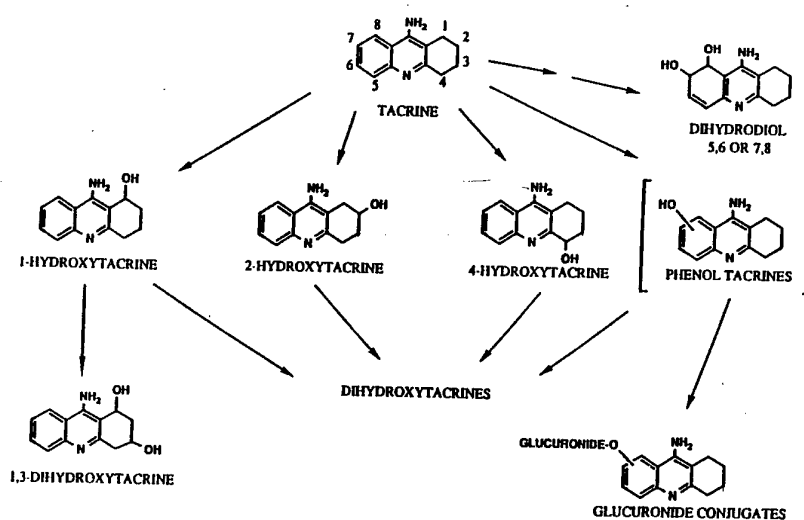




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(54) Title: USE OF CYTOCHROME P450 INHIBITORS FOR INHIBITING THE METABOLISM OF NITROGEN SUBSTITUTED ACRIDINE



Proposed Metabolic Pathway for Tacrine in Man

(57) Abstract

Described is a method of inhibiting the enzymatic metabolism of nitrogen substituted acridines comprising co-administering with the acridine an effective oxidase inhibiting amount of a P450 1A2 inhibitor. The oxidase is one whose activity is induced by beta-naphthaflavone, 3-methylcholanthrene, arochlor, 2,3,7,8-tetrachlorodibenzo-p-dioxin and isosafrole. The oxidase inhibitor may be a naphthyridine, a xanthine, a phenoxy amino alkane, a carbamoyl imidazole, a heterocyclic guanidine, a quinoline or a trifluoromethyl oxime ether.

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USE OF CYTOCHROME P450 INHIBITORS FOR INHIBITING THE METABOLISM OF NITROGEN SUBSTITUTED ACRIDINE

Reference To Cross-Related Cases

5 The present application is a continuation-
in-part of U.S. application Serial No. 943,323, filed
September 10, 1992.

Technical Field

10 The present invention is concerned with
nitrogen substituted acridines and, in particular,
their effect on cytochrome P450.

Background Art

15 Nitrogen substituted acridine have been
proposed for use in treatment of senile dementia such
as Alzheimer's disease. Such materials are described
in U.S. Patents 4,631,286; 4,695,573; 4,754,050;
4,816,456; 4,835,275; 4,839,364; 4,999,430; and
British Patent Appln. 2,091,249, all of which are
hereby incorporated by reference.

20 Clinical studies have been performed on
patients suffering from Alzheimer's disease by
utilizing tacrine or 1,2,3,4-tetrahydro-9-acridinamine
monohydrate monohydrochloride (THA). Serum
determinations of patients given THA indicated the
25 very rapid formation of THA metabolites. It has also
been indicated that elevations of liver enzymes are

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found in some patients after THA administration, which reportedly can be controlled by adjustment of medication. W.K. Summers, T.H. Tachiki and A. Kling: "Tacrine In The Treatment Of Alzheimer's Disease", EUR. NEUROL. 29 (Supp. 3): 28-32 (1989). Various metabolites of THA have been reported. C.A. Truman, J.M. Ford, C.J.C. Roberts: "Comparison of the Chromatographic Characteristics of Metabolites of Tacrine Hydrochloride in Human Serum and Urine with those of In Vitro Metabolic Products From Hepatic Microsomes", BIOCHEMICAL PHARMACOLOGY, Vol. 42, no. 4, pp. 956-959 (1991). THA is extensively metabolized in animals and man to several monohydroxy and dihydroxy metabolites, some of which are excreted as glucuronide derivatives.

Monooxygenation of chemical materials has been ascribed to cytochromes P450 (P450). These hemoprotein containing monooxygenase enzymes displaying a reduced carbon monoxide absorption spectrum maximum near 450 nm have been shown to catalyze a variety of oxidation reactions including hydroxylation of endogenous and exogenous compounds. M. R. Jachau, "Substrates, Specificities and Functions of the P450 Cytochromes", LIFE SCIENCES, Vol. 47, pp. 2385-2394 (1990). An extensive amount of research has been conducted on the mechanism's by which P450's can catalyze oxygen transfer reactions. B. Testa and P. Jenner, "Inhibitors Of Cytochrome P-450s and Their Mechanism of Action", DRUG METABOLISM REVIEWS, 12(1)1-117 (1981); F. P. Guengerich, "Cytochrome P450: Advances and Prospects", FASEB J., Vol. 6, pp. 667-668 (1992); K. Brosen; M. Murray and C.F. Reidy, "Recent Developments In Hepatic Drug Oxidation Implications For Clinical

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5 Pharmacokinetics", CLIN. PHARMACOKINET., 18(3): 220-239, 1990; and M. Murray and G.F. Reidy, "Selectivity in the Inhibition of Mammalian Cytochrome P-450 By Chemical Agents", PHARMACOLOGICAL REVIEWS, 42, 85-101 (1990).

 Murray & Reidy, *supra*, further state that P-450's are ubiquitous enzymes found in the smooth endoplasmic-reticulum as well as mitochondrial fractions of mammalian cells. P450 constitutes a
10 multigene family of enzymes with nearly 150 isoforms identified to date. T. D. Porter and M. J. Coon, "Cytochrome P-450: Multiplicity of Isoforms, Substrates, and Catalytic and Regulatory Mechanisms", J. BIOL. CHEM., Vol. 266, 13469-13472 (1991). The P450
15 reaction cycle proceeds briefly as follows: initial binding of a substrate molecule (RH) to the ferric form of the cytochrome results in the formation of a binary complex and a shift in the spin equilibrium of the ferric enzyme from the low- to high-spin state.
20 Some evidence has been presented that suggests this configuration more readily accepts an electron from the flavoprotein reductase to form the ferrous P450-substrate complex. However, not all P450s exhibit a relationship between high-spin content and reduction
25 rate. Indeed, it has been proposed that several factors, including the nature of the P450 substrate, the topography of the enzyme/substrate complex, and the potentials of oxidizable atoms each play a role in regulation of the reduction rate. Molecular oxygen
30 binds to the ferrous P450-substrate complex to form the ferrous dioxygen complex which is then reduced by a second electron from the P450 reductase (or perhaps, in some cases, from reduced nicotinamide adenine

-4-

dinucleotide via cytochrome b_5 and its reductase).
Dioxygen bond cleavage in the reduced ferrous dioxygen
complex results in the insertion of one atom of oxygen
into the substrate, reduction of the other oxygen atom
5 to water, and restoration of the ferric hemoprotein.

Individual members of the P450 family of
enzymes and associated mixed function oxidase
activities have been described in extrahepatic tissues
including brain, adrenal, kidney, testis, ovary, lung
10 and skin. Individual P450s have likewise been charac-
terized in terms of their inducibility by selected
chemical classes. Induction of specific P450 enzymes,
such as the P450 1A1 and 1A2 subfamily have been
extensively studied with respect to regulatory
15 processes of increased mRNA transcription and
expression of enzymatic activity. It has been
ascertained that materials such as beta-naphthaflavone
(beta-NF), 3-methylcholanthrene (3-MC), arochlor 1254
(ACLR) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
20 are materials that have been categorized as inducers
of P450 enzymes bearing the designated P450 1A
subfamily. At present, two specific P450 enzymes
termed 1A1 (nonhepatic) and 1A2 (hepatic) have been
characterized by several laboratories. Materials that
25 induce the hepatic P450 1A subfamily of enzymes, and
in particular the constitutive 1A2 enzyme include 3-
MC, cigarette smoke, beta-NF, TCDD, and ACLR and, in
addition, isosafrole, and musk xylenes, are
preferential inducers of 1A2. M. Murray and G.F.
30 Reidy, "Selectivity In The Inhibition Of Mammalian -
Cytochromes P450 By Chemical Agents", PHARMACOLOGICAL
REVIEWS, 42, 85-101 (1990); and F. P. Guengerich,
"Characterization of Human Microsomal Cytochrome P-450

-5-

Enzymes", ANNU. REV. PHARMACOL. TOXICOL. Vol, 29, pp. 241-264 (1989).

It is an object of the present invention to improve the metabolic stability of nitrogen substituted acridines in human body fluids (blood, plasma, brain, liver, etc).

It is an object of the present invention to provide in mammalian body fluids a stable concentration of nitrogen substituted acridine.

It is a further object of the present invention to describe and utilize nitrogen substituted acridines in conjunction with inhibitors of cytochrome P450 1A subfamily (1A1 and 1A2) of enzymes.

