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(54) **FUSED HETEROCYCLIC SUCCINIMIDE  
COMPOUNDS AND ANALOGS THEREOF,  
MODULATORS OF NUCLEAR HORMONE  
RECEPTOR FUNCTION**

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(57) **ABSTRACT**

Fused cyclic compounds, methods of using such compounds  
in the treatment of nuclear hormone receptor-associated con-  
ditions such as cancer and immune disorders, and pharma-  
ceutical compositions containing such compounds.

**FUSED HETEROCYCLIC SUCCINIMIDE  
COMPOUNDS AND ANALOGS THEREOF,  
MODULATORS OF NUCLEAR HORMONE  
RECEPTOR FUNCTION**

**[0001]** This application is a continuation of application Ser. No. 10/917,031, filed Aug. 12, 2004, which is a divisional application of application Ser. No. 10/322,077, filed Dec. 18, 2002, now abandoned, which is a continuation-in-part of application Ser. No. 10/025,116, filed Dec. 19, 2001, now abandoned, which is a continuation-in-part of application Ser. No. 09/885,381, filed Jun. 20, 2001, now abandoned, and application Ser. No. 09/885,827, filed Jun. 20, 2001, now U.S. Pat. No. 6,960,474, wherein application Ser. No. 09/885,381 further claims the benefit of provisional Application No. 60/284,438, filed Apr. 18, 2001, provisional Application No. 60/284,730, filed Apr. 18, 2001, and provisional Application No. 60/233,519, filed Sep. 19, 2000, and application Ser. No. 09/885,827 further claims the benefit of provisional Application No. 60/284,438, filed Apr. 18, 2001, provisional Application No. 60/284,730, filed Apr. 18, 2001, and provisional Application No. 60/233,519, filed Sep. 19, 2000, which applications are incorporated herein by reference in their entirety.

**FIELD OF THE INVENTION**

**[0002]** The present invention relates to fused cyclic compounds, to methods of using such compounds in the treatment of nuclear hormone receptor-associated conditions such as cancer, and to pharmaceutical compositions containing such compounds.

**BACKGROUND OF THE INVENTION**

**[0003]** Nuclear hormone receptors (NHR's) constitute a large super-family of ligand-dependent and sequence-specific transcription factors. Members of this family influence transcription either directly, through specific binding to the promoter target genes (Evans, in *Science* 240: 889-895 (1988)), or indirectly, via protein-protein interactions with other transcription factors (Jonat et al., *Cell* 62: 1189-1204 (1990), Schuele et al., *Cell* 62: 1217-1226 (1990), and Yang-Yen et al., *Cell* 62: 1205-1215 (1990)). The nuclear hormone receptor super-family (also known in the art as the "steroid/thyroid hormone receptor super-family") includes receptors for a variety of hydrophobic ligands, including cortisol, aldosterone, estrogen, progesterone, testosterone, vitamin D<sub>3</sub>, thyroid hormone and retinoic acid (Evans, 1988, supra). In addition to these conventional nuclear hormone receptors, the super-family contains a number of proteins that have no known ligands, termed orphan nuclear hormone receptors (Mangelsdorf et al., *Cell* 83: 835-839 (1995), O'Malley et al., *Mol. Endocrinol.* 10: 1293 (1996), Enmark et al., *Mol. Endocrinol.* 10, 1293-1307 (1996) and Giguere, *Endocrin. Rev.* 20, 689-725 (1999)). The conventional nuclear hormone receptors are generally transactivators in the presence of ligand, and can either be active repressors or transcriptionally inert in the absence of ligand. Some of the orphan receptors behave as if they are transcriptionally inert in the absence of ligand. Others, however, behave as either constitutive activators or repressors. These orphan nuclear hormone receptors are either under the control of ubiquitous ligands that have not been identified, or do not need to bind ligand to exert these activities. In common with other transcription factors, the

nuclear hormone receptors have a modular structure, being comprised of three distinct domains: an N-terminal domain of variable size containing a transcriptional activation function AF-1, a highly conserved DNA binding domain and a moderately conserved ligand-binding domain. The ligand-binding domain is not only responsible for binding the specific ligand but also contains a transcriptional activation function called AF-2 and a dimerisation domain (Wurtz et al., *Nature Struc. Biol.* 3, 87-94 (1996), Parker et al., *Nature Struc. Biol.* 3, 113-115 (1996) and Kumar et al., *Steroids* 64, 310-319 (1999)). Although the overall protein sequence of these receptors can vary significantly, all share both a common structural arrangement indicative of divergence from an ancestral archetype, and substantial homology (especially, sequence identity) at the ligand-binding domain.

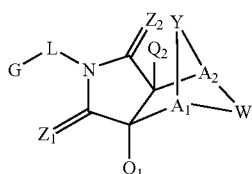
**[0004]** The steroid binding nuclear hormone receptors (SB-NHR's) comprise a sub-family of nuclear hormone receptors. These receptors are related in that they share a stronger sequence homology to one another, particularly in the ligand binding domain (LBD), than to the other members of the NHR super-family (Evans, 1988, supra) and they all utilize steroid based ligands. Some examples of this sub-family of NHR's are the androgen receptor (AR), the estrogen receptor (ER), the progesterone receptor (PR), the glucocorticoid receptor (GR), the mineralocorticoid receptor (MR), the aldosterone receptor (ALDR) and the steroid and xenobiotic receptor (SXR) (Evans et al., WO 99/35246). Based on the strong sequence homology in the LBD, several orphan receptors may also be members of the SB-NHR sub-family. Consistent with the high sequence homology found in the LBD for each of the SB-NHR's, the natural ligands for each is derived from a common steroid core. Examples of some of the steroid based ligands utilized by members of the SB-NHR's include cortisol, aldosterone, estrogen, progesterone, testosterone and dihydrotestosterone. Specificity of a particular steroid based ligand for one SB-NHR versus another is obtained by differential substitution about the steroid core. High affinity binding to a particular SB-NHR, coupled with high level specificity for that particular SB-NHR, can be achieved with only minor structural changes about the steroid core (e.g., Waller et al., *Toxicol. Appl. Pharmacol.* 137, 219-227 (1996) and Mekenyan et al., *Environ. Sci. Technol.* 31, 3702-3711 (1997), binding affinity for progesterone towards the androgen receptor as compared to testosterone). Numerous synthetically derived steroidal and non-steroidal agonists and antagonists have been described for the members of the SB-NHR family. Many of these agonist and antagonist ligands are used clinically in man to treat a variety of medical conditions. RU486 is an example of a synthetic agonist of the PR, which is utilized as a birth control agent (Vegeto et al., *Cell* 69: 703-713 (1992)), and Flutamide is an example of an antagonist of the AR, which is utilized for the treatment of prostate cancer (Neri et al., *Endo.* 91, 427-437 (1972)). Tamoxifen is an example of a tissue specific modulator of the ER function, that is used in the treatment of breast cancer (Smigel, *J. Natl. Cancer Inst.* 90, 647-648 (1998)). Tamoxifen can function as an antagonist of the ER in breast tissue while acting as an agonist of the ER in bone (Grese et al., *Proc. Natl. Acad. Sci. USA* 94, 14105-14110 (1997)). Because of the tissue selective effects seen for Tamoxifen, this agent and agents like it are referred to as "partial-agonist" or partial-antagonist". In addition to synthetically derived non-endogenous ligands, non-endogenous ligands for NHR's can be obtained from food sources (Regal et al., *Proc. Soc.*

*Exp. Biol. Med.* 223, 372-378 (2000) and Hempstock et al., *J. Med. Food* 2, 267-269 (1999)). The flavanoid phytoestrogens are an example of an unnatural ligand for SB-NHR's that are readily obtained from a food source such as soy (Quella et al., *J. Clin. Oncol.* 18, 1068-1074 (2000) and Banz et al., *J. Med. Food* 2, 271-273 (1999)). The ability to modulate the transcriptional activity of individual NHR by the addition of a small molecule ligand, makes them ideal targets for the development of pharmaceutical agents for a variety of disease states. As mentioned above, non-natural ligands can be synthetically engineered to serve as modulators of the function of NHR's. In the case of SB-NHR's, engineering of an unnatural ligand can include the identification of a core structure which mimics the natural steroid core system. This can be achieved by random screening against several SB-NHR's or through directed approaches using the available crystal structures of a variety of NHR ligand binding domains (Bourguet et al., *Nature* 375, 377-382 (1995), Brzozowski, et al., *Nature* 389, 753-758 (1997), Shiau et al., *Cell* 95, 927-937 (1998) and Tanenbaum et al., *Proc. Natl. Acad. Sci. USA* 95, 5998-6003 (1998)). Differential substitution about such a steroid mimic core can provide agents with selectivity for one receptor versus another. In addition, such modifications can be employed to obtain agents with agonist or antagonist activity for a particular SB-NHR. Differential substitution about the steroid mimic core can result in the formation of a series of high affinity agonists and antagonists with specificity for, for example, ER versus PR versus AR versus GR versus MR. Such an approach of differential substitution has been reported, for example, for quinoline based modulators of steroid NHR in *J. Med. Chem.*, 41, 623 (1999); WO 9749709; U.S. Pat. No. 5,696,133; U.S. Pat. No. 5,696,130; U.S. Pat. No. 5,696,127; U.S. Pat. No. 5,693,647; U.S. Pat. No. 5,693,646; U.S. Pat. No. 5,688,810; U.S. Pat. No. 5,688,808 and WO 9619458, all incorporated herein by reference.

[0005] The compounds of the present invention comprise a core which serves as a steroid mimic, and are useful as modulators of the function of steroid binding nuclear hormone receptors, as well as other NHR as described following.

#### SUMMARY OF THE INVENTION

[0006] The present invention provides fused cyclic compounds of the following formula I and salts thereof, which compounds are especially useful as modulators of nuclear hormone receptor function:



[0007] As used in formula I, and throughout the specification, the symbols have the following meanings unless otherwise indicated, and are, for each occurrence, independently selected:

[0008] G is an aryl or heterocyclo (e.g., heteroaryl) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with hydrogen, alkyl or substituted alkyl, alkenyl or sub-

stituted alkenyl, alkynyl or substituted alkynyl, halo, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo, arylalkyl or substituted arylalkyl, heterocycloalkyl or substituted heterocycloalkyl, CN, R<sup>1</sup>OC=O, R<sup>1</sup>C=O, R<sup>1</sup>C=S, R<sup>1</sup>HNC=O, R<sup>1</sup>R<sup>2</sup>NC=O, HO-CR<sup>3</sup>R<sup>3'</sup>, nitro, R<sup>1</sup>OCH<sub>2</sub>, R<sup>1</sup>O, NH<sub>2</sub>, NR<sup>4</sup>R<sup>5</sup>, SR<sup>1</sup>, S=OR<sup>1</sup>, SO<sub>2</sub>R<sup>1</sup>, SO<sub>2</sub>OR<sup>1</sup>, SO<sub>2</sub>NR<sup>1</sup>R<sup>1'</sup>, (R<sup>1</sup>O)(R<sup>1</sup>O)P=O, oxo, (R<sup>1</sup>)(R<sup>1'</sup>)P=O, or (R<sup>1</sup>)(NHR<sup>1</sup>)P=O;

[0009] Z<sub>1</sub> is O, S, NH, or NR<sup>6</sup>;

[0010] Z<sub>2</sub> is O, S, NH, or NR<sup>6</sup>;

[0011] A<sub>1</sub> is CR<sup>7</sup> or N;

[0012] A<sub>2</sub> is CR<sup>7</sup> or N;

[0013] Y is J-J'-J'' where J is (CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub> and n=0-3, J' is a bond or O, S, S=O, SO<sub>2</sub>, NH, NR<sup>7</sup>, C=O, OC=O, NR<sup>1</sup>C=O, CR<sup>7</sup>R<sup>7'</sup>, C=CR<sup>8</sup>R<sup>8'</sup>, R<sup>2</sup>P=O, R<sup>2</sup>P=S, R<sup>2</sup>OP=O, R<sup>2</sup>NHP=O, OP=OOR<sup>2</sup>, OP=ONHR<sup>2</sup>, OP=OR<sup>2</sup>, OSO<sub>2</sub>, C=NR<sup>7</sup>, NHNH, NHNR<sup>6</sup>, NR<sup>6</sup>NH, N=N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo or aryl or substituted aryl, and J'' is (CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub> and n=0-3, where Y is not a bond (i.e., if J' is a bond, then in at least one of J or J'' (each defined as (CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub>), n is not zero);

