

## ANTIPARASITIC ARTEMISININ DERIVATIVES (ENDOPEROXIDES)

This invention relates to the use of certain  
5 C-10 substituted derivatives of artemisinin in the  
treatment and/or prophylaxis of diseases caused by  
infection with a parasite, certain novel C-10  
substituted derivatives of artemisinin, processes for  
their preparation and pharmaceutical compositions  
10 containing such C-10 substituted derivatives.

Malaria is the most important human parasitic  
disease in the world today. Approximately 270 million  
people throughout the world are infected with malaria,  
with about 2 million dying each year. The ability of  
15 parasites to produce a complex survival mechanism by  
expressing variant antigens on the surface of infected  
erythrocytes makes it possible for the parasites to  
escape from the destructive action of the host immune  
response against these antigens. In addition, the  
20 increasing rate of malaria infection is due to the  
spread of chloroquine-resistant strains of Plasmodium  
falciparum and the other multi-drug resistant strains.

In the field of animal health, parasitic diseases  
are a major problem, especially those diseases which  
25 are functionally related to malaria. For instance,  
neosporosis is a term used to describe diseases caused  
by parasites of the species Neospora, especially  
Neospora caninum, in animals. Neospora infections are  
known to occur in dogs, cattle, sheep, goats and  
30 horses.

The final host for Neospora spp., including  
Neospora caninum, is unknown and, in addition, the  
complete cycle of development of the parasite is not  
understood. The asexual phases of reproduction, known  
35 as schizogony, and the behaviour of the unicellular  
tachyzoite/bradyzoite stage have been clarified,  
however. Tachyzoites are infectious unicellular  
parasite stages of about 3-7 x 1-5 mm in size formed

after intracellular reproduction termed endodyogeny. Reproduction via tachyzoites takes place preferentially in organelles such as muscle or nerve cells. Pathological symptoms invoked after an  
5 infection are associated mainly in those tissues. Some five to six weeks after natural infection in a dog, symptoms of the disease are hypersensitivity caused by inflammation of neuronal cells and  
10 increasing tendency to hyperextension of the hind legs. Histopathological lesions are apparent in the nervous system, preferentially in the brain and spinal cord. Extensive non-suppurative inflammations, glial excrescences and perivascular infiltrations of  
15 mononuclear cells (macrophages, lymphocytes, plasma cells) dominate, and are also partly apparent in eosinophils and neutrophils. In the muscular system, macroscopically observable necroses and degenerative changes appear. Apart from the more or less strongly developed atrophy, long pale longitudinal stripes are  
20 evident.

In California and Australia, Neospora caninum infections appear to be the main cause for abortion in cattle. Symptoms of the disease in cattle are similar to those in the dog. Ataxia is apparent, joint  
25 reflexes are weakened and pareses at the hind legs, partly in all four legs, can be observed. The histological picture is similar to that of the dog; mainly non-suppurative meningitis and myelitis.

Data on in vivo activity of compounds suitable  
30 against neosporosis are rare because adequate in vivo test systems still have to be developed. Sulfadiazin (administered via drinking water) is effective in experimentally infected mice, only if the treatment was prophylactic, that is, the treatment was started  
35 before infection. In dogs, treatment with sulfadiazin and clindamycin is only successful if it is started early, that is, at the appearance of first clinical

symptoms as a result of neuronal inflammation.

Coccidiosis, an infection of the small intestine, is relatively rarely diagnosed in humans, where it is caused by Isospora belli. However, humans are also the final host of at least two cyst-forming coccidial species (Sarcocystis suihominis and S. bovi hominis). Consumption of raw or inadequately cooked pork or beef containing such cysts can lead to severe diarrhoea, the cause of which is probably seldom diagnosed correctly. Coccidia (phylum Apicomplexa, suborder Eimeriina) are one of the most successful groups of parasitic protozoans, having conquered virtually every class of Metazoa. The ones that are of particular importance for man are the 60-100 species which parasitise domestic animals and which in some instances can cause very severe losses, especially in poultry, although also in lambs, calves, piglets, rabbits and other animals (see Table A).

Table A: Causatives of intestinal coccidiosis in domestic animals

| Animal                                  | number of <u>Eimeria</u> and/or <u>Isospora</u> species*) | most pathogenic and/or very common species ( <u>E=Eimeria</u> , <u>I=Isospora</u> )                                                                                                      |
|-----------------------------------------|-----------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| chicken ( <u>Gallus gallus</u> )        | 7                                                         | <u>E.tenella</u> , <u>E.necatrix</u> , <u>E.maxima</u> , <u>E.acervulina</u>                                                                                                             |
| turkey ( <u>Meleagris gallopavo</u> )   | 7                                                         | <u>E.meleagridis</u> , <u>E.adenoides</u>                                                                                                                                                |
| goose ( <u>Anser anser</u> )            | 6                                                         | <u>E.anseris</u> , <u>E.truncata</u> , <u>E.nocens</u> , <u>E.kotlani</u>                                                                                                                |
| duck ( <u>Anas platyhynchos</u> )       | 3                                                         | <u>Tyzzzeria pernicioso</u> , <u>E.anatis</u>                                                                                                                                            |
| pigeon ( <u>Columba livia</u> )         | 2                                                         | <u>E.columbarum</u> , <u>E.labbeana</u>                                                                                                                                                  |
| rabbit ( <u>Oryctolagus cuniculus</u> ) | 11(12)                                                    | <u>E.intestinalis</u> , <u>E.flavescens</u> , <u>E.stiedai</u> , <u>E.magna</u> , <u>E.perforans</u>                                                                                     |
| sheep ( <u>Ovis aries</u> )             | 11(16)                                                    | <u>E.ovinoidalis</u> , <u>E.ashata</u> , <u>E.ovina</u>                                                                                                                                  |
| goat ( <u>Capra hircus</u> )            | 12(15)                                                    | <u>E.ninakhlyakimovae</u> , <u>E.arloingi</u>                                                                                                                                            |
| cattle ( <u>Bos taurus</u> )            | 12(15)                                                    | <u>E.zuernii</u> , <u>E.bovis</u> , <u>E.auburnensis</u>                                                                                                                                 |
| pig ( <u>Sus scrofa</u> )               | 7(14)                                                     | <u>I.suis</u> , <u>E.debliecki</u> , <u>E.scabra</u>                                                                                                                                     |
| dog ( <u>Canis familiaris</u> )         | 5                                                         | <u>I.canis</u> , <u>I.(Cystispora) burrowsi</u>                                                                                                                                          |
| cat ( <u>Felis catus</u> )              | 2+6                                                       | <u>I.felis</u> , <u>I.rivolta</u> as final host: <u>Sarcocystis bovifelis</u> , <u>S.ovifelis</u> , <u>S.fusiiformis</u> , <u>S.muris</u> , <u>S.cuniculi</u> , <u>Toxoplasma gondii</u> |

\*) regarding to Pellerdy (1974), Eckert et al. (1995b, Levine and Ivens (1970) and Mehlhorn 1988)

Most of the pathogenic species are strictly host-specific. They have a complex life cycle with two asexual reproduction phases (schizogony or merogony, and sporogony) and a sexual development phase (gametogony). In view of the major importance of coccidiosis, numerous reviews are available, for instance, by Davies et al. (1963), Hammond and Long

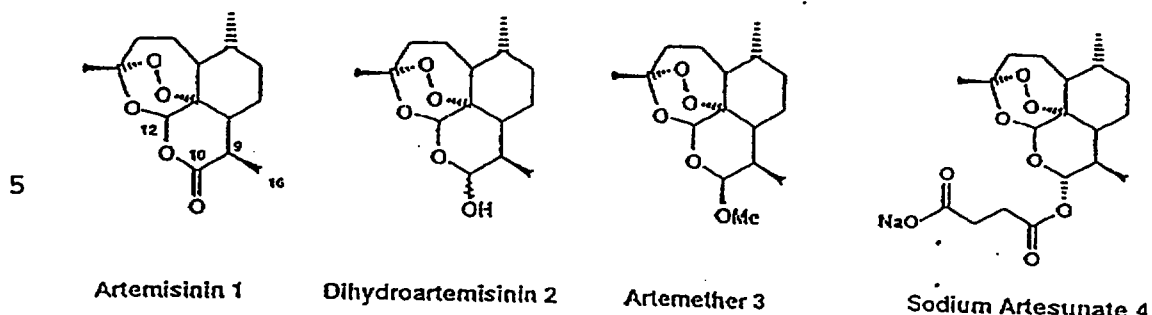
-5-

(1973), Long (1982, 1990), and Pellerdy (1974). The economically important species sometimes differ very considerably in their sensitivity to medicinal active ingredients. The sensitivity of the different  
5 developmental stages to medicinal agents also varies enormously.

As far as the use of drugs is concerned, prophylaxis is the main approach in poultry, in which symptoms do not appear until the phase of increased  
10 morbidity, and therapy is the principal strategy in mammals (McDougald 1982). Polyether antibiotics and sulfonamides, among other drugs, are currently used for such treatment and prophylaxis. However, drug-resistant strains of Eimeria have emerged and drug-  
15 resistance is now a serious problem. New drugs are therefore urgently required. Given the multiplicity of pathogens and hosts, there is no "ideal model" for identifying and testing anticoccidial agents. For example, most of the many substances used for  
20 preventing coccidiosis in poultry are insufficiently effective or even completely ineffective against mammalian coccidia (Haberkorn and Mundt; 1989; Haberkorn 1996). Numerous works and sets of instructions have been published on testing of active  
25 ingredients in animals for anticoccidial efficacy, for immunisation, etc. One particularly important and comprehensive example is the survey of current methods published by Eckert et al. (1995a).

The compound artemisinin, also known as qinghaosu  
30 (1), is a tetracyclic 1,2,4-trioxane occurring in Artemisia annua. Artemisinin and its derivatives dihydroartemisinin (2), artemether (3) and sodium artesunate (4) have been used for the treatment of malaria.

35



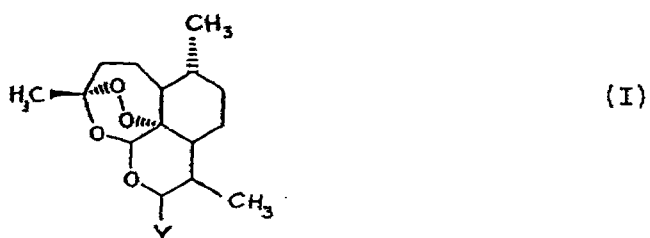
Different modes of action have been proposed by various groups to account for the action of artemisinin and its derivatives in treating malaria (Posner et al., *J. Am. Chem. Soc.* 1996, 118, 3537; Posner et al., *J. Am. Chem. Soc.* 1995, 117, 5885; Posner et al., *J. Med. Chem.* 1995, 38, 2273). However, irrespective of actual mode of action, all current derivatives suffer from poor oral bioavailability and poor stability (Meshnick et al., *Parasitology Today* 1996, 12, 79), especially the 'first generation' ethers and esters artemether and sodium artesunate obtained from dihydroartemisinin. Extensive chemical studies carried out on artemisinin and derivatives indicate that a cause of instability is the facile opening of the trioxane moiety in artemisinin itself, or in the metabolite common to all currently used derivatives artemether, arteether and artesunate, namely dihydroartemisinin. Ring opening will provide the free hydroperoxide, which is susceptible to reduction. Removal of this group ensures destruction of drug activity with the reduction products being transformed into desoxo metabolites. In order to render ring-opening less facile, the oxygen atom at C-10 can be either removed to provide 10-deoxydihydroartemisinin, or replaced by other groups, and this has provided the basis for the so-called 'second generation' compounds which are generally 10-deoxy artemisinin derivatives. In addition, derivatives of artemisinin have also been prepared with a variety of substituents at C-9.

-7-

Artemisinin derivatives are also known in which the oxygen atom at C-10 has been replaced by an amine group. For instance, Yang et al (Biorg. Med. Chem. Lett., 1995, 5, 1791-1794) synthesised ten new  
5 artemisinin derivatives in which the oxygen atom at C-10 was replaced by a group -NHAr where Ar represents a phenyl, 3-chlorophenyl, 4-chlorophenyl, 3-bromophenyl, 4-bromophenyl, 4-iodophenyl, 4-methylphenyl, 4-methoxyphenyl, 3-carboxylphenyl or 4-carboxylphenyl  
10 group. These compounds were tested for in vivo activity against the K173 strain of Plasmodium berghei and found to be active.

Whilst the current artemisinin derivatives are successful, there are problems associated with  
15 stability, bioavailability and potential neurotoxicity. There is also a need for artemisinin derivatives which exhibit a broad spectrum of activity against a variety of parasites.

It has now been discovered that certain C-10  
20 substituted derivatives of artemisinin are effective in the treatment of diseases caused by infection with a parasite. These compounds are particularly effective in the treatment of diseases caused by infection with a parasite of the genera Plasmodium,  
25 Neospora or Eimeria, especially Plasmodium falciparum, Neospora caninum and Eimeria tenella which cause malaria, neosporosis and coccidiosis respectively. According to the present invention there is therefore provided a compound of the general  
30 formula I



35

or a salt thereof,

in which

Y represents a halogen atom, an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or

5 heterocyclylalkyl group or a group  $-NR^1R^2$ ; where

$R^1$  represents a hydrogen atom or an optionally substituted alkyl, alkenyl or alkynyl group;

$R^2$  represents an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group;

10 or

$R^1$  and  $R^2$  together with the interjacent nitrogen atom represent an optionally substituted heterocyclic group or an amino group derived from an optionally substituted amino acid ester;

15 for use in the treatment and/or prophylaxis of a disease caused by infection with a parasite other than an organism of the genus Plasmodium.

Suitable salts include acid addition salts and these may be formed by reaction of a suitable compound of formula I with a suitable acid, such as an organic acid or a mineral acid. Acid addition salts formed by reaction with a mineral acid are particularly preferred, especially salts formed by reaction with hydrochloric or hydrobromic acid. Compounds of

20 formula I in which Y represents a group  $-NR^1R^2$  where  $R^1$  and  $R^2$  are as defined above are particularly suitable for the formation of such acid addition salts.

Any alkyl, alkenyl or alkynyl group, unless otherwise specified, may be linear or branched and may contain up to 12, preferably up to 6, and especially up to 4 carbon atoms. Preferred alkyl groups are methyl, ethyl, propyl and butyl. It is preferred that any alkenyl or alkynyl group is not an alk-1-enyl or alk-1-ynyl group. In other words, there should preferably be at least one methylene group  $-CH_2-$  or similar  $sp^3$ -hybridised centre between a carbon atom forming part of the double or triple C-C bond and the

30

35

nitrogen atom to which the group is attached.

Preferred alkenyl and alkynyl groups include propenyl, butenyl, propynyl and butynyl groups. When an alkyl moiety forms part of another group, for example the  
5 alkyl moiety of an aralkyl group, it is preferred that it contains up to 6, especially up to 4, carbon atoms. Preferred alkyl moieties are methyl and ethyl.

An aryl group may be any aromatic hydrocarbon group and may contain from 6 to 24, preferably 6 to  
10 18, more preferably 6 to 16, and especially 6 to 14, carbon atoms. Preferred aryl groups include phenyl, naphthyl, anthryl, phenanthryl and pyryl groups, especially a phenyl or naphthyl, and particularly a phenyl, group. When an aryl moiety forms part of  
15 another group, for example the aryl moiety of an aralkyl group, it is preferred that it is a phenyl, naphthyl, anthryl, phenanthryl or pyryl, especially phenyl or naphthyl, and particularly a phenyl, moiety.

An aralkyl group may be any alkyl group  
20 substituted by an aryl group. A preferred aralkyl group contains from 7 to 30, particularly 7 to 24 and especially 7 to 18, carbon atoms, particularly preferred aralkyl groups being benzyl, naphthylmethyl, anthrylmethyl, phenanthrylmethyl and pyrylmethyl  
25 groups. A particularly preferred aralkyl group is a benzyl group.

A cycloalkyl group may be any saturated cyclic hydrocarbon group and may contain from 3 to 12, preferably 3 to 8, and especially 3 to 6, carbon  
30 atoms. Preferred cycloalkyl groups are cyclopropyl, cyclopentyl and cyclohexyl groups.

A heteroaryl group may be any aromatic monocyclic or polycyclic ring system which contains at least one heteroatom. Preferably, a heteroaryl group is a 5-  
35 18- membered, particularly a 5- to 14-membered, and especially a 5- to 10-membered, aromatic ring system containing at least one heteroatom selected from

oxygen, sulphur and nitrogen atoms. Preferred heteroaryl groups include pyridyl, pyrylium, thiopyrylium, pyrrolyl, furyl, thienyl, indolinyl, isoindolinyl, indolizinyll, imidazolyl, pyridonyl, pyronyl, pyrimidinyl, pyrazinyl, oxazolyl, thiazolyl, purinyl, quinolinyl, isoquinolinyl, quinoxalinyl, pyridazinyl, benzofuranyl, benzoxazolyl and acridinyl groups. A C-linked heteroaryl group is therefore a heteroaryl group as defined above which is linked to the tetracyclic 1,2,4-trioxane moiety of a compound of general formula I via a carbon atom in the heteroaromatic ring system.

A heterocyclic group may be any monocyclic or polycyclic ring system which contains at least one heteroatom and may be unsaturated or partially or fully saturated. The term "heterocyclic" thus includes heteroaryl groups as defined above as well as non-aromatic heterocyclic groups. Preferably, a heterocyclic group is a 3- to 18- membered, particularly a 3- to 14-membered, especially a 5- to 10-membered, ring system containing at least one heteroatom selected from oxygen, sulphur and nitrogen atoms. Preferred heterocyclic groups include the specific heteroaryl groups named above as well as pyranyl, piperidinyl, pyrrolidinyl, dioxanyl, piperazinyl, morpholinyl, thiomorpholinyl, morpholinosulphonyl, tetrahydroisoquinolinyl and tetrahydrofuranly groups.

A heterocyclalkyl group may be any alkyl group substituted by a heterocyclic group. Preferably, the heterocyclic moiety is a 3- to 18- membered, particularly a 3- to 14-membered, and especially a 5- to 10-membered, heterocyclic group as defined above and the alkyl moiety is a C<sub>1-6</sub> alkyl, preferably C<sub>1-4</sub> alkyl, and especially methyl, group.

An amino acid may be any  $\alpha$ -amino acid, such as glycine, alanine, valine, leucine, isoleucine, serine,

threonine, cysteine, cystine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, hydroxylysine, arginine, histidine, phenylalanine, tyrosine, tryptophan, proline, hydroxyproline or phenylglycine, and includes both D- and L- configurations. An amino acid ester may be any ester of such an amino acid; alkyl esters, particularly C<sub>1-4</sub> alkyl esters, being especially preferred.

When any of the foregoing substituents are designated as being optionally substituted, the substituent groups which are optionally present may be any one or more of those customarily employed in the development of pharmaceutical compounds and/or the modification of such compounds to influence their structure/activity, stability, bioavailability or other property. Specific examples of such substituents include, for example, halogen atoms, nitro, cyano, hydroxyl, cycloalkyl, alkyl, alkenyl, haloalkyl, alkoxy, haloalkoxy, amino, alkylamino, dialkylamino, formyl, alkoxycarbonyl, carboxyl, alkanoyl, alkylthio, alkylsulphinyl, alkylsulphonyl, alkylsulphonato, arylsulphinyl, arylsulphonyl, arylsulphonato, carbamoyl, alkylamido, aryl, aralkyl, optionally substituted aryl, heterocyclic and alkyl- or aryl-substituted heterocyclic groups. When any of the foregoing substituents represents or contains an alkyl or alkenyl substituent group, this may be linear or branched and may contain up to 12, preferably up to 6, and especially up to 4, carbon atoms. A cycloalkyl group may contain from 3 to 8, preferably from 3 to 6, carbon atoms. An aryl group or moiety may contain from 6 to 10 carbon atoms, phenyl groups being especially preferred. A heterocyclic group or moiety may be a 5- to 10-membered ring system as defined above. A halogen atom may be a fluorine, chlorine, bromine or iodine atom and any group which contains a halo moiety, such as a haloalkyl group, may thus

contain any one or more of these halogen atoms.

In one aspect, it is preferred that Y represents a halogen atom, particularly a fluorine or bromine, and especially a fluorine, atom.

5 In another preferred aspect Y may represent a C<sub>3-8</sub> cycloalkyl group, a C<sub>6-18</sub> aryl group, a 5- to 10-membered C-linked heteroaryl group or a 5- to 10-membered heterocyclyl-C<sub>1-6</sub> alkyl group, each group being optionally substituted by one or more  
10 substituents selected from the group consisting of halogen atoms, hydroxyl, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> alkoxy, amino, C<sub>1-4</sub> alkylamino, di(C<sub>1-4</sub> alkyl)amino, carboxyl, C<sub>6-10</sub> aryl, 5 to 10-membered heterocyclic and C<sub>1-4</sub> alkyl- or phenyl-substituted 5-  
15 to 10-membered heterocyclic groups. Preferably Y represents a C<sub>6-18</sub> aryl group optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> haloalkoxy,  
20 amino, C<sub>1-4</sub> alkylamino, di(C<sub>1-4</sub> alkyl)amino and carboxyl groups. In particular, Y may represent a phenyl, naphthyl, anthryl or phenanthryl group, each group being optionally substituted by one or more substituents selected from the group consisting of  
25 halogen atoms and hydroxyl, methyl, vinyl, C<sub>1-4</sub> alkoxy and carboxyl groups.

In a particularly preferred sub-group of compounds, Y represents a phenyl, fluorophenyl, chlorophenyl, bromophenyl, trimethylphenyl,  
30 vinylphenyl, methoxyphenyl, dimethoxyphenyl, trimethoxyphenyl, carboxylphenyl, naphthyl, hydroxynaphthyl, methoxynaphthyl, anthryl or phenanthryl group. Compounds in which Y represents a phenyl or trimethoxyphenyl group are especially  
35 preferred.

In a further preferred aspect, Y may represent a group -NR<sup>1</sup>R<sup>2</sup> where R<sup>1</sup> represents a hydrogen atom or a

- C<sub>1-6</sub> alkyl group and R<sup>2</sup> represents a C<sub>1-6</sub> alkyl, C<sub>3-6</sub> cycloalkyl, C<sub>6-10</sub> aryl or C<sub>7-16</sub> aralkyl group, or R<sup>1</sup> and R<sup>2</sup> together with the interjacent nitrogen atom represent a 5- to 10-membered heterocyclic group or an amino group derived from a C<sub>1-6</sub> alkyl ester of an amino acid, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> haloalkyl, C<sub>1-6</sub> alkoxy carbonyl, phenyl, halophenyl, C<sub>1-4</sub> alkylphenyl, C<sub>1-4</sub> haloalkylphenyl, C<sub>1-4</sub> alkoxyphenyl, benzyl, pyridyl and pyrimidinyl groups. In particular, Y may represent a group -NR<sup>1</sup>R<sup>2</sup> where R<sup>1</sup> represents a hydrogen atom or a C<sub>1-4</sub> alkyl group and R<sup>2</sup> represents a C<sub>1-4</sub> alkyl, C<sub>3-6</sub> cycloalkyl, phenyl or benzyl group, or R<sup>1</sup> and R<sup>2</sup> together with the interjacent nitrogen atom represent a 6- to 10-membered heterocyclic group or an amino group derived from a C<sub>1-4</sub> alkyl ester of an amino acid, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> alkoxy carbonyl, phenyl, halophenyl, C<sub>1-4</sub> alkylphenyl, C<sub>1-4</sub> haloalkylphenyl, C<sub>1-4</sub> alkoxyphenyl, benzyl, pyridyl and pyrimidinyl groups.
- In a particularly preferred sub-group of these compounds, Y represents a propylamino, cyclopentylamino, cyclohexylamino, phenylamino, fluorophenylamino, chlorophenylamino, bromophenylamino, iodophenylamino, methoxycarbonylphenylamino, biphenylamino, benzylamino, fluorobenzylamino, bis(trifluoromethyl)-benzylamino, phenylethylamino, phenylmethoxycarbonylmethylamino, diethylamino, morpholinyl, thiomorpholinyl, morpholinosulphonyl, indolinyl, tetrahydroisoquinolinyl, phenylpiperazinyl, fluorophenylpiperazinyl, chlorophenylpiperazinyl, methylphenylpiperazinyl, trifluoromethylphenyl-

piperazinyl, methoxyphenylpiperazinyl, benzylpiperazinyl, pyridylpiperazinyl and pyrimidinylpiperazinyl group. Compounds in which Y represents a propylamino, phenylamino, 5 bromophenylamino, iodophenylamino, biphenylamino, benzylamino, bis(trifluoromethyl)benzylamino, phenylethylamino, phenyl-methoxycarbonylmethylamino or morpholinyl group are especially preferred.