It is a further object of the present invention to describe nitrogen substituted acridines co-administered with a naphthyridine.

It is a further object of the present invention to describe nitrogen substituted acridines co-administered with a xanthine.

It is a further object of the present invention to describe nitrogen substituted acridines co-administered with a phenoxy amino alkane.

It is a further object of the present invention to describe nitrogen substituted acridines co-administered with a carbamoyl imidazole.

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It is a further object of the present invention to describe nitrogen substituted acridines co-administered with a guanidine imidazole, e.g. cimetididine (N-cyano-N'-methyl-N''-[2[[[5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]guanidine)

It is a further object of the present invention to describe nitrogen substituted acridines co-administered with a quinoline, e.g. chloroquine (7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline) and primaquine (8-(4-amino-1-methylbutylamino)-6-methoxyquinoline).

It is a further object of the present invention to describe nitrogen substituted acridines co-administered with a trifluoromethyl oxime ether, e.g., fluvoxamine, also known as 5-methoxy-1-[4-(trifluoromethyl)-phenyl]-1 pentanone O-(2-aminoethyl) oxime.

None of the references disclose techniques for maintaining the stability, i.e., non-metabolism of nitrogen substituted acridines, so that they may suitably be effective as agents for treatment of senile dementia.

Summary Of The Invention

The above objects are accomplished by the invention described herein.

Described is a method of inhibiting the enzymatic metabolism of nitrogen substituted acridines

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by co-administering an effective oxidase inhibiting amount of a P450 1A subfamily inhibitor.

5 A further embodiment of the invention comprises co-administering with the nitrogen substituted acridine an effective oxidase inhibiting amount of a naphthyridine.

10 A further embodiment of the invention comprises co-administering with the nitrogen substituted acridine an effective oxidase inhibiting amount of a xanthine.

A further embodiment of the invention comprises co-administering with the nitrogen substituted acridine an effective oxidase inhibiting amount of a phenoxy amino alkane.

15 A further embodiment of the invention comprises co-administering with the nitrogen substituted acridine an effective oxidase inhibiting amount of a carbamoyl imidazole.

20 A further embodiment of the invention comprises co-administering with the nitrogen substituted acridine an effective oxidase inhibiting amount of a guanidine imidazole.

25 A further embodiment of the invention comprises co-administering with the nitrogen substituted acridine an effective oxidase inhibiting amount of a quinoline.

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A further embodiment of the invention comprises co-administering with the nitrogen substitute acridine an effective oxidase inhibiting amount of a trifluoromethyl oxime ether.

5 A further embodiment of the invention comprises the use of the aforementioned compounds or compositions for the manufacturing of pharmaceutical compositions for the mentioned methods or treatments. The compositions are supplied to those mammals who
10 have a need for them.

Brief Description Of The Drawings

FIGURE 1 is a proposed metabolic pathway for tacrine in man; and

FIGURE 2 is a proposed pathway for
15 irreversible binding of tacrine in human liver microsomes.

Description Of The Preferred Embodiments

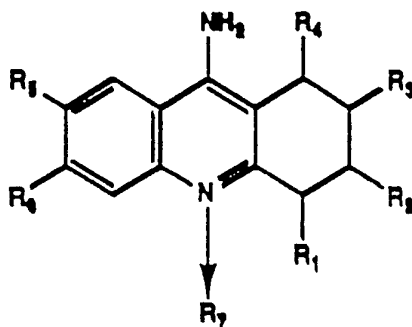
The present invention is concerned with nitrogen substituted acridines to decrease the amount
20 of or to prevent the metabolism thereof. It has been determined that nitrogen substituted acridines are metabolized by P450 (cytochrome P450) monooxygenase enzymes and, in particular, by the type 1A2 enzyme.

P450s that metabolize the nitrogen
25 substituted or amino acridines as described herein are those enzymes that are induced by materials such as isosafrole, 3-MC, cigarette smoke, beta-NF, TCDD, and

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ACLR. These P450s belonging to the 1A subfamily, which are hemoprotein containing oxygenases, have their enzymatic activity increased or induced by the aforementioned chemical materials. Therefore, it is
5 desired that these P450 1A2 enzymes located in the liver are hemoproteins whose activity needs to be inhibited in order to prevent the metabolism of the amino acridines as described herein. Applicant, therefore, has characterized the applicable oxygenase
10 P450s both by the general terminology P450 1A2 (cytochrome P450 1A2) but also by the chemical materials that cause or induce the action of these enzymes. Characterization of the specific amino acid sequence for the P450 1A subfamilies in rat and man has been
15 reported. P. Soucek and I. Gut, "Cytochromes P-450 In Rats; Structures, Functions, Properties and Relevant Human Forms", XENOBIOTICA, Vol. 22, pp. 83-103 (1992).

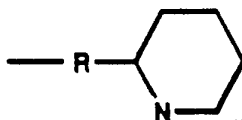
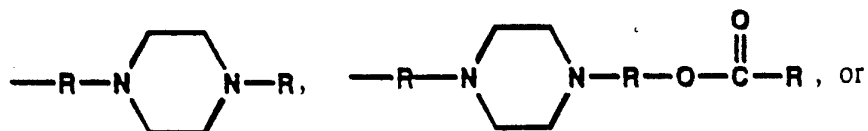
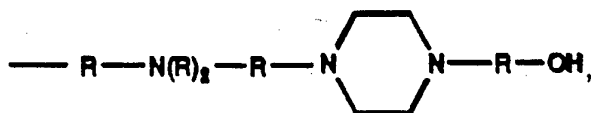
The nitrogen substituted amino acridines which the present invention is concerned and the
20 metabolism which is sought to be decreased or eliminated can be described in U.S. Patent 4,816,456, hereby incorporated by reference. See Formula 1 below:



(1)

-10-

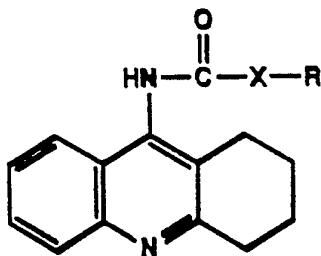
wherein R_1 is related from the group consisting of hydrogen, hydroxy, methyl, methoxy, ethyl and ethoxy; R_1 and R_2 together may form a double bond, R_3 and R_4 together may form a double bond, or R_1 , R_2 , R_3 and R_4 are all hydrogen; R_5 is related from the group consisting of hydrogen, hydroxy, methoxy and ethoxy; R_6 is related from the group consisting of hydrogen, hydroxy, methoxy, and ethoxy; and R_7 represents no radical; an N-oxy radical; a C_1 - C_{20} alkyl radical or a radical selected from the group consisting of



wherein each R is independently selected from C_1 - C_{20} alkyl; and pharmaceutically acceptable salts thereof.

Nitrogen substituted acridines can also be characterized by the materials described in U.S. Patent 4,999,430, hereby incorporated by reference. See Formula 2.

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(2)

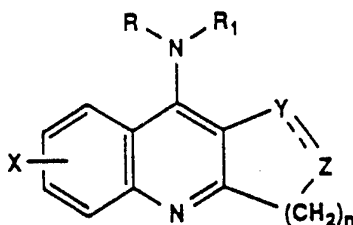
wherein

X is selected from the group consisting of oxygen and CH_2 ; and

5 R is alkyl of from one to twenty carbon atoms and $-(\text{CH}_2)_n$ -phenyl,

wherein n is zero or an integer of one to twenty; or a pharmaceutically acceptable acid addition salt thereof.

10 The nitrogen substituted acridines can also be characterized from the materials described in U.S. Patent 4,631,286 and 4,695,573 hereby incorporated by reference. See Formula 3.



(3)

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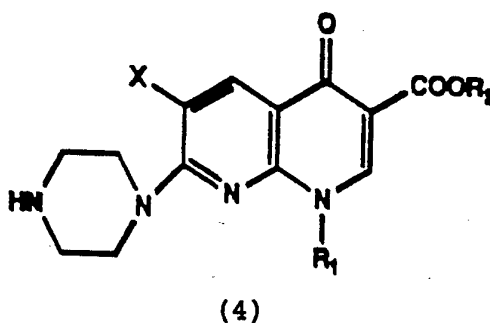
wherein n is 1, 2 or 3; X is hydrogen, lower alkyl, lower alkoxy, halogen, hydroxy, nitro, trifluoromethyl, -NHCOR₂ wherein R₂ is lower alkyl, or a group of the formula -NR₃R₄ wherein R₃ and R₄ are independently hydrogen or lower alkyl; R and R₁ are independently hydrogen, lower alkyl, di-lower alkylamino lower alkyl, aryl lower alkyl, diaryl lower alkyl, furyl lower alkyl, thienyl lower alkyl, oxygen bridged aryl lower alkyl, oxygen bridged diaryl lower alkyl, oxygen bridged furyl lower alkyl, oxygen bridged thienyl lower alkyl, aryl lower alkoxy wherein the aryl group may be unsubstituted or substituted by lower alkyl, lower alkoxy, halogen, hydroxy, trifluoromethyl or diphenyl lower alkyl, unsubstituted or substituted diphenyl lower alkyl wherein the substituents on the phenyl group may be lower alkyl, lower alkoxy, halogen, hydroxy, or trifluoromethyl; Y is -C=O or -C(R₅)OH wherein R₅ is hydrogen or lower alkyl; Z is -CH₂- or C=C(R₆)(R₇) wherein R₆ and R₇ are independently hydrogen or lower alkyl; or Y and Z taken together is CR₅-CH wherein C(R₅) and CH correspond to Y and Z respectively; an optical antipode thereof, or a pharmaceutically acceptable acid addition salt thereof,

Applicant now wishes to describe the P450 inhibitors that are useful in the present case although it is deemed within the invention herein that any P450 type 1A2 inhibitor may be used in conjunction

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with the nitrogen substituted acridine as described above.