[0014] W is CR<sup>7</sup>R<sup>7'</sup>-CR<sup>7</sup>R<sup>7'</sup>, CR<sup>8</sup>=CR<sup>8'</sup>, CR<sup>7</sup>R<sup>7'</sup>-C=O, C=O-C=O, CR<sup>7</sup>R<sup>7'</sup>-C=CH<sub>2</sub>, C=CH<sub>2</sub>-C=CH<sub>2</sub>, CR<sup>7</sup>R<sup>7'</sup>-C=NR<sup>1</sup>, C=NR<sup>1</sup>C=NR<sup>1</sup>, NR<sup>9</sup>-CR<sup>7</sup>R<sup>7'</sup>, N=CR<sup>8</sup>, N=N, NR<sup>9</sup>-NR<sup>9'</sup>, S-CR<sup>7</sup>R<sup>7'</sup>, SO-CR<sup>7</sup>R<sup>7'</sup>, SO<sub>2</sub>-CR<sup>7</sup>R<sup>7'</sup>, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein when W is not NR<sup>9</sup>-CR<sup>7</sup>R<sup>7'</sup>, N=CR<sup>8</sup>, N=N, NR<sup>9</sup>-NR<sup>9'</sup>, S-CR<sup>7</sup>R<sup>7'</sup>, SO-CR<sup>7</sup>R<sup>7'</sup>, SO<sub>2</sub>-CR<sup>7</sup>R<sup>7'</sup>, or heterocyclo or substituted heterocyclo, then J' must be O, S, S=O, SO<sub>2</sub>, NH, NR<sup>7</sup>, OC=O, NR<sup>1</sup>C=O, OP=OOR<sup>2</sup>, OP=ONHR<sup>2</sup>, OSO<sub>2</sub>, NHNH, NHNR<sup>6</sup>, NR<sup>6</sup>NH, or N=N; or

[0015] when W is CR<sup>7</sup>R<sup>7'</sup>-CR<sup>7</sup>R<sup>7'</sup>, the R<sup>7</sup> and R<sup>7'</sup> substituents in each occurrence may be taken together to form a substituted or unsubstituted carbocyclic or substituted or unsubstituted heterocyclic ring system which can be formed by any combination of R<sup>7</sup> and R<sup>7'</sup> attached to the same carbon atom;

[0016] Q<sub>1</sub> is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, R<sup>1</sup>OC=O, R<sup>4</sup>C=O, R<sup>5</sup>R<sup>6</sup>NC=O, HO-CR<sup>7</sup>R<sup>7'</sup>, nitro, R<sup>1</sup>OCH<sub>2</sub>, R<sup>1</sup>O, NH<sub>2</sub>, C=OSR<sup>1</sup>, SO<sub>2</sub>R<sup>1</sup> or NR<sup>4</sup>R<sup>5</sup>;

[0017] Q<sub>2</sub> is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, R<sup>1</sup>OC=O, R<sup>4</sup>C=O, R<sup>5</sup>R<sup>6</sup>NC=O, HO-CR<sup>7</sup>R<sup>7'</sup>, nitro, R<sup>1</sup>OCH<sub>2</sub>, R<sup>1</sup>O, NH<sub>2</sub>, C=OSR<sup>1</sup>, SO<sub>2</sub>R<sup>1</sup> or NR<sup>4</sup>R<sup>5</sup>;

[0018] L is a bond, (CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub>, NH, NR<sup>5</sup>, NH(CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub>, or NR<sup>5</sup>(CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub>, where n=0-3;

[0019]  $R^1$  and  $R^{1'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

[0020]  $R^2$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

[0021]  $R^3$  and  $R^{3'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, hydroxylamine, hydroxamide, alkoxy or substituted alkoxy, amino,  $NR^2$ , thiol, alkylthio or substituted alkylthio;

[0022]  $R^4$  is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl,  $R^1C=O$ ,  $R^1OC=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ ,  $SO_2OR^1$  or  $SO_2NR^1R^{1'}$ ;

[0023]  $R^5$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl,  $R^1C=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ ,  $SO_2OR^1$ , or  $SO_2NR^1R^{1'}$ ;

[0024]  $R^6$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH,  $OR^1$ ,  $R^1C=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ ,  $SO_2OR^1$ , or  $SO_2NR^1R^{1'}$ ;

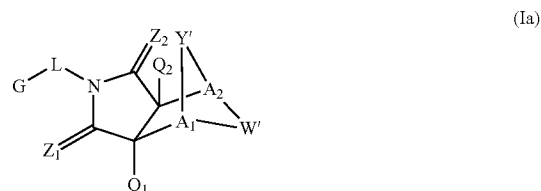
[0025]  $R^7$  and  $R^{7'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN,

$OR^4$ , nitro, hydroxylamine, hydroxylamide, amino,  $NHR^4$ ,  $NR^2R^5$ ,  $NR^5R^5$ ,  $NOR^1$ , thiol, alkylthio or substituted alkylthio,  $HOC=O$ ,  $R^1C=O$ ,  $R^1(C=O)O$ ,  $R^1OC=O$ ,  $R^1NHC=O$ ,  $NH_2C=O$ ,  $SO_2R^1$ ,  $SOR^1$ ,  $PO_3R^1R^{1'}$ ,  $R^1R^1NC=O$ ,  $C=OSR^1$ ,  $SO_2R^1$ ,  $SO_2OR^1$ , or  $SO_2NR^1R^{1'}$ , or, wherein  $A_1$  or  $A_2$  contains a group  $R^7$  and W contains a group  $R^7$ , said  $R^7$  groups of  $A_1$  or  $A_2$  and W together form a heterocyclic ring;

[0026]  $R^8$  and  $R^{8'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, nitro, halo, CN,  $OR^1$ , amino,  $NHR^4$ ,  $NR^2R^5$ ,  $NOR^1$ , alkylthio or substituted alkylthio,  $C=OSR^1$ ,  $R^1OC=O$ ,  $R^1C=O$ ,  $R^1NHC=O$ ,  $R^1R^1NC=O$ ,  $SO_2OR^1$ ,  $S=OR^1$ ,  $SO_2R^1$ ,  $PO_3R^1R^{1'}$ , or  $SO_2NR^1R^{1'}$ ; and

[0027]  $R^9$  and  $R^{9'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH,  $OR^1$ ,  $R^1C=O$ ,  $R^1OC=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ ,  $SO_2OR^1$ , or  $SO_2NR^1R^{1'}$ .

[0028] Compounds within formula I are novel, a preferred subgenus of which is the following formula Ia:



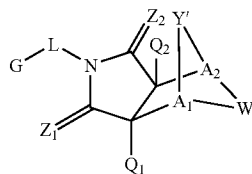
[0029] where G, L,  $Z_1$ ,  $Z_2$ ,  $A_1$ ,  $A_2$ ,  $Q_1$  and  $Q_2$  are as defined above;

[0030]  $Y'$  is  $J-J'-J''$  where J is  $(CR^7R^{7'})_n$  and  $n=0-3$ ,  $J'$  is a bond or O, S,  $S=O$ ,  $SO_2$ , NH,  $NR^7$ ,  $CR^7R^{7'}$ ,  $R^2P=O$ ,  $R^2P=S$ ,  $R^2OP=O$ ,  $R^2NHP=O$ ,  $OP=OOR^2$ ,  $OP=ONHR^2$ ,  $OSO_2$ ,  $NHNH$ ,  $NHNR^6$ ,  $NR^6NH$ ,  $N=N$ , cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and  $J''$  is  $(CR^7R^{7'})_n$  and  $n=0-3$ , where Y is not a bond; and

[0031]  $W'$  is  $CR^7R^{7'}-CR^7R^{7'}$ ,  $CR^7R^{7'}-C=O$ ,  $C=O-C=O$ ,  $CR^7R^{7'}-C=CH_2$ ,  $C=CH_2-C=CH_2$ ,  $CR^7R^{7'}-C=NR^1$ ,  $C=NR^1-C=NR^1$ ,  $NR^9-CR^7R^{7'}$ ,  $N=CR^8$ ,  $N=N$ ,  $NR^9-NR^9$ , cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein when  $W'$  is not  $NR^9-CR^7R^{7'}$ ,  $N=CR^8$ ,  $N=N$ ,  $NR^9-NR^9$ , or heterocyclo or substituted heterocyclo, then  $J'$  must be O, S,  $S=O$ ,  $SO_2$ , NH,  $NR^7$ ,  $OP=OOR^2$ ,  $OP=ONHR^2$ ,  $OSO_2$ ,  $NHNH$ ,  $NHNR^6$ ,  $NR^6NH$ , or  $N=N$ ; or

- [0032] when  $W'$  is  $CR^7R''-CR^7R''$ , the  $R^7$  and  $R''$  substituents in each occurrence may be taken together to form a substituted or unsubstituted carbocyclic or substituted or unsubstituted heterocyclic ring system which can be formed by any combination of  $R^7$  and  $R''$  attached to the same carbon atom; or alternatively,
- [0033]  $Y'$  is  $NR^7-CR^7R''$  and  $W'$  is  $CR^8=CR^8$ ; or, alternatively,
- [0034]  $Y'$  is  $CR^7R''-C=O$  and  $W'$  is  $NR^9-CR^7R''$ ;
- [0035] where  $R^2$ ,  $R^6$ ,  $R^7$ ,  $R''$ ,  $R^8$ ,  $R^9$  and  $R''$  are as defined above and with the provisos that
- [0036] (1) when  $Y'$  is  $-O-$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is  $-CH_2-CH_2-$ , and  $A_1$  and  $A_2$  are  $CH$ , then G-L is not phenyl, monosubstituted phenyl or phenyl which is substituted with two or more of the following groups: methoxy, halo,  $NO_2$ , methyl,  $CH_3-S-$ ,  $OH$ ,  $CO_2H$ , trifluoromethyl,  $-C(O)-C_6H_5$ ,  $NH_2$ , 4-7-epoxy, hexahydro-1H-isindole-1,3 (2H)dione, or  $-C(O)-CH_3$ ;
- [0037] (2) when  $Y'$  is  $-O-$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is  $CH_2-CH_2-$ , and one of  $A_1$  and  $A_2$  is  $CH$  and the other is  $CR^7$ , then G-L is not unsubstituted phenyl;
- [0038] (3) when  $Y'$  is  $-O-$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is  $CH_2-CH_2-$ , and one of  $A_1$  and  $A_2$  is  $CH$  and the other is  $C-CH_3$ , then G-L is not phenyl substituted with chloro and/or methyl;
- [0039] (4) when  $Y'$  is  $-O-$  or  $-S-$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is  $CH_2-CH_2-$ , and one of  $A_1$  and  $A_2$  is  $CH$  and the other is  $CH$  or  $C$ -alkyl, then G-L is not N-substituted piperazine-alkyl- or N-substituted imidazolidine-alkyl-;
- [0040] (5) when  $Y'$  is  $-O-$ ;  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is  $CH_2-CH_2-$ , and  $A_1$  and  $A_2$  are  $CH$ , then G-L is not oxazole or triazole;
- [0041] (6) when  $Y'$  is  $-O-$ ;  $Q_1$  and  $Q_2$  are hydrogen or methyl,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is  $CH_2-CH_2-$ , and  $A_1$  and  $A_2$  are  $CH$  or  $C-CH_3$ , then G-L is not thiazole or substituted thiazole (in addition such compounds where G-L is optionally substituted thiadiazole or partially saturated thiazole are optionally removed by proviso where  $A_1$  and  $A_2$  are both  $CH$ );
- [0042] (7) when  $Y'$  contains a group  $J'$  selected from  $S$ ,  $S=O$ ,  $SO_2$ ,  $NH$ ,  $NR^7$ ,  $R^2P=O$ ,  $R^2P=S$ ,  $R^2OP=O$ ,  $R^2NHP=O$ ,  $OP=OOR^2$ ,  $OP=ONHR^2$ ,  $OSO_2$ ,  $NHNH$ ,  $NHR^6$ ,  $NR^7NH$  or  $N=N$ ,  $W'$  is  $CR^7R''-CR^7R''$ , and  $Z_1$  and  $Z_2$  are  $O$ , then G-L is not unsubstituted phenyl;
- [0043] (8) when  $Y'$  is  $NR^7$ ,  $W'$  is unsubstituted or substituted phenyl, and  $Q_1$  and  $Q_2$  are hydrogen, then  $Z_1$  and  $Z_2$  are not  $O$ ;
- [0044] (9) when  $Y'$  is  $-O-$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is dihydroisoxazole bearing an optionally substituted phenyl group, and  $A_1$  and  $A_2$  are  $CH$ , then G-L is not unsubstituted phenyl or dichlorophenyl;
- [0045] (10) when  $Y'$  is  $O$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is ethylene oxide, and  $A_1$  and  $A_2$  are  $CH$ , then G-L is not methylphenyl or chlorophenyl;
- [0046] (11) when  $Y'$  is  $NR^7-CR^7R''$ ,  $W'$  is  $CR^8=CR^8$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $A_1$  and  $A_2$  are  $CH$ ,  $C-CH_3$ ,  $C-CH_2-C_6H_5$  or  $C-CH_2-CH_3$ , and  $Z_1$  and  $Z_2$  are  $O$ , then G-L is not unsubstituted phenyl, monosubstituted phenyl or methylpivindyl;