Preferably, the parasite is an organism of the 10 genus Neospora or the genus Eimeria.

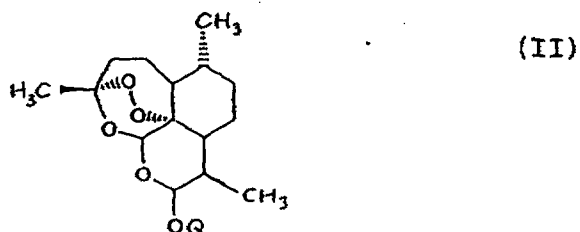
The present invention also provides the use of a compound of the general formula I as defined above for the manufacture of a medicament for the treatment and/or prophylaxis of a disease caused by infection 15 with a parasite other than an organism of the genus Plasmodium. Preferably, the parasite is an organism of the genus Neospora or the genus Eimeria.

Certain compounds of the general formula I are novel and the invention therefore further provides a 20 compound of the general formula I as defined above, with the proviso that, when Y is a group  $-NR^1R^2$  and  $R^2$  represents a phenyl, 3-chlorophenyl, 4-chlorophenyl, 3-bromophenyl, 4-bromophenyl, 4-iodophenyl, 4-methylphenyl, 4-methoxyphenyl, 3-carboxylphenyl or 4- 25 carboxylphenyl group, then  $R^1$  is an optionally substituted alkyl group.

It should also be appreciated that the compounds of general formula I are capable of existing as different geometric and optical isomers. The present 30 invention thus includes both the individual isomers and mixtures of such isomers.

The present invention also provides a process for the preparation of a novel compound of the general formula I as defined in the ante-preceding paragraph 35 which comprises reacting a compound of the general formula II

-15-



5 in which Q represents a hydrogen atom or trimethylsilyl group, with a suitable halogenating agent to form a compound of the general formula I in which Y represents a halogen atom; and, if desired, reacting the compound of general formula I thus formed either with a Grignard reagent of the general formula YMgX where Y is an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group and X is a halogen atom to form a compound of general formula I in which Y represents an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group or with an amine of the general formula HNR<sup>1</sup>R<sup>2</sup> where R<sup>1</sup> and R<sup>2</sup> are as defined above to form a compound of general formula I in which Y represents a group -NR<sup>1</sup>R<sup>2</sup> where R<sup>1</sup> and R<sup>2</sup> are as defined above.

Suitable halogenating agents for forming compounds of the general formula I in which Y represents a halogen atom include diethylaminosulphur trifluoride, chlorotrimethylsilane, bromotrimethylsilane and iodotrimethylsilane. In particular, compounds of the general formula I in which Y represents a chlorine, bromine or iodine atom may be prepared by reacting a compound of the general formula II in which Q represents a trimethylsilyl group with a suitable chlorinating, brominating or iodinating agent respectively, such as chlorotrimethylsilane, bromotrimethylsilane or iodotrimethylsilane respectively. This reaction may be conveniently carried out in the presence of a solvent. Suitable solvents include halogenated hydrocarbons, especially chlorinated hydrocarbons, such as dichloromethane.

Preferably, the reaction is carried out at a temperature of  $-30^{\circ}\text{C}$  to  $+10^{\circ}$ , particularly  $-5^{\circ}\text{C}$  to  $+5^{\circ}\text{C}$ , about  $0^{\circ}\text{C}$  being especially preferred.

Compounds of the general formula I in which Y represents a fluorine atom may be conveniently prepared by reacting a compound of the general formula II in which Q represents a hydrogen atom with a suitable fluorinating agent, such as diethylaminosulphur trifluoride. This reaction may be conveniently carried out in the presence of a solvent, suitable solvents including halogenated hydrocarbons, especially chlorinated hydrocarbons, such as dichloromethane. Preferably, the reaction is carried out at  $-5^{\circ}\text{C}$  to room temperature, that is,  $-5$  to  $+35^{\circ}\text{C}$ , preferably  $0$  to  $30^{\circ}\text{C}$ . The reaction may also be carried out under an inert atmosphere, such as nitrogen.

Suitable Grignard reagents for forming compounds of the general formula I in which Y is an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group include compounds of the general formula  $\text{YMgX}$  where X represents a chlorine, bromine or iodine atom. However, it is particularly preferred that X represents a bromine atom. The reaction of a compound of the general formula I in which Y represents a halogen, preferably a bromine, atom with a Grignard reagent may be conveniently carried out in the presence of a solvent. Suitable solvents include ethers, such as diethyl ether. Preferably, the reaction is carried out under an inert atmosphere, such as nitrogen, at a temperature of  $-5^{\circ}\text{C}$  to  $+5^{\circ}\text{C}$ ,  $0^{\circ}\text{C}$  being especially preferred. This method produces a single pure isomer of the final product.

The reaction of an amine with a compound of the general formula I in which Y represents a halogen, preferably a bromine, atom to form a compound of the general formula I in which Y represents a group  $-\text{NR}^1\text{R}^2$

where  $R^1$  and  $R^2$  are as defined above may be conveniently carried out in the presence of a solvent. Suitable solvents include halogenated hydrocarbons, especially chlorinated hydrocarbons, such as dichloromethane, and ethers, such as tetrahydrofuran. Preferably, the reaction is carried out at a temperature of  $-5^\circ\text{C}$  to  $+5^\circ\text{C}$ ,  $0^\circ\text{C}$  being especially preferred.

When a compound of the general formula I in which Y represents a bromine atom is to be further reacted with a Grignard reagent or an amine to form a compound of the general formula I in which Y represents an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group or a group  $-\text{NR}^1\text{R}^2$  where  $R^1$  and  $R^2$  are as defined above, it is preferred that the compound of the general formula I in which Y represents a bromine atom is generated in situ by reacting a compound of the general formula II in which Q represents a trimethylsilyl group with bromotrimethylsilane.

A compound of the general formula II in which Q represents a trimethylsilyl group may be prepared by reacting dihydroartemisinin, that is, the compound of general formula II in which Q represents a hydrogen atom, with chlorotrimethylsilane in the presence of a base, such as pyridine or triethylamine. Preferably, the reaction is carried out at room temperature, that is,  $15$  to  $35^\circ\text{C}$ , preferably  $20$  to  $30^\circ\text{C}$ .

Dihydroartemisinin, that is, the compound of general formula II in which Q represents a hydrogen atom, is a known compound and can be prepared by known processes.

Compounds of the general formula I in which Y represents an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group can also be prepared by reacting 9,10-anhydroartemisinin with a compound of the general formula  $\text{Y-H}$ , where Y is

as defined above, in the presence of a suitable Lewis acid. This method produces a mixture of isomers in the final product.

Suitable Lewis acids include boron trifluoride dietherate and trifluoromethanesulphonic acid. The reaction may be conveniently carried out in the presence of a solvent. Suitable solvents include halogenated hydrocarbons, especially chlorinated hydrocarbons, such as dichloromethane. Preferably, the reaction is carried out under an inert atmosphere, such as nitrogen, at room temperature, that is, 15 to 35°C, preferably 20 to 30°C.

9,10-Anhydroartemisinin may be conveniently prepared by reacting dihydroartemisinin with trifluoroacetic anhydride. The reaction may be conveniently carried out in the presence of a solvent, preferably a halogenated hydrocarbon, and especially a chlorinated hydrocarbon, such as dichloromethane. It is also preferred that the reaction is carried out in the presence of a base, such as pyridine or a derivative thereof, for example, dimethylaminopyridine. Preferably, the reaction is carried out under an inert atmosphere, such as nitrogen, at a temperature of -5°C to +5°C, preferably 0°C, with the reaction mixture being subsequently allowed to warm to room temperature, that is, 15 to 35°C, preferably 20 to 30°C.

Compounds of the general formula I in which Y represents an optionally substituted aryl or C-linked heteroaryl group can also be prepared by reacting 10-trichloroacetimidoyl-10-deoxoartemisinin with a compound of the general formula Y-H, where Y is as defined above, in the presence of a suitable Lewis acid, such as boron trifluoride diethyl etherate. It is preferred that the 10-trichloroacetimidoyl-10-deoxoartemisinin is generated in situ by reacting a compound of the general formula II in which Q

represents a hydrogen atom with trichloroacetonitrile in the presence of a suitable base, such as 1,8-diazabicyclo[5.4.0]undecane. Preferably, the reaction to form 10-trichloroacetimidoyl-10-deoxoartemisinin is carried out at room temperature, that is, 15 to 35°C, preferably 20 to 30°C. The reaction may be conveniently carried out in the presence of a solvent. Suitable solvents include halogenated hydrocarbons, especially chlorinated hydrocarbons, such as dichloromethane. Preferably, the remainder of the reaction is carried out under an inert atmosphere, such as nitrogen. Preferably, the remainder of the reaction is carried out at a temperature of -60 to -20°C, particularly -55 to -30°C, and especially -40 to -50°C.

Compounds of the general formula I in which Y represents an optionally substituted aryl or C-linked heteroaryl group can also be prepared by reacting a 10-acyloxyartemisinin compound in which the acyloxy group is of formula  $A(C=O)-O-$ , where A represents an optionally substituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclic or polycyclic group, with a compound of the general formula  $Y-H$ , where Y is as defined above, in the presence of a suitable Lewis acid. Suitable Lewis acids include boron trifluoride diethyl etherate, tin(IV) chloride, copper(II)-trifluoromethanesulfonate and trifluoromethanesulphonic acid. It is preferred that the Lewis acid is boron trifluoride diethyl etherate.

When A represents an optionally substituted alkyl group, unless otherwise specified, this may be linear or branched and may contain up to 12, preferably up to 6, and especially up to 4 carbon atoms. Preferred alkyl groups are methyl, ethyl, propyl and butyl.

When A represents an optionally substituted aryl group, this may be any aromatic hydrocarbon group and may contain from 6 to 24, preferably 6 to 18, more preferably 6 to 16, and especially 6 to 14, carbon

atoms. Preferred aryl groups include phenyl, naphthyl, anthryl, phenanthryl and pyryl groups, especially phenyl, naphthyl and anthryl groups. When an aryl moiety forms part of another group, for example the aryl moiety of an aralkyl group, it is preferred that it is a phenyl, naphthyl, anthryl, phenanthryl or pyryl, especially a phenyl or naphthyl, and particularly a phenyl, moiety.

When A represents an optionally substituted aralkyl group, this may be any alkyl group substituted by an aryl group. A preferred aralkyl group contains from 7 to 30, particularly 7 to 24, more particularly 7 to 18, and especially 7 to 10, carbon atoms, particularly preferred aralkyl groups being benzyl, naphthylmethyl, anthrylmethyl, phenanthrylmethyl and pyrylmethyl groups, a benzyl group being especially preferred.

When A represents an optionally substituted cycloalkyl group, this may be any saturated or partially unsaturated cyclic hydrocarbon group and may contain from 3 to 12, preferably 3 to 8, and especially 3 to 6, carbon atoms. Preferred cycloalkyl groups are cyclopropyl, cyclopentyl and cyclohexyl groups.

When A represents an optionally substituted polycyclic group, this may be any saturated or partially unsaturated hydrocarbon group which contains more than one ring system. Such ring systems may be "fused", that is, adjacent rings have two adjacent carbon atoms in common, "bridged", that is, the rings are defined by at least two common carbon atoms (bridgeheads) and at least three acyclic chains (bridges) connecting the common carbon atoms, or "spiro" compounds, that is, adjacent rings are linked by a single common carbon atom. It is also envisaged that a polycyclic group may contain more than one of these types of ring system. Polycyclic groups

preferably contain from 4 to 30, particularly 4 to 26, and especially 6 to 18, carbon atoms. Bicyclic, tricyclic and tetracyclic groups are particularly preferred. Preferred bicyclic groups contain from 4 to 14, especially 6 to 10, carbon atoms. Preferred tricyclic groups contain from 5 to 20, especially 6 to 14, carbon atoms with anthraquinone groups being especially preferred. Preferred tetracyclic groups contain from 6 to 26, especially 6 to 18, carbon atoms.

Optional substituents for the substituent A may be any of those previously identified as suitable in this respect.

The reaction may be conveniently carried out in the presence of a solvent. Suitable solvents include halogenated hydrocarbons, especially chlorinated hydrocarbons, such as dichloromethane. Preferably, the reaction is carried out under an inert atmosphere, such as nitrogen. Preferably, the reaction is carried out at a temperature of -60 to -20°C, particularly -55 to -30°C, and especially -40 to -50°C.

Compounds of formula I in which Y represents a substituted aryl group where at least one of the substituents is a hydroxyl group can also be prepared by rearrangement of the corresponding C-10 ether linked artemisinin derivative so that the oxygen atom of the ether link becomes the oxygen atom of the hydroxyl group in the substituted aryl group of the desired product. Such a rearrangement can be effected by reacting the corresponding C-10 ether linked artemisinin derivative with a Lewis acid, such as a boron trifluoride dietherate. The reaction is conveniently carried out in the presence of a solvent such as dichloromethane at a temperature of -5°C to +5°C, preferably 0°C.

Certain compounds of the general formula I may also be prepared by conversion of another compound of

general formula I. For instance, 10-(4-vinylphenyl)-dihydroartemisinin may be converted to 10-(4-carboxyphenyl)dihydroartemisinin by reaction with an oxidising agent, such as potassium permanganate.

5 Also, compounds of general formula I which contain a heterocyclic moiety having at least one sulphur atom in the ring system may be oxidised to form compounds of general formula I in which the or each sulphur atom has been converted to a sulphinyl or sulphonyl group  
10 by reaction with a suitable oxidising agent. Suitable oxidising agents include 4-methylmorpholine N-oxide (NMO), tetrapropylammonium perruthenate (TPAP) and mixtures thereof. The reaction may be conveniently carried out in the presence of a solvent,  
15 suitable solvents including halogenated hydrocarbons, especially chlorinated hydrocarbons, such as dichloromethane. Preferably, the reaction is carried out at room temperature, that is, 15 to 35°C, preferably 20 to 30°C. The reaction may also be  
20 carried out under an inert atmosphere, such as nitrogen.

The invention also provides a pharmaceutical composition which comprises a carrier and, as active ingredient, a novel compound of the general formula I  
25 as defined above.

A pharmaceutically acceptable carrier may be any material with which the active ingredient is formulated to facilitate administration. A carrier  
30 may be a solid or a liquid, including a material which is normally gaseous but which has been compressed to form a liquid, and any of the carriers normally used in formulating pharmaceutical compositions may be  
35 used. Preferably, compositions according to the invention contain 0.5 to 95% by weight of active ingredient.

The compounds of general formula I can be formulated as, for example, tablets, capsules,

suppositories or solutions. These formulations can be produced by known methods using conventional solid carriers such as, for example, lactose, starch or talcum or liquid carriers such as, for example, water, fatty oils or liquid paraffins. Other carriers which may be used include materials derived from animal or vegetable proteins, such as the gelatins, dextrans and soy, wheat and psyllium seed proteins; gums such as acacia, guar, agar, and xanthan; polysaccharides; alginates; carboxymethylcelluloses; carrageenans; dextrans; pectins; synthetic polymers such as polyvinylpyrrolidone; polypeptide/protein or polysaccharide complexes such as gelatin-acacia complexes; sugars such as mannitol, dextrose, galactose and trehalose; cyclic sugars such as cyclodextrin; inorganic salts such as sodium phosphate, sodium chloride and aluminium silicates; and amino acids having from 2 to 12 carbon atoms such as a glycine, L-alanine, L-aspartic acid, L-glutamic acid, L-hydroxyproline, L-isoleucine, L-leucine and L-phenylalanine.

Auxiliary components such as tablet disintegrants, solubilisers, preservatives, antioxidants, surfactants, viscosity enhancers, colouring agents, flavouring agents, pH modifiers, sweeteners or taste-masking agents may also be incorporated into the composition. Suitable colouring agents include red, black and yellow iron oxides and FD & C dyes such as FD & C blue No. 2 and FD & C red No. 40 available from Ellis & Everard. Suitable flavouring agents include mint, raspberry, liquorice, orange, lemon, grapefruit, caramel, vanilla, cherry and grape flavours and combinations of these. Suitable pH modifiers include citric acid, tartaric acid, phosphoric acid, hydrochloric acid and maleic acid. Suitable sweeteners include aspartame, acesulfame K and thaumatin. Suitable taste-masking agents include

sodium bicarbonate, ion-exchange resins; cyclodextrin inclusion compounds, adsorbates or microencapsulated actives.

For treatment of and prophylaxis against  
5 coccidiosis and related parasites, for instance, in poultry, especially in chickens, ducks, geese and turkeys, 0.1 to 100 ppm, preferably 0.5 to 100 ppm of the active compound may be mixed into an appropriate, edible material, such as nutritious food. If desired,  
10 the amounts applied can be increased, especially if the active compound is well tolerated by the recipient. Accordingly, the active compound can be applied with the drinking water.

For the treatment of a single animal, for  
15 instance, for the treatment of coccidiosis in mammals or toxoplasmosis, amounts of 0.5 to 100 mg/kg body weight active compound are preferably administered daily to obtain the desired results. Nevertheless, it may be necessary from time to time to depart from the  
20 amounts mentioned above, depending on the body weight of the experimental animal, the method of application, the animal species and its individual reaction to the drug or the kind of formulation or the time or interval in which the drug is applied. In special  
25 cases, it may be sufficient to use less than the minimum amount given above, whilst in other cases the maximum dose may have to be exceeded. For a larger dose, it may be advisable to divide the dose into several smaller single doses.

30 The invention also includes a novel compound of the general formula I as defined above for use in the treatment and/or prophylaxis of a disease caused by infection with a parasite of the genus Plasmodium and use of a novel compound of the general formula I as  
35 defined above for the manufacture of a medicament for the treatment and/or prophylaxis of a disease caused by infection with a parasite of the genus Plasmodium.

Preferred compounds in this respect include compounds of the general formula I in which Y represents a fluorine atom, Y represents a phenyl, dimethoxyphenyl or trimethoxyphenyl group or Y represents a  
5 propylamino, fluorophenylamino, biphenylamino, benzylamino, phenylethylamino, phenyl-methoxycarbonylmethylamino or diethylamino group.

The invention also provides a method for treating a disease caused by infection with a parasite other  
10 than an organism of the genus Plasmodium which comprises administering to a host in need of such treatment a therapeutically effective amount of a compound of the general formula I as first defined above. Preferably, the parasite is an organism of the  
15 genus Neospora or the genus Eimeria. A method for treating a disease caused by infection with a parasite of the genus Plasmodium is also provided which comprises administering to a host in need of such  
20 treatment a therapeutically effective amount of a novel compound of the general formula I as defined above.

The invention is further illustrated by the following examples.

25 Example 1

Preparation of 10 $\beta$ -fluoro-10-deoxo-10-dihydro-artemisinin (10 $\beta$ -fluoro-10-deoxodihydroartemisinin)  
(Formula I: Y = F)

A solution of dihydroartemisinin (1.136 g, 4 mmol) in  
30 dichloromethane (24 ml) was cooled to 0°C under nitrogen and diethylaminosulphur trifluoride (DAST) (0.6 ml, 4.8 mmol) was added. The reaction mixture was allowed to warm up to room temperature and then stirred under nitrogen for 24 hours. The yellow  
35 solution was cooled again to 0°C, Na<sub>2</sub>CO<sub>3</sub> solution (5%, 20 ml) was added and the mixture was stirred for 2 hours at room temperature. After this the two phases

-26-

were separated and the organic layer was washed with 1 molar HCl, 5% NaHCO<sub>3</sub> and water and dried over MgSO<sub>4</sub>. Immediately after evaporating the solvent, the residue was purified twice by flash column chromatography (10% ethyl acetate/hexane), followed by recrystallisation from hexane (289 mg, 50.5%); <sup>1</sup>H NMR(300 MHz, CDCl<sub>3</sub>): δ ppm 0.97 (d, J<sub>6-Me,6</sub>=6.1 Hz, 3 H, 6-CH<sub>3</sub>), 1.00 (d, J<sub>9-Me,9</sub>=7.4 Hz, 3 H, 9-CH<sub>3</sub>), 1.13-1.47 (m, 3 H), 1.44 (s, 3 H, 3-CH<sub>3</sub>), 1.47-1.72 (m, 4 H), 1.82-1.96 (m, 2 H), 2.05 (ddd, J=14.6 Hz, J=4.9 Hz, J=3.0 Hz, 1 H), 2.39 (td, J=13.5 Hz, J=4.0 Hz, 1 H), 2.64 (dm, J<sub>9,F</sub>=36.1 Hz, 1 H, H-9), 5.60 (dd, J<sub>10,F</sub>=54.4 Hz, J<sub>10,9</sub>=2.4 Hz, 1 H, H-10), 5.56 (d, J=1.83 Hz, 1 H, H-12); <sup>19</sup>F NMR(282 MHz, CDCl<sub>3</sub>): δ (ppm) = - 136.43 (dd, J<sub>F,10</sub>=54.1 Hz, J<sub>F,9</sub>=36.0 Hz); MS (CI,NH<sub>3</sub>): m/z (%) = 304 [M<sup>+</sup>+NH<sub>4</sub><sup>+</sup>] (18), 286 [M<sup>+</sup>], 284 [304-HF] (100), 267 (64), 256 (28), 239 (16), 221 (12), 163 (8), 52 (28).

### Example 2

Preparation of 10β-phenyl-10-deoxo-10-dihydro-artemisinin (10β-(phenyl)dihydroartemisinin) (Formula I : Y = phenyl)

(a) Preparation of 10-(trimethylsiloxy)dihydro-artemisinin (Formula II : O = -Si(CH<sub>3</sub>)<sub>3</sub>)

#### Method 1

To a solution of dihydroartemisinin (1.51 g, 5.32 mmol) in pyridine (20 ml) at 0°C under nitrogen was added dropwise chlorotrimethylsilane (5.20 ml, mmol). The mixture was stirred at room temperature for a further 1 hour and poured into ice-water mixture. The solution was extracted with diethyl ether (3x15 ml), dried (MgSO<sub>4</sub>) and concentrated in vacuo. The residue was purified by flash chromatography (SiO<sub>2</sub>; 5% ethyl acetate/hexanes) to give 10-(trimethylsiloxy)dihydro-artemisinin as a white solid (1.47 g, 78%). δ<sub>H</sub> 5.49 (1H, s, H-12), 5.19 (1H, d, J = 3.05 Hz, H-10), 2.52-2.62 (1H, m, H-9), 2.39 (1H, ddd, J = 17.5, 13.4,

-27-

4.01 Hz), 2.04 (1H, ddd,  $J = 14.5, 4.84, 3.05$  Hz),  
1.20-1.97 (9H, m), 1.45 (3H, s, H-14), 0.97 (3H, d,  $J = 6.24$  Hz, H-16), 0.87 (3H, d,  $J = 7.29$  Hz, H-15),  
0.17 (9H, s,  $(CH_3)_3Si$ ).