The first class of the P450 inhibitors that may be utilized are naphthyridines of the type
5 described in U.S. Patent 4,359,578, hereby incorporated by reference. See Formula 4.



wherein X is a halogen atom, especially a
fluorine atom, R₁ is an ethyl or vinyl group,
10 and R₂ is a hydrogen atom or a lower alkyl
group; and nontoxic pharmaceutically accept-
able salts thereof.

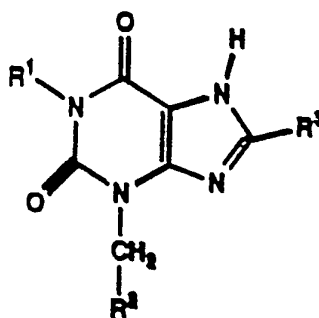
The preparation of the aforementioned
naphthyridines is disclosed in U.S. Patent No.
15 4,359,878, the working examples and column 5, line 16
to column 8, line 61.

A potential therapeutic regimen could
involve dosing with enoxacin (400 to 800 milligrams
per day) for 1 to 2 days (steady state enoxacin plasma
concentrations) prior to initiation of tacrine therapy
20 (20-160 mg per day) with coadministration thereafter
of tacrine and enoxacin in either a composition
product or individual dosage form. Enoxacin is

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described in MERCK INDEX 11th Ed. (1989), reference No. 3540 as 1-ethyl-6 fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1, 8-naphthyridine-3-carboxylic acid, herein incorporated by reference.

5 A second class of desirable P450 inhibitors are xanthines described in British specification 2091249, hereby incorporated by reference. See Formula 5.



(5)

10 wherein R¹ and R³ each independently represent an alkyl group containing from 1 to 6 (preferably at most 4) carbon atoms, and R² represents a cyclohexenyl, furyl, tetrahydrofuryl or thienyl group, and
15 pharmacologically acceptable salts thereof such as that formed with an alkali metal or a nitrogen-containing organic base.

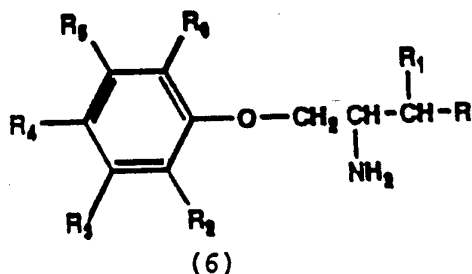
The preparation of the aforementioned xanthine derivatives are described in British Patent

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2,091,249 on page 1, line 38 and following over to page 2, line 44 as well as the working examples.

A potential therapeutic regime could involve dosing with a preferred xanthine, furafylline (1H-purine-2,6-dione,3-(2-furanylmethyl)-3,7-dihydro-1,8-dimethyl; 300-800 milligrams per day) for 1-2 days (steady state furafylline plasma concentrations) prior to initiation of the preferred amino acridine, tacrine therapy (20-160 milligrams per day) with coadministration thereafter of tacrine and furafylline in either a composition product or individual dosage form.

A further embodiment of a P450 1A2 inhibitor are phenoxy amino alkanes described in U.S. Patent 3,659,019, hereby incorporated by reference. See Formula 6.



wherein

R is hydrogen or alkyl of 1 to 3 carbon atoms,

R₁ is hydrogen or alkyl of 1 to 2 carbon atoms, and

R₂ through R₆, which may be identical to or different from each other, are each hydrogen or alkyl of 1 to 5 carbon atoms, but preferably 1 to 2 carbon atoms;

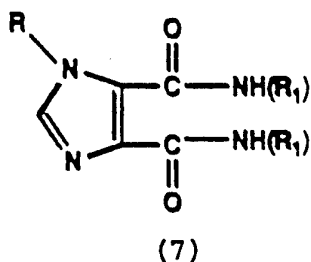
-16-

preferably, however, at least one of R_1 through R_6 is other than hydrogen and, if R_1 and R_4 are both methyl, at least one of the remaining substituents R , R_2 , R_5 , and R_6 is other than hydrogen; or a non-toxic, pharmacologically acceptable acid addition salt thereof.

The method of preparing these compounds is described in U.S. Patent 3,659,019, in particular, Column 2 and Column 3 and the working examples therein which are all herein incorporated by reference.

A potential therapeutic regime could involve dosing with a preferred phenoxy amino alkane such as mexiletine (100-800 milligrams per day) for 1-2 days (steady state mexiletine plasma concentrations) prior to initiation with the preferred amino acridine, namely tacrine (20-160 milligrams per day) with coadministration thereafter of tacrine and mexiletine in either a composition product or individual dosage form. Mexiletine is described in MERCK INDEX, 11th Ed., reference No. 6094 as 1-(2,6-dimethylphenoxy)-2-propanamine, herein incorporated by reference.

A fourth P450 type 1A2 inhibitor that is desirable herein is the carbamoyl imidazole of Formula 7.



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wherein

R is alkyl of from 1 to 20 carbon atoms,
preferably lower alkyl of from 1 to 4 carbon
atoms, most preferably -CH₃; and

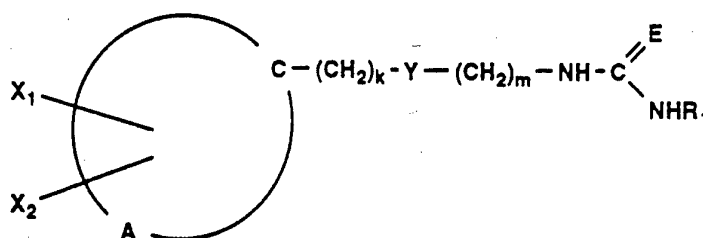
- 5 R₁ is independently alkyl of from 1 to 20
carbon atoms, preferably lower alkyl of from
1 to 4 carbon atoms, most preferably -CH₃.

- The method of preparing these compounds is
described in N.B. Vinogradova and N.U. Khromov-
10 Borisov, Zhur.Obshchei Khim. 31, 1466-70 (1961)
(Chemical Abstracts 55 23501i (1961); and N.B.
Vinogradova and N.U. Khromov-Borisov, Med. Prom. SSSR
19(6), 7-13 (1965) (Chemical Abstracts 63 11538d
(1965).

- 15 A potential therapeutic regime could involve
dosing with a preferred carbamoyl imidazole such as
ethimizol (100-800 milligrams per day) for 1-2 days
(steady state ethimizol plasma concentrations) prior
to initiation with the preferred amino acridine,
20 namely tacrine (20-160 milligrams per day) with
coadministration thereafter of tacrine and ethimizol
in either a composition product or individual dosage
form. Ethimizol is known as 1-ethyl-4,5-bis
(methylcarbamoyl) imidazole.

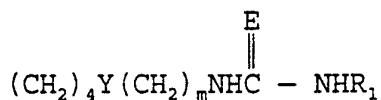
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An additional P450 type 1A2 inhibitor that is desirable herein is a heterocyclic guanidine of Formula 8:



(8)

- 5 wherein A is such that there is formed together with the carbon atom shown an unsaturated heterocyclic nucleus, which comprises at least one nitrogen and may comprise a further hetero atom such as sulphur and oxygen, said unsaturated heterocyclic nucleus, being
 10 an imidazole, pyrazole, pyrimidine, pyrazine, pyridazine, thiazole, isothiazole, oxazole, isoxazole, triazole, thiadiazole, benzimidazole or 5,6,7,8-tetrahydroimidazo[1,5-a]pyridine ring; X₁ is hydrogen, lower alkyl, hydroxyl, trifluoromethyl, benzyl,
 15 halogen, amino or



- 20 X₂ is hydrogen or when X₁ is lower alkyl, lower alkyl or halogen; k is 0 to 2 and m is 2 or 3, provided that the sum of k and m is 3 or 4; Y is oxygen, sulphur or

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NH; E is NR_2 ; R_1 is hydrogen, lower alkyl or di-lower alkylamino-lower alkyl; and R_2 is hydrogen, nitro or cyano or a pharmaceutically acceptable addition salt thereof. Y is preferably oxygen or sulphur, most
5 advantageously sulphur. Preferably A is such that the nitrogen atom is adjacent to the carbon atom shown and, more preferably, such that it forms with the said carbon atom an imidazole, thiazole or isothiazole ring. Preferably, X_1 is hydrogen, methyl, bromo, amino
10 or hydroxyl and X_2 is hydrogen. One group of preferred compounds within the present invention is that wherein Y is sulphur, k is 1, m is 2 and R_1 is methyl. Specific compounds which are found to be particularly useful are N-cyano-N'-methyl-N"-[2-((4-methyl-5-imidazolyl)-
15 methylthio)-ethyl]guanidine, N-cyano-N'-ethyl-N"-[2-((4-methyl-5-imidazolyl)methylthio)ethyl]guanidine, N-cyano-N'-methyl-N"-[2-((4-bromo-5-imidazolyl)-methylthio)ethyl]guanidine. N-cyano-N'-methyl-N"-[2-(2-thiazolylmethylthio)ethyl]-guanidine and N-cyano-N'-
20 methyl-N"-[2-(3-isothiazolylmethylthio)ethyl]-guanidine.

