52 (> 10^{-4.3}) and 5-fluorouracil 3/57 (> 10^{-2.6}).

Rankings of potencies of the compounds in series 3-7: The first investigation involved comparing the IC₅₀ values of those compounds in series 3-7 which had the same substituents in the arylidene aryl ring. The scores for the compounds in each of the Molt 4/C8, CEM and L1210 cell lines were obtained as follows. The compound having the highest potency was assigned a value of 5, the compound with the next lowest IC₅₀ figures was given a rating of 4 and so forth; the amide with lowest potency was given a figure of 1. The standard deviations for each IC₅₀ value were taken into consideration. The scores available for equipotent compounds were divided equally and a total of 15 points were awarded for each of the comparisons. The second analysis was designed to find the optimal substitution pattern in the arylidene aryl rings in the three cell lines. Thus in each of the series 3-7, the most potent compound was given a rating of 4, the second most potent analog 3 and so forth. A total of ten points were used in each comparison. Standard deviations were taken into consideration and the scores for compounds having the same potencies were divided equally.

Determination of MDR-reversal properties: The methodology used in evaluating the capacity of the compounds to reverse multiple drug resistance (MDR) has been described previously (Kawase, M.; Sakagami, H.; Motohashi, N.; Hauer, H.; Chatterjee, S.S.; Spengler, G.; Vigyikanne, A.V.; Molnár, A.; Molnár, J. *In Vivo* **2005**, 19, 705-712). In brief, both the L-5178 MDR and parental cells were grown in McCoy's 5A medium containing heat-inactivated horse serum (10%), L-glutamine and antibiotics. A solution of the test compound (2 mg/ml, 10 µl) in dimethylsulfoxide was added to aliquots of the cell suspension and incubated at room temperature for 10 minutes. Then 10 µl of rhodamine 123 in dimethylsulfoxide was added so that its final concentration was 5.2 µM and the cells were incubated for a further 20 minutes at 37° C. The cells were washed twice and resuspended in PBS (pH 7.4) after which the fluorescence was measured using a Beckton Dickinson FACScan instrument. The fluorescence of the cells was measured in treated MDR cells (F1), untreated MDR cells (F2), treated parental cells (F3) and the FAR values were obtained from the following equation, viz (F1 / F2)/(F3 / F4).

Cytotoxicity evaluations towards various cell lines: The CC₅₀ (cytotoxicity dose) values of the compounds in series 1 and 3 to 7 towards HSC-2, HSC-4, HGF, HPC, HL-60 and HPLF cells were determined using a literature method involving the MTT assay (Kawase, M.; Sakagami, H.; Motohashi, N.; Hauer, H.; Chatterjee, S.S.; Spengler, G.; Vigyikanne, A.V.; Molnár, A.; Molnár, J. *In Vivo* **2005**, 19, 705-712; and Takeuchi, R.; Hoshijima, H.; Onuki, N.; Nagasaka, H.; Chowdhury, S.A.; Kawase, M.; Sakagami, H. *Anticancer Res.* **2005**, 25, 4037-4042) (MTT assay is a laboratory test for measuring cellular proliferation

(cell growth). MTT stands for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The cells were incubated with varying concentrations of the compounds along with untreated cells for 24h and the absorbance at 540 nm measured. In the case of the HL-60 assay, the trypan blue exclusion method was followed and cell viability was determined using a haemocytometer. The CC₅₀ values were obtained from dose-response curves (Sakagami, H.; Satoh, K.; Ohata, H.; Takahashi, H.; Yoshida, H.; Iida, M.; Kuribayashi, N.; Sakagami, T.; Momose, K.; Takeda, M. *Anticancer Res.* 1996, 16, 2635-2644). The general methods used in culturing the cells employed in these cytotoxicity experiments have been described previously (Kawase, M.; Sakagami, H.; Motohashi, N.; Hauer, H.; Chatterjee, S.S.; Spengler, G.; Vigyikanne, A.V.; Molnár, A.; Molnár, J. *In Vivo* 2005, 19, 705-712; and Takeuchi, R.; Hoshijima, H.; Onuki, N.; Nagasaka, H.; Chowdhury, S.A.; Kawase, M.; Sakagami, H. *Anticancer Res.* 2005, 25, 4037-4042). In brief, the malignant cells were obtained from Professor Nagumo, Showa University, Japan. The HGF, HPC and HPLF cells were prepared from human periodontal tissue after obtaining informed consent according to the guidelines of Meikai University Ethics Committee, Japan (No. 0206). Since normal cells have a short lifespan, cells at the 3-7 population doubling time were used. With the exception of the HL-60 neoplasm, the cells were cultured in DMEM containing heat-inactivated FBS (10%) at 37° C in a humidified carbon dioxide atmosphere and subcultured by trypsinization. The HL-60 cells were cultured in a similar fashion except that RPMI 1640 media was employed.