5 Method 2

Preparation of 10 $\alpha$ -(trimethylsiloxy)dihydro-  
artemisinin (Formula II:  $O = -Si(CH_3)_3$ )

To a solution of dihydroartemisinin (1.51 g, 5.32 mmol) in dichloromethane (40 ml) at 0°C under nitrogen  
10 was added dropwise triethylamine (0.94 ml, 6.65 mmol) and chlorotrimethylsilane (0.84 ml, 6.65 mmol). The mixture was stirred at room temperature for a further 1 hour and poured into ice-water mixture. The aqueous solution was extracted with dichloromethane (2x20 ml).  
15 The combined organic layers were dried ( $MgSO_4$ ) and concentrated in vacuo. The residue was purified by flash chromatography ( $SiO_2$ ; 5% ethyl acetate/hexanes) to give 10 $\alpha$ -(trimethylsiloxy)dihydro-artemisinin as a white solid (1.48 g, 78%).  $\delta_H$  5.32 (1H, s, H-12), 4.76  
20 (1H, d,  $J = 9.00$  Hz, H-10), 2.25-2.45 (2H, m, H-8, H-9), 2.01 (1H, m, H-4), 1.89 (1H, m, H-5), 1.18-1.79 (8H, m, H-2a, H-2b, H-3a, H-3b, H-6a, H-6b, H-7a, H-7b), 1.31 (3H, s, 1- $CH_3$ ) 0.95 (3H, d,  $J = 5.88$  Hz, 9- $CH_3$ ), 0.86 (3H, d,  $J = 7.14$  Hz, 5- $CH_3$ ), 0.20 (9H, s, Me<sub>3</sub>Si) ppm.  
25

(b) Preparation of 10-bromo-10-deoxo-10-  
dihydroartemisinin (10-bromoartemisinin) (Formula  
I :  $Y = Br$ )

30 A solution of 10 $\alpha$ -(trimethylsiloxy)dihydroartemisinin (372 mg, 1.04 mmol) prepared as described in (a) Method 2 above in dichloromethane (5 ml) at 0° C was treated dropwise with bromotrimethylsilane (140  $\mu$ l, 1.06 mmol). The mixture was stirred at 0°C for a further 30 minutes  
35 to produce 10-bromoartemisinin in situ.

(c) Preparation of 10 $\beta$ -phenyl-10-deoxo-10-dihydro

-artemisinin (10 $\beta$ -(phenyl)dihydroartemisinin)  
(Formula I : Y = phenyl).

The solution prepared in (b) above was concentrated in vacuo. The residue was dissolved in diethyl ether (5  
5 ml). To this solution was added phenylmagnesium bromide (1.40 ml, 2.38 mmol, 1.7M) at 0°C under nitrogen. The mixture was then stirred at 0°C and then allowed to reach room temperature overnight. The solution was then quenched with saturated ammonium  
10 chloride solution, dried (MgSO<sub>4</sub>) and concentrated in vacuo. The residue was purified by flash chromatography (SiO<sub>2</sub>; 8% ethyl acetate/hexanes to give 10 $\beta$ -phenyl-10-deoxo-10-dihydroartemisinin (10 $\beta$ -(phenyl)dihydroartemisinin) (159mg, 45%) as a white solid.  
15 Recrystallisation from ether/hexane mixture gave a colourless rectangular crystal. M.p. 122°C;  $[\alpha]_D^{20}$  -36.0° (c 0.47/CHCl<sub>3</sub>);  $\nu_{max}$  (film) 2938, 2874, 1494, 1452, 1376, 1208, 1112, 1076, 1058, 1038, 1010, 954, 944, 904, 882, 852, 820, 740, 700;  
20  $\delta_H$  7.19-7.34 (5H, m, Ar-H), 5.75 (1H, d, J = 6.70 Hz, H-10), 5.60 (1H, s, H-12), 2.71-2.84 (1H, m, H-9), 2.31-2.42 (1H, m), 1.65-2.12 (5H, m), 1.28-1.60 (5H, m), 1.41 (3H, s, H-14), 1.01 (1H, d, J = 5.77 Hz, H-16), 0.54 (1H, d, J = 7.68 Hz, H-15);  $\delta_C$  141.03,  
25 127.67, 126.24, 126.09, 102.22, 90.82, 81.10, 72.99, 51.46, 43.45, 37.46, 36.64, 34.16, 32.08, 25.68, 24.88, 24.71, 19.85, 13.62; m/z (CI, CH<sub>4</sub>) 345 (M<sup>+</sup>+1, 14%), 327 (14), 299 (100); Anal.Calc. for C<sub>21</sub>H<sub>28</sub>O<sub>4</sub> : C, 73.26; H, 8.14; Found: C, 73.58; H, 8.32.  
30 nOe-difference experiment: irradiation of the doublet signal of H-10 at  $\delta$  5.75 gave 10% enhancement in the multiplet signal of H-9 at  $\delta$  2.75; this showed that the stereochemistry of H-10 and H-9 are syn to each other.

35

Example 3Preparation of 10 $\alpha$ -(4'-fluorobenzylamino)-10-deoxo-10-

dihydroartemisinin (10 $\alpha$ -(4'-fluorobenzylamino)dihydro-  
artemisinin) (Formula I : Y = -NR<sup>1</sup>R<sup>2</sup>; R<sup>1</sup> = H; R<sup>2</sup> = 4-F  
benzyl)

5 (a) Preparation of 10 $\alpha$ -(trimethylsiloxy)dihydro-  
artemisinin (Formula II : O = -Si(CH<sub>3</sub>)<sub>3</sub>)

To a solution of dihydroartemisinin (1.51 g, 5.32 mmol)  
in dichloromethane (40 ml) at 0°C under nitrogen was  
added dropwise triethylamine (0.94 ml, 6.65 mmol) and  
chlorotrimethylsilane (0.84 ml, 6.65 mmol). The  
10 mixture was stirred at room temperature for a further 1  
hour and poured into ice-water mixture. The aqueous  
solution was extracted with dichloromethane (2x20 ml).  
The combined organic layers were dried (MgSO<sub>4</sub>) and  
concentrated in vacuo. The residue was purified by  
15 flash chromatography (SiO<sub>2</sub>; 5% ethyl acetate/hexanes)  
to give 10 $\alpha$ -(trimethylsiloxy)dihydroartemisinin as a  
white solid (1.48 g, 78%).  $\delta_H$  5.32 (1H, s, H-12), 4.76  
(1H, d, J 9.00 Hz, H-10), 2.25-2.45 (2H, m, H-8, H-9),  
2.01 (1H, m, H-4), 1.89 (1H, m, H-5), 1.18-1.79 (8H, m,  
20 H-2a, H-2b, H-3a, H-3b, H-6a, H-6b, H-7a, H-7b), 1.31  
(3H, s, 1-CH<sub>3</sub>) 0.95 (3H, d, J 5.88 Hz, 9-CH<sub>3</sub>), 0.86  
(3H, d, J 7.14 Hz, 5-CH<sub>3</sub>), 0.20 (9H, s, Me<sub>3</sub>Si) ppm.

25 (b) Preparation of 10 $\alpha$ -(4'-fluorobenzylamino)-10-  
deoxo-10-dihydroartemisinin (10 $\alpha$ -(4'-fluoro-  
benzylamino)dihydroartemisinin) (Formula I : Y = -  
NR<sup>1</sup>R<sup>2</sup>; R<sup>1</sup>=H; R<sup>2</sup> =4-F-benzyl)

A solution of 10 $\alpha$ -(trimethylsiloxy)dihydroartemisinin  
(214 mg, 0.600 mmol) prepared as described in (a) above  
30 in dichloromethane (5 ml) at 0°C was treated dropwise  
with bromotrimethylsilane (80  $\mu$ l, 0.600 mmol). The  
mixture was stirred at 0°C for a further 30 minutes  
after which it was then transferred by cannula into a  
solution of 4-fluorobenzylamine (140  $\mu$ l 1.20 mmol) in  
35 tetrahydrofuran (5 ml) at 0°C. The mixture was stirred  
at 0°C and then allowed to reach room temperature  
overnight. The suspension was washed with saturated

-30-

NaHCO<sub>3</sub> solution, dried (MgSO<sub>4</sub>) and concentrated in vacuo. The residue was purified by flash chromatography (SiO<sub>2</sub>; 15% ethyl acetate/hexanes) to give 10 $\alpha$ -(4'-fluorobenzylamino)-10-deoxo-10-dihydroartemisinin (10 $\alpha$ -(4'-fluorobenzylamino)-dihydroartemisinin) (76.9 mg, 33%) and 9,10-anhydro-10-deoxoartemisinin (9,10-anhydro-dehydroartemisinin) (84.7mg, 53%), both as white solids. M.p. 45.2-46.3°C;  $[\alpha]_D^{20}$ -18.2° (c 0.055 CHCl<sub>3</sub>);  $\delta_H$  7.32-7.37 (2H, m, Ar-H), 6.95-7.02 (2H, m, Ar-H), 5.29 (1H, s, H-12), 4.10 (1H, d, J = 13.8 Hz, H-1'), 4.08 (1H, d, J = 9.76 Hz, H-10), 3.91 (1H, d, J = 13.8 Hz, H-1'), 2.33-2.42 (2H, m), 1.85-2.07 (3H, m), 1.65-1.77 (2H, m), 1.03-1.75 (5H, m), 1.46 (3H, s, H-14), 0.96 (3H, d, J = 6.02 Hz, H-16), 0.93 (3H, d, J = 7.19 Hz, H-15);  $\delta_C$  136.42 (d, J = 3.10 Hz), 129.30 (d, J = 7.97 Hz), 114.75 (d, J = 21.1 Hz), 103.90, 91.35, 85.47, 80.60, 51.66, 47.50, 45.82, 37.23, 36.26, 34.03, 32.72, 26.03, 24.61, 21.70, 20.15, 14.06;  $\delta_F$  -118; m/z (CI, CH<sub>4</sub>) 392 (M<sup>+</sup>+1, 90%), 374 (54), 346 (100), 328 (20), 267 (16), 209 (16), 165 (26), 109 (18). Anal. Calc. for C<sub>22</sub>H<sub>30</sub>NO<sub>4</sub>F: C, 67.50; H, 7.72; N, 3.58; Found: C, 67.51; H, 7.77; N, 3.49.

#### 25 Example 4

Preparation of 10-(2',4'-dimethoxyphenyl)-10-deoxo-10-dihydroartemisinin (10-(2',4'-dimethoxyphenyl)dihydroartemisinin (Formula I : Y = 2,4-dimethoxyphenyl)

#### 30 (a) Preparation of 9,10-anhydro-10-deoxoartemisinin (9,10-anhydroartemisinin)

To a solution of dihydroartemisinin (500mg, 1.86 mmol) in dichloromethane (28 ml) at 0°C under nitrogen was added 4-(N,N-dimethylamino)pyridine (37mg) and trifluoroacetic anhydride (0.79ml, 5.58 mmol). The mixture was allowed to warm to room temperature and stirred overnight. The solution was then concentrated in vacuo. The residue was purified by flash

-31-

chromatography ( $\text{SiO}_2$ ; ether:hexane from 0.5:9.5 to 1.5:8.5) to give 9,10-anhydro-10-deoxoartemisinin (9,10-anhydroartemisinin) (180 mg, 25%) as a white solid. M.p.  $100^\circ\text{C}$ ;  $[\alpha]_D^{20.5} + 155.74^\circ$  (c.0.0101 in  $\text{CHCl}_3$ );  $\nu_{\text{max}}$  (film): 2948, 2922, 2862, 2850, 1684, 1432, 1372, 1334, 1198, 1178, 1158, 1142, 1114, 1078, 1028, 1016, 992, 954, 944, 904, 880, 828, 812;  $\delta_{\text{H}}$ : 6.18 (1H, s, H-10), 5.54 (1H, s, H-12), 2.40 (1H, ddd,  $J = 17.1$ , 13.2, 4.14 Hz, H-9), 2.00-2.09 (2H, m), 1.88-1.95 (1H, m), 1.07-1.73 (8H, m), 1.58 (3H, d,  $J = 1.37$  Hz, H-16), 1.42 (3H, s, H-14), 0.98 (3H, d,  $J = 5.98$  Hz, H-15);  $m/z$  (EI): 380 ( $M^+$ ); Anal. Calc. for  $\text{C}_{15}\text{H}_{22}\text{O}_4$ : C, 67.67; H, 8.27; Found: C, 67.63; H, 8.51

(b) Preparation of 10-(2',4'-dimethoxyphenyl)-10-deoxo-10-dihydroartemisinin (10-(2',4'-dimethoxyphenyl)-dihydroartemisinin) (Formula I : Y = 2,4-dimethoxyphenyl)

To a solution of 9,10-anhydro-10-deoxoartemisinin (9,10-anhydroartemisinin) (191 mg, 0.71 mmol) prepared as described in (a) above and 1,3-dimethoxybenzene (130  $\mu\text{l}$ , 1.00 mmol) in dichloromethane (10 ml) at room temperature under nitrogen was added boron trifluoride diethyl etherate (2 drops). The solution was stirred for a further 1 hour, and then quenched with 20% hydrochloric acid solution (5 ml). The mixture was extracted with diethyl ether (3 x 20 ml), and the ether extracts were dried ( $\text{MgSO}_4$ ) and concentrated in vacuo. The residue was purified by flash chromatography ( $\text{SiO}_2$ ; 15% ethyl acetate/hexanes) to give 10-(2',4'-dimethoxyphenyl)-10-deoxo-10-dihydroartemisinin (10-(2',4'-dimethoxyphenyl) dihydroartemisinin) (89.5, 44%) as a white solid.  $\delta_{\text{H}}$  7.56 (1H, brd,  $J = 8.4$  Hz, Ar-H), 6.40-6.58 (2H, m, Ar-H), 5.43 (1H, s, H12), 5.42 (1H, s, H-12'), 5.16 (1H, d,  $J = 10.8$  Hz, H-10), 4.96 (1H, d,  $J = 10.3$  Hz, H-10'), 3.82, 3.78 (OMe), 2.37-2.48 (2H, m), 1.05-2.07 (10H, m), 1.63 (3H, s, H-14), 1.34 (3H, s, H-14'), 1.00 (3H, d,  $J = 6.22$  Hz,

-32-

H-16'), 0.90-0.93 (3H, m, H-15 & H-16); 0.59 (3H, d, J = 7.22 Hz, H-15'); m/z (CI, NH<sub>3</sub>) 422 (M+NH<sub>4</sub><sup>+</sup>, 26%), 406 (84), 405 (M<sup>+</sup>+1, 54), 389 (80), 359 (100), 330 (30), 317 (40), 300 (14). Anal.Calc. for C<sub>23</sub>H<sub>32</sub>O<sub>6</sub>: C, 68.29; H, 7.97%; Found: C, 68.34; H, 8.09.

#### Example 5

#### Preparation of 10 $\alpha$ -(2'-hydroxy-1'-naphthyl)dihydroartemisinin (Formula I:Y = 2-OH naphthyl)

##### 10 (a) Preparation of 10 $\beta$ -(2'-naphthoxy)-dihydroartemisinin

To a solution of dihydroartemisinin (568mg, 2.00 mmol) and 2-naphthol (288 mg, 2.00 mmol) in tetrahydrofuran (10 ml) was added triphenylphosphine (524 mg, 4.00 mmol) and diethyl azodicarboxylate (330  $\mu$ l, 2.00 mmol) at °C under nitrogen. The mixture was stirred at room temperature overnight. The yellow solution was then concentrated in vacuo and the residue purified by flash chromatography (SiO<sub>2</sub>; 5% ethyl acetate/hexanes) to give 10 $\beta$ -(2'-naphthoxy)dihydroartemisinin (185mg, 23%) as a white solid.

##### 20 (b) Preparation of 10 $\alpha$ -(2'-hydroxy-1'-naphthyl)-dihydroartemisinin

To a solution of 10 $\beta$ -(2'-naphthoxy)dihydroartemisinin (232mg, 0.564 mmol) prepared as described in (a) above in dichloromethane (10 ml) was added boron trifluoride dietherate (220  $\mu$ l) at 0°C. The mixture was allowed to warm to room temperature and stirred for a further 30 minutes. The solution was washed with 10% sodium hydrogen carbonate solution (2 x 5 ml), dried (MgSO<sub>4</sub>) and concentrated in vacuo. The residue was then purified by flash chromatography (SiO<sub>2</sub>; 10% ethyl acetate/hexanes) to give 10 $\alpha$ -(2'-hydroxy-1'-naphthyl)-dihydroartemisinin as a white solid (72.7 mg).  $\delta_H$  8.91 (1H, s, OH), 7.28-7.91 (6H, m, Ar-H), 5.57 (1H, s, H-12), 3.11-3.19 (1H, m), 1.28-2.55 (11H, m), 1.51 (3H, s, H-14), 1.04 (3H, d, J = 5.96 Hz, H-16), 0.63

-33-

(3H, d, J = 7.23 Hz, H-16).

#### Example 6

Preparation of 10 $\alpha$ -(4'-thiomorpholino-1'-yl)-10-deoxo-10-dihydroartemisinin (10 $\alpha$ -(thiomorpholino)dihydroartemisinin)

(Formula I : Y =thiomorpholino)

Reaction of bromide prepared from 10 $\alpha$ -(trimethylsiloxy)dihydroartemisinin (356 mg, 1.00 mmol) as described in Example 3(b) above with thiomorpholine (300  $\mu$ l, 3.00 mmol) afforded 10 $\alpha$ -(thiomorpholino)-dihydroartemisinin (243 mg, 66%) as a white solid after flash chromatography (8% ethyl acetate/hexanes). M.p. 147.0-147.6°C;  $[\alpha]_D^{20} + 17^\circ$  (c 0.021/CHCl<sub>3</sub>);  $\nu_{\max}$  (film) 2924, 2872, 1454, 1418, 1376, 1326, 1278, 1226, 1198, 1184, 1154, 1130, 1100, 1056, 1038, 1018, 988, 940, 926, 880, 850, 828, 756;  $\delta_H$  5.23 (1H, s, H-12), 3.93 (1H, d, J=10.21 Hz, H-10), 3.20-3.28 (2H, m), 2.85-2.93 (2H, m), 2.53-2.68 (5H, m), 2.25-2.36 (1H, m), 1.93-2.01 (1H, m), 1.78-1.86 (1H, m), 1.63-1.70 (2H, m), 1.14-1.52 (5H, m), 1.36 (3H, s, H-14), 0.90-1.04 (1H, m), 0.91 (3H, d, J=6.14 Hz, H-16), 0.76 (3H, d, J=7.18 Hz, H-15);  $\delta_C$ : 103.70, 92.28, 91.42, 80.11, 51.54, 50.39, 45.66, 37.19, 36.14, 34.12, 28.15, 25.84, 24.59, 21.44, 20.15, 13.41; m/z (CI, NH<sub>3</sub>) 370 (M<sup>+</sup>+1, 100), 324 (70), 310 (10); Anal. Calc. for C<sub>19</sub>H<sub>31</sub>NO<sub>4</sub>S: C, 61.76; H, 8.46; N, 3.79%; found C, 62.04; H, 8.39; N, 3.65.

#### Example 7

Preparation of 10 $\alpha$ -(4'-(S,S-dioxothiomorpholin-1'-yl))-10-deoxo-10-dihydroartemisinin (10 $\alpha$ -(4'-morpholino-sulphonyl)dihydroartemisinin) (Formula I : Y = 4'-(S,S-dioxothiomorpholin-1'-yl) (4-morpholinosulphonyl)

To a solution of 10 $\alpha$ -(4'-thiomorpholino)-10-deoxo-10-dihydroartemisinin (10 $\alpha$ -(thiomorpholino)-dihydroartemisinin) (388 mg, 1.05 mmol) prepared as

-34-

described in Example 6 above in dichloromethane (10 ml) at room temperature under nitrogen was added NMO (369 mg, 3.15 mmol), powdered molecular sieve (525 mg, 4 Å), and TPAP (18.5 mg, cat.). The mixture was stirred at room temperature overnight after which it was filtered through a pad of SiO<sub>2</sub> and the residue was washed with ethyl acetate (3x15 ml). The filtrate was concentrated in vacuo. The residue was then purified by flash chromatography (SiO<sub>2</sub>; 35% ethyl acetate/hexanes) to give 10α-(4'-(S,S-dioxothiomorpholin-1'-yl)-10-deoxo-10-dihydroartemisinin (10α-(4'-morpholinosulphonyl)-dihydroartemisinin) as a white solid (421 mg, 100%). M.p. 152.3-152.7°C;  $[\alpha]_D^{20} + 13^\circ$  (c 0.035/CHCl<sub>3</sub>);  $\nu_{\max}$  (film) 2928, 2872, 1454, 1378, 1308, 1270, 1228, 1198, 1124, 1040, 1018, 976, 940, 878, 846, 826, 752, 704, 666;  $\delta_H$ : 5.27 (1H, s, H-12), 4.21 (1H, d, J=10.30 Hz, H-10), 3.18-3.46, (8H, m), 2.54-2.62 (1H, m), 2.28-2.36 (1H, m), 1.20-2.02 (9H, m), 1.35 (3H, s, H-14), 0.92-1.06 (1H, m), 0.93 (3H, d, J=5.99 Hz, H-15), 0.78 (3H, J=7.13 Hz, H-16);  $\delta_C$ : 174.20, 104.09, 91.92, 90.84, 90.04, 51.74, 51.27, 46.88, 45.46, 37.29, 36.02, 34.04, 28.91, 25.76, 24.66, 21.45, 20.10, 13.31; m/z (CI, NH<sub>3</sub>) 402 (M<sup>+</sup>+1, 100), 373 (30), 356 (64), 342 (16), 356 (20); Anal. Calc. for C<sub>19</sub>H<sub>31</sub>NO<sub>6</sub>S: C, 56.84; H, 7.78; N, 3.49; found: C, 56.83; H, 7.82; N, 3.37.

#### Example 8

#### Preparation of 10α-(4'-benzylpiperazin-1'-yl)-10-deoxo-10-dihydroartemisinin (Formula I: Y = 4'-benzyl-1'-piperazinyl)

Reaction of the bromide prepared from 10β-(trimethylsiloxy)dihydroartemisinin (356 mg, 1.00 mmol) as described in Example 3(b) with 1-benzylpiperazine (212.1 μl, 1.22 mmol) afforded 10α-(4'-benzylpiperazin-1'-yl)-10-deoxo-10-dihydroartemisinin (144.3 mg, 40%) as a white solid after flash chromatography (40% ethyl acetate/hexane). M.p. 105-106°C;  $[\alpha]_D^{20} + 10.3^\circ$

-35-

(c. 0.909 CHCl<sub>3</sub>);  $\nu_{\max}$  (film): 2954, 2920, 2860, 2802, 1494, 1454, 1376, 1344, 1294, 1270, 1204, 1132, 1114, 1062, 1042, 1016, 986, 942, 924, 880, 852, 824, 738, 694 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$  7.43-7.30 (5H, m, Ar-H), 5.35 (1H, s, H-12), 4.10 (1H, d, J = 10.2 Hz, H-10), 3.62 (1H, d, J = 13.1 Hz, benzylic-H), 3.55 (1H, d, J = 13.1 Hz, benzylic-H), 3.11-3.06 (2H, m), 2.80-2.70 (2H, m), 2.70-2.30 (7H, m), 2.15-2.02 (1H, m), 2.02-1.85 (1H, m), 1.85-1.70 (2H, m), 1.70-1.20 (9H, m), 1.20-1.00 (4H, m), 0.88 (3H, d, J = 7.2 Hz, 6-methyl) ppm; <sup>13</sup>C NMR (76 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$  138.3, 129.13, 128.1, 126.9, 103.8, 91.6, 90.4, 80.3, 63.1, 53.5, 51.7, 45.9, 37.4, 36.3, 34.3, 28.5, 26.0, 24.8, 21.6, 20.3, 13.4 ppm; MS (CI, CH<sub>4</sub>) m/e 443 (M<sup>+</sup>+1, 10). Anal. Calcd. for C<sub>26</sub>H<sub>38</sub>N<sub>2</sub>O<sub>4</sub>: C, 70.56, H, 8.65, N, 6.33; Found: C, 70.24, H, 8.67, N, 6.28.

#### Example 9

#### Preparation of 10 $\alpha$ -(2'-furyl)-10-deoxo-10-dihydroartemisinin (Formula I: Y = 2-furyl)

##### Method 1:

To a solution of dihydroartemisinin (284 mg, 1.0 mmol) in dichloromethane (10 mL) at 20 °C was added trichloroacetonitrile (2.0 mL, 20.0 mmol) and one drop of 1,8-diazabicyclo[5.4.0]undecane. The mixture was stirred at 20 °C for 2 hours after which it was concentrated in vacuo at 20 °C. The residue was then taken up in dichloromethane (10 mL) at 0 °C and cooled to -40 °C. The solution was treated sequentially with furan (1.09 mL, 15.0 mmol) and boron trifluoride diethyl etherate (123  $\mu$ l, 1.0 mmol), and the resulting mixture was stirred at -40 °C for another 30 min. The mixture was quenched with saturated NaHCO<sub>3</sub> solution and extracted with dichloromethane (2 x 10 mL). The extracts were dried (MgSO<sub>4</sub>) and concentrated in vacuo. The residue was purified by flash chromatography (SiO<sub>2</sub>; 15% ethyl acetate/hexanes) to give the captioned

compound (11.0 mg, 3.3%) as a colourless oil.