- [0047] (12) when  $Y'$  is  $CR^7R^7-C=O$ ,  $W'$  is  $NR^9-CR^7R^7$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $A_1$  and  $A_2$  are  $CH$ , and  $Z_1$  and  $Z_2$  are  $O$ , then G-L is not unsubstituted phenyl;
- [0048] (13) when  $Y'$  is  $CHR^7-NR^7$  where  $R^7$  is unsubstituted phenyl, methoxy or ethoxy and  $R^7$  is unsubstituted phenyl, methyl or  $-C(O)-C_6H_5$ ,  $W'$  is dimethoxyphenylene or unsubstituted phenylene,  $Z_1$  and  $Z_2$  are  $O$ ,  $Q_1$  and  $Q_2$  are hydrogen, and  $A_1$  and  $A_2$  are  $CH$ ,  $C-CN$ ,  $C-C(O)-C_6H_5$ , or  $-C(O)-$  dimethoxyphenyl, then G-L is not unsubstituted phenyl;
- [0049] (14) the compound of formula Ia is not 6,10-epithio-4H-thieno-[3',4':5,6]cyclooct[1,2-j]isoindole-7,9(5H,8H)-dione, 8-(3,5-dichlorophenyl)-6,6 $\alpha$ , 9a,10,11,12,-hexahydro-1,3,6,10-tetramethyl-2,2,13-trioxide, (6R,6aR,9aS,10S);
- [0050] (15) when  $Y'$  is  $O$ ,  $W'$  is  $-CH_2-CH_2-$ ,  $Q_1$  and  $Q_2$  are methyl,  $Z_1$  and  $Z_2$  are  $O$ , and  $A_1$  and  $A_2$  are  $CH$ , then G-L is not unsubstituted phenyl, phenyl substituted with methoxy, phenyl-alkyl-, or morpholine-alkyl, nor is the compound bridged to itself through a group L which is alkylene to form a bis compound;
- [0051] (16) when  $Y'$  is  $-O-$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is  $CR^7R^7-CR^7R^7$ , and  $A_1$  and  $A_2$  are  $CH$ , then G-L is not an unsubstituted phenyl group; and
- [0052] (17) when  $Y'$  is  $-O-$ ,  $Q_1$  and  $Q_2$  are hydrogen,  $Z_1$  and  $Z_2$  are  $O$ ,  $W'$  is cyclopentyl, cyclohexyl, 3-phenyl-2-isoxazoline or  $CR^7R^7-CR^7R^7$  where  $R^7$  and  $R^7$  are each independently defined as  $Cl$ ,  $Br$ ,  $H$  and 4-butyrolactone and  $R^7$  and  $R^7$  are not all simultaneously  $H$ , and  $A_1$  and  $A_2$  are  $CH$ , then G-L is not an unsubstituted naphthyl ring or a monosubstituted phenyl ring, where said substituent is methoxy,  $Br$ ,  $Cl$ ,  $NO_2$ , methyl, ethyl,  $CH_2$ -phenyl,  $S$ -phenyl, or  $O$ -phenyl.
- [0053] Preferably, compounds of formula I are monomeric, and are not comprised within other oligomers or polymers.
- [0054] Another preferred novel subgenus is that of the following formula Ib:



Ib

- [0055]** where G, Z<sub>1</sub>, Z<sub>2</sub>, Q<sub>1</sub> and Q<sub>2</sub> are as defined above;  
**[0056]** Y' is J-J'-J'' where J is (CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub> and n=0-3, J' is a bond or O, S, S=O, SO<sub>2</sub>, NH, NR<sup>7</sup>CR<sup>7</sup>R<sup>7'</sup>, R<sup>2</sup>P=O, R<sup>2</sup>P=S, R<sup>2</sup>OP=O, R<sup>2</sup>NHP=O, OP=OOR<sup>2</sup>, OP=ONHR<sup>2</sup>, OSO<sub>2</sub>, NHNH, NHNHR<sup>6</sup>, NR<sup>6</sup>NH, N=N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and J'' is (CR<sup>7</sup>R<sup>7'</sup>)<sub>n</sub> and n=0-3, where Y is not a bond; and  
**[0057]** W' is CR<sup>7</sup>R<sup>7'</sup>—CR<sup>7</sup>R<sup>7'</sup>, CR<sup>7</sup>R<sup>7'</sup>—C=O, C=O—C=O, CR<sup>7</sup>R<sup>7'</sup>—C=CH<sub>2</sub>, C=CH<sub>2</sub>—C=CH<sub>2</sub>, CR<sup>7</sup>R<sup>7'</sup>—C=NR<sup>1</sup>, C=NR<sup>1</sup>—C=NR<sup>1</sup>, NR<sup>9</sup>—CR<sup>7</sup>R<sup>7'</sup>, N=CR<sup>8</sup>, N=N, NR<sup>9</sup>—NR<sup>9</sup>, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein when W' is not NR<sup>9</sup>—CR<sup>7</sup>R<sup>7'</sup>, N=CR<sup>8</sup>, N=N, NR<sup>9</sup>

NR<sup>9</sup>, or heterocyclo or substituted heterocyclo, then J' must be O, S, S=O, SO<sub>2</sub>, NH, NR<sup>7</sup>, OP=OOR<sup>2</sup>, OP=ONHR<sup>2</sup>, OSO<sub>2</sub>, NHHN, NHNR<sup>6</sup>, NR<sup>6</sup>NH, or N=N; or

**[0058]** when W' is CR<sup>7</sup>R<sup>7'</sup>—CR<sup>7</sup>R<sup>7'</sup>, the R<sup>7</sup> and R<sup>7'</sup> substituents in each occurrence may be taken together to form a substituted or unsubstituted carbocyclic or substituted or unsubstituted heterocyclic ring system which can be formed by any combination of R<sup>7</sup> and R<sup>7'</sup> attached to the same carbon atom; or alternatively,

**[0059]** Y' is CR<sup>7</sup>R<sup>7'</sup>—C=O and W' is NR<sup>9</sup>—CR<sup>7</sup>R<sup>7'</sup>;

**[0060]** L is a bond; and

**[0061]** A<sub>1</sub> and A<sub>2</sub> are as defined above, especially where A<sub>1</sub> and/or A<sub>2</sub> are alkyl or optionally substituted alkyl (preferred such optional substituents being one or more groups V<sup>1</sup> defined below), with the proviso that, when Y'=O and W'=CH<sub>2</sub>—CH<sub>2</sub>—, then at least one of A<sub>1</sub> or A<sub>2</sub> is not CH;

with the further provisos (2), (3), (6), (7) and (8) above.

**[0062]** The compounds of formula I and salts thereof comprise a core which can serve as a steroid mimic (and do not require the presence of a steroid-type (e.g., cyclo-pentanoperhydrophenanthrene analog) structure).

#### FURTHER DESCRIPTION OF THE INVENTION

**[0063]** The following are definitions of terms used in the present specification. The initial definition provided for a group or term herein applies to that group or term throughout the present specification individually or as part of another group, unless otherwise indicated.

**[0064]** The terms “alkyl” and “alk” refers to a straight or branched chain alkane (hydrocarbon) radical containing from 1 to 12 carbon atoms, preferably 1 to 6 carbon atoms. Exemplary such groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, and the like. “Substituted alkyl” refers to an alkyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include but are not limited to one or more of the following groups: halo (e.g., a single halo substituent or multiple halo substituents forming, in the latter case, groups such as a perfluoroalkyl group or an alkyl group bearing Cl<sub>3</sub> or CF<sub>3</sub>), alkoxy, alkylthio, hydroxy, carboxy (i.e., —COOH), alkoxy-carbonyl, alkylcarbonyloxy, amino (i.e., —NH<sub>2</sub>), carbamoyl or substituted carbomoyl, carbamate or substituted carbamate, urea or substituted urea, amidinyl or substituted amidinyl, thiol (i.e., —SH), aryl, heterocycle, cycloalkyl, heterocycloalkyl, —S-aryl, —S-heterocycle, —S=O-aryl, —S=O-heterocycle, arylalkyl-O—, —S(O)<sub>2</sub>-aryl, —S(O)<sub>2</sub>-heterocycle, —NHS(O)<sub>2</sub>-aryl, —NHS(O)<sub>2</sub>-heterocycle, —NHS(O)<sub>2</sub>NH-aryl, —NHS(O)<sub>2</sub>NH-heterocycle, —P(O)<sub>2</sub>-aryl, —P(O)<sub>2</sub>-heterocycle, —NHP(O)<sub>2</sub>-aryl, —NHP(O)<sub>2</sub>-heterocycle, —NHP(O)<sub>2</sub>NH-aryl, —NHP(O)<sub>2</sub>NH-heterocycle, —O-aryl, —O-heterocycle, —NH-aryl, —NH-heterocycle, —NHC=O-aryl, —NHC=O-alkyl, —NHC=O-heterocycle, —OC=O-aryl, —OC=O-heterocycle, —NHC=ONH-aryl, —NHC=ONH-heterocycle, —OC=OO-aryl, —OC=OO-heterocycle, —OC=ONH-aryl, —OC=ONH-heterocycle, —NHC=OO-aryl, —NHC=OO-heterocycle, —C=ONH-aryl, —C=ONH-heterocycle, —C=OO-aryl, —C=OO-heterocycle, —N(alkyl)S(O)<sub>2</sub>-aryl, —N(alkyl)S

(O)<sub>2</sub>-heterocycle, —N(alkyl)S(O)<sub>2</sub>NH-aryl, —N(alkyl)S(O)<sub>2</sub>NH-heterocycle, —N(alkyl)P(O)<sub>2</sub>-aryl, —N(alkyl)P(O)<sub>2</sub>-heterocycle, —N(alkyl)P(O)<sub>2</sub>NH-aryl, —N(alkyl)P(O)<sub>2</sub>NH-heterocycle, —N(alkyl)-aryl, —N(alkyl)-heterocycle, —N(alkyl)C=O-aryl, —N(alkyl)C=O-heterocycle, —N(alkyl)C=ONH-aryl, —N(alkyl)C=ONH-heterocycle, —OC=ON(alkyl)-aryl, —OC=ON(alkyl)-heterocycle, —N(alkyl)C=OO-aryl, —N(alkyl)C=OO-heterocycle, —C=ON(alkyl)-aryl, —C=ON(alkyl)-heterocycle, —NHS(O)<sub>2</sub>N(alkyl)-aryl, —NHS(O)<sub>2</sub>N(alkyl)-heterocycle, —NHP(O)<sub>2</sub>N(alkyl)-aryl, —NHP(O)<sub>2</sub>N(alkyl)-heterocycle, —NHC=ON(alkyl)-aryl, —NHC=ON(alkyl)-heterocycle, —N(alkyl)S(O)<sub>2</sub>N(alkyl)-aryl, —N(alkyl)S(O)<sub>2</sub>N(alkyl)-heterocycle, —N(alkyl)P(O)<sub>2</sub>N(alkyl)-aryl, —N(alkyl)P(O)<sub>2</sub>N(alkyl)-heterocycle, —N(alkyl)C=ON(alkyl)-aryl, and —N(alkyl)C=ON(alkyl)-heterocycle. In the aforementioned exemplary substituents, in each instance, groups such as “alkyl”, “aryl” and “heterocycle” can themselves be optionally substituted; for example, “alkyl” in the group “NCH=OO-alkyl” recited above can be optionally substituted so that both “NHC=OO-alkyl” and “NHC=OO-substituted alkyl” are exemplary substituents. Exemplary alkyl substituents also include groups such as “T” and “T-R<sup>12</sup>” (which are defined below), especially for substituted alkyl groups within A<sub>1</sub> or A<sub>2</sub>.