All references mentioned herein are incorporated by reference.

Table 1. Potencies of the compounds in series **1-7** and **10** towards human Molt 4/C8 and CEM T-lymphocytes and murine L1210 cells^a

Compound ID	IC ₅₀ (μM)					
	Molt 4/C8 cells	SD	CEM cells	SD	L1210 cells	SD
1a		0.15		0.02		0.11
1b	13.4	4.0		0.48	41.4	0.3
1c		0.75		2.28	32.9	4.2
1d		0.09		0		0.14
2a		0.07		0.03	12.0	0
2b		0.73		3.33	37.10	3.7
3a	0.58	0.09	1.24	0.57	10.2	0.10
3b		0.57	1.09	0.67		1.30
3c	0.314	0.035	0.319	0.013		0.71
3d		0.21	0.884	0.532		133
4a		0.15		0.15		0.43
4b	13.3	14.9	12.3	5.9	47.3	4.7
4c	0.31	0.01	0.34	0.01		0.20
4d	1.32	0.45	1.33	0.30		0.08
5a		0.12		0.64		0.22
5b	34.0	19.5		2.99	103	47
5c	0.33	0.011	0.315	0.002		2.47
5d	0.91	0.45		0.73		1.64
6a		0.16		0.09	222	13
6b	18.10	10.1		1.00	81.4	54.6
6c	0.343	0.013	0.379	0.032		1.08
6d	1.72	0.21	1.36	0.13	21.10	9.6
7a	124	82	110	12	160	30
7b	55.10	20.6	71.2	43.6	729	230
7c	40.2	2.9	39.3	5.2	65.0	0.9
7d	12.4	2.6	23.7	4.3	18.7	1.5
8a	>500	N/a	298	262	179	30
9a	7.14	0.69	5.63	2.32	8.34	0.01
10	>500	n/a	>500	n/a	>500	n/a
Melphalan ^b		0.56		0.21		0.02

^a The background shading reflect the potencies expressed as IC₅₀ values, namely black (<1 μM), dark grey (1-10 μM), grey (11-99 μM) and light grey (>100 μM).

Table 2. Cytotoxicity of representative compounds towards human tumor cell lines

Comp	All cell lines		Colon cancer cells, IC ₅₀ (μ M)						Leukemic cells, IC ₅₀ (μ M)					
	IC ₅₀ (μ M)	S.I. ^a	COLO 205	HCT 116	HCT 15	KM 12	SW 620	HCC 2998	K 562	RPMI 8226	HL 60	SR		
1a^b	1.62	93.3	1.55	0.182	0.631	0.794	0.575	2.00	0.490	0.275	0.363	0.209		
3b	1.91	141	1.70	6.03	0.295	1.74	0.209	0.263	0.331	0.098	1.41	0.126		
3d	1.15	39.8	1.02	1.38	0.912	0.776	0.562	0.229	1.82	0.224	1.32	0.479		
4a	<1.38	>2455	2.63	<0.01	1.17	1.74	1.35	-	0.043	<0.01	-	-		
5b	>11.5	>66.1	15.8	47.9	49.0	19.1	3.55	1.58	4.37	0.759	20.4	2.29		
5d	1.38	93.3	6.92	1.58	1.07	0.148	0.339	-	0.589	0.123	0.933	-		
6a	>2.63	>457	22.9	0.339	1.45	0.129	0.191	-	0.158	0.123	0.562	-		
6b	<0.562	>2188	-	7.25	2.19	2.29	1.45	<0.005	6.76	0.132	5.50	-		
6d	<0.708	>1738	1.15	2.45	0.832	1.12	1.02	-	0.275	0.115	1.10	0.245		
7a	>41.7	>4.47	20.4	>50.1	>50.1	43.7	30.2	28.2	43.7	17.4	>50.1	38.0		
7d	7.76	166	8.91	14.1	9.33	9.33	10.5	6.17	1.35	1.26	11.8	22.9		
5-Fluoro-uracil	>29.5	>4365	3.39	2.04	6.61	7.94	18.6	5.75	126	3.55	85.1	60.3		
Melphalan	26.9	118	66.1	30.2	36.3	43.7	38.9	41.7	43.7	66.1	2.04	1.86		