Analytical sample was obtained from recrystallization from hexanes.

Method 2:

5 (a) Preparation of 10 $\beta$ -benzoyloxy-10-dihydroartemisinin (10 $\beta$ -dihydroartemisinyl benzoate)

To a solution of dihydroartemisinin (568 mg, 2.00 mmol) and benzoic acid (244mg, 2.00 mmol) in tetrahydrofuran at 0°C under nitrogen was added triphenylphosphine  
10 (524mg, 2.00 mmol) and diethyl azodicarboxylate (ml). The mixture was allowed to warm to room temperature and stirred overnight. The solution was concentrated in vacuo. Flash chromatography (SiO<sub>2</sub>; 10% ethyl acetate/hexanes) gave 10 $\beta$ -dihydroartemisinyl benzoate  
15 as a white solid (419mg, 53%). M.p. 151.4-153.0°C;  $[\alpha]_D^{20} +119^\circ$  (c 0.19/CHCl<sub>3</sub>);  $\nu_{\max}$  (film): 2942, 2872, 1724, 1452, 1378, 1268, 1176, 1114, 1064, 1024, 976, 902, 858, 832, 754, 712;  $\delta_H$  7.43-8.03 (5H, m, Ar-H), 6.52 (1H, d, J = 3.43, H-10), 5.58 (1H, s, H-12),  
20 2.91-3.01 (1H, m, H-9), 2.42 (1H, ddd, J = 17.4, 13.3, 3.91 Hz), 1.33-2.10 (10H, m), 1.45 (3H, s, H-14), 1.02 (3H, d, J = 6.11 Hz, H-15), 0.98 (3H, d, J = 7.35 Hz, H-14);  $\delta_C$  : 165.31, 133.03, 129.96, 129.48, 128.39, 104.30, 95.29, 88.66, 88.63, 80.42, 52.27, 43.84,  
25 37.44, 36.10, 34.43, 29.98, 25.78, 24.50, 24.25, 20.14, 12.50; m/z (EI) : 388 (M<sup>+</sup>).

(b) Preparation of 10 $\alpha$ -(2'-furyl)-10-deoxo-10-dihydroartemisinin

30 (Formula I : Y = 2-furyl)

A solution of 10 $\beta$ -benzoyloxy-10-dihydroartemisinin (193 mg, 0.50 mmol) in dichloromethane (5 mL) at -45 °C was treated sequentially with furan (542  $\mu$ l, 7.5 mmol) and boron trifluoride diethyl etherate (123  $\mu$ l, 1.0 mmol).  
35 The resulting mixture was stirred at -45 °C for another 1 hr. The mixture was quenched with saturated NaHCO<sub>3</sub> solution and extracted with dichloromethane (3 x 10

-37-

mL). The extracts were dried ( $\text{MgSO}_4$ ) and concentrated in vacuo. The residue was purified by flash chromatography ( $\text{SiO}_2$ ; 15% ethyl acetate/hexanes) to give the captioned compound (53.7 mg, 32%) as a colourless oil.

5 M.p. 96-97°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  7.38 (1H, m, H-5'), 6.34-6.30 (2H, m, H-3' & H-4'), 5.38 (1H, s, H-12), 4.46 (1H, d,  $J = 10.9$  Hz, H-10), 2.84 (1H, m), 2.60-2.20 (2H, m), 2.20-1.20 (9H, m), 1.20-0.80 (6H, m), 0.62 (3H, d,  $J = 7.2$  Hz, 6-methyl) ppm;  $^{13}\text{C}$  NMR (76

10 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  153.2, 142.0, 110.0, 108.3, 104.2, 92.2, 80.4, 76.6, 71.1, 52.0, 45.7, 37.4, 36.3, 34.1, 31.5, 26.1, 24.7, 21.3, 20.3, 13.7 ppm; MS (CI,  $\text{CH}_4$ )  $m/e$  335 ( $\text{M}^+ + 1$ , 43).

15 Example 10

Preparation of 10 $\alpha$ -(Pyrrol-2'-yl)-10-deoxo-10-dihydroartemisinin (Formula I : Y = 2-pyrrolyl)

A solution of 10 $\beta$ -benzoyloxy-10-deoxoartemisinin (700.8 mg, 1.80 mmol) prepared as described in Example 9,

20 Method 2(a) in dichloromethane (30 mL) at -50 °C was treated sequentially with pyrrole (624  $\mu\text{l}$ , 9.00 mmol) and boron trifluoride diethyl etherate (332  $\mu\text{l}$ , 2.70 mmol), and then stirred at -50 °C for 1 hr. The mixture was quenched with saturated  $\text{NaHCO}_3$  solution,

25 and extracted with dichloromethane (3 x 10 mL). The extracts were dried ( $\text{MgSO}_4$ ) and concentrated in vacuo. The residue was purified by flash chromatography ( $\text{SiO}_2$ ; 30% diethyl ether/hexanes) to give the captioned compound (486.6 mg, 81%) as a colourless

30 oil.  $[\alpha]_{\text{D}}^{20} + 198.7^\circ$  (c 0.105  $\text{CHCl}_3$ );  $\nu_{\text{max}}$  (film): 2924, 2854, 1460, 1376, 1066, 1024, 722  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  8.80 (1H, br s, NH), 6.71 (1H, m, H-5'), 6.04 (2H, m, H-3' & H-4'), 5.39 (1H, s, H-12), 4.47 (1H, d,  $J = 10.8$  Hz), 2.58 (1H, m), 2.50-2.10 (2H, m), 2.10-

35 1.95 (1H, m), 1.93 (1H, m), 1.80-1.68 (2H, m), 1.68-1.15 (7H, m), 1.15-0.80 (4H, m), 0.93 (3H, d,  $J = 7.1$  Hz, 6-methyl) ppm;  $^{13}\text{C}$  NMR (76 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  129.9,

-38-

117.6, 107.2, 106.7, 104.1, 91.9, 80.5, 71.9, 60.2, 51.8, 45.7, 37.2, 36.2, 34.0, 32.9, 25.9, 24.6, 21.2, 20.1, 14.0, 13.9 ppm; MS (CI, butane) m/e 334 ( $M^+ + 1$ , 100). Anal. Calcd. for  $C_{19}H_{27}NO_4$ : C, 68.44, H, 8.16, N, 4.20; Found: C, 68.77, H, 8.56, N, 3.85.

### Example 11

#### Preparation of 10 $\alpha$ -(4'-Benzyl-4'-methylpiperazin-1'-yl)-10-deoxo-10-dihydroartemisinin Iodide Salt

10 (Formula I: Y = 4'-benzyl-4'-methylpiperazin-1'-yl)  
A solution of 10 $\alpha$ -(4'-benzylpiperazin-1'-yl)-10-deoxo-10-dihydroartemisinin (272 mg, 0.62 mmol) prepared as described in Example 8 above in a mixture of dichloromethane (1.8 mL) and diethyl ether (5.4 mL)  
15 under nitrogen atmosphere at 0 °C was treated dropwise with iodomethane (36.7  $\mu$ l, 0.59 mmol). The mixture was agitated and allowed to warm to 20 °C gradually overnight. The precipitate was collected and washed with diethyl ether (2 x 5 mL) and dried in high vacuum.  
20 It was further purified by recrystallization from methanol/diethyl ether to yield rectangular-plate shaped crystals (87 mg, 24%). M.p. 159-161 °C;  $[\alpha]_D^{20} + 18.4^\circ$  (c 0.436  $CHCl_3$ );  $\nu_{max}$  (film): 3448, 2928, 2196, 1457, 1378, 1210, 1133, 1099, 1041, 982, 918, 880,  
25 852, 828, 766, 732, 642  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta_H$  8.00-7.60 (2H, d, J = 6.2 Hz, H-2" & H-6"), 7.60-7.35 (3H, m, Ar-H), 5.32 (1H, s, H-12), 5.25-5.05 (2H, m, benzylic-H), 4.13 (1H, d, J = 10.2 Hz, H-10), 3.95-3.55 (4H, m), 3.55-2.90 (9H, m), 2.65-2.20 (2H, m), 2.20-  
30 1.15 (14H, m), 1.15-0.87 (4H, m), 0.80 (3H, d, J = 6.9 Hz, 6-methyl) ppm;  $^{13}C$  NMR (76 MHz,  $CDCl_3$ )  $\delta_C$  133.4, 130.6, 129.1, 126.5, 104.0, 91.5, 90.1, 80.1, 67.4, 59.5, 59.3, 51.5, 45.5, 37.2, 36.1, 34.0, 28.4, 25.9, 24.5, 21.5, 20.1, 13.3 ppm

35

#### Examples 12 to 61

By processes similar to those described in Examples 1

to 11 above, further compounds according to the invention were prepared as detailed in Table I below. In this table, the compounds are identified by reference to formula I.

Table I

| Ex. No. | Y                                                                            | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------|------------------------------------------------------------------------------|----------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 12      | 2,4,6-(-OCH <sub>3</sub> ) <sub>3</sub><br>phenyl<br>(mixture of<br>isomers) | -              | -              | White foam. $[\alpha]_D^{20} +49.51^\circ$ (c. 0.053 in CHCl <sub>3</sub> ); $\nu_{\max}$ (Neat): 2936, 2872, 1608, 1496, 1456, 1420, 1374, 1330, 1278, 1224, 1204, 1152, 1120, 1050, 1040, 974, 954, 930, 902, 880, 856, 834, 814, 734, 702 cm <sup>-1</sup> ; $\delta_H$ 6.10-6.18 (2H, m, Ar-H), 5.46 (1H, s, H12), 5.38 (1H, s, H-12'), 5.31 (1H, d, J = 10.4 Hz, H-10), 5.07 (1H, d, J = 10.9 Hz, H-10'), 3.88, 3.81, 3.80, 3.76 (OMe), 3.36-3.42 (1H, m), 2.35-2.41 (1H, m), 1.05-2.15 (10H, m), 1.63 (3H, s, H-14), 1.39 (3H, s, H-14'), 0.99 (3H, d, J = 6.27 Hz, H-16'), 0.88-0.93 (3H, m, H-15 & H-16), 0.58 (3H, d, J = 7.26 Hz, H-15'); m/z (CI, NH <sub>3</sub> ) 452 (M+NH <sub>4</sub> <sup>+</sup> , 4%), 436 (16), 435 (M <sup>+</sup> +1, 12), 419 (100), 389 (74), 347 (28). Anal. Calc. for C <sub>24</sub> H <sub>34</sub> O <sub>7</sub> : C, 66.36; H, 7.83; Found: C, 66.42; H, 7.89                    |
| 13      | 2-naphthyl<br>(10 $\beta$ -isomer)                                           | -              | -              | White solid. M.p. 145-146°C; $[\alpha]_D^{20}$ : -67.8° (c 0.027/CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 2950, 2874, 1510, 1452, 1376, 1208, 1106, 1074, 1040, 1010, 954, 936, 886, 854, 824, 786, 750; $\delta_H$ 7.80-7.85 (5H, m, Ar-H), 7.42-7.51 (3H, m, Ar-H), 5.93 (1H, d, J = 6.59 Hz, H-10), 5.67 (1H, s, H-12), 2.81-2.94 (1H, m, H-9), 2.33-2.48 (1H, m), 0.86-2.13 (10H, m), 1.42 (3H, s, H-14), 1.02 (1H, d, J = 6.09 Hz, H-16), 0.55 (1H, d, J = 7.66 Hz, H-15); $\delta_C$ 134.85, 127.82, 127.42, 127.12, 125.65, 125.17, 124.84, 124.26, 90.92, 73.04, 51.48, 43.44, 37.48, 36.64, 34.15, 32.10, 25.69, 24.89, 24.77, 19.85, 13.65; m/z (CI, CH <sub>4</sub> ) 395 (M <sup>+</sup> +1, 16%), 394 (M <sup>+</sup> , 32), 362 (44), 349 (84), 331 (16), 304 (20), 291 (26), 182 (100), 168 (60). Anal. Calcd. for C <sub>25</sub> H <sub>30</sub> O <sub>4</sub> : C, 76.11; H, 7.66; found: C, 76.24; H, 7.69. |

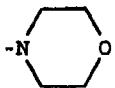
| Ex. NO. | Y                                               | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|---------|-------------------------------------------------|----------------|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 14      | 2-OCH <sub>3</sub> phenyl<br>(10β-isomer)       | -              | -              | Colourless oil. δ <sub>H</sub> 6.83-7.50 (4H, m, Ar-H), 5.94 (1H, d, J = 6.65 Hz, H-10), 5.58 (1H, s, H-12), 3.84 (3H, s, OCH <sub>3</sub> ), 2.86-2.99 (1H, m, H-9), 2.30-2.40 (1H, m), 1.19-2.11 (10H, m), 1.39 (3H, s, H-14), 1.01 (1H, d, J = 5.77 Hz, H-16), 0.43 (1H, d, J = 7.64 Hz, H-15); δ <sub>C</sub> 134.85, 127.00, 126.37, 120.02, 109.19, 90.86, 68.63, 55.19, 51.30, 43.39, 37.53, 36.72, 34.21, 29.87, 25.68, 24.97, 24.75, 19.83, 13.45; m/z (CI, CH <sub>4</sub> ) 375 (M <sup>+</sup> +1, 12%), 374 (M <sup>+</sup> , 16), 342 (100), 329 (48), 311 (14), 284 (28), 182 (56), 148 (76), 137 (60), 121 (48). Anal. Calc. for C <sub>22</sub> H <sub>20</sub> O <sub>5</sub> : C, 70.56; H, 8.07; Found C, 70.78; H, 8.28                                                                                                                                                                                                                                                                                                                                                         |
| 15      | -NR <sup>1</sup> R <sup>2</sup><br>(10α-isomer) | -H             | phenyl         | White solid. M.p. 159-160°C; [α] <sub>D</sub> <sup>20</sup> -51.4° (c 0.35/CHCl <sub>3</sub> ); ν <sub>max</sub> (film) 3348, 2924, 2872, 1604, 1502, 1444, 1376, 1314, 1270, 1196, 1152, 1116, 1098, 1040, 1012, 994, 944, 926, 878, 856, 826, 748, 690; δ <sub>H</sub> 7.17-7.22 (2H, m, Ar-H), 6.75-6.87 (3H, m, Ar-H), 5.45 (1H, s, H-12), 4.85 (1H, dd, J = 9.86, 9.81 Hz, H-10), 4.32 (1H, d, J = 9.81 Hz, NH), 2.49-2.61 (1H, m, H-9), 2.35-2.45 (1H, m), 2.00-2.08 (1H, m), 1.74-1.92 (4H, m), 1.26-1.65 (7H, m), 1.42 (3H, s, H-14), 1.05-1.10 (1H, m), 1.01 (3H, d, J = 6.18 Hz, H-16), 0.95 (3H, d, J = 7.18 Hz, H-15); δ <sub>C</sub> 128.99, 118.56, 114.02, 91.08, 80.70, 80.39, 51.71, 45.76, 37.18, 36.26, 34.03, 32.71, 25.97, 24.60, 21.79, 20.17, 13.80; m/z (CI, CH <sub>4</sub> ) 360 (M <sup>+</sup> +1, 56%), 359 (M <sup>+</sup> , 56), 342 (98), 324 (20), 314 (100), 296 (98), 267 (50), 249 (22), 221 (80), 163 (40), 133 (100), 94 (38). Anal. Calc. for C <sub>21</sub> H <sub>20</sub> NO <sub>4</sub> : C, 70.17; H, 8.13; N, 3.90; Found: C, 70.25; H, 8.24; N, 3.73 |

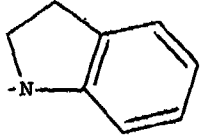
| Ex. No. | Y                                                        | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---------|----------------------------------------------------------|----------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 16      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 4-F phenyl     | White solid. M.p. 170.1°C; $\nu_{\max}$ (Nujol): 3358 ( $\nu_{\text{NH}}$ ), 2924, 2854, 1512, 1460, 1378, 1264, 1216, 1194, 1116, 1099, 1046, 1022, 942, 924, 880, 846, 832, 810, 780 cm <sup>-1</sup> ; $\delta_{\text{H}}$ 6.66-6.92 (4H, m, Ar-H), 5.44 (1H, s, H-12), 4.76 (1H, dd, J = 10.0, 10.0 Hz, H-10), 4.32 (1H, d, J = 10.0 Hz, NH), 2.49-2.61 (1H, m, H-9), 2.40 (1H, ddd, J = 17.3, 13.4, 3.93 Hz), 2.05 (1H, ddd, J = 14.6, 4.79, 3.07 Hz), 1.05-1.97 (9H, m), 1.42 (3H, s, H-14), 1.00 (3H, d, J = 6.11 Hz, H-16), 0.95 (3H, d, J = 7.18 Hz, H-15); $\delta_{\text{C}}$ 141.95, 115.34 (d, J = 17.7 Hz), 115.15 (d, J = 2.69 Hz), 104.13, 91.10 (d, J = 2.22 Hz), 81.41, 80.41, 51.69, 45.73, 37.29, 36.25, 34.01, 32.60, 25.94, 24.60, 21.79, 20.17, 13.81; m/z (CI; CH <sub>4</sub> ) 378 (M <sup>+</sup> +1, 44%), 377 (M <sup>+</sup> , 100), 358 (70), 314 (14), 267 (26), 221 (18), 163 (34), 151 (42), 111 (6). Anal. Calc. for C <sub>21</sub> H <sub>28</sub> FNO <sub>4</sub> : C, 66.82; H, 7.48; N, 3.71; Found C, 67.06; H, 7.60; N, 3.51 |
| 17      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 4-Cl phenyl    | White solid. M.p. 179.0°C; $[\alpha]_{\text{D}}^{20}$ -63.5° (c 0.20/CHCl <sub>3</sub> ); $\nu_{\max}$ (film): 3346, 2926, 2874, 1604, 1514, 1494, 1454, 1378, 1268, 1196, 1152, 1094, 1040, 1012, 992, 944, 926, 878, 818, 756; $\delta_{\text{H}}$ 7.09-7.14 (2H, m, Ar-H), 6.66-6.71 (2H, m, Ar-H), 5.44 (1H, s, H-12), 4.78 (1H, brs, H-10), 4.42 (1H, brs, NH), 2.49-2.61 (1H, m, H-9), 2.40 (1H, ddd, J = 17.4, 13.5, 3.98 Hz), 2.05 (1H, ddd, J = 14.6, 4.78, 3.12 Hz), 1.05-1.97 (9H, m), 1.41 (3H, s, H-14), 1.00 (3H, d, J = 6.12 Hz, H-16), 0.94 (3H, d, J = 7.18 Hz, H-15); $\delta_{\text{C}}$ 144.31, 128.76, 123.20, 115.28, 104.15, 91.09, 80.76, 80.38, 51.66, 45.67, 37.28, 36.23, 33.99, 32.56, 25.93, 24.59, 21.78, 20.16, 13.73; m/z (CI, CH <sub>4</sub> ) 393 (M <sup>+</sup> +1, 16%), 376 (8), 347 (20), 330 (16), 267 (10), 221 (16), 167 (100), 127 (8). Anal. Calc. for C <sub>21</sub> H <sub>28</sub> ClNO <sub>4</sub> : C, 64.03; H, 7.16; N, 3.55; Found C, 64.16; H, 7.40; N, 3.45.                                                   |

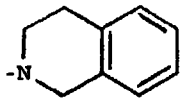
| Ex. No. | Y                                                        | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---------|----------------------------------------------------------|----------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 18      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 4-Br phenyl    | White solid. M.p. 183.1°C; $[\alpha]_D^{20}$ -60.0° (c 0.23/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 3346, 2924, 2872, 1598, 1514, 1492, 1452, 1378, 1268, 1196, 1152, 1122, 1094, 1040, 1012, 992, 926, 878, 816, 756; $\delta_H$ 7.20-7.25 (2H, m, Ar-H), 6.61-6.66 (2H, m, Ar-H), 5.44 (1H, s, H-12), 4.78 (1H, dd, J = 10.0, 9.95 Hz, H-10), 4.48 (1H, d, J = 10.0 Hz, NH), 2.49-2.61 (1H, m, H-9), 2.40 (1H, ddd, J = 14.0, 13.7, 3.87 Hz), 1.05-2.08 (10H, m), 1.41 (3H, s, H-14), 1.00 (3H, d, J = 6.07 Hz, H-16), 0.94 (3H, d, J = 7.15 Hz, H-15); $\delta_C$ 144.79, 131.61, 115.76, 110.32, 104.17, 91.09, 80.65, 80.39, 51.67, 45.67, 37.29, 36.24, 33.99, 32.54, 25.92, 24.60, 21.78, 20.17, 13.71; m/z (CI, CH <sub>4</sub> ) 439 (M <sup>+</sup> +1, 12%), 422 (14), 392 (100), 376 (36), 267 (14), 221 (50), 154 (34). Anal. Calc. for C <sub>21</sub> H <sub>28</sub> BrNO <sub>4</sub> : C, 57.54; H, 6.44; N, 3.19; Found: C, 57.81; H, 6.64; N, 3.14. |
| 19      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 4-I phenyl     | White solid. $[\alpha]_D^{20}$ -68.8° (c 0.16/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 3346, 2924, 1592, 1510, 1454, 1378, 1268, 1196, 1040, 994, 926, 878, 818, 754; $\delta_H$ 7.36-7.41 (2H, m, Ar-H), 6.51-6.56 (2H, m, Ar-H), 5.43 (1H, s, H-12), 4.78 (1H, dd, J = 10.0, 9.97 Hz, H-10), 4.56 (1H, d, J = 10.0 Hz, NH), 2.34-2.56 (2H, m, H-9), 1.05-2.08 (10H, m), 1.41 (3H, s, H-14), 1.00 (3H, d, J = 6.04 Hz, H-16), 0.93 (3H, d, J = 7.13 Hz, H-15); $\delta_C$ 145.46, 137.45, 116.35, 104.18, 91.09, 80.46, 79.59, 51.66, 45.66, 37.29, 36.25, 34.00, 32.50, 25.91, 24.61, 21.79, 20.19, 13.71; m/z (CI, CH <sub>4</sub> ) 486 (M <sup>+</sup> +1, 4%), 485 (M <sup>+</sup> , 6), 468 (12), 440 (100), 422 (34), 267 (6), 259 (20), 221 (20). Anal. Calc. for C <sub>21</sub> H <sub>28</sub> INO <sub>4</sub> : C, 51.97; H, 5.81; N, 2.89; Found: C, 52.22; H, 5.83; N, 2.57.                                                                             |

| Ex. No. | Y                                                        | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------|----------------------------------------------------------|----------------|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 20      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 4-biphenyl     | White solid. $[\alpha]_D^{20}$ -76.5° (c 0.51/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 3348, 2924, 2872, 1614, 1528, 1488, 1446, 1378, 1268, 1268, 1196, 1152, 1128, 1040, 1012, 992, 926, 878, 826, 760, 698; $\delta_H$ 7.25-7.58 (7H, m, Ar-H), 6.82-6.87 (2H, m, Ar-H), 5.49 (1H, s, H-12), 4.90 (1H, dd, J = 9.85, 9.85 Hz, H-10), 4.50 (1H, d, J = 9.85 Hz, NH), 2.36-2.62 (2H, m), 1.07-2.09 (10H, m), 1.44 (3H, s, H-14), 1.02 (3H, d, J = 6.12 Hz, H-16), 0.98 (3H, d, J = 7.17 Hz, H-15); $\delta_C$ 145.22, 141.16, 131.53, 128.45, 127.71, 126.29, 125.98, 114.30, 104.14, 91.14, 80.67, 80.42, 51.72, 45.76, 37.30, 36.27, 34.04, 21.68, 25.98, 24.62, 21.81, 20.19, 13.81; m/z (CI, CH <sub>4</sub> ) : 436 (M <sup>+</sup> +1, 2%), 412 (100), 395 (42), 379 (8), 284 (2), 267 (2), 170 (2). Anal.Calc. for C <sub>27</sub> H <sub>33</sub> NO <sub>4</sub> : C, 74.45; H, 7.64; N, 3.22; Found: C, 73.51; H, 7.67; N, 3.12.                                                                                 |
| 21      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | benzyl         | White solid. $[\alpha]_D^{20}$ -26.7° (c 0.15/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 3348, 2924, 2870, 1452, 1376, 1158, 1116, 1056, 1042, 1014, 992, 942, 926, 878, 828, 736, 700; $\delta_H$ 7.21-7.42 (5H, m, Ar-H), 5.32 (1H, s, H-12), 4.17 (1H, d, J = 13.9 Hz, H-1'), 4.13 (1H, d, J = 9.63 Hz, H-10), 3.97 (1H, d, J = 13.9 Hz, H-1'), 2.29-2.45 (2H, m), 2.29 (1H, brs, NH), 2.01-2.09 (1H, m), 1.86-1.95 (1H, m), 1.65-1.78 (2H, m), 1.44-1.59 (2H, m), 1.48 (3H, s, H-14), 1.22-1.40 (3H, m), 0.91-1.09 (1H, m), 0.97 (3H, d, J = 5.94 Hz, H-16), 0.96 (3H, d, J = 7.14 Hz, H-15); $\delta_C$ 140.82, 128.03, 127.84, 126.45, 103.91, 91.39, 85.68, 80.65, 51.69, 48.21, 45.86, 37.24, 36.29, 34.07, 32.77, 26.07, 24.63, 21.73, 30.19, 14.12; m/z (CI, CH <sub>4</sub> ) 374 (M <sup>+</sup> +1, 100%), 356 (54), 338 (42), 328 (38), 309 (12), 253 (16), 221 (10), 119 (16). Anal.Calc. for C <sub>22</sub> H <sub>31</sub> NO <sub>4</sub> : C, 70.75; H, 8.37; N, 3.75; Found: C, 70.78; H, 8.82; N, 3.75. |