**[0065]** The term “alkenyl” refers to a straight or branched chain hydrocarbon radical containing from 2 to 12 carbon atoms and at least one carbon-carbon double bond. Exemplary such groups include ethenyl or allyl. “Substituted alkenyl” refers to an alkenyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents.

**[0066]** The term “alkynyl” refers to a straight or branched chain hydrocarbon radical containing from 2 to 12 carbon atoms and at least one carbon to carbon triple bond. Exemplary such groups include ethynyl. “Substituted alkynyl” refers to an alkynyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents.

**[0067]** The term “cycloalkyl” refers to a fully saturated cyclic hydrocarbon group containing from 1 to 4 rings and 3 to 8 carbons per ring. Exemplary such groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc. “Substituted cycloalkyl” refers to a cycloalkyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, nitro, cyano, alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents, and as previously mentioned as preferred aryl substituents in the definition for G. Exemplary substituents also include spiro-attached or fused cyclic substituents, especially cycloalkenyl or substituted cycloalkenyl.

**[0068]** The term “cycloalkenyl” refers to a partially unsaturated cyclic hydrocarbon group containing 1 to 4 rings and 3 to 8 carbons per ring. Exemplary such groups include cyclobutenyl, cyclopentenyl, cyclohexenyl, etc. “Substituted cycloalkenyl” refers to a cycloalkenyl group substituted with one more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include but are not limited to nitro, cyano, alkyl or substituted

alkyl, as well as those groups recited above as exemplary alkyl substituents, and as previously mentioned as preferred aryl substituents in the definition for G. Exemplary substituents also include spiro-attached or fused cyclic substituents, especially cycloalkyl or substituted cycloalkyl.

**[0069]** The terms “alkoxy” or “alkylthio” refer to an alkyl group as described above bonded through an oxygen linkage ( $-\text{O}-$ ) or a sulfur linkage ( $-\text{S}-$ ), respectively. The terms “substituted alkoxy” or “substituted alkylthio” refer to a substituted alkyl group as described above bonded through an oxygen or sulfur linkage, respectively.

**[0070]** The term “alkoxycarbonyl” refers to an alkoxy group bonded through a carbonyl group.

**[0071]** The term “alkylcarbonyl” refers to an alkyl group bonded through a carbonyl group. The term “alkylcarbonyloxy” refers to an alkylcarbonyl group bonded through an oxygen linkage.

**[0072]** The terms “arylalkyl”, “substituted arylalkyl”, “cycloalkylalkyl”, “substituted cycloalkylalkyl”, “cycloalkenylalkyl”, “substituted cycloalkenylalkyl”, “heterocycloalkyl” and “substituted heterocycloalkyl” refer to aryl, cycloalkyl, cycloalkenyl and heterocyclo groups bonded through an alkyl group, substituted on the aryl, cycloalkyl, cycloalkenyl or heterocyclo and/or the alkyl group where indicated as “substituted.”

**[0073]** The term “aryl” refers to cyclic, aromatic hydrocarbon groups which have 1 to 5 aromatic rings, especially monocyclic or bicyclic groups such as phenyl, biphenyl or naphthyl. Where containing two or more aromatic rings (bicyclic, etc.), the aromatic rings of the aryl group may be joined at a single point (e.g., biphenyl), or fused (e.g., naphthyl, phenanthrenyl and the like). “Substituted aryl” refers to an aryl group substituted by one or more substituents, preferably 1,2,3,4 or 5 substituents, at any point of attachment. Exemplary substituents include, but are not limited to, nitro, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, cyano, alkyl- $\text{S}(\text{O})_m$ — ( $m=0, 1$  or  $2$ ), alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents and as previously mentioned as preferred aryl substituents in the definition for G. Exemplary substituents also include fused cyclic substituents, such as heterocyclo or cycloalkenyl, or substituted heterocyclo or cycloalkenyl, groups (e.g., thereby forming a fluoroenyl, tetrahydronaphthalenyl, or dihydroindenyl group).

**[0074]** “Carbamoyl” refers to the group  $-\text{CONH}-$  which is bonded on one end to the remainder of the molecule and on the other to hydrogen or an organic moiety (such as alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, alkylcarbonyl, hydroxyl and substituted nitrogen). “Carbamate” refers to the group  $-\text{O}-\text{CO}-\text{NH}-$  which is bonded on one end to the remainder of the molecule and on the other to hydrogen or an organic moiety (such as those listed above). “Urea” refers to the group  $-\text{NH}-\text{CO}-\text{NH}-$  which is bonded on one end to the remainder of the molecule and on the other to hydrogen or an organic moiety (such as those listed above). “Amidinyl” refers to the group  $-\text{C}(=\text{NH})(\text{NH}_2)-$  “Substituted carbamoyl”, “substituted carbamate”, “substituted urea” and “substituted amidinyl” refer to carbamoyl, carbamate, urea or amidinyl groups as described above in which one more of the hydrogen groups are replaced by an organic moiety (such as those listed above).

**[0075]** The terms “heterocycle”, “heterocyclic” and “heterocyclo” refer to fully saturated, or partially or fully unsaturated, including aromatic (i.e., “heteroaryl”) cyclic groups (for

example, 3 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 16 membered tricyclic ring systems) which have at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, 3, or 4 heteroatoms selected from nitrogen atoms, oxygen atoms and/or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. (The term “heteroaryl” refers to a heteroaryl group bearing a quaternary nitrogen atom and thus a positive charge.) The heterocyclic group may be attached to the remainder of the molecule at any heteroatom or carbon atom of the ring or ring system. It is understood that, where W or W' are cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, that A1 and A2 can be separately bonded to different (such as adjacent) ring atoms on said groups. Exemplary monocyclic heterocyclic groups include ethylene oxide, azetidiny, pyrrolidinyl, pyrrolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazoliny, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazoliny, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, hexahydrodiazepinyl, 4-piperidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, triazolyl, tetrazolyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, and the like. Exemplary bicyclic heterocyclic groups include indolyl, isoindolyl, benzothiazolyl, benzodioxolyl, benzoxazolyl, benzoxadiazolyl, benzothienyl, quinuclidinyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indoliziny, benzofuryl, benzofurazanyl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl] or furo[2,3-b]pyridinyl), dihydrobenzodioxinyl, dihydrodioxidobenzothiophenyl, dihydroisoindolyl, dihydroindolyl, dihydroquinolinyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), triazinylazepinyl, tetrahydroquinolinyl and the like. Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthrolinyl, dibenzofuranyl, acridinyl, phenanthridinyl, xanthenyl and the like.

**[0076]** “Substituted heterocycle”, “substituted heterocyclic”, and “substituted heterocyclo” (such as “substituted heteroaryl”) refer to heterocycle, heterocyclic or heterocyclo groups substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, nitro, oxo (i.e.,  $=\text{O}$ ), cyano, alkyl- $\text{S}(\text{O})_m$ — ( $m=0, 1$  or  $2$ ), alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents, and as previously mentioned as preferred heterocyclo substituents in the definition for G.

**[0077]** The term “quaternary nitrogen” refers to a tetravalent positively charged nitrogen atom including, for example, the positively charged nitrogen in a tetraalkylammonium group (e.g., tetramethylammonium, N-methylpyridinium), the positively charged nitrogen in protonated ammonium species (e.g., trimethyl-hydroammonium, N-hydropyridinium), the positively charged nitrogen in amine N-oxides (e.g.,

N-methyl-morpholine-N-oxide, pyridine-N-oxide), and the positively charged nitrogen in an N-amino-ammonium group (e.g., N-aminopyridinium).

[0078] The terms “halogen” or “halo” refer to chlorine, bromine, fluorine or iodine.

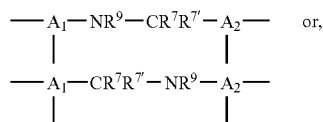
[0079] The terms “hydroxylamine” and “hydroxylamide” refer to the groups  $\text{OH}-\text{NH}-$  and  $\text{OH}-\text{NH}-\text{CO}-$ , respectively.

[0080] When a functional group is termed “protected”, this means that the group is in modified form to mitigate, especially preclude, undesired side reactions at the protected site. Suitable protecting groups for the methods and compounds described herein include, without limitation, those described in standard textbooks, such as Greene, T. W. et al., *Protective Groups in Organic Synthesis*, Wiley, N.Y. (1991).

[0081] When a term such as “(CRR) $n$ ” is used, it denotes an optionally substituted alkyl chain existing between the two fragments to which it is bonded, the length of which chain is defined by the range described for the term  $n$ . An example of this is  $n=0-3$ , implying from zero to three (CRR) units existing between the two fragments, which are attached to the primary and terminal (CRR) units. In the situation where the term  $n$  is set to zero ( $n=0$ ) then a bond exists between the two fragments attached to (CRR).

[0082] Unless otherwise indicated, any heteroatom with unsatisfied valences is assumed to have hydrogen atoms sufficient to satisfy the valences.

[0083] Divalent groups, such as those in the definition of W (e.g.,  $\text{NR}^9-\text{CR}^7\text{R}^{7'}$ ), may be bonded in either direction to the remainder of the molecule (e.g.,



For the aforementioned group within the definition of W).

[0084] Carboxylate anion refers to a negatively charged group  $-\text{COO}^-$ .

[0085] The compounds of formula I form salts which are also within the scope of this invention. Reference to a compound of the formula I herein is understood to include reference to salts thereof, unless otherwise indicated. The term “salt(s)”, as employed herein, denotes acidic and/or basic salts formed with inorganic and/or organic acids and bases. In addition, when a compound of formula I contains both a basic moiety, such as but not limited to a pyridine or imidazole, and an acidic moiety such as but not limited to a carboxylic acid, zwitterions (“inner salts”) may be formed and are included within the term “salt(s)” as used herein. Pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts are preferred, although other salts are also useful, e.g., in isolation or purification steps which may be employed during preparation. Salts of the compounds of the formula I may be formed, for example, by reacting a compound I with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

[0086] The compounds of formula I which contain a basic moiety, such as but not limited to an amine or a pyridine or imidazole ring, may form salts with a variety of organic and inorganic acids. Exemplary acid addition salts include

acetates (such as those formed with acetic acid or trihaloacetic acid, for example, trifluoroacetic acid), adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides, hydrobromides, hydroiodides, hydroxyethanesulfonates (e.g., 2-hydroxyethanesulfonates), lactates, maleates, methanesulfonates, naphthalenesulfonates (e.g., 2-naphthalenesulfonates), nicotines, nitrates, oxalates, pectinates, persulfates, phenylpropionates (e.g., 3-phenylpropionates), phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates (such as those formed with sulfuric acid), sulfonates (such as those mentioned herein), tartrates, thiocyanates, toluenesulfonates such as tosylates, undecanoates, and the like.

[0087] The compounds of formula I which contain an acidic moiety, such but not limited to a carboxylic acid, may form salts with a variety of organic and inorganic bases. Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as benzathines, dicyclohexylamines, hydrabamines (formed with N,N-bis(dehydroabietyl)ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glycamides, t-butyl amines, and salts with amino acids such as arginine, lysine and the like. Basic nitrogen-containing groups may be quaternized with agents such as lower alkyl halides (e.g. methyl, ethyl, propyl, and butyl chlorides, bromides and iodides), dialkyl sulfates (e.g. dimethyl, diethyl, dibutyl, and diamyl sulfates), long chain halides (e.g. decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides), aralkyl halides (e.g. benzyl and phenethyl bromides), and others.

[0088] Prodrugs and solvates of the compounds of the invention are also contemplated herein. The term “prodrug” as employed herein denotes a compound which, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield a compound of the formula I, or a salt and/or solvate thereof. Solvates of the compounds of formula I include, for example, hydrates.