^aThe letters SI refer to the selectivity index, i.e., the ratio of the IC₅₀ figures for the least and most sensitive cell lines to the compound.^bThe hydrochloride salt of **1a** was used in this assay

Table 3. MDR-reversing properties and cytotoxicity of the compounds in series 1 and 3-7

Compound ID	FAR value ^a		CC ₅₀ (μ M) ^b										SI ^d	
	4 μ g/ml	40 μ g/ml	HSC-2	HSC-4	HL-60	ave ^c	HGF	HPC	HPLF					
1a	1.58	2.00	2.2	4.4	1.5	2.7	20	38	24					10
1d	3.26	4.03	3.9	9.3	4.5	5.9	150	200	170					29
1b	1.25	0.91	18	20	22	20	69	130	56					4.3
1c	1.04	0.80	0.90	6.1	3.6	3.5	13	88	39					13
3a	45.5	51.6	3.8	2.1	1.0	2.3	3.6	13	8.9					3.7
3d	83.7	---	1.4	2.1	0.85	1.5	7.9	7.2	3.4					4.3
3b	98.0	98.9	2.3	1.8	1.7	1.9	23	11	4.3					6.6
3c	63.0	3.23	0.93	0.71	0.31	0.7	7.0	5.2	5.9					9.3
4a	49.5	23.8	3.1	2.0	0.61	1.9	4.2	12	6.0					3.9
4d	106	66.6	2.2	2.3	0.79	1.8	3.6	9.3	4.6					3.3
4b	118	99.7	160	34	46	80	230	>400	>400					>4.3
4c	77.5	14.1	0.98	1.1	0.26	0.8	5.0	4.5	5.9					6.6
5a	123	136	3.0	2.5	1.4	2.3	4.6	12	7.4					3.5
5d	179	146	2.9	4.1	2.3	3.1	6.0	15	5.5					2.9
5b	157	133	110	48	63	74	>400	>400	340					>5.2
5c	145	51.5	0.86	0.90	0.29	0.7	8.0	3.9	4.9					8.2
6a	48.5	63.3	2.4	2.4	1.2	2.0	83	95	30					35
6d	95.8	124	1.6	2.0	1.2	1.6	10	49	12					15
6b	76.0	6.88	71	18	36	42	400	>400	360					>9.3
6c	63.3	10.9	0.76	0.74	0.35	0.6	12	8.2	9.3					16
7a	1.37	1.05	130	82	82	98	160	170	79					1.4
7d	1.93	114	17	17	16	17	34	29	8.3					1.4
7b	1.21	1.85	>400	220	64	228	>400	>400	9.4					~1.2
7c	1.03	1.22	67	61	29	52	93	130	13					1.5
Melphalan	---	---	35	81	6	40.7	>200	>200	>200					>4.9