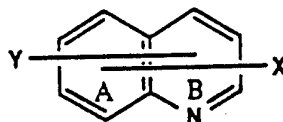
The preparation of the aforementioned compounds is described in U.S. Patent No. 3,950,333 and particularly, in the working examples herein, and are
25 all incorporated by reference.

A potential therapeutic regime could involve dosing with a preferred heterocyclic guanidine such as cimetidine (100-800 milligrams per day) for 1-2 days (steady state cimetidine plasma concentrations) prior
30 to initiation with the preferred amino acridine, namely tacrine (20-160 milligrams per day) with coadminis-

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tration thereafter of tacrine and cimetidine in either a composition product or individual dosage form.

Another preferred P450 Type 1A2 inhibitor that is desirably herein is a quinoline of Formula 9:



5

(9)

wherein:

Y is an amino group substituted by amino lower alkyl, lower alkyl amino lower alkyl, di-lower alkyl amino lower alkyl, lower alkyl amino, di-lower alkyl amino, lower alkyl substituted by a six-membered nitrogen heterocyclic ring, hydroxy lower alkyl, hydroxy aryl, halogenated lower alkyl;

X is lower alkyl, lower alkoxy, thio lower alkyl, hydroxy lower alkyl, aryl, halogen (such as chloro, bromo or iodo), or lower alkyl mercaptan;

wherein Y may be either in ring A or ring B and X may be present in one or both rings.

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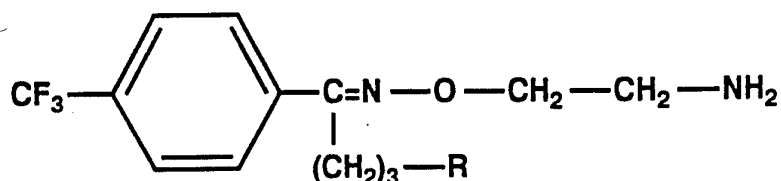
Most preferably, the carbon, alpha to the nitrogen in the quinoline ring, is not substituted by an atom other than hydrogen.

The quinolines and the method of preparation
5 are described in U.S. Patent No. 2,233,970 especially the working examples and page 1, left column, line 35 to the right column, line 40. This patent is hereby incorporated by reference. In addition, see The Merck Index, 11th Ed., published by Merck & Co., Inc.
10 (1989), Reference No. 21634 7751. See also Elderfield et al., J. AM. CHEM. Soc., 68, 1525 (1946); improved procedure: Elderfield et al., *ibid*, 77, 4816 (1955); Review: Olenick in ANTIBIOTICS, Vol. 3, J.W. Corcoran, F.E. Hahn, Eds. (Springer-Verlag, New York 1975), pp.
15 516-520. Preferred materials are chloroquine and primaquine.

A potential therapeutic regime could involve dosing with a preferred quinoline such as chloroquine or primaquine (100-800 milligrams per day) for 1-2
20 days (steady state chloroquine or primaquine plasma concentrations) prior to initiation with the preferred amino acridine, namely tacrine (20-160 milligrams per day) with coadministration thereafter of tacrine and chloroquine or primaquine in either a composition
25 product or individual dosage form.

Another preferred P450 type 1A2 inhibitor that is desirable herein is a trifluoromethyl oxime ether of Formula 10:

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(10)

and salts thereof with pharmaceutically acceptable acids, in which formula R is a cyano group, a cyanomethyl group, a methoxymethyl group or an ethoxymethyl group. A preferred material is fluvoxamine. Other preferred materials are 5-methoxy-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime maleate (1:1); 5-ethoxy-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime fumarate (1:1); 4-cyano-4'-trifluoromethylbutyrophenone O-(2-aminoethyl) oxime hydrochloride; 5-cyano-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime hydrochloride; 5-cyano-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime hydrochloride; 5-methoxy-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime maleate (1:1); 5-ethoxy-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime fumarate (1:1); 5-cyano-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime hydrochloride; 5-ethoxy-4'-trifluoromethylvalerophenone O-(2-aminoethyl) oxime fumarate (1:1).

The oxime ether compounds and the method of preparation are described in U.S. Patent 4,085,225, especially the working examples. This patent is hereby incorporated by reference. In addition, see MERCK

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INDEX, 11th Ed., published by Merck & Company, Inc.
(1989), Reference No. 4138. See also Inhibition of 5-HT uptake, V. Claassen et al., *Brit. J. Pharmacol.* 60, 505 (1977); HPLC determin in plasma, G.J. De Jong, *J. Chromatog.* 183, 203 (1980); Quantitative EEG, psychometric and pharmacokinetic studies in man, B. Saletu et al., *J. Neurol Transm.* 49, 63 (1980); Use in endogenous depress, J. E. De Wilde, D. P. Doogan, *J. Affective Disord.* 4, 249 (1982); Effects on Clonidine-induced Depression in Mice, J. Maj et al. *J. Neurol. Transm.* 55, 19 (1982); Review: *Brit. J. Clin. Pharmacol.* 15, Suppl. 3, 347S-450S (1983); Review of pharmacology, clinical efficacy, P. Benfield, A. Ward, *Drugs* 32, 313 (1986). Fluvoxamine is also discussed in the literature as a potent inhibitor of cytochrome P450 1A2; *Biochemical Pharm.*, Vol. 45, No. 6, pp. 1211-1214 (1993), in an article entitled *Fluvoxamine Is A Potent Inhibitor Of Cytochrome P450 1A2* by K. Brosen et al.

A potential therapeutic regimen would involve dosing with a preferred trifluoromethyloxime ether such as fluvoxamine (10 to 800 mg. per day) for one to two days (steady state fluvoxamine plasma concentrations) prior to initiation with the preferred amino acridine, namely, tacrine (20-160 mg. per day) with co-administration thereafter of tacrine and fluvoxamine and either a composition product or individual dosage form.

Listed below are exemplifications of the invention wherein all parts are parts by weight and all degrees are degrees centigrade unless otherwise indicated.

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Example 1 - In Vivo Metabolism of Tacrine

A single-dose pharmacokinetic and metabolic disposition study was conducted in human male volunteers given [^{14}C]tacrine HCl at two dose levels.

5 [^{14}C]Tacrine HCl was administered orally to healthy male volunteers at 10-mg (100 μCi) followed by a 40-mg (100 μCi) oral dose 1 month later. Plasma and red blood cells (RBC) were collected predose and for 48 hours postdose. Urine and feces were collected
10 predose and for 96 hours postdose. Urine and plasma samples were analyzed directly by liquid scintillation spectrometry while RBC and fecal samples were solubilized prior to counting. Plasma was assayed for tacrine, 1-hydroxytacrine, 2-hydroxytacrine and 4-
15 hydroxytacrine by a validated HPLC/fluorescence method. Metabolic profiling of urine was performed by HPLC radioactivity detection. Metabolites were identified by HPLC/photodiode array/mass spectrometry.

After oral administration of the 10-mg
20 [^{14}C]tacrine HCl dose, mean cumulative urinary and fecal recovery of ^{14}C activity averaged 56.5% and 23.2% of dose, respectively. Mean total recovery was 79.4%. After a 40-mg dose mean cumulative urinary and fecal recovery of ^{14}C activity averaged 54.1% and 20.8%,
25 respectively. Mean total recovery was 74.9%.

Time to maximum plasma concentration (t_{max}) values for plasma radioactivity, tacrine, and each metabolite were approximately 2 hours after administration of both tacrine doses. Mean plasma
30 total radioactivity area under the curve (time zero to last detectable concentration) ($\text{AUC}(0-t_{\text{ldc}})$) increased

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in a dose proportional manner while mean AUC(O-tldc) values for 1-hydroxytacrine, 2-hydroxytacrine, and tacrine increased greater than dose proportionally. Mean AUC(O-tldc) for tacrine, 1-, 2-, and 4-
5 hydroxytacrine comprised approximately 3% and 4% of the total mean plasma radioactivity AUC(O-tldc) for the 10- and 40-mg doses, respectively. In all volunteers, elimination rate of 1-hydroxytacrine from plasma appeared to be limited by the rate of
10 formation.