[0089] Compounds of the formula I, and salts thereof, may exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present invention.

[0090] All stereoisomers of the present compounds (for example, those which may exist due to asymmetric carbons on various substituents), including enantiomeric forms and diastereomeric forms, are contemplated within the scope of this invention. Individual stereoisomers of the compounds of the invention may, for example, be substantially free of other isomers (e.g., as a pure or substantially pure optical isomer having a specified activity), or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the present invention may have the S or R configuration as defined by the IUPAC 1974 Recommendations. The racemic forms can be resolved by physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomeric derivatives or separation by chiral column chromatography. The individual optical isomers can be obtained from the racemates by any suitable method, including without limitation, conventional



methods, such as, for example, salt formation with an optically active acid followed by crystallization.

**[0091]** All configurational isomers of the compounds of the present invention are contemplated, either in admixture or in pure or substantially pure form. The definition of compounds of the present invention embraces both cis (Z) and trans (E) alkene isomers, as well as cis and trans isomers of cyclic hydrocarbon or heterocyclic rings. In certain cases, for example, the exo or endo conformation can be preferred for the fused ring system bonded to G-L in formula I. For example, for androgen receptor antagonists (or selective androgen receptor modulators), where Y is O or NR<sup>7</sup>, the exo configuration can be preferred, while for most other definitions of Y, the endo configuration can be preferred. As can be appreciated, the preferred configuration can be a function of the particular compound and its preferred activity. Separation of configurational isomers can be achieved by any suitable method, such as column chromatography.

**[0092]** Throughout the specifications, groups and substituents thereof may be chosen to provide stable moieties and compounds.

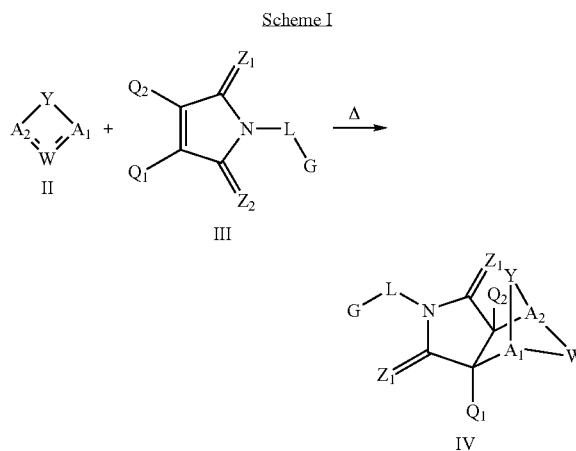
**[0093]** Embodiments indicated herein as exemplary or preferred are intended to be illustrative and not limiting.

#### METHODS OF PREPARATION

**[0094]** The compounds of the present invention may be prepared by methods such as those illustrated in the following Schemes I to XI. Solvents, temperatures, pressures, and other reaction conditions may readily be selected by one of ordinary skill in the art. Starting materials are commercially available or readily prepared by one of ordinary skill in the art. Combinatorial techniques may be employed in the preparation of compounds, for example, where the intermediates possess groups suitable for these techniques. See the following which describe other methods which may be employed in the preparation of compounds of the present invention: Li, et al., *Eur. J. Org. Chem.* 9, 1841-1850 (1998); Li, Y-Q, *Synlett.* 5, 461-464 (1996); Thiemann, et al., *Bull. Chem. Soc. Jpn.* 67, 1886-1893 (1994); Tsuge et al., *Heterocycles* 14, 423-428 (1980); Ward et al., *Can. J. Chem.* 75, 681-693 (1997); Ward et al., *Can. J. Chem.* 69, 1487-1497 (1991); Ward et al., *Tetrahedron Lett.* 31, 845-848 (1990); Fleming et al., *J. Org. Chem.* 44, 2280-2282 (1979); Jankowski et al., *J. Organomet. Chem.* 595, 109-113 (2000); Keglevich et al., *J. Organomet. Chem.* 579, 182-189 (1999); Keglevich et al., *J. Organomet. Chem.* 570, 49-539 (1998); Jankowski et al., *Heterocycl. Chem.* 7, 369-374 (1996); Jankowski et al., *J. Am. Chem. Soc.* 113, 7011-7017 (1991); Quin et al., *Tetrahedron Lett.* 31, 6473-6476 (1990); Quin et al., *J. Org. Chem.* 59, 120-129 (1994); Quin et al., *J. Org. Chem.* 58, 6212-6216 (1993); Quin et al., *Phosphorous, Sulfur Silicon Relat. Elem.* 63, 349-362 (1991); Quin et al., *Heterocycl. Chem.* 2, 359-367 (1991); Hussong et al., *Phosphorus Sulfur* 25, 201-212 (1985); Quin et al., *J. Org. Chem.* 51, 3341-3347 (1986); Myers et al., *J. Am. Chem. Soc.* 114, 5684-5692 (1992); Myers et al., *J. Am. Chem. Soc.* 113, 6682-6683 (1991); Shen et al., U.S. Pat. No. 5,817,679; Cordone et al., *J. Am. Chem. Soc.* 111, 5969-5970 (1989); Jung et al., *J. Chem. Soc. Commun.* 630-632 (1984); Lay et al., *J. Am. Chem. Soc.* 104, 7658-7659 (1982); Gonzalez et al., *J. Am. Chem. Soc.* 117, 3405-3421 (1995); Kreher et al., *Chem. Ber.* 125, 183-189 (1992); Simig et al., *Synlett.* 7, 425-426 (1990); Sha et al., *J. Org. Chem.* 55, 2446-2450 (1990); Drew et al., *J. Chem. Soc., Perkin Trans.* 17, 1277-1284 (1985); Kreher et al., *Anorg. Chem., Org. Chem.* 31B, 599-604 (1976); Avalos

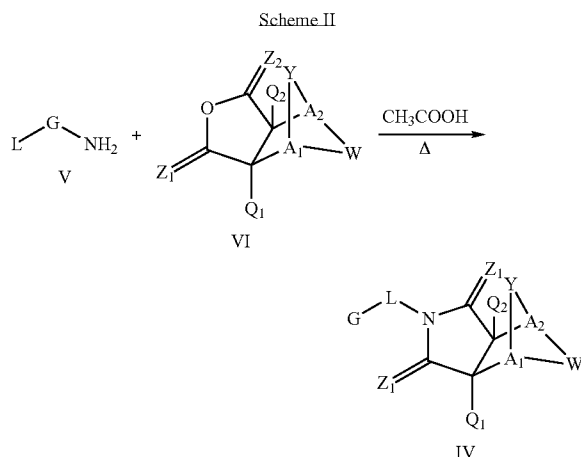
et al., *Tetrahedron Lett.* 39, 9301-9304 (1998); Gousse et al., *Macromolecules* 31, 314-321 (1998); Mikhailuchenko et al., *Khim. Geterotsikl. Soedin.* 6, 751-758 (1993); Lubowitz et al., U.S. Pat. No. 4,476,184; Padwa et al., *J. Org. Chem.* 61, 3706-3714 (1996); Schlessinger et al., *J. Org. Chem.* 59, 3246-3247 (1994); Buchmeiser et al., WO Publication No. 9827423; Tanabe et al., Japanese Patent Document JP 07144477; Mochizuki et al., Japanese Patent Document JP 63170383; Hosoda et al., Japanese Patent Document JP 62053963; Onaka et al., Japanese Patent Document JP 62053964; Kato et al., Japanese Patent Document JP 53086035; Kato et al., Japanese Patent Document JP 51088631; Tottori et al., Japanese Patent Document JP 49124225; Augustin et al., German Patent Document DD101271; Title et al., French Patent Document FR 2031538; Gousse et al., *Polym. Int.* 48, 723-731 (1999); Padwa et al., *J. Org. Chem.* 62, 4088-4096 (1997); Theurillat-Moritz et al., *Tetrahedron: Asymmetry* 7, 3163-3168 (1996); Mathews et al., *J. Carbohydr. Chem.* 14, 287-97 (1995); Srivastava et al., *Natl. Acad. Sci. Lett. (India)* 15, 41-44 (1992); Mayorga et al., *Rev. Cubana Quim.* 4, 1-6 (1988); Kondoli et al., *J. Chem. Res., Synop.* 3, 76 (1987); Primelles et al., *Cent. Azucar* 7-14 (1985); Solov'eva et al., *Khim. Geterotsikl. Soedin.* 5, 613-15 (1984); Liu et al., *Yaoxue Xuebao* 18, 752-759 (1983); Joshi et al., *Indian J. Chem., Sect. B.* 22B, 131-135 (1983); Amos et al., WO Publication No. 9829495; Odagiri et al., U.S. Pat. No. 4,670,536; Gallucci et al., European Patent Document EP 355435; Redmore, D. U.S. Pat. No. 3,821,232; Nakano et al., *Heterocycles* 35, 37-40 (1993); Tomisawa et al., *Chem. Pharm. Bull.* 36, 1692-1697 (1988); Krow et al., *J. Heterocycl. Chem.* 22, 131-135 (1985); Krow et al., *J. Org. Chem.* 47, 1989-1993 (1982); Liu et al., *Yaoxue Xuebao* 18, 752-759 (1983); Nishikawa et al., *Yaoxue Xuebao* JP 01061457; and/or Rice et al., *J. Med. Chem.* 11, 183-185 (1968).

**[0095]** All documents cited in the present specification, such as those cited in this "Methods of Preparation" as well as other sections herein, are incorporated herein by reference in their entirety. Reference to any document herein is not to be construed as an admission that such document is prior art.



**[0096]** As illustrated in Scheme I, a diene of formula II can be reacted with a dienophile of formula III, under conditions readily selected by one skilled in the art (such as by the addition of heat ("Δ")), to obtain a compound of formula IV,

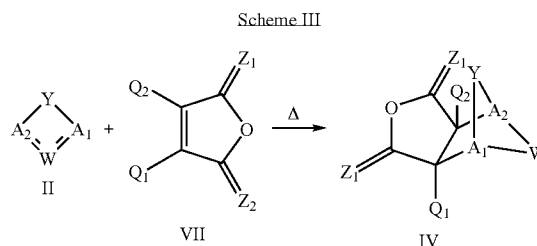
which is a compound of formula I. An intermediate diene of formula II can be obtained from commercial sources or readily made by one skilled in the art, for example, in accordance with the following literature documents and the references found therein: Hofman et al., *J. Agric. Food Chem.* 45, 898-906 (1997); Baciocchi et al., *J. Chem. Soc., Perkin Trans.* 28, 821-824 (1975); Wu et al., *J. Heterocycles* 38, 1507-1518 (1994); Yin et al., *Tetrahedron Lett.* 38, 5953-5954 (1997); Mic'ovic' et al., *Tetrahedron* 20, 2279-2287 (1964); Gorbunova et al., *J. Org. Chem.* 35, 1557-1566 (1999); Rassu et al., *Chem. Soc. Rev.* 29, 109-118 (2000); Kaberdin et al., *Russ. Chem. Rev.* 68, 765-779 (1999); Barluenga et al., *Aldrichimica Acta* 32, 4-15 (1999); Bogdanowicz-Szwed et al., *Pol. Wiad. Chem.* 52, 821-842 (1998); Casiraghi et al., *Adv. Asymmetric Synth.* 3, 113-189 (1998); and/or Baekvall et al., *Chem. Rev.* 98, 2291-2312 (1998). An intermediate dieneophile of formula III can be obtained from commercial sources or readily made by one skilled in the art, for example, in accordance with the following literature references and the references found therein: Deshpande et al., *Heterocycles* 51, 2159-2162 (1999); Seijas et al., *J. Chem. Res., Synop.* 7, 420-421 (1999); Langer et al., *Eur. J. Org. Chem.* 7, 1467-1470 (1998); Kita et al., Japanese Patent Document JP 09194458; Lopez-Alvarado et al., *J. Org. Chem.* 61, 5865-5870 (1996); Condon et al., U.S. Pat. No. 5,523,277; Sasaki-hara et al., Japanese Patent Document JP 04290868; Igarashi et al., Japanese Patent Document JP 04149173; Aoyama et al., Japanese Patent Document JP 04134063; Aoyama et al., Japanese Patent Document JP 04134062; Pastor et al., *J. Org. Chem.* 53, 5776-5779 (1988); and/or Takahashi et al., *Chem. Lett.* 6, 1229-1232 (1987).