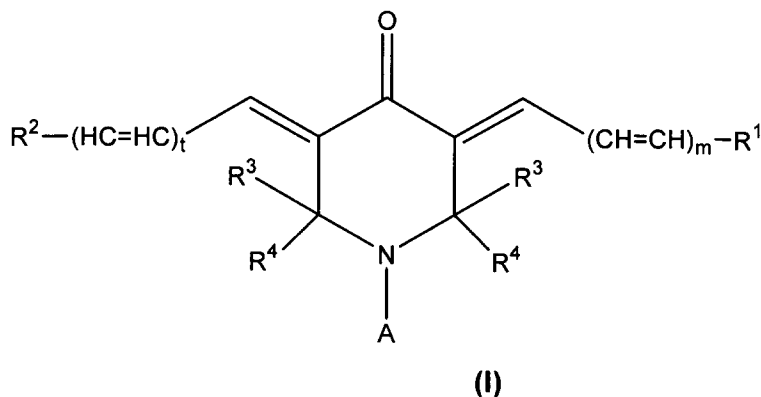
^aThe fluorescence activity ratio (FAR) values are the ratios of the fluorescent intensities of rhodamine 123 in treated and untreated murine L-5178 cells. A reference drug verapamil had a FAR value of 5.61 using a concentration of 10 μ g/ml. ^bThe CC₅₀ values are the concentration of the compounds which kill 50% of the cells and are the average of two independent determinations. ^cThese figures are the average CC₅₀ figures for the HSC-2, HSC-4 and HL-60 malignant cells. ^dThe letters SI refer to the selectivity index which was computed by dividing the average CC₅₀ figures of the HGF, HPC and HPLF cells by the average CC₅₀ values of the HSC-2, HSC-4 and HL-60 neoplasms. ^eA concentration of 40 μ g/ml of **3d** was toxic to the cells.

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What is Claimed:

1. A 4-piperidone compound represented by structural formula (I):



wherein

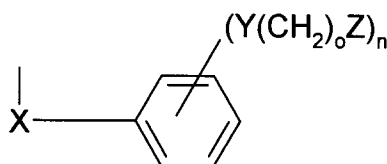
R^1 and R^2 independently represent alkyl, alkoxy, aryl, or heteroaryl;

R^3 and R^4 independently represent H, alkyl, hydroxyalkyl, or carboxylate ester,

wherein each R^3 and each R^4 may be the same or different;

m and t independently represent an integer from 0 to 3;

A represents H or



wherein

n represents an integer from 0 to 4 and indicates the number of $Y(CH_2)_oZ$ substituents on the benzene ring;

o represent an integer from 0 to 4;

X represents $(CH_2)_r$, CO, COO, SO, SO₂, or O, where r represents an integer

from 0 to 4;

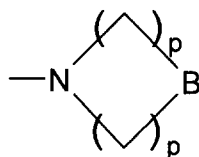
Y represents O, S, NH, N(alkyl), N(aryl), or $(CH_2)_s$, where s represents an integer from 0 to 4;

Z represents H, N(alkyl)₂, NH(alkyl), N(aryl)₂, NH(aryl), alkyl, substituted alkyl,

+N(alkyl)₃, or

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where B represents O, S, $(CH_2)_q$, NH, N-alkyl, or N-aryl, and p and

q

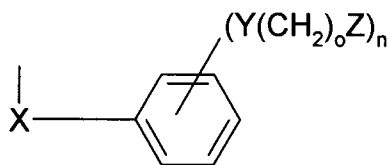
independently represent an integer from 0 to 4;

or a pharmaceutically acceptable salt thereof,

with the proviso that when $A=H$ or $H\cdot HCl$, R^3 and $R^4 = H$, and m and t are 0, R^1 and R^2 are not both phenyl, both 4-chlorophenyl, or both 4-nitrophenyl.

2. The compound according to claim 1, wherein A represents hydrogen, m and t are 0, and R^3 and R^4 are hydrogen.

3. The compound of claim 1, wherein A represents



4. The compound of claim 3, wherein $Y(CH_2)_oZ$ is located at the 2, 3, or 4 position of the benzene ring.

5. The compound of claim 4, wherein $Y(CH_2)_oZ$ is located at the 4 position of the benzene ring.

6. The compound of any of claims 3 to 5, wherein R^3 and R^4 are hydrogen.

7. The compound of any one of claims 3 to 6, wherein m and t are 0.

8. The compound of claim 3, wherein $Y(CH_2)_oZ$ is located at the 4 position of the benzene ring, R^3 and R^4 are hydrogen, X is CO, m and t are 0, n is 1, Y is O, and o is 2.