HPLC radioactivity profiling of urine through 24 hours postdose indicated that tacrine is extensively metabolized with only trace amounts of unchanged drug excreted. Present in the chromatograms
15 were several polar radioactive components accounting for 67% of urinary radioactivity (33% of the dose) with 1-, 4-, and 2-hydroxytacrine accounting for less than 5%, 1%, and 0.5% of the dose, respectively, after administration of either 10 or 40 mg tacrine doses.
20 No apparent differences exist in metabolic profiles at these dose levels and no single metabolite comprised greater than 5% of the dose. Figure 1 displays a proposed metabolic pathway for tacrine in man. In this same study, a correlation of decreased excretion
25 and lower 1-hydroxytacrine plasma concentrations was observed in subjects who were cigarette smokers as compared to nonsmokers. Human hepatic P450 1A2 has been shown to be inducible by cigarette smoke. D. Sesardic, A. R. Boobis, R. J. Edwards, and D. S.
30 Davies, " A Form of Cytochrome P450 in Man, Orthologous to Form d in the Rat, Catalyses the O-deethylation of Phenacetin and is Inducible by

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Cigarette Smoking", BR. J. CLIN. PHARMAC., Vol. 26, pp. 363-372 (1988).

Cimetidine is an inhibitor of P450 enzymes including P450 1A2. A. Somogyi and M. Muirhead,
5 "Pharmacokinetic Interactions of Cimetidine", CLIN. PHARMACOKIN., Vol. 12, pp. 321-366 (1987). In a clinical drug interaction study involving administration of a 40 mg tacrine dose and 300-mg four times a day cimetidine dose, approximately 40% higher plasma
10 concentrations of tacrine and 1-hydroxytacrine were observed as compared to subjects administered tacrine alone.

Concomitant administration of a single 158-mg dose of theophylline with repeated 20-mg capsule
15 doses of tacrine has resulted in approximately a two-fold increase in theophylline elimination half-life. The major route of theophylline clearance involves metabolism by P450 1A2. M. E. Campbell, D. M. Grant, T. Inaba, and W. Kalow, "Biotransformation of
20 Caffeine, Paraxanthine, Theophylline, and Theobromine by Polycyclic Aromatic Hydrocarbon-Inducible Cytochrome(s) P-450 in Human Liver Microsomes", DRUG METAB. Dispos. Vol. 15, pp. 237-249 (1987). Therefore, a
25 potential explanation for the theophylline-tacrine clinical interaction is competition for the same P450 1A2 enzymes.

Example 2 - In Vitro Metabolism of Tacrine

A series of in vitro metabolism studies have been conducted using human and rat hepatic tissues to
30 investigate the metabolic fate of tacrine as well as

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to explore the effect of various inducers and inhibitors on tacrine disposition. Incubations of ¹⁴C-tacrine (0.5 μ M) were conducted for 1 hour with microsomal preparations (app. 1 μ M P450) in the presence of NADPH (0.5 mM) and a generating system comprised of 4.0 mM glucose-6-phosphate, 2.0 mM MgCl₂, and 1 unit of glucose-6-phosphate dehydrogenase at 37°C in 0.1 M potassium phosphate buffer (pH 7.4). Total reaction volume was 3mL. Reactions were stopped by freezing in liquid nitrogen or dry-ice acetone. Post reaction incubates were analyzed by HPLC radioactivity detection for tacrine biotransformation products and unchanged drug following precipitation of microsomal protein with either methanol or ethanol (3 volumes). Results are summarized in Table 1. In human liver preparations B and C, tacrine was completely metabolized within 60 min under these incubation conditions. The major product detected was 1-hydroxytacrine with minor amounts of the 2- and 4-hydroxytacrine regioisomers also observed. Incubations with human liver preparation D affected only partial tacrine turnover. Results with rat liver microsomes showed more 1-hydroxytacrine produced as compared to human preparations. Incubations with rat microsomes from phenobarbital (PB) pretreated rats had only a minimal effect on 1-hydroxytacrine formation suggesting that formation of this metabolite is not by the P450 2B (cytochrome P450 IIB or CYP2B) subfamily. D. J. Waxman and L. Axaroff, "Phenobarbital induction of cytochrome P-450 Gene Expression", BIOCHEM. J., Vol. 281, pp. 577-592 (1992); P. Soucek and I. Gut, supra. Isoniazid (I), an inducer of cytochrome P450 2E1 (cytochrome P450 IIE1 or CYP2E1) (D. J. Waxman and L. Axaroff, supra; R.C. Lind, A.J. Gadolfi, P. de la M.

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Hall: "Isoniazid Potentiation of a Guinea Pig Model of Halothane-Associated Hepatotoxicity" J. TOXICOL 10(3): 161-165 (1990); S.A. Rice. L. Sbordone, R.I.

5 Mazze: "Metabolism by Rat Hepatic Microsomes of Fluorinated Ether Anesthetics Following Isoniazid Administration" ANESTHESIOLOGY 53: 489-493 (1980)
markedly increased 1-hydroxytacrine formation compared to control rat liver microsomes. Less 1-
hydroxytacrine was observed after incubations with MC
10 (P450 1A1 (CYP1A1) and P450 1A2 (CYP1A2) inducer)
induced rat liver microsomes as compared to control, reflecting induction of sequential metabolism (see proposed metabolic pathway in Figure 1). The amount of 1-hydroxytacrine detected after a 1 hour incubation
15 with MC induced rat microsomes was similar to that observed in human preparations B and C. In a supplementary study, 1-hydroxytacrine metabolism by P4501A2 was found to be inhibited by co-incubation with tacrine, thereby indicating that sequential
20 tacrine metabolism is also mediated by this specific P450.

25 TABLE 1. Tacrine and metabolites present in the deproteinized microsomal supernatant fraction after a 60 min incubation of ¹⁴C-tacrine (0.5 μM) catalyzed by human and rat liver microsomes (1 μM P450) in presence of NADPH regenerating system at 37°C

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		Tacrine nmole equivalents					
		POLAR UNKS	2-OH	1-OH	UNKS	4-OH	TAC
5	Noninduced Rat						
	w/ NADPH	0.022	0.088	0.994	ND	NQ	ND
	w/out NADPH	ND	ND	ND	ND	ND	1.22
	PB induced Rat						
10	w/ NADPH	0.026	0.144	1.15	NQ	NQ	ND
	w/out NADPH	ND	ND	ND	ND	ND	1.20
	I induced Rat						
15	w/ NADPH	0.083	NQ	1.57	NQ	NQ	ND
	w/out NADPH	ND	ND	ND	ND	ND	1.14
	MC induced Rat						
20	w/ NADPH	0.009	NQ	0.790	0.091	0.018	ND
	w/out NADPH	ND	ND	ND	ND	ND	1.16
	Human Prep B						
	w/ NADPH	0.039	NQ	0.750	0.146	0.039	ND
	w/out NADPH	ND	ND	ND	ND	ND	1.34
25	Human Prep C						
	w/ NADPH	0.035	0.095	0.599	0.139	NQ	ND
	w/out NADPH	ND	ND	ND	ND	ND	1.07
	Human Prep D						
30	w/ NADPH	ND	ND	0.201	ND	ND	0.803
	w/out NADPH	ND	ND	ND	ND	ND	1.086
Pb = Phenobarbital							
I = Isoniazid							
35	MC = 3-Methylcholanthrene						
	NADPH = nicotinamide adenine diphosphate hydride						
	ND = Not Detectable						

**Example 3 - Proposed Pathway For Irreversible
Binding Of Tacrine To Human Liver Microsomal Protein**

40 Irreversible (nonextractable, presumably
covalent) binding of tacrine-derived radioactivity was

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measured by a slight modification of the method of Martin and Garner. C.N. Martin and R.C. Garner: "Covalent Binding In Vitro and In Vivo" in BIOCHEMICAL TOXICOLOGY: A PRACTICAL APPROACH, Eds. K. Snell and B. Mullock, IRL, Wash. D.C. pp. 109-126 (1987). Results following exhaustive extraction are displayed in Table 2. Clearly, a high percentage of tacrine was metabolically activated to a reactive intermediate capable of binding to microsomal protein. MC induced rat displayed nearly a 3 fold increase in binding compared to control rat. PB and I pretreatment had little or no effect on binding. The binding of tacrine-derived radioactivity to microsomal protein therefore is not increased by induction of the P450 2B or 2E1 enzymes, respectively. ¹⁴C tacrine incubations which included glutathione (GSH) resulted in a dramatic reduction in irreversible binding whereas incubations with epoxide hydrolase (EH) had only a slight decrease in binding (Table 3). Both GSH and EH failed to produce detectable adducts with the reactive intermediate. Attempts to detect a hydroxylamine metabolite in postreaction incubates with and without ascorbic acid were unsuccessful. These data do not support either an epoxide or hydroxylamine mechanism for activation of tacrine to a reactive species capable of irreversible or covalent binding. A time course study through 1 hour showed tacrine to be rapidly metabolized not only to 1-hydroxytacrine but also to 7-hydroxytacrine. 7-Hydroxytacrine levels rise then fall over time. Thus, 7-hydroxytacrine appears to be a metabolic intermediate which is further metabolized to a putative reactive species capable of irreversible binding to microsomal protein. Based on all of the above information, a potential

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mechanism responsible for binding is shown in Figure 2. The reactive quinonemethides formed from either 7-hydroxytacrine or 1,7-dihydroxytacrine are the most likely chemical species capable of the observed irreversible binding to microsomal protein.