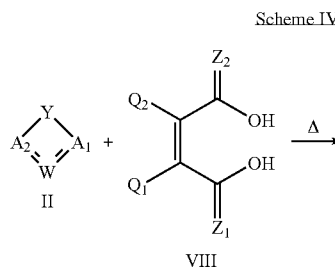
**[0097]** As illustrated in Scheme II, compounds of formula I can be obtained by reaction of a primary amine of formula V with a substituted anhydride-like intermediate of formula VI, for example, in a solvent such as acetic acid with or without heating, to yield a compound of formula IV, which is a compound of formula I. Primary amines of formula V can be obtained from commercial sources or readily synthesized by one skilled in the art. Anhydride-like agents of formula VI can be obtained from commercial sources or readily synthesized by one skilled in the art. The documents listed following describe exemplary approaches for the synthesis of intermediates of formula VI as well as synthetic approaches which can be applied to the synthesis of compounds of formula IV (all incorporated herein by reference in their entirety):

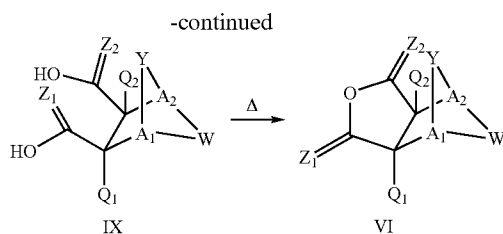
Kohler, E. P.; Tishler, M.; Potter, H.; Thompson, H. T. *J. Am. Chem. Soc.* 1939, 1057-1061; Yur'ev, Y. K.; Zefirov, N. S. *J. Gen. Chem. U.S.S.R. (Engl. Transl.)* 1961, 31, 772-5; Norman G. Gaylord U.S. Pat. No. 3,995,099; Schueler, P. E.; Rhodes, Y. E. *J. Org. Chem.* 1974, 39, 2063-9; Ishitobi, H.; Tanida, H.; Tsuji, T. *Bull. Chem. Soc. Japan* 1971, 44, 2993-3000; Stájer, G.; Virág, M.; Szabó, A. E.; Bernáth, G.; Sohár, P.; Sillanpää, R. *Acta. Chem. Scand.* 1996, 50, 922-30; Hart, H.; Ghosh, T. *Tetrahedron Lett.* 1988, 29, 881-884; Kato, M.; Yamamoto, S.; Yoshihara, T.; Furuichi, K.; Miwa, T. *Chem. Lett.* 1987, 1823-1826; Kottwitz, J.; Vorbrüggen, H. *Synthesis* 1995, 636-637; Creary, X. *J. Org. Chem.* 1975, 40, 3326-3331; Alder, K.; Ache, H.-J.; Flock, F. H. *Chem. Ber.* 1960, 93, 1888-1895; Toder, B. H.; Branca, S. J.; Dieter, R. K.; Smith, A. B. III *Synth. Commun.* 1975, 5, 435-439; Sprague, P. W.; Heikes, J. E.; Gougoutas, J. Z.; Malley, M. F.; Harris, D. N.; and/or Greenberg, R. *J. Med. Chem.* 1985, 28, 1580-1590.

**[0098]** The aforementioned approach(es) can be applied in a combinatorial fashion, for example, by utilizing a multi-well reaction block such as is described in Waldemar Ruediger, Wen-Jeng Li, John W., Allen Jr., and Harold N. Weller III, U.S. Pat. No. 5,961,925, Apparatus for Synthesis of Multiple Organic Compounds With Pinch Valve Block (incorporated herein by reference in its entirety). By utilizing the above-mentioned multi-well reaction block, one can, for example, perform multiples of 96 reactions at a time. Solvent can then be removed from the reaction tubes without removal from the reaction block and the crude products can be precipitated using a base such as sodium bicarbonate. The precipitates can be collected by filtration of the reaction block and then the desired products can be transferred directly to 96 well plates for screening. In this fashion, a large array of compounds of formula I can be synthesized, and tests conducted as desired by an automated approach.



**[0099]** Scheme III describes a method for preparing an intermediate compound of formula VI which can be used to synthesize a compound of formula I, as described in Scheme II. As described in Scheme III, a diene of formula II can be reacted with a dieneophile of formula VII to yield the intermediate of formula VI. The methods applied to obtain such a transformation are analogous to those described in Scheme I.

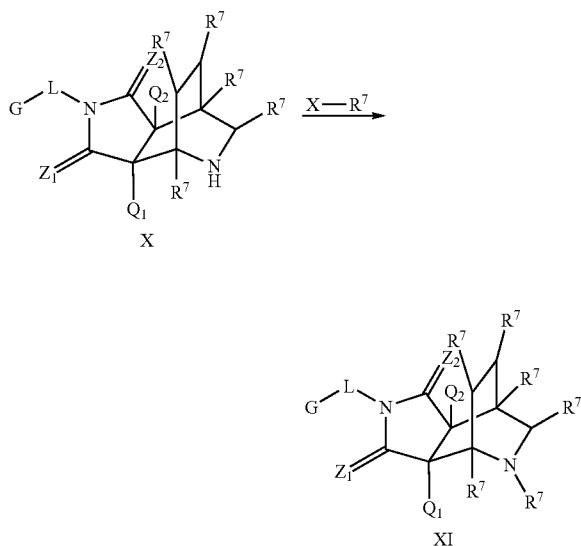




**[0100]** Scheme IV describes a method for preparing an intermediate compound of formula VI which can be used to synthesize a compound of formula I, as described in Scheme II. As shown in Scheme IV, a diene of formula II can be reacted with a dienophile of formula VIII to yield the intermediate of formula IX. The intermediate of formula IX can be dehydrated to an anhydride-like intermediate of formula VI. Dehydration of the bis-acid intermediate of formula IX to can be achieved by a variety of methods, such as those known to one skilled in the art and described in the following documents and the references embodied therein: Sprague et al., *J. Med. Chem.* 28, 1580-1590 (1985); and/or Retemi et al., *J. Org. Chem.* 61, 6296-6301 (1996).

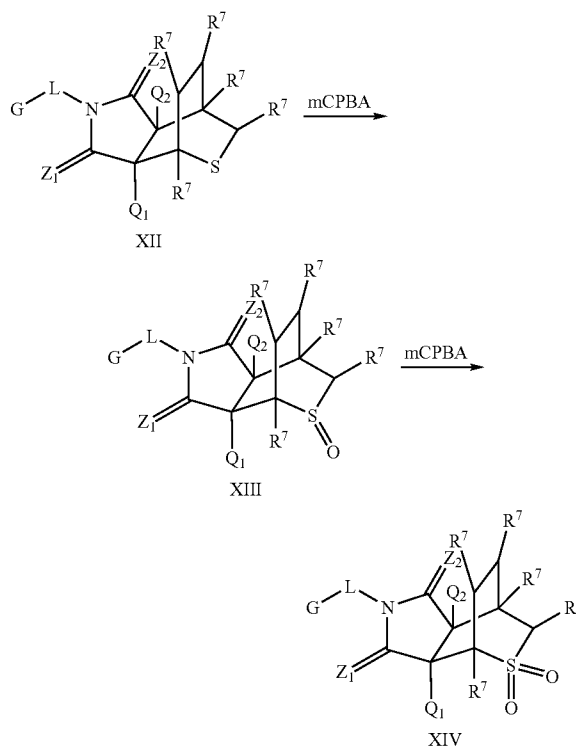
**[0101]** Schemes I to IV describe general methods for the synthesis of compounds of formula I, and intermediates thereof, in which substitution about the ring system is incorporated directly, for example, at the level of the intermediate diene, dienophile, anhydride-like intermediate and amine groups. In addition to these approaches, additional substitution can be incorporated onto an already-prepared compound of formula I by a variety of approaches to prepare other compounds of the formula I. Exemplary methods for further substitution are described in Schemes V to XI.

Scheme V



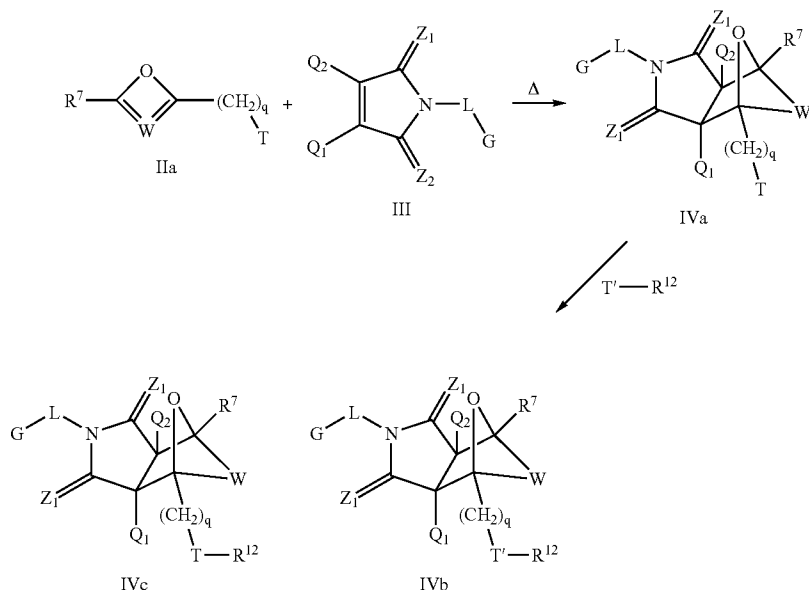
**[0102]** Scheme V describes one such approach to incorporating additional substitution into a structure of formula I. As illustrated in Scheme V, a compound of formula X, which is a compound of formula I where  $A_1$  and  $A_2$  are  $CR^7$ , W is  $NH-CHR^7$  and Y is  $CHR^7-CHR^7$ , can be functionalized at the free amine of the group W by reaction with any of a variety of electrophilic agents such as acid halides or alkyl halides in the presence of base, for example, by methods known by one skilled in the art. In Scheme V, X is a leaving group, and a compound of formula XI is a compound of formula I where  $A_1$  and  $A_2$  are  $CR^7$ , W is  $NR^7-CHR^7$  and Y is  $CHR^7-CHR^7$ .

Scheme VI



**[0103]** Scheme VI describes an additional approach for further incorporating substitution onto a compound of formula I. As illustrated in Scheme VI, a compound of formula XII, which is a compound of formula I where  $A_1$  and  $A_2$  are  $CR^7$ , W is  $S-CHR^7$  and Y is  $CHR^7-CHR^7$ , can be partially oxidized with an oxidizing agent such as mCPBA or other agents such as those known to one skilled in the art, to give the sulfoxide analog of formula XIII, which is a compound of formula I where  $A_1$  and  $A_2$  are  $CR^7$ , W is  $SO-CHR^7$  and Y is  $CHR^7-CHR^7$ . Further treatment of a compound of formula XIII with an oxidizing agent such as mCPBA or other agents such as those known to one skilled in the art, can yield the sulfone analog of formula XIV, which is a compound of formula I where  $A_1$  and  $A_2$  are  $CR^7$ , W is  $SO_2-CHR^7$  and Y is  $CHR^7-CHR^7$ . Alternatively, a compound of formula XII can be converted directly to a compound of formula XIV by prolonged treatment with an oxidizing agent, such as mCPBA, or with other agents such as those known to one skilled in the art.

