METHODS OF INHIBITING MELANOMA

Background of the Invention

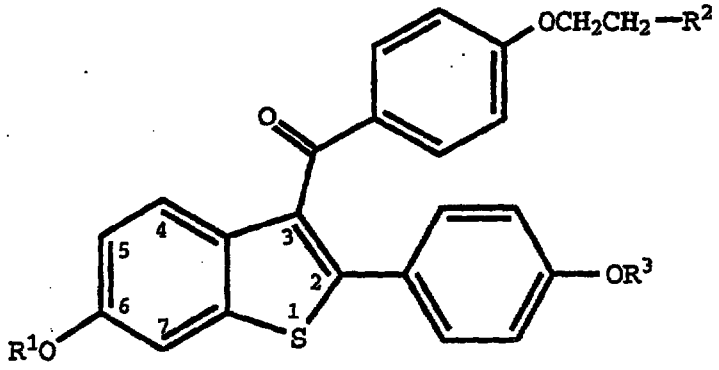
Melanoma is a malignant tumor of melanocytes largely located in the skin. Melanoma occurs primarily in adults and commonly arises from a benign pigmented nevus. Any increase in size, change in coloration, or onset of itching are all early signs that may indicate a malignant change. Malignant melanoma currently accounts for approximately 1% of all cancer deaths. The rate of incidence, however, of melanoma is increasing at a faster rate then any other neoplasm, except for lung cancer in women.

The greatest danger with melanoma is metastases by local extension through lymphatics and by hematogenous routes to distant sites. While any organ may be involved by metastases, the lung and liver are common sites. Melanoma that has spread to distant sites is infrequently curable with standard therapy, although long-term survival is occasionally achieved by resection of the metastases. Many time such patients are appropriate candidates for clinical trials exploring new forms of chemotherapy or biological response modifiers.

Currently, after approximately 20 years of experimentation dacarbazine (DTIC) is the only agent approved by the FDA for treatment of metastatic melanoma, and no combinations of chemotherapeutic agents have been shown to be conclusively more effective than dacarbazine alone. Dacarbazine therapy is associated with the response rate of 15-25%, with only 5% of patients achieving a complete response. Therefore, there is strong interest in finding a more beneficial treatment regimen and additional melanoma-inhibiting agents.

SUMMARY OF THE INVENTION

This invention provides methods for inhibiting melanoma comprising administering to a human in need thereof an effective amount of a compound of formula I



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$, $-C-(C_1-C_6\ alkyl)$, or $-C-Ar$, wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidino, hexamethyleneimino, and piperidino; and pharmaceutically acceptable salts and solvates thereof, alone or in combination with one or more melanoma-inhibiting agents.

DETAILED DESCRIPTION OF THE INVENTION

The current invention concerns the discovery that a select group of 2-phenyl-3-aroylbenzothiophenes (benzothiophenes), those of formula I, are useful for inhibiting melanoma.

The methods of use provided by this invention are practiced by administering to a human in need thereof a dose of a compound of formula I or a pharmaceutically acceptable salt or solvate thereof, that is effective to inhibit melanoma, alone or in combination with one or more melanoma inhibiting agents.

The term "inhibit" includes its generally accepted meaning which includes prohibiting, preventing, restraining, and slowing, stopping or reversing. As such,

the present method includes both medical therapeutic and/or prophylactic administration, as appropriate.

Raloxifene, a compound of this invention wherein it is the hydrochloride salt of a compound of formula 1, R^1 and R^3 are hydrogen and R^2 is 1-piperidinyl, is a nuclear regulatory molecule. Raloxifene has been shown to bind to the estrogen receptor and was originally thought to be a molecule whose function and pharmacology was that of an anti-estrogen in that it blocked the ability of estrogen to activate uterine tissue and estrogen dependent breast cancers. Indeed, raloxifene does block the action of estrogen in some cells; however in other cell types, raloxifene activates the same genes as estrogen does and displays the same pharmacology, e.g., osteoporosis, hyperlipidemia. As a result, raloxifene has been referred to as an anti-estrogen with mixed agonist-antagonist properties. The unique profile which raloxifene displays and differs from that of estrogen is now thought to be due to the unique activation and/or suppression of various gene functions by the raloxifene-estrogen receptor complex as opposed to the activation and/or suppression of genes by the estrogen-estrogen receptor complex. Therefore, although raloxifene and estrogen utilize and compete for the same receptor, the pharmacological outcome from gene regulation of the two is not easily predicted and is unique to each.