TABLE 2. Irreversible protein binding of ^{14}C -tacrine-derived radioactivity ($0.5\ \mu\text{M}$) catalyzed by human and rat liver microsomes ($1\ \mu\text{M}$ P450) in presence of NADPH regenerating system after a 60 min incubation at 37°C ($N = 3$).

Tacrine equivalents irreversibly bound to 1 mg microsomal protein (nmoles)			
Noninduced Rat	with NADPH	0.041 ± 0.001	5.97 ± 0.68
	without NADPH	0.003	
PB induced Rat	with NADPH	0.034 ± 0.006	4.49 ± 0.85
	without NADPH	0.002	
I induced Rat	with NADPH	0.042 ± 0.004	4.62 ± 0.73
	without NADPH	0.003	
MC induced Rat	with NADPH	0.139 ± 0.010	14.5 ± 0.37
	without NADPH	0.003	
Human Prep B	with NADPH	0.207 ± 0.014	26.9 ± 4.09
	without NADPH	0.003	
Human Prep C	with NADPH	0.226 ± 0.005	28.8 ± 1.21
	without NADPH	0.003	
Human Prep D	with NADPH	0.027 ± 0.002	2.96 ± 0.30
	without NADPH	0.003	
Percent Bound = Total tacrine mole equivalents bound divided by total substrate times 100			
Pb = Phenobarbital			
I = Isoniazid			
MC = 3-Methylcholanthrene			

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TABLE 3. Glutathione (GSH) (5 mM) and human liver epoxide hydrolase (EH) 100 (ug) effect on irreversible protein binding of ^{14}C -tacrine (0.5 μM) catalyzed by human liver preparation B and MC induced rat liver microsomes (1 μM P450) in presence of NADPH regenerating system after a 60 min incubation (N = 3) at 37°C.

Tacrine equivalents irreversibly bound to 1 mg microsomal protein

(nmoles)

MC induced Rat			
	NADPH	0.139 $\pm$ 0.010	--
15	with GSH	0.058 $\pm$ 0.005	58.3
	with EH	0.116 $\pm$ 0.007	16.6
Human Prep B			
	NADPH	0.207 $\pm$ 0.014	--
	with GSH	0.031 $\pm$ 0.001	85.0
20	with EH	0.134 $\pm$ 0.026	35.3

Deletion of NADPH from the incubation mixture resulted in no detectable irreversible binding of ^{14}C -tacrine-derived radioactivity to microsomal protein.

MC = 3-Methylcholanthrene

Example 4

To determine if tacrine is a substrate for the polymorphic P450 2D6 enzyme.

A series of experiments were conducted to determine the potential of tacrine to be a substrate for the polymorphic P450 2D6 enzyme. U. A. Meyer, J. Gut, T. Kronbach, C. Skoda, U. T. Meier, and T. Catin, "The Molecular Mechanisms of Two Common Polymorphisms of Drug Oxidation- Evidence for Functional Changes in Cytochrome P-450 Isozymes Catalysing Bufuralol and Mephenytoin Oxidation", XENOBIOTICA, Vol. 16, pp. 449-464 (1986). Microsomes prepared from intact human

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liver tissue when incubated up to 60 minutes with ^{14}C tacrine alone or ^{14}C tacrine co-incubated with quinidine produced the following results shown in Table 4. Quinidine is an inhibitor of P450 2D6. K. Brosen and L. F. Gram, "Quinidine Inhibits the 2-Hydroxylation of Imipramine and Desipramine but not the Demethylation of Imipramine", EUR. J. CLIN. PHARMACOL., Vol. 37, pp. 155-160 (1989).

Table 4

	nMoles per 3mL incubate			
	Tacrine		1-Hydroxytacrine	
Quinidine	-	+	-	+
Time (min)				
0	2.13	2.02	0	0
5	1.24	1.22	0.416	0.383
10	0.831	0.816	0.716	0.606
20	0.302	0.401	1.03	1.067
40	0	0.037	1.34	1.192
60	0	0.033	1.43	1.209

Tacrine was metabolized to mainly 1-hydroxy-tacrine with 7-hydroxytacrine also being observed at incubation times through 40 min. The presence of quinidine did not inhibit the conversion of tacrine to 1-hydroxytacrine. In addition, the irreversible binding of tacrine to human hepatic microsomal proteins was also not effected by this co-incubation. Therefore, the metabolism and irreversible binding of tacrine to microsomal protein is not catalyzed by P450 2D6.

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Example 5

To demonstrate the P450 1A2 inhibiting activity, enoxacin, a 1,8 naphthyridine, was tested.

5 To test the hypothesis that the high
affinity (K_m less than 5 μM) saturable component of
tacrine metabolism in human liver microsomes is by the
P450 1A2 enzyme, a time course co-incubation study of
10 ^{14}C tacrine (0.5 μM) with enoxacin (50 μM), a selective
inhibitor of P450 1A2, T. Hasegawa, M. Nadai, T.
Kuzuya, I. Muraoka, R. Apichartpichean, K. Takagi, and
K. Miyamoto, "The Possible Mechanism of Interaction
between Xanthines and Quinolone", J. PHARM. PHARMACOL.,
Vol. 42, pp. 767-772 (1990) was conducted. The
15 results of this study are shown in Table 5. At all
time points, the extent of irreversible binding was
inhibited by up to 73%. In addition, tacrine's rate
of biotransformation was markedly inhibited (5 to 6
fold). Therefore, a specific P450 1A2 inhibitor can
20 not only decrease the rate of irreversible binding but
also inhibit the overall rate of tacrine biotrans-
formation. The effect of various enoxacin concentra-
tions (8, 20, and 50 μM) on the extent of irreversible
binding was examined in a separate study. Results are
25 presented in Table 6. The greatest inhibition of
tacrine irreversible binding and biotransformation was
achieved with co-incubation with 50 μM enoxacin.

Since 1-hydroxytacrine was found to bind
irreversibly (nonextractable, presumably covalent) to
30 human microsomal protein, the role of P450 1A2 in the
metabolic activation step was examined by co-
incubating ^{14}C 1-hydroxytacrine with various concentra-

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tions of enoxacin (8, 20, and 50 μM). Table 6 displays results confirming the inhibitory effect of enoxacin on the binding of 1-hydroxytacrine which supports the involvement of P450 1A2 in its activation pathway.

TABLE 5. The effect of 50 μM enoxacin on the irreversible binding of 0.5 μM tacrine-derived radioactivity to human microsomal (1 μM P450) protein with time in the presence of a NADPH regenerating system at 37°C.

Tacrine Equivalents irreversibly bound to 1 mg microsomal protein			
	Time (min)	(nmoles)	Percent Inhibition
Tacrine	5	0.022 $\pm$ 0.005 *, **	
	10	0.048 $\pm$ 0.006	
	20	0.096 $\pm$ 0.006	
	45	0.148 $\pm$ 0.024	
Tacrine + Enoxacin	5	0.009 $\pm$ 0.002	59.1
	10	0.017 $\pm$ 0.003	64.5
	20	0.026 $\pm$ 0.002	72.9
	45	0.052 $\pm$ 0.006	64.9

* N = 3

** SD

TABLE 6. Irreversible binding of ^{14}C -tacrine or ^{14}C -1-hydroxytacrine-derived radioactivity to human microsomal protein (1 μM cytochrome P450) in the presence of various concentrations of enoxacin and a NADPH regenerating system following a 20 min incubation at 37°C.

Tacrine or 1-hydroxytacrine equivalents
irreversibly bound to 1 mg microsomal protein

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Tacrine (0.3 μ M)	Enoxacin (μ M)		
	0.0	0.040	0.001*, --
	8.0	**	14.4
	20.0	0.036 $\pm$ 0.008	29.8
	50.0	0.028 $\pm$ 0.001	43.4
		0.023 $\pm$ 0.002	
1-Hydroxytacrine (0.5 μ M)			
	0.0	0.018 $\pm$ 0.001	--
	8.0	0.017 $\pm$ 0.001	8.2
	20.0	0.014 $\pm$ 0.001	22.2
	50.0	0.009 $\pm$ 0.001	51.7

5 Deletion of NADPH from the incubation mixture resulted in no irreversible binding of 14 C-tacrine derived radioactivity or 14 C-1-hydroxytacrine to microsomal protein.

* N = 3

10 ** SD

Example 6

The potential for other P450 1A2 inhibitors, namely furafylline and ethimizol to inhibit the irreversible binding of 14 C tacrine and 14 C 1-hydroxytacrine were examined in a MC induced rat hepatocyte study. Results in terms of percent of tacrine-derived radioactivity binding as determined in tacrine alone incubates are presented in Table 7. Furafylline was the most effective inhibitor of irreversible binding for both tacrine and 1-hydroxytacrine while ethimizol and enoxacin had less of an effect. These data support the concept that other specific P450 1A2 inhibitors besides enoxacin can affect the rate of tacrine and 1-hydroxytacrine metabolism as well as irreversible binding.