| Ex. No. | Y                                                        | R <sup>1</sup> | R <sup>2</sup>                             | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|---------|----------------------------------------------------------|----------------|--------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 22      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 2-F benzyl                                 | White solid. M.p. 47.4-48.7°C, $[\alpha]_D^{20}$ -16.9° (c 1.46/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 3336, 2924, 2872, 1584, 1486, 1454, 1376, 1226, 1196, 1158, 1116, 1056, 1042, 1014, 994, 926, 878, 826, 756; $\delta_H$ 6.99-7.50 (4H, m, Ar-H), 5.34 (1H, s, H-12), 4.21 (1H, d, J = 14.5 Hz, H-1'), 4.15 (1H, d, J = 6.72 Hz, H-10), 3.99 (1H, d, J = 14.5 Hz, H-1'), 2.35-2.45 (2H, m), 0.90-2.08 (10H, m), 1.47 (3H, s, H-14), 0.98 (3H, d, J = 5.99 Hz, H-16), 0.94 (3H, d, J = 7.16 Hz, H-15); $\delta_C$ 129.63 (d, J = 4.79 Hz), 127.94 (d, J = 8.05 Hz), 123.64 (d, J = 3.40 Hz), 114.89 (d, J = 21.6 Hz), 103.90, 91.35, 86.03, 80.59, 51.69, 45.86, 41.96 (d, J = 3.53 Hz), 37.26, 36.28, 34.08, 32.66, 26.02, 24.62, 21.72, 20.17, 14.00; $\delta_F$ -120; m/z (CI, CH <sub>4</sub> ) 392 (M <sup>+</sup> +1, 24%), 374 (46), 346 (100), 328 (34), 267 (2), 221 (4), 209 (6), 165 (82), 154 (50), 109 (42). Anal. Calcd. for C <sub>22</sub> H <sub>30</sub> NO <sub>4</sub> F: C, 67.50; H, 7.72; N, 3.58; found C, 67.75, H, 7.92; N, 3.49. |
| 23      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 3,5-(CF <sub>3</sub> ) <sub>2</sub> benzyl | Colourless oil. M.p. 51.0-52.8°C; $[\alpha]_D^{20}$ -27° (c 0.027 CHCl <sub>3</sub> ); $\delta_H$ 7.88 (2H, brs, Ar-H), 7.56 (1H, brs, Ar-H), 5.31 (1H, s, H-12), 4.24 (1H, d, J = 15.1 Hz, H-1'), 4.12 (1H, d, J = 15.1 Hz, H-1'), 4.06 (1H, d, J = 9.82 Hz, H-10), 2.34-2.45 (2H, m), 0.90-2.09 (10H, m), 1.47 (3H, s, H-14), 0.98 (3H, d, J = 7.26 Hz, H-15), 0.97 (3H, d, J = 4.94 Hz, H-16); $\delta_F$ -64.1; m/z (CI, CH <sub>4</sub> ) 510 (M <sup>+</sup> +1, 48%), 490 (100), 464 (74), 441 (38), 283 (24), 267 (30), 244 (10), 221 (20), 163 (22). Anal. Calc. for C <sub>24</sub> H <sub>30</sub> NO <sub>4</sub> F <sub>6</sub> : C, 56.58; H, 5.74; N, 2.75; Found: C, 56.75; H, 5.76; N, 2.70.                                                                                                                                                                                                                                                                                                                                                     |

| Ex. No. | Y                                                                                                                         | R <sup>1</sup> | R <sup>2</sup>                               | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|---------|---------------------------------------------------------------------------------------------------------------------------|----------------|----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 24      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer)                                                                  | -H             | - <sup>n</sup> C <sub>3</sub> H <sub>7</sub> | White solid. M.p. 96.1-97.3°C (changed colour before melting); [ $\alpha$ ] <sub>D</sub> <sup>20</sup> +24.8° (c 0.33/CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 3304, 2952, 2924, 2870, 1492, 1454, 1378, 1208, 1160, 1118, 1042, 1012, 974, 942, 922, 878, 844, 828, 754; $\delta_H$ 5.31 (1H, s, H-12), 4.11 (1H, d, J = 9.78 Hz, H-10), 2.95 (1H, ddd, J = 11.4, 8.07, 6.55 Hz, CH <sub>2</sub> NH), 2.61 (1H, ddd, J = 11.4, 8.07, 6.45 Hz, CH <sub>2</sub> NH), 2.26-2.43 (2H, m), 2.03 (1H, ddd, J = 14.5, 4.54, 2.49 Hz), 1.84-1.93 (1H, m), 1.00-1.83 (10H, m), 1.44 (3H, s, H-14), 0.97 (3H, d, J = 6.10 Hz, H-16), 0.92 (3H, t, J = 7.36 Hz, CH <sub>3</sub> ), 0.89 (3H, d, J = 7.18 Hz, H-15); $\delta_C$ 103.82, 91.34, 86.17, 51.69, 46.27, 45.88, 37.27, 36.27, 34.10, 32.49, 26.04, 24.61, 23.49, 21.72, 20.19, 14.03, 11.62; m/z (CI, CH <sub>4</sub> ) 326 (M <sup>+</sup> +1, 100%), 308 (56), 280 (48), 221 (16), 163 (18). Anal. Calcd. for C <sub>13</sub> H <sub>11</sub> NO: C, 66.43; H, 9.60; N, 4.36; Found: C, 66.17, H, 9.68; N, 4.20. |
| 25      | morpholino<br><br>(10 $\alpha$ -isomer) | -              | -                                            | White solid. M.p. 121.2°C; [ $\alpha$ ] <sub>D</sub> <sup>20</sup> +15.3° (c 0.30/CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 2924, 2850, 1450, 1376, 1294, 1258, 1202, 1158, 1110, 1056, 984, 930, 880, 846, 826, 744; $\delta_H$ 5.29 (1H, s, H-12), 3.99 (1H, d, J = 10.23 Hz, H-10), 3.63-3.76 (4H, m, O(CH <sub>2</sub> ) <sub>2</sub> ), 2.96-3.03 (2H, m, CH <sub>2</sub> NCH <sub>2</sub> ), 2.64-2.71 (2H, m, CH <sub>2</sub> NCH <sub>2</sub> ), 2.53-2.61 (1H, m, H-9), 2.31-2.41 (1H, m), 1.00-2.06 (10H, m), 1.41 (3H, s, H-14), 0.96 (3H, d, J = 6.14 Hz, H-16), 0.83 (3H, d, J = 7.18 Hz, H-15); $\delta_C$ 103.74, 91.48, 90.51, 80.16, 67.25, 51.57, 47.52, 45.66, 37.25, 36.16, 34.14, 28.04, 25.84, 24.62, 21.50, 20.14, 13.25; m/z (EI) 353 (M <sup>+</sup> , 6), 294 (4), 236 (4), 221 (16), 209 (12), 163 (14), 127 (32), 116 (100), 88 (24). Anal. Calcd. for C <sub>13</sub> H <sub>11</sub> NO: C, 64.56; H, 8.84; N, 3.96%; Found: C, 64.67; H, 9.10; N, 3.90.                                                                              |

| Ex. No. | Y                                                                                                                       | R <sup>1</sup>                 | R <sup>2</sup>                 | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|---------|-------------------------------------------------------------------------------------------------------------------------|--------------------------------|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 26      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer)                                                                | -C <sub>2</sub> H <sub>5</sub> | -C <sub>2</sub> H <sub>5</sub> | White solid. $\delta_H$ 5.37 (1H, s, H-12), 4.76 (d, J = 7.54 Hz, H-10), 2.80-3.03 (4H, m, N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> ), 2.29-2.44 (1H, m, H-9), 0.94-1.89 (11H, m), 1.53 (3H, s, H-14), 1.14 (6H, dd, J = 7.20, 7.12 Hz, N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> ), 1.04 (3H, d, J = 7.23 Hz, H-15), 0.91 (3H, d, J = 5.72 Hz, H-16); $\delta_C$ 105.33, 96.11, 81.43, 51.68, 45.23, 41.48, 35.12, 34.52, 33.96, 29.56, 23.77, 22.24, 21.88, 18.52, 15.56, 11.50. m/z (CI, CH <sub>4</sub> ) : 340 (M <sup>+</sup> +1, 52%), 251 (100), 221 (26).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 27      | indolinyl<br><br>(10 $\alpha$ -isomer) | -                              | -                              | White solid. M.p. 147.8-148.2°C; $[\alpha]_D^{20}$ -11.6° (c 0.19/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 2926, 2872, 1606, 1488, 1462, 1376, 1258, 1200, 1158, 1126, 1040, 1010, 926, 880, 828, 746, 718; $\delta_H$ 7.03-7.09 (2H, m, Ar-H), 6.60-6.71 (2H, m, Ar-H), 5.44 (1H, s, H-12), 4.98 (1H, d, J = 10.4 Hz, H-10), 3.79 (1H, apparent dt, J = 10.4, 9.08 Hz, ArCH <sub>2</sub> ), 3.56 (1H, apparent dt, J = 9.08, 4.36 Hz, ArCH <sub>2</sub> ), 2.94-3.12 (2H, m, NCH <sub>2</sub> ), 2.67-2.79 (1H, m, H-9), 2.39 (1H, ddd, J = 14.3, 13.3, 3.94 Hz), 2.03 (1H, ddd, J = 14.5, 4.73, 2.97 Hz), 1.75-1.95 (4H, m), 1.06-1.69 (6H, m), 1.38 (3H, s, H-14), 1.00 (3H, d, J = 6.17 Hz, H-16), 0.94 (3H, d, J = 7.16 Hz, H-15); $\delta_C$ 150.51, 130.18, 126.92, 124.57, 118.19, 107.47, 103.90, 91.46, 81.53, 80.05, 51.58, 45.61, 44.83, 37.28, 36.20, 34.12, 29.75, 27.99, 25.83, 24.61, 21.51, 20.16, 13.30; m/z (CI, CH <sub>4</sub> ) 386 (M <sup>+</sup> +1, 100), 340 (50), 326 (14), 267 (6). Anal. Calcd. for C <sub>23</sub> H <sub>21</sub> NO <sub>4</sub> ; C, 71.66; H, 8.10; N, 3.63; Found: C, 71.45; H, 8.07; N, 3.57. |

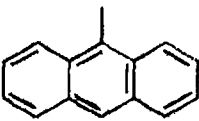
| Ex. No. | Y                                                                                                                                                          | R <sup>1</sup> | R <sup>2</sup>              | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------|------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 28      | 1,2,3,4-tetrahydro-<br>isoquinoliny1<br><br><br><br>(10 $\alpha$ -isomer) | -              | -                           | White solid. M.p. 125.3-126.6°C; $[\alpha]_D^{20}$ +14.7° (c 0.19/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 2924, 2870, 1452, 1376, 1278, 1200, 1154, 1130, 1100, 1040, 1014, 982, 926, 880, 826, 742; $\delta_H$ 7.07-7.15 (4H, m, Ar-H), 5.36 (1H, s, H-12), 4.26 (1H, d, J = 10.2 Hz, H-10), 4.20 (1H, d, J = 15.2 Hz, ArCH <sub>2</sub> N), 3.97 (1H, d, J = 15.2 Hz, ArCH <sub>2</sub> N), 3.26-3.36 (1H, m, ArCH <sub>2</sub> ), 2.70-3.00 (4H, m, 3H-CH <sub>2</sub> CH <sub>2</sub> N & 1H), 2.40 (1H, ddd, J = 14.4, 13.6, 3.93 Hz), 2.00-2.07 (1H, m), 1.86-1.95 (1H, m), 1.71-1.81 (2H, m), 1.19-1.65 (4H, m), 1.41 (3H, s, H-14), 1.02-1.12 (1H, m), 0.99 (3H, d, J = 6.09 Hz, H-16), 0.87 (3H, d, J = 7.19 Hz, H-15); $\delta_C$ 135.74, 134.94, 128.55, 126.59, 125.45, 125.21, 103.76, 91.61, 90.60, 80.31, 51.64, 49.18, 45.82, 45.65, 37.29, 36.21, 34.20, 29.89, 28.66, 25.88, 24.67, 21.53, 20.19, 13.46; m/z (CI, CH <sub>4</sub> ) 400 (M <sup>+</sup> +1, 100), 398 (22), 354 (54), 340 (20), 267 (4), 162 (44), 134 (14). Anal. Calc. for C <sub>24</sub> H <sub>23</sub> NO <sub>4</sub> : C, 72.15; H, 8.33; N, 3.51; Found: C, 71.98; H, 8.36; N, 3.36. |
| 29      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ ,1'-S-isomer)                                                                                              | -H             | -CH(CH <sub>3</sub> )phenyl | White solid. M.p. 55.4-57.5°C; $\delta_H$ 7.20-7.42 (5H, m, Ar-H), 5.13 (1H, s, H-12), 4.45 (1H, q, J = 6.62 Hz, H-1'), 3.77 (1H, d, J = 9.79 Hz, H-10), 2.23-2.43 (2H, m), 2.03 (1H, ddd, J = 14.5, 4.73, 3.08 Hz), 0.96-1.88 (9H, m), 1.48 (3H, s, H-14), 1.31 (3H, d, J = 6.62 Hz, CH <sub>3</sub> ), 0.91 (3H, d, J = 5.94 Hz, H-16), 0.91 (3H, d, J = 7.14 Hz, H-15); $\delta_C$ 146.07, 128.01, 126.96, 126.39, 103.81, 91.38, 83.80, 80.70, 52.37, 51.71, 45.82, 37.08, 36.30, 33.95, 33.40, 26.11, 25.53, 24.57, 21.64, 20.14, 14.22; m/z (CI, CH <sub>4</sub> ) 388 (M <sup>+</sup> +1, 100%), 370 (22), 342 (64), 267 (8), 221 (10). Anal. Calc. for C <sub>23</sub> H <sub>23</sub> NO <sub>4</sub> : C, 71.29; H, 8.58; N, 3.61; Found C, 71.20; H, 8.72; N, 3.62.                                                                                                                                                                                                                                                                                                                                                                                                  |

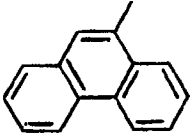
| Ex. No. | Y                                                            | R <sup>1</sup> | R <sup>2</sup>                  | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|---------|--------------------------------------------------------------|----------------|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 30      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ ,1'R-isomer) | -H             | -CH(CH <sub>3</sub> )phenyl     | White solid. $\delta_H$ 7.20-7.43 (5H, m, Ar-H), 5.36 (1H, s, H-12), 4.44 (1H, q, J = 6.41 Hz, H-1'), 4.31 (1H, d, J = 9.70 Hz, H-10), 2.21-2.40 (2H, m), 2.00-2.08 (1H, m), 1.02-1.95 (9H, m), 1.45 (3H, s, H-14), 1.31 (3H, d, J = 6.41 Hz, CH <sub>3</sub> ), 0.99 (3H, d, J = 6.18 Hz, H-16), 0.93 (3H, d, J = 7.16 Hz, H-15); $\delta_C$ 147.01, 128.06, 127.01, 126.59, 103.85, 91.33, 83.03, 80.56, 51.72, 51.51, 45.92, 37.29, 36.28, 34.14, 33.09, 26.01, 24.66, 22.35, 21.83, 20.21, 14.22; m/z (CI, CH <sub>4</sub> ) 388 (M <sup>+</sup> +1, 100%), 370 (56), 342 (42), 309 (30), 267 (18), 253 (32), 221 (20), 119 (34).                                                                                                                                                                                                                                        |
| 31      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ ,1'R-isomer) | -H             | -CH(CO-OCH <sub>3</sub> )phenyl | Colourless oil. $\nu_{max}$ (Neat): 3342 ( $\nu_{NH}$ ), 2926, 2827, 1742 ( $\nu_{C=O}$ , ester), 1602, 1494, 1452, 1376, 1246, 1198, 1158, 1130, 1042, 1014, 994, 928, 880, 844, 826, 736, 700 cm <sup>-1</sup> ; <sup>1</sup> H nmr (300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 7.21-7.50 (5H, m, Ar-H), 5.13 (1H, s, H-12), 4.94 (1H, s, H-2'), 3.85 (1H, d, J 9.81 Hz, H-10), 3.65 (3H, s, OMe), 2.74 (1H, br.s., NH), 2.35 (1H, m, H-9), 1.05-2.07 (11H, m, H-2 x 2, H-3 x 2, H-4, H-5, H-6 x 2, H-7 x 2, H-8), 1.47 (3H, s, 1-CH <sub>3</sub> ), 0.90 (6H, d x 2, signals overlap, 5-CH <sub>3</sub> , 9-CH <sub>3</sub> ) ppm; m/z (CI, CH <sub>4</sub> ) 432 (M <sup>+</sup> +1, 96%), 386 (100), 372 (44), 312 (14), 267 (24), 221 (28), 166 (96). Anal. Calcd. for C <sub>24</sub> H <sub>31</sub> NO: C, 66.80; H, 7.71; N, 3.24; Found: C, 66.98; H, 7.53; N, 3.05. |
| 32      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ ,1'S-isomer) | -H             | -CH(CO-OCH <sub>3</sub> )phenyl | <sup>1</sup> H nmr (300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 7.28-7.51 (5H, m, Ar-H), 5.22 (1H, s, H-12), 5.04 (1H, s, H-2'), 4.28 (1H, d, J 9.81 Hz, H-10), 3.68 (3H, s, OMe), 2.57 (1H, br.s., NH), 2.29-2.44 (1H, m, H-9), 0.96-2.44 (11H, m, H-2 x 2, H-3 x 2, H-4, H-5, H-6 x 2, H-7 x 2, H-8), 1.43 (3H, s, 1-CH <sub>3</sub> ), 0.97 (3H, d, J 6.11 Hz, H-15), 0.90 (3H, d, J 7.16 Hz, H-16) ppm; m/z (CI, CH <sub>4</sub> ) 432 (M <sup>+</sup> +1, 90%), 386 (100), 372 (50).                                                                                                                                                                                                                                                                                                                                                                                        |

| Ex. No. | Y                                                        | R <sup>1</sup> | R <sup>2</sup>                 | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|---------|----------------------------------------------------------|----------------|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 33      | -NR <sup>1</sup> R <sup>2</sup><br>(10 $\alpha$ -isomer) | -H             | 4-(CO-OCH <sub>3</sub> )phenyl | White solid. M.p. 117.7-118.5°C; [ $\alpha$ ] <sub>D</sub> <sup>20</sup> -84.1 (c 0.82/CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 3344, 2948, 1710, 1608, 1528, 1434, 1378, 1270, 1178, 1110, 1040, 1012, 926, 878, 842, 768; $\delta_H$ 6.66-7.76 (4H, m, Ar-H), 5.46 (1H, s, H-12), 5.06 (1H, d, J=9.96 Hz, NH), 4.88 (1H, dd, J=9.89, Hz, H-10), 3.83 (3H, s, OMe), 2.56-2.60 (1H, m), 2.33-2.42 (1H, m), 0.85-2.04 (10H, m), 1.39 (3H, s, H-14), 0.99 (3H, d, J=6.09 Hz, H-15), 0.92 (3H, d, J=7.11 Hz, H-16); $\delta_C$ : 167.06, 149.99, 131.04, 119.64, 113.01, 104.30, 91.21, 80.49, 80.02, 51.70, 51.40, 45.67, 37.34, 36.20, 34.04, 32.50, 25.92, 24.66, 21.84, 20.94, 20.22, 14.10, 13.67; m/z (CI, CH <sub>4</sub> ) 418 (M <sup>+</sup> +1, 32), 400 (6), 372 (100), 358 (8), 221 (28), 152 (26). Anal. Calc. for C <sub>23</sub> H <sub>31</sub> NO <sub>6</sub> : C, 66.17; H, 7.48; N, 3.35; Found: C, 65.57; H, 7.57; N, 3.36. |
| 34      | -NR <sup>1</sup> R <sup>2</sup><br>( $\alpha$ -isomer)   | -H             | cyclopentyl                    | White solid. M.p. 114.1-114.9°C; [ $\alpha$ ] <sub>D</sub> <sup>20</sup> -1.6° (c 0.98/CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 3314, 2950, 2870, 1446, 1376, 1198, 1154, 1114, 1098, 1042, 1014, 976, 944, 924, 878, 860, 826, 754; $\delta_H$ : 5.29 (1H, s, H-12), 4.08 (1H, d, J=9.76 Hz, H-10), 3.52-3.60 (1H, m, H-1'), 2.23-2.41 (2H, m), 1.24-2.05 (17H, m), 1.43 (3H, s, H-14), 0.84-1.11 (1H, m), 0.96 (3H, d, J=6.15 Hz, H-15), 0.85 (3H, d, J=7.17 Hz, H-16); $\delta_C$ : 103.91, 91.44, 85.08, 80.77, 54.33, 51.84, 46.01, 37.38, 36.40, 34.23, 34.20, 33.11, 32.70, 26.17, 24.74, 23.63, 21.89, 20.31, 14.30; m/z (CI, CH <sub>4</sub> ) 352 (M <sup>+</sup> +1, 14), 334 (10), 306 (100), 288 (14), 251 (4), 221 (4), 125 (10); Anal. Calc. for C <sub>20</sub> H <sub>33</sub> NO <sub>4</sub> : C, 68.34, H, 9.46; N, 3.98; Found: C, 67.89; H, 9.46; N, 3.92.                                                               |