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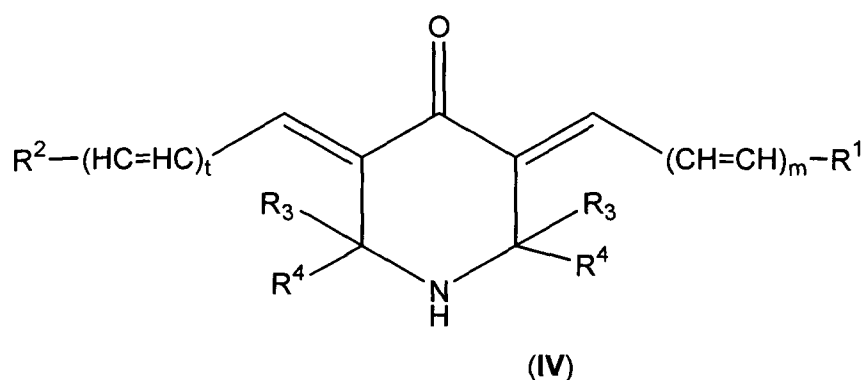
9. The compound of claim 3, wherein $Y(CH_2)_oZ$ is located at the 4 position of the benzene ring, R^3 and R^4 are hydrogen, X is CO, m and t are 0, n is 1, Y is O, and o is 0.

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10. The compound of claim 3, wherein $Y(CH_2)_oZ$ is H, m and t are 0, and R^3 and R^4 are each H.

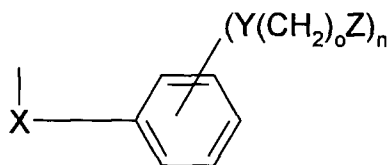
11. A method of preparing the compound of claim 3, comprising the steps of reacting

10



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- with a compound of formula T-A' (V), in the presence of a base, where T represents a halogen, and A' represents



(A').

12. The method of claim 11, wherein the solvent is a polar aprotic solvent or a halogenated hydrocarbon solvent.

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13. The method of claim 12, wherein the polar aprotic solvent is tetrahydrofuran or acetonitrile.

14. The method of claim 12, wherein the halogenated hydrocarbon solvent is chloroform, dichloromethane, or 1, 2-dichloroethane.

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15. A pharmaceutical composition comprising the compound of any one of claims 1 to 10, together with a pharmaceutically acceptable diluent or carrier.
16. The pharmaceutical composition of claim 15, for the treatment of cancer.
17. A method of treating cancer comprising administering a therapeutically effective amount of the compound of any one of claims 1 to 10, to a patient in need thereof.
18. The method of claim 17, wherein the cancer is colon cancer or leukemia.
19. The pharmaceutical composition of claim 15, for reversing multi-drug resistance.
20. A method of reversing multi-drug resistance comprising administering a therapeutically effective amount of the compound of any one of claims 1 to 10, to a patient in need thereof.