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TABLE 7. The effect of enoxacin, ethimizol, furafylline, and glutathione on the irreversible binding of ¹⁴C-tacrine and ¹⁴C-1-hydroxytacrine-derived radioactivity to hepatocytes from MC induced male rats.

		Percent Control Value

10	Tacrine (10 uM)	
	+ Enoxacin (50 uM)	78.9
	+ Ethimizol (50 uM)	80.1
	+ Furafylline (50 uM)	31.6
	+ Glutathione (5 mM)	62.6
15	1-Hydroxytacrine (10 uM)	
	+ Enoxacin (50 uM)	79.8
	+ Ethimizol (50 uM)	65.6
	+ Furafylline (50 uM)	31.7
	+ Glutathione (5 mM)	49.1
20	-----	

Example 7

The antimalarial agents chloroquine and primaquine were examined in rat liver microsomes by Back et al. (1983) and found to be inhibitors of ethoxyresorufin O-deethylase activity. D. J. Back, H. S. Purba, C. Staiger, M. L. Orme, A. M. Breckenridge, "Inhibition of Drug Metabolism by the Anti Malarial Drugs Chloroquine and Primaquine in the Rat", BIOCHEM. PHARMACOL., Vol. 32, pp. 257-264 (1983).

Ethoxyresorufin O-deethylation is selectively catalyzed by P450 1A2. C. Gleizes, C. Eeckhoutte, T. Pineau, M. Alvinerie, and P. Galtier, "Inducing Effect of Oxfendazole on Cytochrome P4501A2 in Rabbit Liver", BIOCHEM. PHARMACOL. VOL. 41, pp. 1813-1820 (1991). Incubation of ¹⁴C-tacrine (5 μ M) and chloroquine (100 μ M) in primary suspensions of rat hepatocytes resulted in inhibition of tacrine irreversible binding as well as metabolism. Table 8 displays time course data for inhibition of binding.

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Table 8. Effect of chloroquine (100 μ M) on irreversible binding of tacrine-derived radioactivity to rat hepatocytes.

5	nmoles tacrine-equivalents bound per mg hepatic protein		
	Time (min)	Tacrine	Tacrine+Chloroquine

	5	0.020*	0.005
10	15	0.037	0.012
	30	0.061	0.016
	60	0.128	0.034
	90	0.183	0.039
	120	0.294	0.041
15	180	0.377	0.046

* N = 2

The compositions of the present invention can be prepared and administered in a wide variety of oral and parenteral dosage forms. Thus, the compositions of the present invention can be administered by injection, that is, intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally. Also, the compositions of the present invention can be administered by inhalation, for example, intranasally. Additionally, the compositions of the present invention can be administered transdermally.

For preparing pharmaceutical compositions from the compositions of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents,

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flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

5 In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component.

In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

10 The powders and tablets preferably contain from five or ten to about seventy percent of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, 15 sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with 20 or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

25 For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogenous mixture is then poured into convenient 30 sized molds, allowed to cool, and thereby to solidify.

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Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water propylene glycol solutions. For parenteral injection liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing and thickening agents as desired.

Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

The pharmaceutical preparation is preferably in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as

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packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

5 The quantity of active component in a unit dose preparation may be varied or adjusted according to the particular application and the potency of the active component. The composition can, if desired, also contain other compatible therapeutic agents.

10 In therapeutic use, the compounds utilized in the pharmaceutical method of this invention are administered at the dosage previously indicated. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the
15 condition being treated, and the compound being employed. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound.
20 Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

25 While the forms of the invention herein disclosed constitute presently preferred embodiments, many others are possible. It is not intended herein to mention all of the possible equivalent forms or ramifications of the invention. It is understood that terms
30 used herein are merely descriptive rather than limiting

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and that various changes may be made without departing from the spirit or scope of the invention.

What Is Claimed Is:

1. A method of inhibiting the enzymatic metabolism of a nitrogen substituted acridine selected from the group consisting of Formula 1, 2 and 3 comprising co-administering with the acridine an effective oxidase inhibiting amount of a P450 1A inhibitor.

2. A method of inhibiting the enzymatic metabolism of nitrogen substituted acridines selected from the group consisting of Formula 1, 2 and 3 by co-administering with the acridine an effective amount of an inhibitor of P450 oxidase whose enzymatic activity is induced by beta-naphthaflavone, 3-methylcholanthrene, arochlor, 2,3,7,8-tetrachloro- dibenzo-p-dioxin and isosafrole.

3. The method of claims 1 or 2 wherein the inhibitor is a naphthyridine of Formula 4.

4. The method of claims 1 or 2 wherein the inhibitor is a xanthine of Formula 5.

5. The method of claims 1 or 2 wherein the inhibitor is a phenoxy amino alkane of Formula 6.

6. The method of claims 1 or 2 wherein the inhibitor is a carbamoyl imidazole of Formula 7..

7. The method of claims 1 or 2 wherein the inhibitor is a heterocyclic guanidine of Formula 8.

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8. The method of claims 1 or 2 wherein the inhibitor is a quinoline of Formula 9.

9. The method of claims 1 or 2 wherein the inhibitor is a trifluoromethyloxime ether of formula 10.

5 10. The method of claim 1 wherein the acridine is of Formula 1.

11. The method of claim 1 wherein the acridine is of Formula 2.

10 12. The method of claim 1 wherein the acridine is of Formula 3.

13. The method of claim 2 wherein the acridine is of Formula 1.

14. The method of claim 2 wherein the acridine is of Formula 2.

15 15. The method of claim 2 wherein the acridine is of Formula 3.

16. The method of claims 1 or 2 wherein the inhibitor is of Formula 4 wherein X is fluorine.

20 17. The method of claims 1 or 2 wherein the inhibitor is of Formula 5 wherein R² is furyl.

18. The method of claims 1 or 2 wherein the inhibitor is of Formula 5 wherein R² is tetrahydrofuryl.

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19. The method of claims 1 or 2 wherein the inhibitor is of Formula 6 wherein R and R₁ are hydrogen.

20. The method of claims 1 or 2 wherein the inhibitor is of Formula 6 wherein R₃, R₄ and R₅ are hydrogen.

21. The method of claims 1 or 2 wherein the inhibitor is of Formula 7 wherein R and R₁ are a lower alkyl group.

22. The method of claims 1 or 2 wherein the inhibitor is a material of Formula 8 wherein the heterocyclic nucleus comprises at least one nitrogen heterocyclic ring having 5 or 6 members in the ring.

23. The method of claims 1 or 2 wherein the inhibitor is a material of Formula 8 wherein the heterocyclic nucleus is an imidazole ring.

24. The method of claims 1 or 2 wherein the inhibitor is a compound of Formula 9 wherein Y is in ring B and X is chlorine.

25. The method of claims 1 or 2 wherein the inhibitor is a compound of Formula 9 wherein Y is in ring A and an alkoxy is also substituted in ring A.

26. The method of claims 1 or 2 wherein the inhibitor is a compound of Formula 10 wherein R is a cyano group.

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27. The method of claims 1 or 2 wherein the inhibitor is a compound of Formula 10 wherein R is a cyanomethyl group.

5 28. The method of claims 1 or 2 wherein the inhibitor is a compound of Formula 10 wherein R is a methoxymethyl group.

29. The method of claims 1 or 2 wherein the inhibitor is a compound of Formula 10 wherein R is an ethoxymethyl group.

10 30. The method of claims 1 or 2 wherein the inhibitor is furafylline.

31. The method of claims 1 or 2 wherein the inhibitor is mexiletine.

15 32. The method of claims 1 or 2 wherein the inhibitor is enoxacin.

33. The method of claims 1 or 2 wherein the inhibitor is ethimizol.

34. The method of claims 1 or 2 wherein the inhibitor is chloroquine.

20 35. The method of claims 1 or 2 wherein the inhibitor is primaquine.

36. The method of claims 1 or 2 wherein the inhibitor is cimetidine.

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37. The method of claims 1 or 2 wherein the inhibitor is glutathione.

38. The method of claims 1 or 2 wherein the inhibitor is fluvoxamine.

5 39. The method of claims 1 or 2 wherein the nitrogen substituted acridine is tacrine.

10 40. A composition comprising a nitrogen substituted acridine selected from the group consisting of Formula 1, 2 and 3 and an effective oxidase inhibiting amount of a P450 1A2 inhibitor.

15 41. A composition comprising a nitrogen substituted acridine selected from the group consisting of Formula 1, 2 and 3 and an effective amount of an inhibitor of P450 oxidase whose enzymatic activity is induced by beta-naphthaflavone, 3-methylcholanthrene, arochlor, 2,3,7,8-tetrachlorodibenzo-p-dioxin and isosafrole.