Scheme VII

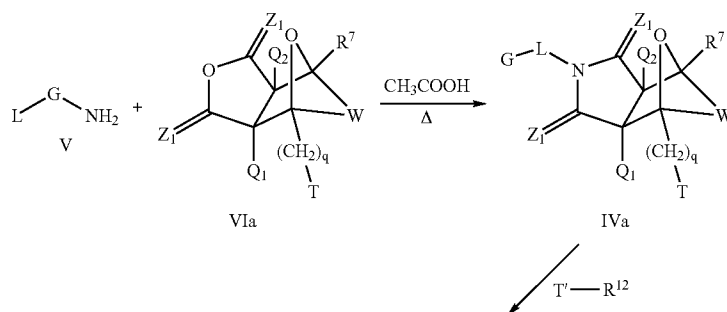


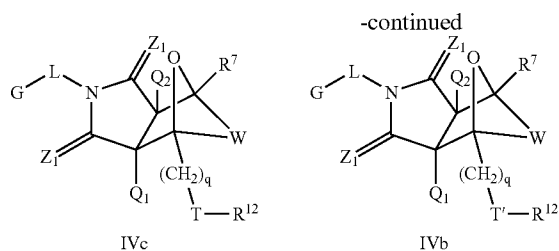
**[0104]** Scheme VII describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme VII, a diene of formula IIa can be reacted with a dienophile of formula III, as described in Scheme I, to yield a compound of formula I where Y is O,  $A_2$  is  $CR^7$  and  $A_1$  is  $C-(CH_2)_q-T$ . The compound of formula IVa can be reacted with a reagent of formula  $R^{12}-T'$  to obtain a compound of formula IVb or IVc which are compounds of formula I where Y is O,  $A_2$  is  $CR^7$  and  $A_1$  is  $C-(CH_2)_q-T'-R^{12}$  or  $C-(CH_2)_q-T-R^{12}$ , respectively. The reagent  $R^{12}-T'$  can be obtained from commercial sources or can readily be prepared by one skilled in the art.

**[0105]** In the above Scheme,  $R^{12}$  has the same definition as  $R^7$  defined earlier, q is zero or an integer from 0-8, and T is defined either as (1) a nucleophilic center such as, but not limited, to a nitrogen, oxygen or sulfur-containing group, capable of undergoing a nucleophilic substitution reaction with the leaving group  $T'$  or (2) a leaving group capable

undergoing a nucleophilic substitution reaction with a nucleophilic group  $T'$  (such as, but not limited, to a nitrogen, oxygen or sulfur-containing nucleophilic group).  $T'$  has the same definition as T. In the present case, for example, a nucleophilic substitution reaction occurs when the attacking reagent (the nucleophile) brings an electron pair to the substrate, using this pair to form the new bond, and the leaving group (the nucleofuge) comes away with the electron pair, leaving as an anionic intermediate. For a detailed discussion of the mechanism of aliphatic nucleophilic substitutions and a review of specific aliphatic nucleophilic substitution reactions see *Advanced Organic Chemistry, Reactions, Mechanisms, and Structure, 4<sup>th</sup> Edition*. Jerry March (Ed.), John Wiley & Sons, New York (1992) 293-500 and the references therein. Compounds of the formulae IVa, IVb, or IVc may, of course, be employed in the methods described herein (especially, in the treatment of nuclear hormone receptor-associated conditions) without undergoing further reaction of T or  $T'$ .

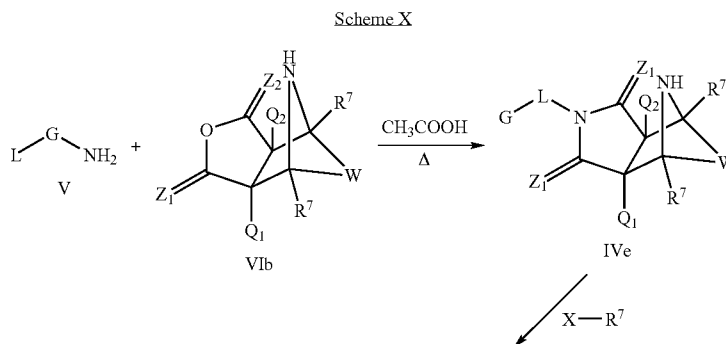
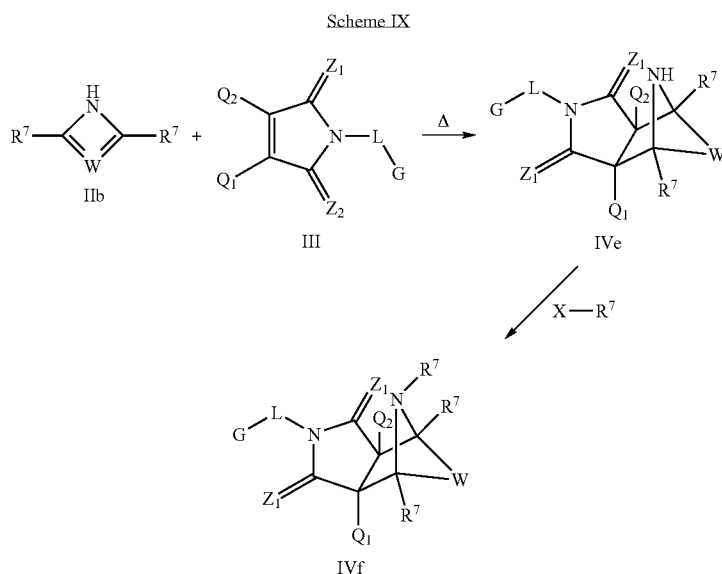
Scheme VIII

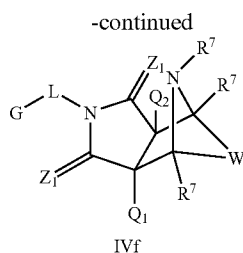




**[0106]** An alternate approach to compounds of formula IVa, IVb and IVc is illustrated in Scheme VIII. For this approach, techniques such as those described in Schemes II, III and IV can be applied to the preparation of an intermediate of formula VIa, where T and q are as defined in Scheme VII. The intermediate of formula VIa can be reacted with a substituted amine of formula V, as described in Scheme II, to yield the compound of formula IVa, which is a compound of formula I where Y is O, A<sub>2</sub> is CR<sup>7</sup> and A<sub>1</sub> is C—(CH<sub>2</sub>)<sub>q</sub>—T. The compound of formula IVa can be treated in the manner described in Scheme VII to obtain compounds of formula IVb or IVc which are compounds of formula I where Y is O, A<sub>2</sub> is CR<sup>7</sup> and A<sub>1</sub> is C—(CH<sub>2</sub>)<sub>q</sub>—T'-R<sup>12</sup> or C—(CH<sub>2</sub>)<sub>q</sub>—T-R<sup>12</sup>, respectively.

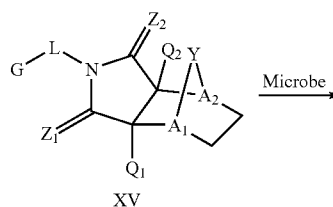
**[0107]** Scheme IX describes another approach to incorporating further substitution onto a compound of formula I. As illustrated in Scheme IX (where X is a leaving group), a diene of formula IIb can be reacted with a dienophile of formula III, as described in Scheme I, to yield a compound of formula IVe, which is a compound of formula I where Y is NH, and A<sub>1</sub> and A<sub>2</sub> are CR<sup>7</sup>. The compound of formula IVe can be functionalized at the free amine by reacting with a variety of electrophilic agents such as acid halides or alkyl halides in the presence of base, for example by methods known by one skilled in the art and described in Scheme V, to yield a compound of formula IVf, which is a compound of formula I where Y is NR<sup>7</sup> and A<sub>1</sub> and A<sub>2</sub> are CR<sup>7</sup>.



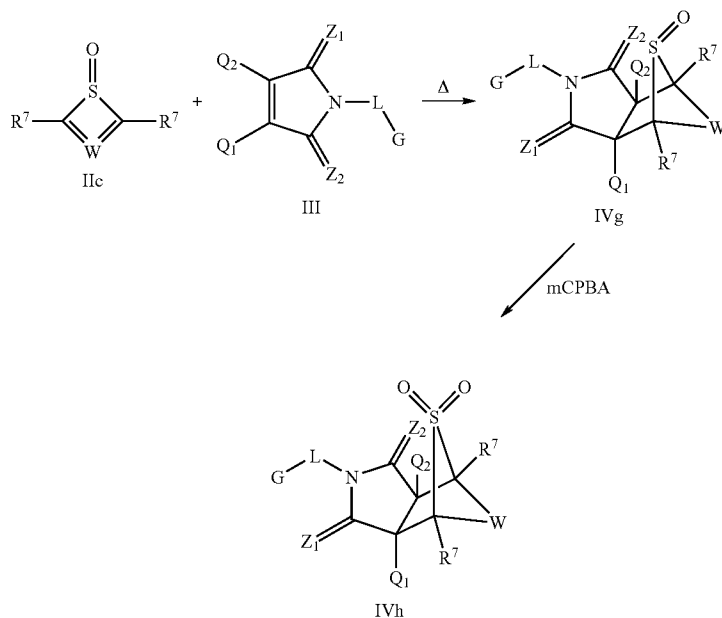


**[0108]** An alternate approach to compounds of formula IVe and IVf is illustrated in Scheme X. For this approach, techniques as described in Schemes II, III and IV can be applied to the preparation of an intermediate of formula VIb. The intermediate of formula VIb can be reacted with a substituted amine of formula V, as described in Scheme II, to yield a compound of formula IVe, which is a compound of formula I where Y is NH, and A<sub>1</sub> and A<sub>2</sub> are CR<sup>7</sup>. The latter intermediate can be treated in the manner described in Scheme V to obtain a compound of formula IVf, which is a compound of formula I where Y is NR<sup>7</sup>, and A<sub>1</sub> and A<sub>2</sub> are CR<sup>7</sup>.

Scheme XII

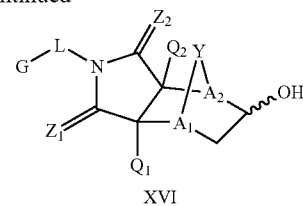


Scheme XI



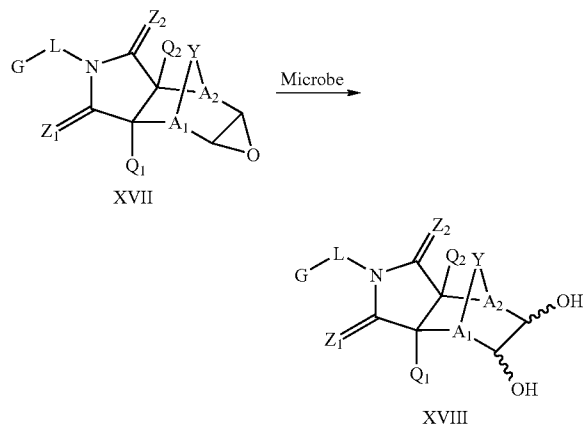
**[0109]** Scheme XI describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme XI, a diene of formula IIc can be reacted with a dienophile of formula III, as described in Scheme I, to yield a compound of formula IVg, which is a compound of formula I where Y is SO and A<sub>1</sub> and A<sub>2</sub> are CR<sup>7</sup>. A compound of formula IVg can be treated with an oxidizing agent such as mCPBA, as described in Scheme VI, to yield a compound of formula IVh, which is a compound of formula I where Y is SO<sub>2</sub> and A<sub>1</sub> and A<sub>2</sub> are CR<sup>7</sup>.

-continued



[0110] Scheme XII describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme XII, a compound of formula XV, which can be prepared in accordance with the above Schemes, can be incubated in the presence of a suitable enzyme or microorganism resulting in the formation of a hydroxylated analog of formula XVI. Such a process can be employed to yield regiospecific as well as enantiospecific incorporation of a hydroxyl group into a molecule of formula XV by a specific microorganism or by a series of different microorganisms. Such microorganisms can, for example, be bacterial, yeast or fungal in nature and can be obtained from distributors such as ATCC or identified for use in this method such as by methods known to one skilled in the art. Compound XVI is a compound of formula I where Y is as described above and  $A_1$  and  $A_2$  are preferably  $CR^7$ .

Scheme XIII

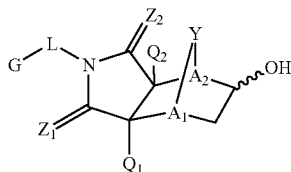


[0111] Scheme XIII describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme XIII, a compound of formula XVII, which can be prepared in accordance with the above Schemes, can be incubated in the presence of a suitable enzyme or microorganism resulting in the formation of a diol analog of formula XVIII. Such a process can be employed to yield regiospecific as well as enantiospecific transformation of a compound of formula XVII to a 1-2 diol of formula XVIII by a specific microorganism or by a series of different microorganisms. Such microorganisms can, for example, be bacterial, yeast or fungal in nature and can be obtained from distributors such as ATCC or identified for use in this method such as by methods known to one skilled in the art. Compound XVIII is a compound of formula I where Y is as described above and  $A_1$  and  $A_2$  are preferably  $CR^7$ .