| Ex. No. | Y                                                            | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------|--------------------------------------------------------------|----------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 35      | -NR <sup>1</sup> R <sup>2</sup><br>( $\alpha$ -isomer)       | -H             | cyclohexyl     | White solid. $\delta_H$ 5.28 (1H, s, H-12), 4.17 (1H, d, J= 9.69 Hz, H-10), 2.93-3.00 (1H, m H-1'), 2.16-2.41 (2H, m), 0.84-2.03 (19H, m), 1.42 (3H, s, H-14), 0.95 (3H, d, J=6.11 Hz, H-15), 0.85 (3H, d, J=7.18 Hz, H-16); $\delta_C$ 103.87, 91.36, 83.36, 80.70, 51.84, 50.94, 46.04, 37.36, 36.38, 34.58, 34.21, 33.18, 32.79, 26.29, 26.12, 24.75, 24.71, 24.27, 21.88, 20.29, 14.29, 14.39; m/z (CI, CH <sub>4</sub> ) 366 (M <sup>+</sup> +1, 10), 348 (10), 329 (100), 318 (12), 221 (4), 139 (8); Anal.Calcd. for C <sub>21</sub> H <sub>33</sub> NO <sub>4</sub> ; C, 69.01; H, 9.65; N, 3.83; found: C, 68.85, H, 9.85; N, 3.80.                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 36      | N-methyl-<br>piperazino<br><br><br><br>(10 $\alpha$ -isomer) | -              | -              | Brownish yellow solid. M.p. 112-114°C; $[\alpha]_D^{20}+12.95^\circ$ (c.0.0149 in CHCl <sub>3</sub> ); $\nu_{max}$ (Neat): 2934, 2872, 2792, 1454, 1376, 1286, 1226, 1192, 1162, 1132, 1102, 1054, 1014, 984, 926, 880, 830 cm <sup>-1</sup> ; <sup>1</sup> H nmr (300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 5.26 (1H, s, H-12), 4.02 (1H, d, J 10.20 Hz, H-10), 3.03 (2H, m, H-6'a, H-6'b), 2.70 (2H, m, H-2'a, H-2'b), 2.59 (1H, m, H-9), 2.30-2.50 (5H, m, H-8, H-3'a, H-3'b, H-5'a, H-5'b), 2.28 (3H, s, N-Me), 1.18-2.05 (10H, m, H-2a, H-2b, H-3a, H-3b, H-4, H-5, H-6a, H-6b, H-7a, H-7b), 1.36 (3H, s, 1-CH <sub>3</sub> ), 0.94 (3H, d, J 6.12 Hz, 9-CH <sub>3</sub> ), 0.80 (3H, d, J 7.17 Hz, 5-CH <sub>3</sub> ) ppm; $\delta_C$ 104.46, 92.16, 90.96, 90.98, 56.18, 52.36, 46.82, 38.05, 36.96, 34.97, 29.16, 26.55, 25.46, 22.30, 20.96, 14.03; m/z (CI, CH <sub>4</sub> ) 367 ([M+1] <sup>+</sup> , 100), 321 ([M-3CH <sub>3</sub> ] <sup>+</sup> , 26). m/z (CI, CH <sub>4</sub> ) 367 ([M+1] <sup>+</sup> , 100), 321 ([M-3CH <sub>3</sub> ] <sup>+</sup> , 26). |

| Ex. No. | Y                             | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------|-------------------------------|----------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 37      | 4-vinylphenyl<br>(10E-isomer) | -              | -              | White solid. $[\alpha]_D^{25}$ -64.6° (c 0.028/CHCl <sub>3</sub> ); $\nu_{max}$ (film) 2948, 2876, 1630, 1512, 1452, 1406, 1376, 1200, 1116, 1074, 1010, 944, 904, 882, 844, 788, 756; $\delta_H$ : 7.37 (2H, d, J=8.26 Hz, Ar-H), 7.27 (2H, d, J=8.26 Hz, Ar-H), 6.71 (1H, dd, J=17.62, 10.90 Hz, vinyl-H), 5.69-5.76 (2H, m, vinyl-H, H-10), 5.57 (1H, s, H-12), 5.20 (1H, d, J=10.90 Hz, vinyl-H), 2.71-2.78 (1H, m), 2.28-2.38 (1H, m), 1.17-2.09 (9H, m), 1.38 (3H, s, H-14), 0.83-0.99 (1H, m), 0.98 (3H, d, J=5.74 Hz, H-15), 0.54 (3H, d, J=7.67 Hz, H-16); $\delta_C$ : 140.91, 136.75, 135.74, 126.36, 125.72, 113.09, 102.40, 90.89, 81.24, 73.07, 51.58, 43.58, 37.58, 36.73, 34.26, 32.19, 25.80, 24.98, 24.80, 19.97, 13.75. Anal. Calcd. for C <sub>23</sub> H <sub>30</sub> O <sub>4</sub> : C, 74.56; H, 8.16; found: C, 74.58; H, 8.26.                                                                                                                                                                                                                 |
| 38      | 4-Br phenyl<br>(10E-isomer)   | -              | -              | White rectangular crystal. M.p. 156-159°C; $[\alpha]_D^{20.5}$ -45.14° (c 0.0216 in CHCl <sub>3</sub> ); $\nu_{max}$ (Nujol): 2924, 1492, 1454, 1374, 1112, 1008, 942, 902, 882, 840, 780 cm <sup>-1</sup> ; <sup>1</sup> H nmr (300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 7.43 (2H, d, J 8.40 Hz, H-3', H-5'), 7.19 (2H, d, J 8.40 Hz, H-2', H-6'), 5.70 (1H, d, J 6.60 Hz, H-10), 5.55 (1H, s, H-12), 2.72 (1H, m, H-9), 2.33 (1H, m, H-8), 1.19-2.10 (10H, m, H-2a, H-2b, H-3a, H-3b, H-4, H-5, H-6a, H-6b, H-7a, H-7b), 1.40 (3H, s, 1-CH <sub>3</sub> ), 0.98 (3H, d, J 5.70 Hz, 9-CH <sub>3</sub> ), 0.48 (3H, d, J 7.80 Hz, 5-CH <sub>3</sub> ) ppm; m/z (CI, CH <sub>4</sub> ) 453 ([M(Br <sup>81</sup> )+2CH <sub>4</sub> ] <sup>+</sup> , 18), 451 ([M(Br <sup>79</sup> )+2CH <sub>4</sub> ] <sup>+</sup> , 20), 425 ([M(Br <sup>81</sup> )+1] <sup>+</sup> , 51), 423 ([M(Br <sup>79</sup> )+1] <sup>+</sup> , 53), 407 (40), 405 (32), 392 (35), 390 (48), 379 (100), 377 (88), 335 (20), 333 (28), 267 (32), 221 (41), 209 (78), 191 (78), 191 (26), 163 (59). |

| Ex. No. | Y                                                                                                                       | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------|-------------------------------------------------------------------------------------------------------------------------|----------------|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 39      | 4-Cl phenyl<br>(10 $\beta$ -isomer)                                                                                     | -              | -              | White rectangular crystal. M.p. 161-146°C; $[\alpha]_D^{20.5}$ -10.35° (c 0.0508 in CHCl <sub>3</sub> ); $\nu_{max}$ (Nujol): 2924, 1494, 1456, 1374, 1114, 1008, 942, 902, 840, 782 cm <sup>-1</sup> ; <sup>1</sup> H nmr (300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 7.30 (2H, d, J 8.16 Hz, H-3', H-5'), 7.24 (2H, d, J 8.16 Hz, H-2', H-6'), 5.69 (1H, d, J 6.60 Hz, H-10), 5.55 (1H, m, H-12), 2.71 (1H, m, H-9), 2.32 (1H, m, H-8), 1.21-2.08 (10H, m, H-2a, H-2b, H-3a, H-3b, H-4, H-5, H-6a, H-6b, H-7a, H-7b), 1.36 (3H, s, 1-CH <sub>3</sub> ), 0.98 (3H, d, J 5.76 Hz, 9-CH <sub>3</sub> ), 0.49 (3H, d, J 7.68 Hz, 5-CH <sub>3</sub> ) ppm; $\delta_C$ 140.37, 132.75, 128.66, 128.31, 103.10, 91.64, 81.91, 73.28, 52.23, 44.16, 38.28, 37.41, 34.93, 32.80, 26.47, 25.53, 20.63, 14.36; m/z (CI, CH <sub>4</sub> ) 407 ([M(CI <sup>37</sup> )+2CH <sub>4</sub> ] <sup>+</sup> , 6), 405 ([M(CI <sup>35</sup> )+2CH <sub>4</sub> ] <sup>+</sup> , 5), 379 ([M(CI <sup>37</sup> )-1] <sup>+</sup> , 97), 377 ([M(CI <sup>35</sup> )-1] <sup>+</sup> , 100), 355 (14), 333 (26), 182 (12). Anal. calc. for C <sub>21</sub> H <sub>22</sub> O <sub>4</sub> Cl C, 66.57; H, 7.18; found C, 66.42; H, 7.05. |
| 40      | 9-anthryl<br><br>(10 $\beta$ -isomer) | -              | -              | White solid. $\delta_H$ 9.00-9.05 (1H, m, Ar-H), 8.31-8.41 (2H, m, Ar-H), 7.05-8.04 (2H, m, Ar-H), 7.39-7.57 (4H, m, Ar-H), 7.23 (1H, d, J=7.51 Hz, H-10), 5.81 (1H, s, H-12), 3.10-3.23 (1H, m), 0.86-2.49 (11H, m), 1.39 (3H, s, H-14), 1.09 (3H, d, J=5.81 Hz, H-15), 0.57 (3H, d, J=7.72 Hz, H-16); $\delta_C$ : 134.12, 131.70, 131.05, 130.88, 129.59, 129.19, 128.73, 128.47, 127.61, 126.05, 124.55, 124.51, 124.32, 123.71, 102.64, 91.22, 81.42, 72.62, 51.50, 44.18, 37.69, 36.87, 34.33, 33.02, 25.71, 25.09, 25.00, 19.94, 13.81.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

| Ex. No. | Y                                                                                                                  | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------|--------------------------------------------------------------------------------------------------------------------|----------------|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 41      | 9-phenanthryl<br><br>(10β-isomer) | -              | -              | White solid. M.p. 89-89.1°C; $[\alpha]_D^{20}$ : -68.8° (c 0.016 CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 2922, 2874, 2362, 1498, 1450, 1376, 1246, 1220, 1110, 1040, 1010, 956, 930, 906, 886, 832, 794, 748, 726; $\delta_H$ : 8.68-8.81 (2H, m, Ar-H), 7.91-8.10 (3H, m, Ar-H), 7.57-7.72 (4H, m, Ar-H), 6.50 (1H, d, J=6.54 Hz, H-10), 5.75 (1H, s, H-12), 3.06-3.19 (1H, m), 2.37-2.48 (1H, m), 2.00-2.16 (3H, s), 1.73-1.84 (2H, m), 0.86-1.60 (5H, m), 1.41 (3H, s, H-14), 1.06 (3H, d, J=5.67 Hz, H-15), 0.39 (3H, d, J=7.61 Hz, H-16); $\delta_C$ : 135.21, 131.68, 130.14, 129.96, 129.59, 128.84, 126.66, 126.52, 126.04, 126.01, 123.84, 123.68, 123.19, 122.33, 102.47, 91.34, 81.42, 69.92, 51.45, 43.77, 37.71, 36.89, 34.27, 31.55, 25.79, 25.11, 24.95, 19.96, 13.22; m/z (CI, CH <sub>4</sub> ) 445 (M <sup>+</sup> +1, 22), 444 (100), 398 (40), 384 (16), 352 (16), 328 (44), 267 (6), 218 (84), 203 (48), 178 (60), 163 (44), 138 (70), 107 (62). |
| 42      | 2-OCH <sub>3</sub> phenyl<br>(10β-isomer)                                                                          | -              | -              | White solid. M.p. 61°C; $[\alpha]_D^{20}$ : -14.4° (c 0.049 CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 2928, 2874, 1590, 1492, 1462, 1374, 1284, 1240, 1178, 1110, 1102, 1052, 1010, 944, 882, 854, 754; $\delta_H$ : 6.83-7.50 (4H, m, Ar-H), 5.94 (1H, d, J=6.65, Hz, H-10), 5.58 (1H, s, H-12), 3.84 (3H, s, OCH <sub>3</sub> ), 2.86-2.99 (1H, m, H-9), 2.30-2.40 (1H, m), 1.19-2.11 (10H, m), 1.39 (3H, s, H-14), 1.01 (1H, d, J = 5.77 Hz, H-16), 0.43 (1H, d, J=7.64 Hz, H-15); $\delta_C$ : 134.85, 127.00, 126.37, 120.02, 109.19, 90.86, 68.63, 55.19, 51.30, 43.39, 37.53, 36.72, 34.21, 29.87, 25.68, 24.97, 24.75, 19.83, 13.45; m/z (CI, CH <sub>4</sub> ) 375 (M <sup>+</sup> +1, 12%), 374 (M <sup>+</sup> , 16), 342 (100), 329 (48), 311 (14), 284 (28), 182 (56), 148 (76), 137 (60), 121 (48); Anal. Calcd. for C <sub>22</sub> H <sub>20</sub> O <sub>5</sub> : C, 70.56; H, 8.07; found: C, 70.78; H, 8.28.                                        |

| Ex. No. | Y                                                                | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------|------------------------------------------------------------------|----------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 43      | 2,4-(OCH <sub>3</sub> ) <sub>2</sub> phenyl<br>(10S-isomer)      | -              | -              | White snow-like crystal. M.p. 62°C;<br>[α] <sub>D</sub> <sup>20.5</sup> - 64.21° (c. 0.0114 in CHCl <sub>3</sub> );<br>(Found C, 68.55; H, 8.14 C <sub>23</sub> H <sub>20</sub> O <sub>6</sub> requires<br>C, 68.29; H, 7.97%); ν <sub>max</sub> (Nujol): 2920,<br>1614, 1590, 1506, 1464, 1376, 1286, 1258,<br>1208, 1156, 1120, 1040, 1010, 946, 880,<br>832, 780, 726 cm <sup>-1</sup> ; <sup>1</sup> H nmr (300 MHz, CDCl <sub>3</sub> )<br>δ <sub>H</sub> 7.33 (1H, d, J 8.40 Hz, H-6'), 6.47<br>(1H, dd, J 8.40, 2.40 Hz, H-5'), 6.42 (1H,<br>d, J 2.40 Hz, H-3'), 5.84 (1H, d, J 6.60<br>Hz, H-10), 5.54 (1H, s, H-12), 3.80, 3.79<br>(6H, 2xs, 2xOMe), 2.84 (1H, m, H-9), 2.32<br>(1H, m, H-8), 1.20-2.10 (10H, m, H-2a, H-<br>2b, H-3a, H-3b, H-4, H-5, H-6a, H-6b, H-<br>7a, H-7b), 1.37 (3H, s, 1-CH <sub>3</sub> ), 1.00 (3H,<br>d, J 5.70 Hz, 9-CH <sub>3</sub> ), 0.40 (3H, d, J 7.50<br>Hz, 5-CH <sub>3</sub> ) ppm; m/z (CI, CH <sub>4</sub> ) 405 ([M+1] <sup>+</sup> ,<br>15), 359 ([-3CH <sub>3</sub> ] <sup>+</sup> , 100), 317 (6), 275<br>(28), 221 (8), 154 (22). Anal. Calc. for<br>C <sub>23</sub> H <sub>20</sub> O <sub>6</sub> : C, 68.29; H, 7.97%; found C,<br>68.55; H, 8.14; C, 68.47; H, 8.37. |
| 44      | 2,4,6-(OCH <sub>3</sub> ) <sub>3</sub><br>phenyl<br>(10S-isomer) | -              | -              | Colourless oil. [α] <sub>D</sub> <sup>22</sup> +10.6° (c 0.016/<br>CHCl <sub>3</sub> ); ν <sub>max</sub> (film) 2938, 1608, 1456, 1204,<br>1154, 1126, 1006, 954; δ <sub>H</sub> : 6.16 (1H, d,<br>J=8.09 Hz, H-10), 6.13 (2H, s, Ar-H), 5.52<br>(1H, s, H-12), 3.81 (3H, s, OMe), 3.78<br>(2x3H, s, OMe), 2.64-2.72 (1H, m), 2.29-<br>2.38 (1H, m), 1.97-2.08 (2H, m), 1.68-1.84<br>(4H, m), 1.20-1.57 (3H, m), 1.40 (3H, s,<br>H-14), 0.84-1.11 (1H, m), 1.00 (3H, d,<br>J=5.73 Hz, H-15), 0.72 (3H, d, J=7.70 Hz,<br>H-16); m/z (CI, CH <sub>4</sub> ) 435 (M <sup>+</sup> +1, 10), 417<br>(8), 389 (100), 371 (6), 347 (10), 329<br>(16), 221 (8): Anal. Calcd. for C <sub>24</sub> H <sub>24</sub> O <sub>7</sub> : C,<br>66.34; H, 7.89; found: C 66.57; H, 8.04.                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

| Ex. No. | Y                                                               | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------|-----------------------------------------------------------------|----------------|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 45      | 2,4,6-(CH <sub>3</sub> ) <sub>3</sub><br>phenyl<br>(10β-isomer) | -              | -              | Colourless oil. [α] <sub>D</sub> <sup>22</sup> +13.7 (c 0.019/<br>CHCl <sub>3</sub> ); ν <sub>max</sub> (film) 2938, 2874, 1452, 1376,<br>1208, 1106, 1076, 1008, 958, 942, 896,<br>880, 848, 780, 756, 724; δ <sub>H</sub> : 6.81 (2H, s,<br>Ar-H), 6.05 (1H, d, J=7.57 Hz, H-10), 5.55<br>(1H, s, H-12), 2.74-2.85 (1H, m), 2.48<br>(3H, s, Me), 2.26-2.40 (1H, m), 2.32 (3H,<br>s, Me), 2.27 (3H, s, Me), 2.05-2.11 (2H,<br>m), 1.64-1.90 (4H, m), 1.29-1.50 (3H, m),<br>1.41 (3H, s, H-14), 0.84-1.04 (1H, m),<br>1.03 (3H, d, J=5.91 Hz, H-15), 0.64 (3H,<br>d, J=7.84 Hz, H-16); δ <sub>C</sub> : 137.22, 133.56,<br>135.21, 133.52, 130.81, 128.37, 102.30,<br>90.71, 80.94, 71.82, 51.32, 43.92, 37.59,<br>36.79, 34.24, 30.44, 25.72, 25.04, 24.46,<br>22.28, 20.70, 20.63, 19.87, 13.22; m/z<br>(CI, CH <sub>4</sub> ) 387 (M <sup>+</sup> +1, 6), 386 (8), 385 (10),<br>341 (100), 327 (8), 299 (8), 267 (14), 221<br>(10), 209 (4), 163 (8), 133 (8);<br>Anal.Calcd. C <sub>22</sub> H <sub>24</sub> O <sub>4</sub> : C, 74.58; H, 8.87;<br>found: C, 74.49; H, 8.86.                                                                                |
| 46      | 2,4,5-(CH <sub>3</sub> ) <sub>3</sub><br>phenyl<br>(10β-isomer) | -              | -              | Colourless Oil. M.P. 141°C; [α] <sub>D</sub> <sup>20</sup> :55.6°<br>(c 0.068/CHCl <sub>3</sub> ); ν <sub>max</sub> (film) 2922, 2874,<br>1502, 1452, 1374, 1278, 1220, 1202, 1180,<br>1120, 1100, 1056, 1040, 1000, 978, 954,<br>934, 896, 880, 820, 754; δ <sub>H</sub> : 7.32 (1H, s,<br>Ar-H), 6.99 (1H, s, Ar-H), 5.94 (1H, d,<br>J=6.71 Hz, H-10), 5.67 (1H, s, H-12),<br>2.80-2.90 (1H, m), 2.38-2.48 (1H, m), 2.33<br>(2x3H, s, Me), 2.31 (3H, s, Me), 2.10-2.19<br>(2H, m), 1.78-2.00 (3H, m), 1.40-1.55 (4H,<br>m), 1.47 (3H, s, H-14), 0.97-1.11 (1H, m),<br>1.11 (3H, d, J=5.75 Hz, H-15), 0.55 (3H,<br>d, J=7.68 Hz, H-16); δ <sub>C</sub> : 136.62, 134.02,<br>133.13, 131.04, 130.76, 127.09, 102.11,<br>91.04, 81.07, 70.00, 51.33, 43.49, 37.57,<br>36.73, 34.23, 29.89, 25.57, 25.01, 24.81,<br>19.89, 19.17, 18.73, 13.65; m/z (CI, CH <sub>4</sub> )<br>387 (M <sup>+</sup> +1, 10), 386 (M <sup>+</sup> , 44), 354 (60), 341<br>(84), 296 (6), 282 (18), 109 (20, 182<br>(28), 160 (100), 149 (56), 133 (38), 121<br>(30); Anal.Calcd. for C <sub>22</sub> H <sub>24</sub> O <sub>4</sub> : C, 74.58; H,<br>8.87; found: C, 74.63, H, 8.73. |

| Ex. No. | Y                                                    | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---------|------------------------------------------------------|----------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 47      | 4-COOH phenyl<br>(10 $\beta$ -isomer)                | -              | -              | White solid. $[\alpha]_D^{20}$ -63.2° (c 0.019/<br>CHCl <sub>3</sub> ); $\nu_{\max}$ (film) 2954, 2878, 2670, 2546,<br>2252, 1688, 1612, 1578, 1512, 1452, 1424,<br>1376, 1314, 1286, 1222, 1208, 1178, 1116,<br>1074, 1056, 1040, 1012, 980, 968, 954,<br>944, 908, 882, 854, 824, 802, 766, 732; $\delta_H$<br>8.09 (2H, d, J=8.34 Hz, Ar-H), 7.45 (2H,<br>d, J=8.34 Hz, Ar-H), 5.82 (1H, d, J=6.63<br>Hz, H-10), 5.60 (1H, s, H-12), 2.76-2.83<br>(1H, m), 2.31-2.40 (1H, m), 1.23-2.10 (9H,<br>m), 1.41 (3H, s, H-14), 0.87-1.02 (1H, m)<br>1.01 (3H, d, J=5.49 Hz, H-15), 0.51 (3H,<br>d, J=7.62 Hz, H-16); $\delta_C$ 171.66, 147.41,<br>129.71, 127.33, 126.15, 102.29, 90.80,<br>81.07, 72.74, 51.35, 43.29, 37.42, 36.53,<br>34.04, 31.94, 29.05, 25.60, 24.67, 19.78,<br>13.42; m/z (CI, CH <sub>4</sub> ) 389 (M <sup>+</sup> +1, 8), 329<br>(100), 283 (36), 267 (20), 219 (26), 177<br>(80), 129 (64). Anal. Calc. for C <sub>22</sub> H <sub>18</sub> O <sub>4</sub> : C,<br>68.02; H, 7.27; found: C, 67.77; H, 7.31.                                    |
| 48      | 4-Phenyl-<br>piperazino<br><br>(10 $\alpha$ -isomer) | -              | -              | White solid. M.p. 149-150 °C; $[\alpha]_D^{20}$ +<br>16.7° (c 1.24 in CHCl <sub>3</sub> ); $\nu_{\max}$ (film): 2924,<br>1600, 1504, 1450, 1378, 1238, 1206, 1158,<br>1042, 984, 926, 880, 758, 690 cm <sup>-1</sup> ; <sup>1</sup> H NMR<br>(300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 7.34 (2H, m), 7.03<br>(2H, d, J = 8.0 Hz, H-2", H-5"), 6.92 (1H,<br>t, J = 7.3 Hz, H-4"), 5.39 (1H, s, H-12),<br>4.18 (1H, d, J = 10.2 Hz), 3.29-3.21 (6H,<br>m), 2.90 (2H, m), 2.70 (1H, m), 2.45 (1H,<br>m), 2.13 (1H, m), 1.95 (1H, m), 1.75 (2H,<br>m), 1.70-1.20 (8H, m), 1.20-1.00 (4H, m),<br>.93 (3H, d, J = 7.1 Hz, 6-methyl) ppm; <sup>13</sup> C<br>NMR (76 MHz, CDCl <sub>3</sub> ) $\delta_C$ 151.6, 128.9,<br>119.3, 116.0, 103.8, 91.6, 90.4, 80.3,<br>51.7, 49.5, 47.2, 45.8, 37.4, 36.3, 34.3,<br>28.5, 25.9, 24.8, 21.6, 20.3, 13.4 ppm; MS<br>(CI, CH <sub>4</sub> ) m/e 429 (M <sup>+</sup> +1, 88).<br>Anal. Calcd. for C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub> : C, 70.06, H,<br>8.47, N, 6.53; Found: C, 69.74, H, 8.38,<br>N, 6.35. |