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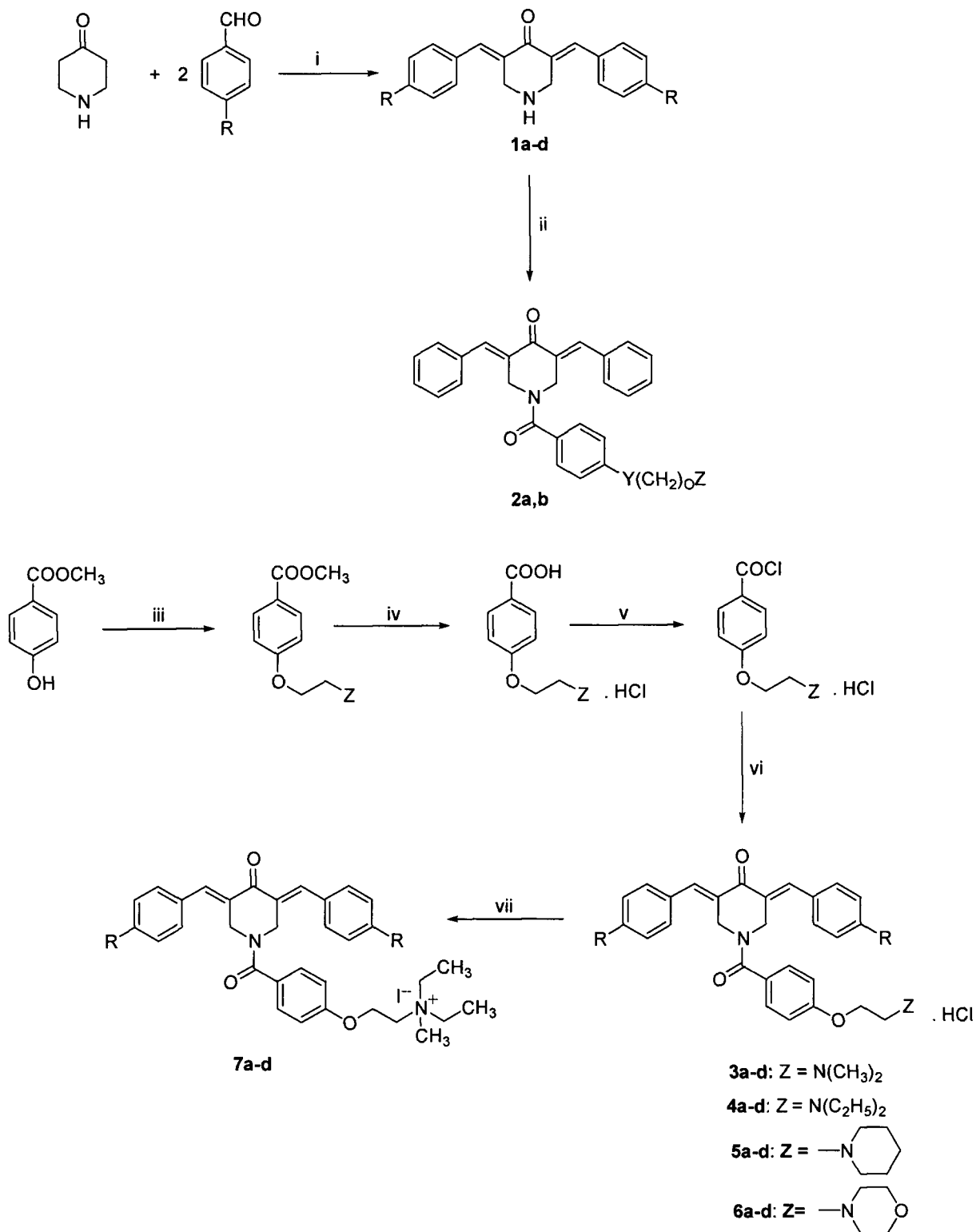


Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001908

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07D 211/44** (2006.01) , **A61K 31/45** (2006.01) , **A61P 35/00** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁸: C07D, A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Canadian Patent Database, HighWire, SCOPUS, Google Scholar, STN CA-PLUS, Depatisnet; Keywords: bimodal, 4-oxo-piperidine, 4-piperidone + 3,5-diarylidene, cytotoxic, antineoplastic, conjugated + enones, multidrug + resistance + related terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5217846 (SMOTHERS, W) Compound in cols 16-17; Sensitizer S-1	1-2
X	DIMMOCK, ET AL., " <i>Cytotoxic evaluation of some 3,5-bis(arylidene)-4-piperidones and various related quaternary ammonium compounds and analogs</i> ", Journal of Pharmaceutical Sciences, Vol. 83, No. 8, pages 1124-1130, August 1994 Compounds 1c-1f, 1h; Tables 1-2; page 1125	1-2, 15-18
X	DIMMOCK, ET AL., " <i>A conformational and structure-activity relationship study of cytotoxic 3,5-bis(arylidene)-4-piperidones and related N-acryloyl analogues</i> ", Journal of Medicinal Chemistry, Vol. 44, pages 586-593, 2001 cited in the application Compounds 1c, 1d, 1f, 1g; Compounds 2a-2g, Table 1; pages 587-588; Tables 4-6, page 590	1-2, 15-18