Generally, the compound is formulated with common excipients, diluents or carriers, and compressed into tablets, or formulated as elixirs or solutions for convenient oral administration, or administered by the intramuscular or intravenous routes. The compounds can be administered transdermally, and may be formulated as sustained release dosage forms and the like.

The compounds used in the methods of the current invention can be made according to established procedures, such as those detailed in U.S. Patent Nos. 4,133,814, 4,418,068, and 4,380,635 all of which are incorporated by

reference herein. In general, the process starts with a benzo[b]thiophene having a 6-hydroxyl group and a 2-(4-hydroxyphenyl) group. The starting compound is protected, acylated, and deprotected to form the formula I compounds. Examples of the preparation of such compounds are provided in the U.S. patents discussed above. Optionally substituted phenyl includes phenyl and phenyl substituted once or twice with C_1-C_6 alkyl, C_1-C_4 alkoxy, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)methyl.

The compounds used in the methods of this invention form pharmaceutically acceptable acid and base addition salts with a wide variety of organic and inorganic acids and bases and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanoic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, ß-hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, teraphthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate,

benzene-sulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like. A preferred salt is the hydrochloride salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

Bases commonly used for formation of salts include ammonium hydroxide and alkali and alkaline earth metal hydroxides, carbonates, as well as aliphatic and primary, secondary and tertiary amines, aliphatic diamines. Bases especially useful in the preparation of addition salts include ammonium hydroxide, potassium carbonate, methylamine, diethylamine, ethylene diamine and cyclohexylamine.

The pharmaceutically acceptable salts generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Pharmaceutical formulations can be prepared by procedures known in the art. For example, the compounds can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl pyrrolidone; moisturizing agents such as glycerol; disintegrating agents

such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes. Additionally, the compounds are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

The regimen and particular dosage of a compound of formula I required to inhibit melanoma according to this invention will depend upon the severity of the condition, the route of administration, and related factors that will be decided by the attending physician. Generally, accepted and effective daily doses will be from about 0.1 to about 1000 mg/day, and more typically from about 50 to about 200 mg/day. Such dosages will be administered to a subject in need thereof from once to about three times each day, or more often as needed, and for a time sufficient to effectively inhibit melanoma. The invention also encompasses combination administration of a compound of formula I along with one or more melanoma-inhibiting agents. Such agents include cisplatin, dacarbazine, and carmustine.

It is usually preferred to administer a compound of formula I in the form of an acid addition salt, as is customary in the administration of pharmaceuticals bearing a basic group, such as the piperidino ring. It is also

advantageous to administer such a compound by the oral route. For such purposes the following oral dosage forms are available.

Formulations

In the formulations which follow, "Active ingredient" means a compound of formula I.

Formulation 1: Gelatin Capsules

Hard gelatin capsules are prepared using the following:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.1 - 1000
Starch, NF	0 - 650
Starch flowable powder	0 - 650
Silicone fluid 350 centistokes	0 - 15

The ingredients are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules.

Examples of specific capsule formulations of raloxifene that have been made include those shown below:

Formulation 2: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	1
Starch, NF	112
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 3: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	5
Starch, NF	108
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 4: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	10
Starch, NF	103
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 5: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	50
Starch, NF	150
Starch flowable powder	397
Silicone fluid 350 centistokes	3.0

The specific formulations above may be changed in compliance with the reasonable variations provided.

A tablet formulation is prepared using the ingredients below:

Formulation 6: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Cellulose, microcrystalline	0 - 650
Silicon dioxide, fumed	0 - 650
Stearate acid	0 - 15

The components are blended and compressed to form tablets.

Alternatively, tablets each containing 0.1 - 1000 mg of active ingredient are made up as follows:

Formulation 7: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60° C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.1 - 1000 mg of medicament per 5 mL dose are made as follows:

Formulation 8: Suspensions

Ingredient	Quantity (mg/5 ml)
Active ingredient	0.1 - 1000 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to	5 mL

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

ASSAYS

A colony forming assay is used to test the activity of the formulated compound. In testing for the effects of short exposure of compound of formula I on the melanoma cells, a single cell suspension of melanoma cells are seeded onto 60 mm tissue culture dishes (Corning, Corning, NY) at 20,000 cells/dish, allowing 2-4 hours for them to attach. Compound is added to the dishes and incubated for 1 hour, then the dishes are washed three times with phosphate buffered saline (Sigma Chemical Co., St. Louis, MO) and the cells are harvested by trypsinization, washed once more to remove drug, and resuspended in 5 ml of complete media containing 0.3% agar solution (Difco,