| Ex. No. | Y                                                            | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|---------|--------------------------------------------------------------|----------------|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 49      | 4-(2'-methoxyphenyl)-piperazino<br><br>(10 $\alpha$ -isomer) | -              | -              | White solid. M.p. 158-159 °C; $[\alpha]_D^{20} + 12.2^\circ$ (c. 0.752 in CHCl <sub>3</sub> ); $\nu_{\max}$ (film): 2936, 1594, 1500, 1448, 1376, 1240, 1180, 1118, 1058, 982, 926, 880, 828, 750 cm <sup>-1</sup> ; <sup>1</sup> H NMR (300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 7.10-6.80 (4H, m, Ar-H), 5.30 (1H, s, H-12), 4.07 (1H, d, J = 10.2 Hz, H-10), 3.85 (3H, s, -OMe), 3.30-2.95 (6H, m), 2.95-2.75 (2H, m), 2.61 (1H, m), 2.35 (1H, m), 2.00 (1H, m), 1.85 (1H, m), 1.70 (2H, m), 1.60-1.15 (9H, m), 1.15-0.90 (4H, m), 0.84 (3H, 7.2 Hz, 6-methyl) ppm; <sup>13</sup> C NMR (76 MHz, CDCl <sub>3</sub> ) $\delta_C$ 152.3, 141.8, 122.5, 120.9, 118.1, 111.2, 103.8, 91.6, 90.4, 80.4, 55.3, 51.8, 51.1, 45.9, 37.4, 36.4, 34.3, 28.5, 26.0, 24.8, 21.7, 21.7, 20.3, 13.4 ppm; MS (CI, CH <sub>4</sub> ) m/e 459 (M <sup>+</sup> +1, 55). Anal. Calcd. for C <sub>26</sub> H <sub>30</sub> N <sub>2</sub> O <sub>5</sub> : C, 68.10, H, 8.35, N, 6.11; Found: C, 67.74, H, 8.35, N, 5.83.                                                                                                                                                                                                              |
| 50      | 4-(4'-fluorophenyl)-piperazino<br><br>(10 $\alpha$ -isomer)  | -              | -              | White solid. M.p. 157-158 °C; $[\alpha]_D^{20} + 23.1^\circ$ (c 0.743 in CHCl <sub>3</sub> ); $\nu_{\max}$ (film): 2933, 2842, 1704, 1689, 1654, 1617, 1560, 1516, 1456, 1381, 1314, 1249, 1227, 1205, 1160, 1135, 1111, 1061, 1047, 1027, 985, 921, 883, 853, 822, 697 cm <sup>-1</sup> ; <sup>1</sup> H NMR (300 MHz, CDCl <sub>3</sub> ) $\delta_H$ 7.00-6.80 (4H, m Ar-H), 5.29 (1H, s, H-12), 4.07 (1H, d, J = 10.2 Hz), 3.15-3.05 (6H, m), 2.83 (2H, m), 2.60 (1H, m), 2.35 (1H, m), 2.00 (1H, m), 1.90 (1H, m), 1.73 (2H, m), 1.70-1.10 (9H, m), 1.10-0.90 (4H, m), 0.83 (3H, d, J = 7.1 Hz, 6-methyl) ppm; <sup>13</sup> C NMR (76 MHz, CDCl <sub>3</sub> ) $\delta_C$ 156.8 (d, <sup>1</sup> J <sub>C-F</sub> = 238 Hz), 148.2 (d, <sup>1</sup> J <sub>C-F</sub> = 1.96 Hz), 117.6 (d, <sup>3</sup> J <sub>C-F</sub> = 7.55 Hz), 115.2 (d, <sup>2</sup> J <sub>C-F</sub> = 21.9 Hz), 103.7, 91.5, 90.3, 80.2, 51.6, 50.4, 47.1, 45.7, 37.3, 36.2, 34.2, 28.4, 25.9, 24.7, 21.5, 20.2, 13.3 ppm; MS (CI, CH <sub>4</sub> ) m/e 447 (M <sup>+</sup> +1, 82). Anal. Calcd. for C <sub>26</sub> H <sub>29</sub> N <sub>2</sub> O <sub>4</sub> F: C, 67.24, H, 7.90, N, 6.27; Found: C, 67.28, H, 8.01, N, 5.95. |

| Ex. No. | Y                                                          | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|---------|------------------------------------------------------------|----------------|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 51      | 4-(2'-pyridyl)-<br>piperazino<br><br>(10 $\alpha$ -isomer) | -              | -              | White solid. M.p. 146-147 °C; [ $\alpha$ ] <sub>D</sub> <sup>20</sup> + 16.4°<br>c 1.34 in CHCl <sub>3</sub> ; $\nu_{\text{max}}$ (film) 2926, 2872,<br>1596, 1564, 1482, 1346, 1378, 1312, 1248,<br>1208, 1160, 1132, 1056, 1026, 980, 926,<br>880, 850, 828, 744, 732 cm <sup>-1</sup> ; <sup>1</sup> H NMR (300<br>MHz, CDCl <sub>3</sub> ) $\delta$ <sub>H</sub> 8.17 (1H, dd, J = 1.3, 5.0<br>Hz, H-6"), 7.44 (1H, td, J = 1.8, 3.4 Hz,<br>H-4"), 6.64-6.55 (2H, m, H-3" & H-5"),<br>5.27 (1H, s, H-12), 4.07 (1H, d, J = 10.3<br>Hz, H-10), 3.52-3.48 (4H, m, H-3' & H-<br>5'), 3.10-3.05 (2H, m), 2.79-2.74 (2H,<br>m), 2.76 (1H, m), 2.32 (1H, m), 2.02-1.80<br>(2H, m), 1.70-1.65 (2H, m), 1.50-1.20<br>(8H, m), 1.10-0.90 (4H, m), 0.83 (3H, d,<br>J = 7.2 Hz, 6-methyl) ppm; <sup>13</sup> C NMR (76<br>MHz, CDCl <sub>3</sub> ) $\delta$ <sub>C</sub> 153.6, 147.8, 137.2, 112.8,<br>107.0, 103.8, 91.6, 90.6, 80.3, 51.7,<br>47.1, 45.8, 45.5, 37.3, 36.3, 34.3, 28.5,<br>25.9, 24.7, 21.6, 20.3, 13.4 ppm; MS (CI,<br>NH <sub>3</sub> ) m/e 430 (M <sup>+</sup> +1, 100); Anal. Calcd. for<br>C <sub>24</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub> : C, 67.11, H, 8.21, N, 9.78;<br>Found: C, 67.01, H, 8.22, N, 9.55. |

| Ex. No. | Y                                                                     | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|---------|-----------------------------------------------------------------------|----------------|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 52      | 4-(3'-trifluoromethyl-phenyl)-piperazino<br><br>(10 $\alpha$ -isomer) |                |                | White solid. M.p. 126-127 °C; [ $\alpha$ ] <sub>D</sub> <sup>20</sup> + 23.0° (c. 0.543 in CHCl <sub>3</sub> ); $\nu_{\max}$ (film): 2928, 2874, 1612, 1588, 1496, 1450, 1412, 1378, 1356, 1320, 1266, 1242, 1208, 1164, 1122, 1100, 1054, 986, 948, 926, 880, 860, 828, 788, 732, 696, 648 cm <sup>-1</sup> ; <sup>1</sup> H NMR (300 MHz, CDCl <sub>3</sub> ) $\delta$ <sub>H</sub> 7.33 (1H, m, H-5"), 7.10-7.02 (3H, m, Ar-H), 5.30 (1H, s, H-12), 4.09 (1H, d, J = 10.2 Hz, H-10), 3.40-3.05 (6H, m), 2.84 (2H, m), 2.65 (1H, m), 2.35 (1H, m), 2.00 (1H, m), 1.85 (1H, m), 1.80-1.60 (2H, m), 1.60-1.15 (10H, m), 1.15-0.90 (4H, m), 0.84 (3H, d, J = 7.1 Hz) ppm; <sup>13</sup> C NMR (76 MHz, CDCl <sub>3</sub> ) $\delta$ <sub>C</sub> 151.7, 129.4, 118.7, 115.4, 111.9, 103.9, 91.6, 90.5, 80.3, 51.7, 49.0, 47.0, 45.8, 37.4, 36.3, 34.3, 28.5, 25.9, 24.8, 21.6, 20.3, 13.4 ppm; <sup>19</sup> F NMR (282 MHz, CDCl <sub>3</sub> ) $\delta$ <sub>F</sub> 63.9 ppm; MS (CI, CH <sub>4</sub> ) m/e 497 (M <sup>+</sup> +1, 58).<br>Anal. Calcd. for C <sub>26</sub> H <sub>32</sub> N <sub>2</sub> O <sub>4</sub> F <sub>3</sub> : C, 62.89, H, 7.10, N, 5.64; Found: C, 62.82, H, 7.27, N, 5.58. |

| Ex. No. | Y                                      | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------|----------------------------------------|----------------|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 53      | 4-Fluorophenyl<br>(10 $\beta$ -isomer) | -              | -              | White solid. M.P. 133.6-134.8 °C; [ $\alpha$ ] <sub>D</sub> <sup>20</sup> -35.66° (c 0.83, CHCl <sub>3</sub> ); $\delta_F$ : -118.00; IR (neat) $\nu_{max}$ : 2952, 2873, 1604, 1510, 1452, 1376, 1222, 1110, 1040, 1010, 944, 906, 882, 838, 782; $\delta_H$ : 7.29-7.24 (2H, m, Ph), 7.04-6.97 (2H, m, Ph), 5.70 (1H, d, H-10, J=6.70 Hz), 5.55 (1H, s, H-12), 2.77-2.65 (1H, m), 2.39-2.28 (1H, m), 2.10-1.97 (2H, m), 1.90-1.82 (1H, m), 1.78-1.64 (2H, m), 1.49-1.17 (8H, m), 0.99 (3H, d, 6-Me, J=5.75 Hz), 0.48 (3H, d, 9-Me, J=7.68 Hz); $\delta_C$ : 162.10 (d, Ph, J <sub>CP</sub> =244.0 Hz), 137.42 (d, Ph, J <sub>CP</sub> =3.09 Hz), 128.30 (d, Ph, J <sub>CP</sub> =7.84 Hz), 115.16 (d, Ph, J <sub>CP</sub> =21.27 Hz), 102.92, 91.55, 81.75, 73.15, 52.09, 44.05, 38.14, 37.30, 34.82, 32.78, 26.35, 25.57, 25.42, 20.52, 14.29; MS (CI positive, NH <sub>3</sub> ) m/z: 382 (MNH <sub>4</sub> <sup>+</sup> , 2 x <sup>13</sup> C, 4%), 381 (MNH <sub>4</sub> <sup>+</sup> , <sup>13</sup> C, 25%), 380 (MNH <sub>4</sub> <sup>+</sup> , base peak), 363 (MH <sup>+</sup> , 6%); Anal Calcd. for C <sub>21</sub> H <sub>27</sub> O <sub>4</sub> F C 69.59, H 7.51; found C 69.51, H 7.62. |

| Ex. No. | Y                                               | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------|-------------------------------------------------|----------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 54      | 1-(2-pyrimidyl)-<br>piperazino<br>(10β-isomer)  | -              | -              | White solid. M.p. 147.1-147.5 °C; $[\alpha]_D^{20}$ : +14.3° (c = 0.86, CHCl <sub>3</sub> ); IR (KBr) $\nu_{max}$ : 2988, 2970, 2948, 2918, 2870, 2854, 1588, 1546, 1502, 1452, 1438, 1430, 1396, 1376, 1308, 1268, 1186, 1160, 1132, 1114, 1102, 1060, 1044, 1022, 980, 940, 926, 880, 852, 826, 798, 742, 694, 640; $\delta_H$ : 8.27 (2H, d, o-Ph, J=4.8 Hz), 6.43 (1H, t, p-Ph, J=4.8 Hz), 5.27 (1H, s, H-12), 4.05 (1H, d, H-10, J=10.2 Hz), 3.87-3.72 (4H, m, 2xNCH <sub>2</sub> ), 3.08-3.01 (2H, m), 2.75-2.59 (4H, 2xNCH <sub>2</sub> ), 2.38-2.27 (1H, m), 2.02-1.37 (8H, m), 1.34 (3H, s, 3-Me), 1.33-1.17 (1H, m), 0.94 (3H, d, 6-Me, J=6.1 Hz), 0.85 (3H, d, 9-Me, J=7.16 Hz); $\delta_C$ : 162.39, 158.31, 110.17, 104.56, 92.34, 91.49, 80.99, 52.44, 47.95, 46.56, 44.76, 38.07, 37.02, 34.99, 29.20, 26.66, 25.44, 22.34, 20.97, 14.22; MS (CI, CH <sub>4</sub> ) m/z: 432 (MH <sup>+</sup> , <sup>13</sup> C, 5%), 431 (MH <sup>+</sup> , 33%); Anal. calcd for C <sub>22</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub> : C 64.16, H 7.96, N 13.01; found C 64.09, H 8.07, N 12.86. |
| 55      | 1-(4-Chlorophenyl)-<br>piperazino<br>10α-isomer | -              | -              | White solid. M.p. 140.7-142.8 °C; $[\alpha]_D^{20}$ : +9.41° (c = 1.01, CHCl <sub>3</sub> ); IR (KBr) $\nu_{max}$ : 2976, 2952, 2934, 2894, 2872, 2842, 1596, 1502, 1454, 1378, 1350, 1316, 1286, 1248, 1206, 1184, 1160, 1136, 1108, 1060, 1046, 1026, 956, 982, 882, 852, 816, 698, 666, 518; $\delta_H$ : 7.20 (2H, d, Ph, J=9.0 Hz), 6.85 (2H, d, Ph, J=9.02 Hz), 5.30 (1H, s, H-12), 4.09 (1H, d, H-10, J=10.23 Hz), 3.20-3.07 (8H, m, 4 x NCH <sub>2</sub> ), 2.86-1.20 (15H, m), 0.96 (3H, d, 6-Me, J=6.09 Hz), 0.84 (3H, d, 9-Me, J=7.18 Hz); $\delta_C$ : 150.93, 129.49, 124.75, 117.83, 104.57, 92.29, 91.11, 81.02, 52.39, 50.18, 47.74, 46.51, 38.08, 36.99, 34.97, 29.21, 26.65, 25.45, 22.33, 20.97, 14.14; MS (CI positive, CH <sub>4</sub> ) m/z: 465 (MH <sup>+</sup> , <sup>37</sup> Cl, 2%), 464 (MH <sup>+</sup> , <sup>13</sup> C, 0.4%), 463 (MH <sup>+</sup> , 6%); Anal. Calcd for C <sub>25</sub> H <sub>25</sub> N <sub>2</sub> O <sub>4</sub> Cl: C 64.85, H 7.62, N 6.05; found: C 64.68, H 7.66, N 5.89.                                                                      |

| Ex. No. | Y                                                    | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------|------------------------------------------------------|----------------|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 56      | 1-(3-Chlorophenyl)-piperazino<br>10 $\alpha$ -isomer | -              | -              | White solid. M.p. 137.3-137.8 °C; $[\alpha]_D^{20}$ : +12.13° (c = 0.89, CHCl <sub>3</sub> ); IR (KBr) $\nu_{max}$ : 2976, 2948, 2922, 2876, 2864, 2848, 2826, 1598, 1566, 1486, 1452, 1430, 1412, 1378, 1360, 1328, 1312, 1284, 1274, 1264, 1244, 1206, 1184, 1158, 1136, 1116, 1102, 1086, 1042, 1028, 988, 942, 928, 880, 854, 832, 782, 774, 680; $\delta_H$ : 7.18-7.12 (1H, m, Ph), 6.88-6.77 (3H, m, Ph), 5.30 (1H, s, H-12), 4.08 (1H, d, H-10, J=10.21 Hz), 3.24-3.12 (6H, m, 3 x NCH <sub>2</sub> ), 2.84-2.78 (2H, m, NCH <sub>2</sub> ), 2.66-2.59 (1H, m), 2.41-2.30 (1H, m), 2.05-1.20 (13H, m), 0.96 (3H, d, 6-Me, J=6.06 Hz), 0.84 (3H, d, 9-Me, J=7.17 Hz); $\delta_C$ : 153.36, 135.54, 130.59, 119.58, 116.33, 114.58, 104.63, 92.34, 91.18, 81.05, 52.44, 49.75, 47.76, 46.55, 38.11, 37.02, 35.00, 29.25, 26.67, 25.48, 22.37, 20.99, 14.16; MS (ES) m/z: 465 (MH <sup>+</sup> , <sup>13</sup> C, 10%), 464 (MH <sup>+</sup> , <sup>13</sup> C, 9%), 463 (MH <sup>+</sup> , 100%); Anal. Calcd for C <sub>25</sub> H <sub>25</sub> N <sub>2</sub> O <sub>4</sub> Cl: C 64.85, H 7.62, N 6.05, found: C 64.91, 7.73, N 6.00. |
| 57      | 1-(2-Chlorophenyl)-piperazino                        | -              | -              | White solid. M. p. 72-75 °C, $[\alpha]_D^{20}$ -5.15° (c 1.01, CHCl <sub>3</sub> ); IR (neat) $\nu_{max}$ : 3062, 2926, 2870, 2360, 1588, 1480, 1450, 1376, 1232, 1206, 1124, 1040, 984, 926, 880, 828, 762, 668; $\delta_H$ : 7.37-6.92 (4H, m, Ph), 5.32 (1H, s, H-12), 4.09 (1H, d, H-10, J=10.21 Hz), 3.23-3.05 (8H, m, 4 x NCH <sub>2</sub> ), 2.67-2.60 (1H, m, H-9), 2.41 (1H, m), 2.37-1.20 (13H, m), 0.96 (3H, d, 6-Me, J=6.14 Hz), 0.85 (3H, d, 9-Me, J=7.19 Hz); $\delta_C$ : 150.37, 131.31, 129.50, 128.11, 124.00, 120.99, 104.62, 92.40, 91.29, 81.07, 52.49, 52.38, 46.61, 38.09, 37.07, 35.02, 29.26, 26.72, 25.47, 22.39, 21.00, 14.21; MS (ES positive) m/z: 465 (MH <sup>+</sup> , <sup>17</sup> Cl, 20%), 464 (MH <sup>+</sup> , <sup>13</sup> C, 19%), 463 (MH <sup>+</sup> , 100%); Anal. Calcd for C <sub>25</sub> H <sub>25</sub> N <sub>2</sub> O <sub>4</sub> Cl: C 64.85, H 7.62, N 6.05; found: C 64.92, H 7.67, N 5.77.                                                                                                                                                                                            |

| Ex. No. | Y                                                     | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|---------|-------------------------------------------------------|----------------|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 58      | 1-(4-Methoxyphenyl)-piperazino                        | -              | -              | White solid. M.p. 147.6-148.4 °C; $[\alpha]_D^{20}$ : +9.07° (c 1.08, CHCl <sub>3</sub> ); IR (KBr) $\nu_{\max}$ 2958, 2924, 2846, 2810, 1512, 1464, 1448, 1420, 1378, 1350, 1326, 1310, 1292, 1264, 1248, 1206, 1182, 1160, 1134, 1108, 1086, 1060, 1038, 1026, 1010, 984, 942, 926, 880, 852, 824, 814, 800; $\delta_H$ : 6.93-6.81 (4H, m, Ph), 5.30 (1H, s, H-12), 4.08 (1H, d, H-10, J=10.20 Hz), 3.77 (3H, s, OMe), 3.20-3.04 (6H, m, 3 x NCH <sub>2</sub> ), 2.86-2.80 (2H, m, NCH <sub>2</sub> ), 2.66-2.59 (1H, m), 2.41-2.31 (1H, m), 2.05-1.21 (13H, m), 0.96 (3H, d, 6-Me, J=6.08 Hz), 0.84 (3H, d, 9-Me, J=7.16 Hz); $\delta_C$ : 154.22, 146.81, 118.81, 115.00, 104.53, 92.27, 91.08, 81.03, 56.22, 52.40, 51.70, 47.93, 46.54, 38.07, 37.00, 34.98, 29.22, 26.64, 25.46, 22.34, 20.98, 14.12; MS (ES positive) m/z: 459 (MH <sup>+</sup> , 100%), 458 (M <sup>+</sup> , 58%) Anal. Calcd for C <sub>26</sub> H <sub>38</sub> N <sub>2</sub> O <sub>5</sub> : C 68.10, H 8.35, N 6.11; found: C 68.16, H 8.42, N 5.97.                                                                                              |
| 59      | 1-(ortho-Tolyl)-piperazino<br><br>10 $\alpha$ -isomer | -              | -              | White solid, m.p. 141.6-142.8°C; $[\alpha]_D^{20}$ +12.55° (c 1.02, CHCl <sub>3</sub> ); IR (neat) $\nu_{\max}$ 3026, 3021, 2926, 2872, 2360, 1598, 1492, 1448, 1376, 1328, 1306, 1258, 1226, 1206, 1180, 1156, 1132, 1118, 1056, 1040, 1026, 982, 958, 926, 880, 850, 828, 762, 722, 668; $\delta_H$ : 7.19-7.14 (2H, m, Ph), 7.07-6.94 (2H, m, Ph), 5.33 (1H, s, H-12), 4.10 (1H, d, H-10, J=10.17 Hz), 3.19-3.14 (2H, m, NCH <sub>2</sub> ), 2.97-2.79 (6H, m, 3 x NCH <sub>2</sub> ), 2.71-2.59 (1H, m, H-9), 2.37 (1H, m), 2.32 (3H, s, PhMe), 2.07-2.00 (1H, m), 1.71-1.17 (11H, m), 0.97 (3H, d, 6-Me, J=6.1 Hz), 0.87 (3H, d, 9-Me, J=7.2 Hz); $\delta_C$ : 174.96, 152.60, 133.43, 131.66, 127.07, 123.47, 119.54, 104.61, 92.45, 91.35, 81.08, 52.84, 52.48, 46.59, 38.07, 37.05, 35.00, 29.25, 26.75, 25.55, 22.38, 20.99, 18.64, 14.22; MS (CI CH <sub>4</sub> ) m/z: 444 (MH <sup>+</sup> , <sup>13</sup> C, 5.1%), 443 (MH <sup>+</sup> , 18%), 442 (M <sup>+</sup> , 7.6%); Anal. Calcd for C <sub>26</sub> H <sub>38</sub> N <sub>2</sub> O <sub>4</sub> : C 70.56, H 8.65, N 6.33; found C 70.43, H 8.54, N 6.28. |

| Ex. No. | Y                                             | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------|-----------------------------------------------|----------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 60      | 4-Benzylpiperidino<br><br>10 $\alpha$ -isomer | -              | -              | White solid. M.p. 137.6-138.9 °C; $[\alpha]_D^{20}$ : +10.82° (c 0.98, CHCl <sub>3</sub> ); IR (neat) $\nu_{max}$ : 2924, 2870, 1452, 1376, 1206, 1132, 1098, 1056, 970, 926, 880, 828, 746, 700; $\delta_H$ : 7.28-7.24 (2H, m, Ph), 7.19-7.12 (3H, m, Ph), 5.25 (1H, s, H-12), 4.00 (1H, d, H-10, J=10.16 Hz), 3.00-2.86 (2H, m), 2.69-2.27 (6H, m), 2.02-1.41 (10H, m), 1.38 (3H, s, 3-Me), 1.33-1.12 (4H, m), 1.09-0.97 (1H, m), 0.93 (3H, d, 6-Me, J=6.12 Hz), 0.78 (3H, d, 9-Me, J=7.18 Hz); $\delta_C$ : 141.60 (Ph), 129.75 (Ph), 128.73 (Ph), 126.28 (Ph), 104.44, 92.40, 91.75, 81.06, 52.46, 46.64, 44.16, 44.03, 38.85, 38.02, 37.02, 34.99, 33.49, 33.18, 29.36, 26.72, 25.41, 22.32, 20.97, 14.20; MS (CI positive, NH <sub>3</sub> ) m/z: 444 (MH <sup>+</sup> , 2 x <sup>13</sup> C, 2%), 443 (MH <sup>+</sup> , <sup>13</sup> C, 12%), 442 (MH <sup>+</sup> , 38%), 441 (M <sup>+</sup> , 1%); Anal Calcd. For C <sub>27</sub> H <sub>31</sub> NO <sub>4</sub> : C 73.44, H 8.90, N 3.17; found C 73.25, H 8.85, N 3.14. |

WO 00/04024

-65-

PCT/GB99/02267

| Ex. No. | Y                                                              | R <sup>1</sup> | R <sup>2</sup> | Physical data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|---------|----------------------------------------------------------------|----------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 61      | 6-Methoxynaphthyl<br><br>10 $\alpha$ - and 10 $\beta$ -isomers | -              | -              | $\delta_H$ : 7.73-7.70 (3H, m, Ph), 7.59-7.55 (1H, m, Ph), 7.13-7.10 (2H, m, Ph), 4.50 (1H, d, H-10, J=10.64 Hz), 3.91 (3H, s, OMe), 2.72-2.65 (1H, m), 2.49-2.43 (1H, m), 2.10-2.02 (1H, m), 1.96-1.87 (1H, m), 1.81-1.74 (1H, m), 1.68-1.53 (4H, m), 1.46 (3H, s, 3-Me), 1.42-1.24 (3H, m), 1.13-1.05 (1H, m), 0.99 (3H, d, 6-Me, J=6.15 Hz), 0.55 (3H, d, 9-Me, J=7.19 Hz); $\delta_C$ : 158.18, 136.70, 135.07, 130.13, 129.28, 127.65, 127.01, 126.46, 119.24, 106.32, 104.92, 92.76, 81.36, 79.15, 55.93, 52.70, 46.78, 38.12, 37.05, 34.93, 34.55, 26.75, 25.51, 22.20, 21.01, 14.80; MS (ES positive) m/z: 424 (MH <sup>+</sup> , 4%). $\delta_H$ : 7.74-7.67 (3H, m, Ph), 7.39-7.35 (1H, m, Ph), 7.14-7.11 (2H, m, Ph), 5.86 (1H, d, H-10, J=3.72 Hz), 5.54 (1H, s, H-12), 3.91 (3H, s, OMe), 2.86-2.78 (1H, m), 2.40-2.30 (1H, m), 2.09-1.58 (5H, m), 1.51-1.23 (7H, m), 0.99 (3H, d, 6-Me, J=5.58 Hz), 0.94-0.88 (1H, m), 0.52 (3H, d, 9-Me, J=7.65 Hz); $\delta_C$ : 157.97, 137.12, 134.08, 130.11, 129.39, 126.83, 126.19, 124.98, 119.23, 106.29, 103.09, 91.72, 81.99, 73.86, 55.94, 52.29, 44.28, 38.29, 37.45, 34.97, 32.96, 26.51, 25.70, 25.57, 20.67, 14.52; MS (CI positive, CH <sub>4</sub> ) m/z: 426 (MH <sup>+</sup> , <sup>13</sup> C, 2%), 425 (MH <sup>+</sup> , 8%), 424 (M <sup>+</sup> , 7%). |

Example 62

The parasitocidal activity of compounds of the invention was investigated by means of the following tests.