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

8 February 2007 (08-02-2007)

Date of mailing of the international search report

13 March 2007 (13-03-2007)

Name and mailing address of the ISA/CA
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DIMMOCK, ET AL., " <i>Cytotoxic Mannich Bases of 1-arylidene-2-tetralones</i> ", Journal of Enzyme Inhib. and Med. Chem., Vol. 18, No. 4, pages 313-324, August 2003 Last para. left hand col. page 315	11-14
X Y	DIMMOCK, ET AL., " <i>Cytotoxic evaluation of some N-acyl and N-acyloxy analogues of 3,5-bis(arylidene)-4-piperidones</i> ", Drug Design and Discovery, Vol. 12, pages 19-28, 1994 cited in the application Compounds 1b, 3a-3e; pages 20-21, procedure in pages 25-26	1-7, 10-12, 14-18 8-9, 13
X Y	DIMMOCK, ET AL., " <i>Evaluation of Some N-acyl analogues of 3,5-Bis(arylidene)-4-piperidones for Cytotoxic activity</i> ", Drug Design and Discovery, Vol. 8, No. 4, pages 291-299, 1992 cited in the application Compound 3a Compounds 1a-1f, 2a-2f, 3a-3d, Table 1	1-2, 15-18 3-7, 10
X Y	DIMMOCK, ET AL., " <i>3,5-Bis-Benzylidene-4-piperidones and related compounds with high activity toward P388 Leukemia cells</i> ", Drug Design and Delivery, Vol. 6, No. 3, pages 183-194, 1990 cited in the application Compound 1b Compounds 1a, 1c-1f, page 184; Table 1; compound 4	1-2, 15-18 3-7, 10
X Y	BUU-HOI, ET AL., " <i>Condensation of aromatic and heterocyclic aldehydes with piperidones: relation between structure and choleric activity of the products of this reaction</i> ", Bulletin de la Societe Chimique de France, Vol. 12, pp. 3096-3099, 1964 Compounds 3, 9-11	1, 3, 6-7 2, 4-10
P,X P,Y	JHA, ET AL., " <i>3,5-bis(arylmethylene)-4-piperidone derivatives as novel anticancer agents</i> ", Indian Journal of Chem., Vol. 45, Issue 10, pages 2313-2320, October 2006 Compounds 1a-1g, 2a-2b, 4a-4f, 5a-5i, 7a-7i, 8-9	1-2, 15-18 3-7, 10
Y	DIMMOCK, ET AL., " <i>Cytotoxic N-[4-(3-aryl-3-oxo-1-propenyl)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidones and related compounds</i> ", Eur. Journal of Med. Chem., Vol. 37, pp. 961-972, 1 December 2002 cited in the application Compound 2; Compounds 3a-3h, Table 1, Figure 1, Scheme iii, page 962-963; Para. 6.1.2 at page 969	1, 3-7, 10-18

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JHA, ET AL., " <i>Synthesis of 4-(3,5-bisphenylmethylene-4-oxo-piperidin-1-yl)-4-oxo-but-2Z-enoic Acid Arylamide as Candidate Cytotoxic Agents</i> ", <i>Synthetic Comm.</i> , Vol. 33, No. 7, pages 1211-1223, 2003 Compound 1, page 1212	1-2, 15-18
Y	DIMMOCK, ET AL., " <i>3,5-bis(phenylmethylene)-1-(N-arylmaleamoyl)-4-piperidones: a novel group of cytotoxic agents</i> ", <i>Journal of Enzyme Inhib. and Med. Chem.</i> , Vol. 18, No. 4, pages 325-332, August 2003 cited in the application Compound 1	1-2
Y	KUETTEL, ET AL., " <i>Piperidone Derivatives. XI. 3-carbethoxy-4-piperidone and 4-piperidone hydrochloride</i> ", <i>Journal of American Chemical Society</i> , Vol. 53, pages 2692-2696, 8 July 1931 Compound IV; page 2692	1-2
Y	EL-SUBBAGH, ET AL., " <i>Synthesis and Biological evaluation of certain α,β-unsaturated ketones and their corresponding fused pyridines as antiviral and cytotoxic agents</i> ", <i>Journal of Med. Chem.</i> , Vol. 43, pages 2915-2921, 2000 Compounds 8-17, page 2916	1-2, 15-18

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 17-18, 20

because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 17-18, 20 are directed to methods of treatment of the human body, the search has been carried out and based on the alleged medicinal effects of the compounds (Rule 39.1 (iv) PCT).

2. ☐ Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. ☐ Claim Nos. :

because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/CA2006/001908

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US5217846	08-06-1993	US5332819 A	26-07-1994