Detroit, MI). The cell suspension is mixed well and then aliquoted at 1 ml/dish, in triplicate, onto preprepared 35 mm dishes containing a basement layer of solidified 1% agarose. The agar containing cell-suspension is allowed to solidify at room temperature and the dishes are incubated at 37°C in humidified 5% CO_2. Colonies greater than 125 µM are counted after 5 days for continuous cell lines or 7-14 days for primary cells.

An assay using continuous exposure of the formulated compound is performed by resuspending melanoma cells in 0.3% agar solution (Difco, Detroit, MI) at 4000 cells/ml, aliquoting this suspension into tubes containing the formulated compound and complete media, e.g., RPMI 1640 (Irving Scientific, Santa Ana, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 50 µg/ml gentamicin (Gemini Bio-Products, Calabasas, CA), 2 mM L-glutamine, 10 nM hydrocortisone, 5 µg/ml insulin, 5 µg/ml human transferin, 10 nM estradiol, and 5 ng/ml selenium (Sigma Chemical Co., St. Louis, MO). The cell suspension is mixed well and then aliquoted at 1 ml/dish, in triplicate, onto preprepared 35 mm dishes containing a basement layer of solidified 1% agarose. The agar containing cell-suspension is allowed to solidify at room temperature, and the dishes are incubated at 37°C in a humidified 5% CO_2. Colonies greater than 125 µM are counted after 5 days for continuous cell lines or 7-14 days for primary cells.

Activity in at least one of the above assays indicates utility for inhibiting melanoma.

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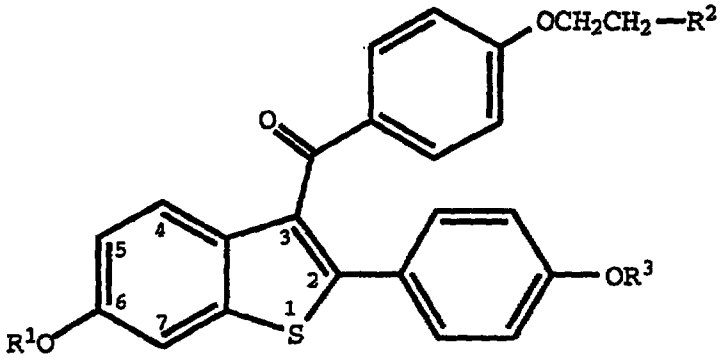
We claim:

1. A method of inhibiting melanoma comprising administering to a human in need thereof an effective amount of a compound having the formula



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$, $-\overset{O}{\overset{\|}{C}}-(C_1-C_6\ alkyl)$, or $-\overset{O}{\overset{\|}{C}}-Ar$, wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidine, hexamethylenemino, and piperidino; or a pharmaceutically acceptable salt or solvate thereof; alone or in combination with one or more melanoma-inhibiting agents.

2. The method of Claim 1 wherein said compound is the hydrochloride salt thereof.

3. The method of Claim 1 wherein said compound is

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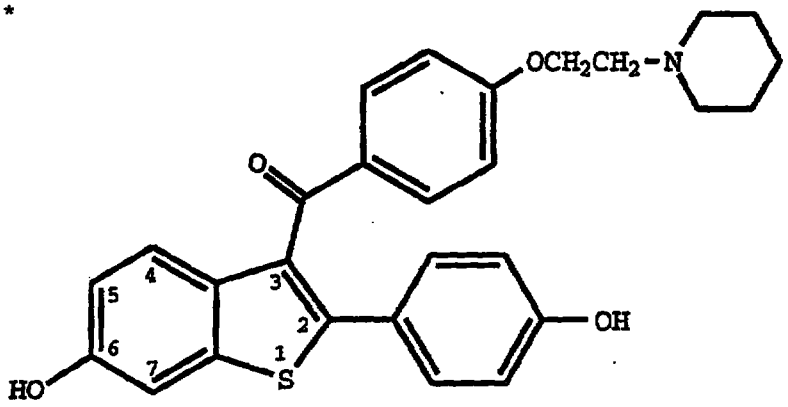

or its hydrochloride salt.