5

Abbreviations used in the examples:

CO<sub>2</sub> = carbon dioxide

DMSO = dimethylsulphoxide

10 ED = dermal cell line of a horse

EDTA = ethylenediaminetetraacetic acid

FCS = fetal calf serum

RPMI = growth medium for cell cultures

rpm = revolutions per minute

15 VERO = kidney cell line of the African green monkey

(a) Screening of compounds against Neospora Caninum cell cultures in vitro.

Screening was conducted in 96-well plates (Falcon 3872). A monolayer of host cells (VERO or ED) were placed on a cell culture plate. Non-infected monolayers of cells were cultured in two 50 ml tissue culture bottles (50 cm<sup>2</sup> cell culture area). The cell layer was detached with trypsin-EDTA (5 ml. Gibco 45300-019) in a CO<sub>2</sub>-culture cupboard at 37°C. After 10 minutes, most of the cells were detached. The cells were transferred with a 5 ml pipette into a 50 ml centrifuge tube (Greiner, B769331) containing about 1 ml warmed fetal calf serum. After centrifugation for 5 minutes at 1500 rpm (Varifuge 3.0, Heraeus), the

-68-

liquid was removed and the cell pellet suspended in RPMI medium (100 ml, 95% RPMI 1640, 2% FCS, 1% L-glutamine, 1% sodium hydrogen carbonate, 1% penicillin/streptomycin). The cell suspension was

5 pipetted into six 96-well plates at 150  $\mu$ l per well. The coated cell culture plates were placed in an incubation cupboard at 37°C under 5% CO<sub>2</sub> for 24 hours. The cells were then infected with Neospora caninum

10 tachyzoites at a concentration of 48,000 tachyzoites per well. This was followed by incubation at 37°C under 5% CO<sub>2</sub> for 24 hours.

The test compounds (0.5 - 1.5 mg) were weighed into 1.5 ml eppendorf vessels and dissolved in 1 ml

15 dimethyl sulphoxide, corresponding to a dilution of about  $1 \times 10^{-3}$  g ml<sup>-1</sup>. The medium used for further dilution consisted of 87% RPMI 1640, 10% FCS, 1% L-glutamine, 1% sodium hydrogen carbonate, 1% penicillin/streptomycin. In the first screening,

20 concentrations of  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  g ml<sup>-1</sup> were used. The diluted preparations were then transferred to the cell culture plates at a volume of 150  $\mu$ l per well

after 24 hour infection with Neospora caninum. For the first row, untreated medium was used; this row contained infected and uninfected cells as controls.

25 The cell plate was incubated at 37°C under 5% CO<sub>2</sub> for 5 days. Microscopic evaluation was conducted 4 days after treatment and 5 days after infection at a magnification of 25 x 10 in an inverse microscope according to the following evaluation scheme.

-69-

|    | <u>Evaluation</u> | <u>Observable effect</u>                                   |
|----|-------------------|------------------------------------------------------------|
|    | 0 = no effect     | monolayer completely destroyed                             |
| 5  | 1 = weak effect   | monolayer partly destroyed,<br>parasite clumps can be seen |
|    | 2 = full effect   | monolayer intact, no tachyzoites<br>observable             |
| 10 | T = cytotoxic     | cells are dead, lysed                                      |

The results are set out in Table II below:-

15

Table II

| Example No. | Dose (g/ml) |           |           |           |
|-------------|-------------|-----------|-----------|-----------|
|             | $10^{-5}$   | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ |
| 2           | 1           | 1         | 0         | -         |
| 15          | T/1         | 1         | 1         | 0         |
| 18          | 2           | 1         | 0         | -         |
| 19          | T           | 0         | -         | -         |
| 20          | T/1         | 1         | 1         | 0         |
| 21          | 2           | 0         | -         | -         |
| 23          | T/2         | 0         | -         | -         |
| 24          | 1           | 0         | -         | -         |
| 25          | T/1         | 1         | 1         | 0         |
| 30          | 1           | 0         | -         | -         |
| 31          | 2           | 1         | 0         | -         |
| 32          | 2           | 0         | -         | -         |
| Artemisinin | 0           | -         | -         | -         |

(b) Screening of Compounds against Eimeria Tenella cell cultures in vitro

Cells from kidneys of 19 day old chicks are cultured as monolayers in 96-well plates (Falcon 3872) in a medium of Hanks lactalbumine hydrolysate, 5% fetal calf serum, 1% glutamine and 1% non-essential amino acids. After two days at 42°C under 5% CO<sub>2</sub>, the culture was infected with excised sporozoites of Eimeria tenella at about 30.00 per well. Test compounds were dissolved in DMSO and diluted with culture medium to a maximum end concentration of 10 µg ml<sup>-1</sup>. The dilution steps were 1:10. On day 5 post infection, the cultures were evaluated under a microscope at 100-fold magnification and the condition of the host cells and the amount of intact schizonts

-71-

and free merozoites was determined. Effectiveness was rated as follows:

|    | <u>Evaluation</u> | <u>Observable effect</u>                        |
|----|-------------------|-------------------------------------------------|
|    | 3 = very active   | no intact parasites/well                        |
| 5  | 2 = active        | 1-6 parasites per well                          |
|    | 1 = weakly active | up to 1 intact schizont/optical field of vision |
|    | 0 = inactive      | > 1 intact schizont/optical field of vision     |
| 10 | T = cytotoxic     | host cells are dead                             |

The results are set out in Table III below:-

Table III

| Example No. | Dose (g/ml) |           |           |           |
|-------------|-------------|-----------|-----------|-----------|
|             | $10^{-5}$   | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ |
| 2           | 2           | 2         | 1         | 0         |
| 15          | 2           | 2         | 1         | 0         |
| 18          | T           | T         | 1         | 0         |
| 19          | T           | T         | 1         | 0         |
| 20          | T           | T/2       | 0         | -         |
| 21          | T/2         | 0         | -         | -         |
| 23          | T           | T/2       | 0         | -         |
| 24          | 2           | 1         | 0         | -         |
| 25          | 2           | 1         | 1         | 0         |
| 30          | T           | 2         | 0         | -         |
| 31          | T           | 1         | 0         | -         |
| 32          | T           | 2         | 0         | -         |
| Artemisinin | 2           | 1         | 0         | -         |

(c) In Vitro Screening against Plasmodium Falciparum

Two parasite strains - W2 resistant to chloroquine, and D6 sensitive to chloroquine but resistant to mefloquine were used. In Table IV below, the best compounds should show no cross resistance between the two strains.

The assay relies on incorporation of radiolabelled

-73-

hypoxanthine by the parasite and inhibition of incorporation is attributed to activity of known or candidate antimalarial drugs. For each assay, proven antimalarials such as chloroquine, mefloquine, 5 quinine, artemisinin and pyrimethamine were used as controls. The incubation period was 66 hours, and the starting parasitemia was 0.2% with 1% hematocrit. The medium was an RPMI-1640 culture with no folate or p-aminobenzoic acid. Albumax rather than 10% normal 10 heat inactivated human plasma was used as, with Albumax, less protein binding is observed, and compounds elicit slightly higher activities in this model. If a compound was submitted with no prior knowledge of activity, it was dissolved directly in 15 dimethyl sulphoxide (DMSO), and diluted 400 fold with complete culture medium. The unknown compound was started at a maximum concentration of 50,000 ng ml<sup>-1</sup> and sequentially diluted 2-fold for 11 times to give a concentration range of 1048 fold. These dilutions 20 were performed automatically by a Biomek 1000 Liquid Handling System in 96-well microtiter plates. The diluted drugs were then transferred to test plates, 200 µl of parasitized erythrocytes were added, and incubated at 37°C in a controlled environment of 5% 25 CO<sub>2</sub>, 5% O<sub>2</sub> and 90% N<sub>2</sub>. After 42 hours, 25 µl of <sup>3</sup>H-hypoxanthine was added, and the plates incubated for an additional 24 hours. After 66 hours, the plates were frozen at -70°C to lyse the red cells, and then thawed and harvested onto glass fiber filter mats in a 30 96-well harvester. The filter mats were then counted in a scintillation counter. For each drug, the concentration response profile was determined and 50%, 90% and 10% inhibitory concentrations (IC<sub>50</sub>, IC<sub>90</sub> and IC<sub>10</sub>) were determined by a non-linear logistic dose 35 response analysis program.

-74-

A prescreen format can be used wherein a 3-dilution assay may be used to determine activity at high medium or low concentrations. The concentrations were selected as 50,000, 500 and 50 ng ml<sup>-1</sup>. These were performed in duplicate on a 96-well format plate with 14 test compounds and one known (standard) compound per plate. The system was automated with a Biomek diluter for mixing and diluting the drugs, and adding drugs and parasites to a test plate.

10 In the prescreen format, if the ANALYSIS FIELD (AF) has a "<", then the compound was "very active" and the IC values are most likely to be below the last dilution value (in nanograms/ml), which is listed next to AF. In most cases, these compounds were run again

15 at lower starting concentration to determine the true IC value. If the AF has a ">", then the IC value is greater than the prescreen dilution value; thus "AF>250" means that the IC value is greater than 250 ng ml<sup>-1</sup> and no further screening is carried out. In

20 such cases, values of 0.00 are entered for IC values.

The results are set out in Table IV below:-

Table IV

| Example No.              | In vitro activity: IC <sub>50</sub> ; IC <sub>90</sub> ; (IC <sub>10</sub> ) ng/ml |                                   |
|--------------------------|------------------------------------------------------------------------------------|-----------------------------------|
|                          | W2 Strain (Chloroquine resistant)                                                  | D6 Strain (Chloroquine sensitive) |
| IA (10 $\alpha$ -isomer) | 0.69; 0.97                                                                         | 0.64; 1.24                        |
| 1B (10 $\beta$ -isomer)  | 0.69; 0.98                                                                         | 0.74; 1.36                        |
| 2                        | 0.31; 0.52; (0.19)                                                                 | 0.73; 0.99; (0.53)                |
| 4                        | 0.84; 1.74; (0.40)                                                                 | 1.05; 2.10; (0.52)                |
| 12                       | 0.78; 1.32; (0.47)                                                                 | 0.77; 1.70; (0.35)                |
| 15                       | 0.66; 0.84; (0.52)                                                                 | 0.61; 0.78; (0.48)                |
| 16                       | 0.64; 0.84; (0.49)                                                                 | 0.61; 0.78; (0.48)                |
| 18                       | 0.23; 0.33; (0.17)                                                                 | 0.28; 0.82; (0.09)                |
| 19                       | 0.33; 0.43; (0.25)                                                                 | 0.39; 0.80; (0.19)                |
| 20                       | 5.81; 12.77; (2.64)                                                                | 9.40; 12.93; (6.84)               |
| 21                       | 0.00; 0.00 250AF<0                                                                 | 1.77; 3.96; (0.79)                |
| 23                       | 0.00; 0.00; AF>250                                                                 | 0.00; 0.00; AF>250                |
| 24                       | 0.77; 1.30; (0.46)                                                                 | 1.17; 2.10; (0.65)                |
| 25                       | 0.11; 0.17; (0.07)                                                                 | 0.09; 0.35; (0.02)                |
| 26                       | 0.00; 0.00 AF<4                                                                    | 9.05; 16.24; (5.05)               |
| 30                       | 0.00; 0.00; 250AF<0                                                                | 11.20; 18.61; (6.74)              |
| 31                       | 0.29; 0.68; (0.12)                                                                 | 1.35; 2.42; (0.75)                |
| 32                       | 0.45; 0.92; (0.22)                                                                 | 2.45; 3.97; (1.51)                |
| 36                       | 0.26; 0.61; (0.11)                                                                 | 0.38; 0.77; (0.19)                |
| 38                       | 1.23; 2.76 (0.55)                                                                  | 0.90; 3.69; (0.22)                |

Table IV (cont)

| Example No. | In vitro activity: IC <sub>50</sub> ; IC <sub>90</sub> ; (IC <sub>10</sub> ) ng/ml |                                   |
|-------------|------------------------------------------------------------------------------------|-----------------------------------|
|             | W2 Strain (Chloroquine resistant)                                                  | D6 Strain (Chloroquine sensitive) |
| 41          | 0.73; 1.7; (0.30)                                                                  | 1.53; 2.04; (1.16)                |
| 44          | 0.3318; 0.8168; (0.13)                                                             | 0.69; 1.67; (0.29)                |

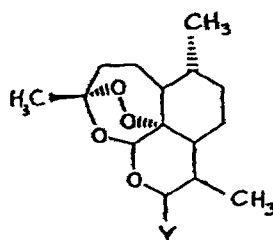
WO 00/04024

-76-

PCT/GB99/02267

CLAIMS

1. A compound of the general formula I



(I)

or a salt thereof,

in which

Y represents a halogen atom, an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclalkyl group or a group  $-NR^1R^2$ ; where

$R^1$  represents a hydrogen atom or an optionally substituted alkyl, alkenyl or alkynyl group;

$R^2$  represents an optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl or aralkyl group; or

$R^1$  and  $R^2$  together with the interjacent nitrogen atom represent an optionally substituted heterocyclic group or an amino group derived from an optionally substituted amino acid ester;

for use in the treatment and/or prophylaxis of a disease caused by infection with a parasite other than an organism of the genus Plasmodium.

2. A compound according to claim 1 in which Y represents a halogen atom.



3. A compound according to claim 1 or claim 2 in which Y represents a fluorine or bromine atom.

4. A compound according to claim 1 in which Y represents a C<sub>3-8</sub> cycloalkyl group, a C<sub>6-18</sub> aryl group, a 5- to 10-membered C-linked heteroaryl group or a 5- to 10-membered heterocyclyl-C<sub>1-6</sub> alkyl group, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> alkoxy, amino, C<sub>1-4</sub> alkylamino, di(C<sub>1-4</sub> alkyl)amino, carboxyl, C<sub>6-10</sub> aryl, 5 to 10-membered heterocyclic and C<sub>1-4</sub> alkyl- or phenyl-substituted 5- to 10-membered heterocyclic groups.

5. A compound according to claim 4 in which Y represents a C<sub>6-18</sub> aryl group optionally substituted by one or more substituents selected from the group consisting of halogen atoms, hydroxyl, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> haloalkoxy, amino, C<sub>1-4</sub> alkylamino, di(C<sub>1-4</sub> alkyl)amino and carboxyl groups.

6. A compound according to claim 4 or claim 5 in which Y represents a phenyl, naphthyl, anthryl or phenanthryl group, each group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms and hydroxyl, methyl, vinyl, C<sub>1-4</sub> alkoxy and carboxyl groups.

7. A compound according to any one of claims 4 to 6 in which Y represents a phenyl, fluorophenyl, chlorophenyl, bromophenyl, trimethylphenyl, vinylphenyl, methoxyphenyl,



dimethoxyphenyl, trimethoxyphenyl, carboxylphenyl, naphthyl, hydroxynaphthyl, methoxynaphthyl, anthryl or phenanthryl group.

5           8. A compound according to any one of claims 4 to 7 in which Y represents a phenyl or trimethoxyphenyl group.

9. A compound according to claim 1 in which Y represents a group  $-NR^1R^2$  where  $R^1$  represents a hydrogen atom or a  $C_{1-6}$  alkyl group and  $R^2$  represents a  $C_{1-6}$  alkyl, 10  $C_{3-8}$  cycloalkyl,  $C_{6-10}$  aryl or  $C_{7-16}$  aralkyl group, or  $R^1$  and  $R^2$  together with the interjacent nitrogen atom represent a 5- to 10-membered heterocyclic group or an amino group derived from a  $C_{1-6}$  alkyl ester of an amino acid, each 15 group being optionally substituted by one or more substituents selected from the group consisting of halogen atoms,  $C_{1-4}$  alkyl,  $C_{1-4}$  haloalkyl,  $C_{1-6}$  alkoxy carbonyl, phenyl, halophenyl,  $C_{1-4}$  alkylphenyl,  $C_{1-4}$  haloalkylphenyl,  $C_{1-4}$  alkoxyphenyl, benzyl, pyridyl and 20 pyrimidinyl groups.

10. A compound according to claim 9 in which Y represents a group  $-NR^1R^2$  where  $R^1$  represents a hydrogen atom or a  $C_{1-4}$  alkyl group and  $R^2$  represents a  $C_{1-4}$  alkyl, 25  $C_{3-6}$  cycloalkyl, phenyl or benzyl group, or  $R^1$  and  $R^2$  together with the interjacent nitrogen atom represent a 6- to 10-membered heterocyclic group or an amino group derived from a  $C_{1-4}$  alkyl ester of an amino acid, each group being optionally substituted by one or more 30 substituents selected from the group consisting of halogen atoms,  $C_{1-4}$  haloalkyl,  $C_{1-4}$  alkoxy carbonyl, phenyl, halophenyl,  $C_{1-4}$  alkylphenyl,  $C_{1-4}$  haloalkylphenyl,  $C_{1-4}$  alkoxyphenyl, benzyl, pyridyl and pyrimidinyl groups.



11. A compound according to claim 9 or claim 10 in which Y represents a propylamino, cyclopentylamino, cyclohexylamino, phenylamino, fluorophenylamino, chlorophenylamino, bromophenylamino, iodophenylamino, methoxycarbonylphenylamino, biphenylamino, benzylamino, 5 fluorobenzylamino, bis(trifluoromethyl)benzylamino, phenylethylamino, phenyl-methoxycarbonylmethylamino, diethylamino, morpholinyl, thiomorpholinyl, morpholinosulphonyl, indolinyl, tetrahydroisoquinolinyl, phenylpiperazinyl, fluorophenylpiperazinyl, 10 chlorophenylpiperazinyl, methylphenylpiperazinyl, trifluoromethylphenylpiperazinyl, methoxyphenylpiperazinyl, benzylpiperazinyl, pyridylpiperazinyl and pyrimidinylpiperazinyl group.

12. A compound according to any one of claims 9 to 11 in which Y represents a propylamino, phenylamino, bromophenylamino, iodophenylamino, biphenylamino, benzylamino, bis(trifluoromethyl)benzylamino, 20 phenylethylamino, phenyl-methoxycarbonylmethylamino or morpholinyl group.

13. A compound according to any one of the preceding claims in which the parasite is an organism of the genus Neospora or the genus Eimeria. 25

14. Use of a compound of the general formula I as defined in any one of claims 1 to 12 for the manufacture of a medicament for the treatment and/or prophylaxis of a 30 disease caused by infection with a parasite other than an organism of the genus Plasmodium.

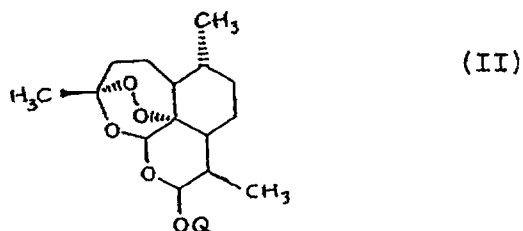


15. Use according to claim 14 in which the parasite is an organism of the genus Neospora or the genus Eimeria.

5 16. A compound of the general formula I as defined in any one of claims 1 to 12, with the proviso that, when Y is a group  $-NR^1R^2$  and  $R^2$  represents a phenyl, 3-chlorophenyl, 4-chlorophenyl, 3-bromophenyl, 4-bromophenyl, 4-iodophenyl, 4-methylphenyl, 4-methoxyphenyl, 3-carboxylphenyl or 4-carboxylphenyl  
10 group, then  $R^1$  is an optionally substituted alkyl group.

17. A process for the preparation of a compound of the general formula I according to claim 16 which comprises reacting a compound of the general formula II

15



20

in which Q represents a hydrogen atom or trimethylsilyl group, with a suitable halogenating agent to form a compound of the general formula I in which Y represents a halogen atom; and, if desired, reacting the compound of  
25 general formula I thus formed either with a Grignard reagent of the general formula  $Y\text{MgX}$  where Y is an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group and X is a halogen atom to form a compound of general formula I in which Y  
30 represents an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group or with an



amine of the general formula  $\text{HNR}^1\text{R}^2$  where  $\text{R}^1$  and  $\text{R}^2$  are as defined in claim 13 to form a compound of general formula I in which Y represents a group  $-\text{NR}^1\text{R}^2$  where  $\text{R}^1$  and  $\text{R}^2$  are as defined above.

5

10

18. A process according to claim 17 in which a compound of the general formula I in which Y represents a bromine atom is generated in situ by reacting a compound of the general formula II in which Q represents a trimethylsilyl group with bromotrimethylsilane.

15

19. A process for the preparation of a compound of the general formula I according to claim 16 in which Y represents an optionally substituted cycloalkyl, aryl, C-linked heteroaryl or heterocyclylalkyl group which comprises reacting 9,10-anhydroartemisinin with a compound of the general formula Y-H, where Y is as defined above, in the presence of a suitable Lewis acid.

20

25

20. A process for the preparation of a compound of the general formula I as defined in claim 1 in which Y represents an optionally substituted aryl or C-linked heteroaryl group which comprises reacting 10-trichloroacetimidoyl-10-deoxoartemisinin with a compound of the general formula Y-H, where Y is defined above, in the presence of a suitable Lewis acid.

30

21. A process according to claim 18 in which the 10-trichloroacetimidoyl-10-deoxoartemisinin is generated in situ by reacting a compound of formula II as defined in claim 17 in which Q represents a hydrogen atom with trichloroacetonitrile in the presence of a suitable base.



22. A process for the preparation of a compound of the general formula I as defined in claim 1 in which Y represents an optionally substituted aryl or C-linked heteroaryl group which comprises reacting a 10-acyloxyartemisinin compound in which the acyloxy group is of formula A-(C=O)-O-, where A represents an optionally substituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclic or polycyclic group, with a compound of the general formula Y-H, where Y is as defined above, in the presence of a Lewis acid.

23. A pharmaceutical composition which comprises a carrier and, as active ingredient, a compound of the general formula I according to claim 16.

15

24. A compound of the general formula I according to claim 16 for use in the treatment and/or prophylaxis of a disease caused by infection with a parasite of the genus Plasmodium.

20

25. Use of a compound of the general formula I according to Claim 16 for the manufacture of a medicament for the treatment and/or prophylaxis of a disease caused by infection with a parasite of the genus Plasmodium.

25

26. A method for treating a disease caused by infection with a parasite other than an organism of the genus Plasmodium which comprises administering to a host in need of such treatment a therapeutically effective amount of a compound of the general formula I as defined in claim 1.

30



-84-

27. A method for treating a disease caused by infection with a parasite of the genus Plasmodium which comprises administering to a host in need of such treatment a therapeutically effective amount of a compound of the general formula I according to claim 16.

5