20 42. The composition of claims 40 or 41 wherein the oxidase inhibitor is a naphthyridine of Formula 4.

 43. The composition of claims 40 or 41 wherein the oxidase inhibitor is a xanthine of Formula 5.

25 44. The composition of claims 40 or 41 wherein the oxidase inhibitor is a phenoxy amino alkane of Formula 6.

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45. The composition of claims 40 or 41 wherein the oxidase inhibitor is a carbamoyl imidazole of Formula 7.

5 46. The composition of claims 40 or 41 wherein the oxidase inhibitor is a heterocyclic guanidine of Formula 8.

47. The composition of claims 40 or 41 wherein the oxidase inhibitor is a quinoline of Formula 9.

10 48. The composition of claims 40 or 41 wherein the oxidase inhibitor is a trifluoromethyl oxime ether of Formula 10.

15 49. The composition of claim 40 wherein the acridine is of Formula 1.

50. The composition of claim 40 wherein the acridine is of Formula 2.

51. The composition of claim 40 wherein the acridine is of Formula 3.

20 52. The composition of claim 41 wherein the acridine is of Formula 1.

53. The composition of claim 41 wherein the acridine is of Formula 2.

25 54. The composition of claim 41 wherein the acridine is of Formula 3.

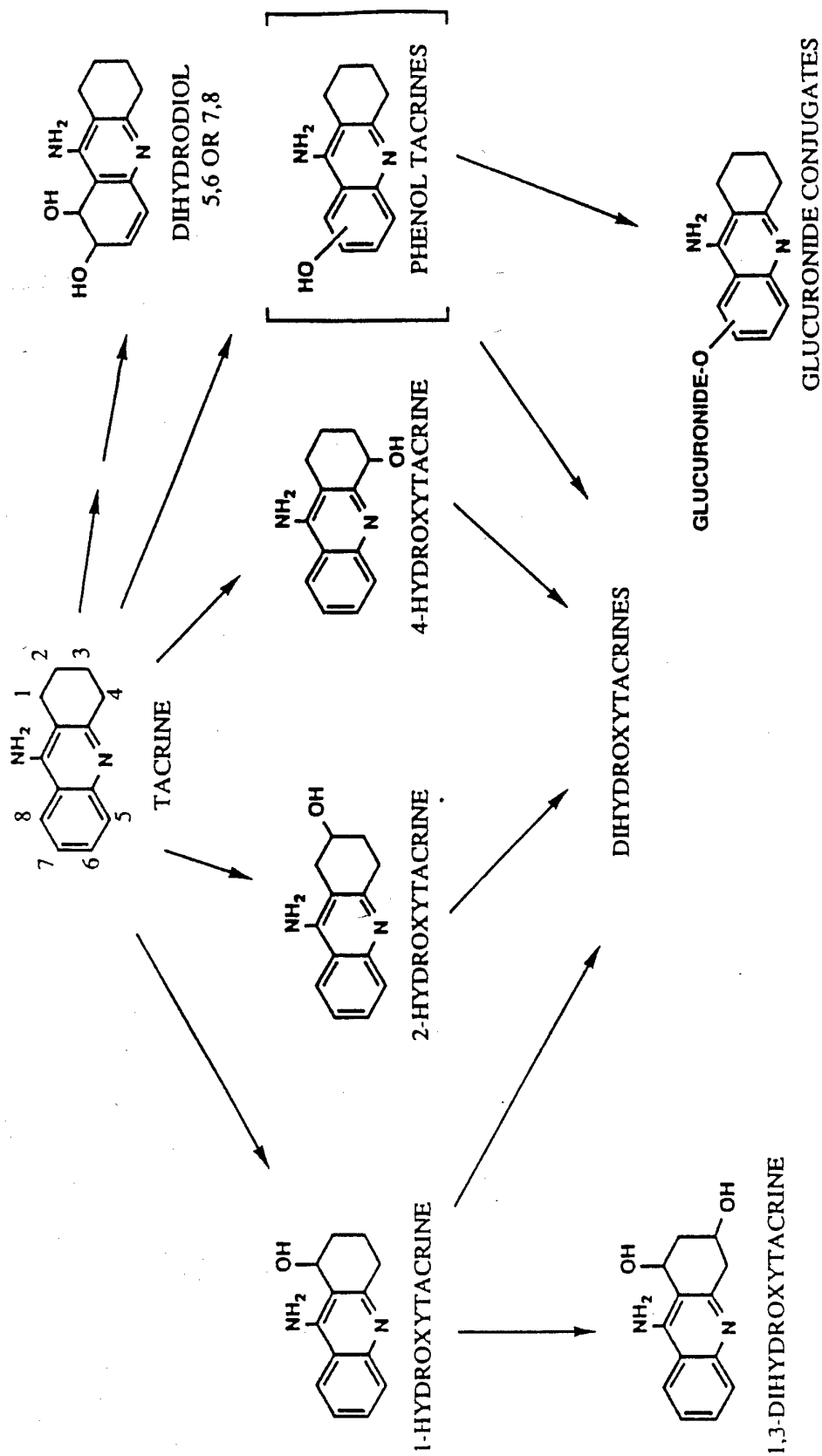


FIGURE 1

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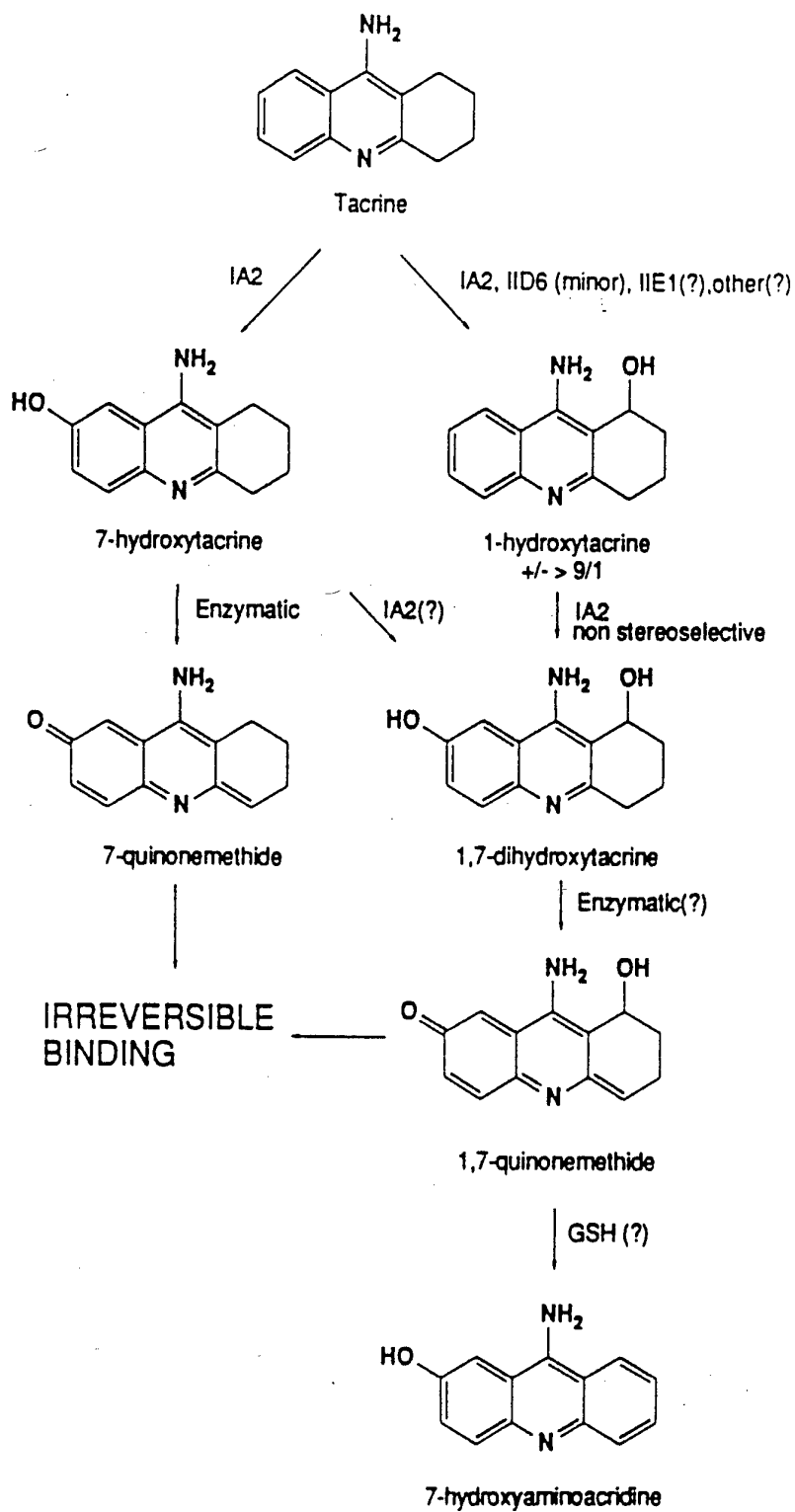


Figure 2

SUBSTITUTE SHEET