4. The method of Claim 1 wherein said administration is in combination with one or more of cisplatin, dacarbazine or carmustine.

5. The method of Claim 1 wherein said melanoma is metastatic.

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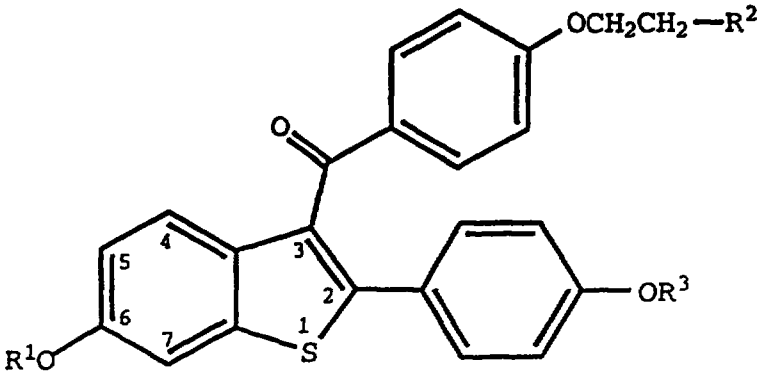
WE CLAIM :

1. A use of a compound of the formula



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$, $-\overset{O}{\overset{\|}{C}}-(C_1\text{-}C_6\ \text{alkyl})$, or $-\overset{O}{\overset{\|}{C}}-AR$, wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidine, hexamethyleneimino, and piperidino; or a pharmaceutically acceptable salt or solvate thereof; in a preparation of a medicament useful for inhibiting susceptible melanoma as cytotoxic agent per se; alone or in combination with one or more melanoma-inhibiting agents.

2. The use of Claim 1 wherein said compound is the hydrochloride salt thereof.

3. The use of Claim 1 wherein said compound is

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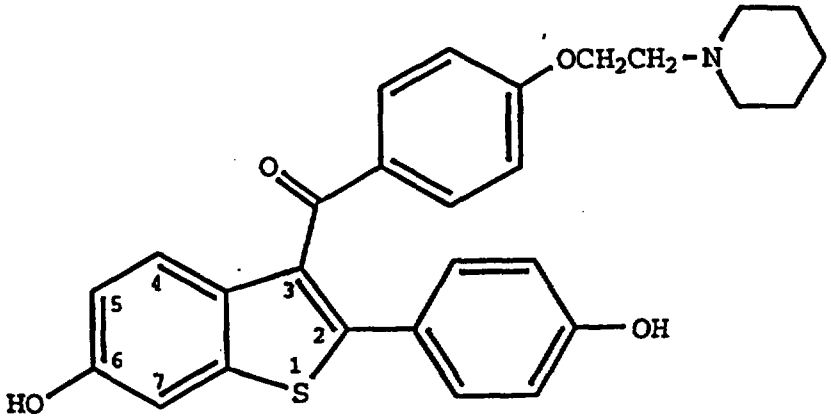

or its hydrochloride salt.

4. The **use** of Claim 1 wherein said administration is in combination with one or more of cisplatin, dacarbazine or carmustine.

5. The **use** of Claim 1 wherein said melanoma is metastatic.

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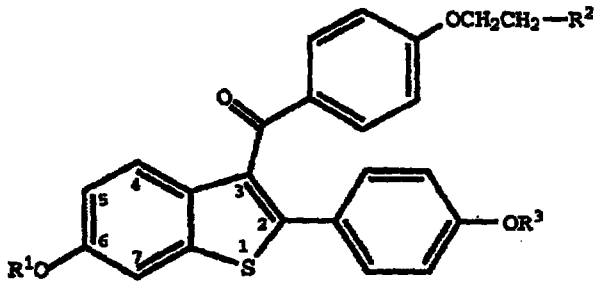

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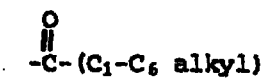

(a)



(b)

(57) Abstract

A method of inhibiting melanoma comprising administering to a human in need thereof an effective amount of a compound having formula (I) wherein R^1 and R^3 are independently hydrogen, $-CH_3$, (a), or (b), wherein Ar is optionally substituted phenyl; R^2 is selected from the group consisting of pyrrolidine, hexamethyleneamino, and piperidino; or a pharmaceutically acceptable salt or solvate thereof; alone or in combination with one or more melanoma-inhibiting agents.