[0112] The present invention also provides the methods of Schemes XII and XIII.

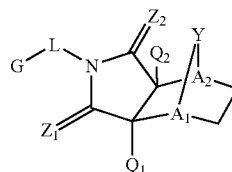
[0113] Thus, in one embodiment, the present invention provides a method for preparation of a compound of the following formula XVI, or salt thereof:

XVI



where the symbols are as defined herein, comprising the steps of contacting a compound of the following formula XV, or salt thereof:

XV

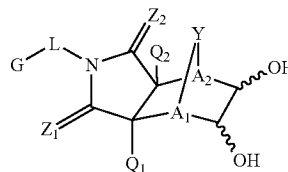


where the symbols are as defined above;

[0114] with an enzyme or microorganism capable of catalyzing the hydroxylation of said compound XV to form said compound XVI, and effecting said hydroxylation.

[0115] In another preferred embodiment, the present invention provides a method for preparation of a compound of the following formula XVIII, or salt thereof:

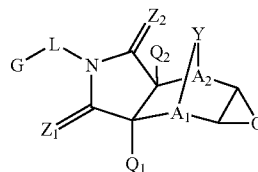
XVIII



where the symbols are as defined herein,

[0116] comprising the steps of contacting a compound of the following formula XVII, or salt thereof:

XVII



where the symbols are as defined above;

[0117] with an enzyme or microorganism capable of catalyzing the opening of the epoxide ring of compound XVII to form the diol of said compound XVIII, and effecting said ring opening and diol formation.

[0118] All stereoconfigurations of the unspecified chiral centers of the compounds of the formulae XV, XVI, XVII and XVIII are contemplated in the methods of the present invention, either alone (that is, substantially free of other stereoisomers) or in admixture with other stereoisomeric forms. Conversion of one isomer selectively (e.g., hydroxylation of the exo isomer preferentially to hydroxylation of the endo isomer) when contacting an isomeric mixture is a preferred embodiment of the invention. Conversion to one isomer selectively (e.g., hydroxylation on the exo face "exo isomer" preferentially to the endo face "endo isomer" or regioselective opening of an epoxide to form only one of two possible regioisomers of a trans diol) is a preferred embodiment of the invention. Hydroxylation of an achiral intermediate to form a

single optical isomer of the hydroxylated product is also a preferred embodiment of the invention. Resolution of a racemic mixture of an intermediate by selective hydroxylation, or epoxide ring opening and diol formation, to generate one of the two possible optical isomers is also a preferred embodiment of the invention. The term "resolution" as used herein denotes partial, as well as, preferably, complete resolution.

**[0119]** The terms "enzymatic process" or "enzymatic method", as used herein, denote a process or method of the present invention employing an enzyme or microorganism. The term "hydroxylation", as used herein, denotes the addition of a hydroxyl group to a methylene group as described above. Hydroxylation can be achieved, for example, by contact with molecular oxygen according to the methods of the present invention. Diol formation can be achieved, for example, by contact with water according to the methods of the present invention. Use of "an enzyme or microorganism" in the present methods includes use of two or more, as well as a single, enzyme or microorganism.

**[0120]** The enzyme or microorganism employed in the present invention can be any enzyme or microorganism capable of catalyzing the enzymatic conversions described herein. The enzymatic or microbial materials, regardless of origin or purity, can be employed in the free state or immobilized on a support such as by physical adsorption or entrapment. Microorganisms or enzymes suitable for use in the present invention can be selected by screening for the desired activity, for example, by contacting a candidate microorganism or enzyme with a starting compound XV or XVII or salt thereof, and noting conversion to the corresponding compound XVI or XVIII or salt thereof. The enzyme may, for example, be in the form of animal or plant enzymes or mixtures thereof, cells of microorganisms, crushed cells, extracts of cells, or of synthetic origin.

**[0121]** Exemplary microorganisms include those within the genera: *Streptomyces* or *Amycolatopsis*. Particularly preferred microorganisms are those within the species *Streptomyces griseus*, especially *Streptomyces griseus* ATCC 10137, and *Amycolatopsis orientalis* such as ATCC 14930, ATCC 21425, ATCC 35165, ATCC 39444, ATCC 43333, ATCC 43490, ATCC 53550, ATCC 53630, and especially ATCC 43491. The term "ATCC" as used herein refers to the accession number of the American Type Culture Collection, 10801 University Blvd., Manassas Va. 20110-2209, the depository for the organism referred to. It should be understood that mutants of these organisms are also contemplated by the present invention, for use in the methods described herein, such as those modified by the use of chemical, physical (for example, X-rays) or biological means (for example, by molecular biology techniques).

**[0122]** Preferred enzymes include those derived from microorganisms, particularly those microorganisms described above. Enzymes may be isolated, for example, by extraction and purification methods such as by methods known to those of ordinary skill in the art. An enzyme may, for example, be used in its free state or in immobilized form. One embodiment of the invention is that where an enzyme is adsorbed onto a suitable carrier, e.g., diatomaceous earth (porous Celite Hyflo Supercel), microporous polypropylene (Enka Accureli® polypropylene powder), or a nonionic polymeric adsorbent such as Amberlite® XAD-2 (polystyrene) or XAD-7 (polyacrylate) from Rohm and Haas Co. When employed to immobilize an enzyme, a carrier may control the enzyme particle size and prevent aggregation of the enzyme

particles when used in an organic solvent. Immobilization can be accomplished, for example, by precipitating an aqueous solution of the enzyme with cold acetone in the presence of the Celite Hyflo Supercel followed by vacuum drying, or in the case of a nonionic polymeric adsorbent, incubating enzyme solutions with adsorbent on a shaker, removing excess solution and drying enzyme-adsorbent resins under vacuum. While it is desirable to use the least amount of enzyme possible, the amount of enzyme required will vary depending upon the specific activity of the enzyme used.

**[0123]** Hydroxylation as described above can occur in vivo. For example, liver enzyme can selectively, relative to the endo isomer, hydroxylate the exo isomer of a compound of the present invention. In conducting the methods of the present invention outside the body, liver microsomal hydroxylase can be employed as the enzyme for catalysis.

**[0124]** These processes may also be carried out using microbial cells containing an enzyme having the ability to catalyze the conversions. When using a microorganism to perform the conversion, these procedures are conveniently carried out by adding the cells and the starting material to the desired reaction medium.

**[0125]** Where microorganisms are employed, the cells may be used in the form of intact wet cells or dried cells such as lyophilized, spray-dried or heat-dried cells, or in the form of treated cell material such as ruptured cells or cell extracts. Cell extracts immobilized on Celite® or Accureli® polypropylene as described earlier may also be employed. The use of genetically engineered organisms is also contemplated. The host cell may be any cell, e.g. *Escherichia coli*, modified to contain a gene or genes for expressing one or more enzymes capable of catalysis as described herein.

**[0126]** Where one or more microorganisms are employed, the enzymatic methods of the present invention may be carried out subsequent to the fermentation of the microorganism (two-stage fermentation and conversion), or concurrently therewith, that is, in the latter case, by in situ fermentation and conversion (single-stage fermentation and conversion).

**[0127]** Growth of the microorganisms can be achieved by one of ordinary skill in the art by the use of an appropriate medium. Appropriate media for growing microorganisms include those which provide nutrients necessary for the growth of the microbial cells. A typical medium for growth includes necessary carbon sources, nitrogen sources, and elements (e.g. in trace amounts). Inducers may also be added. The term "inducer", as used herein, includes any compound enhancing formation of the desired enzymatic activity within the microbial cell.

**[0128]** Carbon sources can include sugars such as maltose, lactose, glucose, fructose, glycerol, sorbitol, sucrose, starch, mannitol, propylene glycol, and the like; organic acids such as sodium acetate, sodium citrate, and the like; and alcohols such as ethanol, propanol and the like.

**[0129]** Nitrogen sources can include N-Z amine A, corn steep liquor, soy bean meal, beef extracts, yeast extracts, molasses, baker's yeast, tryptone, nutrisoy, peptone, yeastamin, amino acids such as sodium glutamate and the like, sodium nitrate, ammonium sulfate and the like.

**[0130]** Trace elements can include magnesium, manganese, calcium, cobalt, nickel, iron, sodium and potassium salts. Phosphates may also be added in trace or, preferably, greater than trace amounts.

**[0131]** The medium employed can include more than one carbon or nitrogen source or other nutrient.



[0132] Preferred media for growth include aqueous media.

[0133] The agitation and aeration of the reaction mixture affects the amount of oxygen available during the conversion process when conducted, for example, in shake-flask cultures or fermentor tanks during growth of microorganisms.

[0134] Incubation of the reaction medium is preferably at a temperature between about 4 and about 60° C. The reaction time can be appropriately varied depending upon the amount of enzyme used and its specific activity. Reaction times may be reduced by increasing the reaction temperature and/or increasing the amount of enzyme added to the reaction solution.

[0135] It is also preferred to employ an aqueous liquid as the reaction medium, although an organic liquid, or a miscible or immiscible (biphasic) organic/aqueous liquid mixture, may also be employed. The amount of enzyme or microorganism employed relative to the starting material is selected to allow catalysis of the enzymatic conversions of the present invention.

[0136] Solvents for the organic phase of a biphasic solvent system may be any organic solvent immiscible in water, such as toluene, cyclohexane, xylene, trichlorotrifluoroethane and the like. The aqueous phase is conveniently of water, preferably deionized water, or a suitable aqueous buffer solution, especially a phosphate buffer solution. The biphasic solvent system preferably comprises between about 10 to 90 percent by volume of organic phase and between about 90 to 10 percent by volume of aqueous phase, and most preferably contains at or about 20 percent by volume of organic phase and at or about 80 percent by volume of the aqueous phase.

[0137] An exemplary embodiment of such processes starts with preparation of an aqueous solution of the enzyme(s) or microbes to be used. For example, the preferred enzyme(s) or microbes can be added to a suitable amount of an aqueous solvent, such as phosphate buffer or the like. This mixture is preferably adjusted to and maintained at a desired pH.

[0138] The compounds XVI and XVIII produced by the processes of the present invention can be isolated and purified, for example, by methods such as extraction, distillation, crystallization, and column chromatography.

#### PREFERRED COMPOUNDS

[0139] A preferred subgenus of the compounds of the present invention includes compounds of the formula I or salts thereof wherein one or more, preferably all, of the following substituents are as defined below:

[0140] G is an aryl or heterocyclo (e.g., heteroaryl) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with hydrogen, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, halo, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo, arylalkyl or substituted arylalkyl, heterocycloalkyl or substituted heterocycloalkyl, CN, R<sup>1</sup>OC=O, R<sup>1</sup>C=O, R<sup>1</sup>HNC=O, R<sup>1</sup>R<sup>2</sup>NC=O, HO-CR<sup>3</sup>R<sup>3</sup>, nitro, R<sup>1</sup>OCH<sub>2</sub>, R<sup>1</sup>O, NH<sub>2</sub>, NR<sup>4</sup>R<sup>5</sup>, S=OR<sup>1</sup>, SO<sub>2</sub>R<sup>1</sup>, SO<sub>2</sub>NR<sup>1</sup>R<sup>1</sup>, (R<sup>1</sup>)(R<sup>1</sup>)P=O, or (R<sup>1</sup>)(NHR<sup>1</sup>)P=O;

[0141] Z<sub>1</sub> is O, S, NH, or NR<sup>6</sup>;

[0142] Z<sub>2</sub> is O, S, NH, or NR<sup>6</sup>;

[0143] A<sub>1</sub> is CR<sup>7</sup> or N;

[0144] A<sub>2</sub> is CR<sup>7</sup> or N;

[0145] Y is J-J'-J'' where J is (CR<sup>7</sup>R<sup>7</sup>)<sub>n</sub> and n=0-3, J' is a bond or O, S, S=O, SO<sub>2</sub>, NH, OC=O, C=O, NR<sup>7</sup>,

CR<sup>7</sup>R<sup>7</sup>, R<sup>2</sup>P=O, R<sup>2</sup>P=S, R<sup>1</sup>O=P=O, R<sup>2</sup>NHP=O, OP=OOR<sup>2</sup>, OP=ONHR<sup>2</sup>, OP=OR<sup>2</sup>, OSO<sub>2</sub>, N=NH, N=NR<sup>6</sup>, NR<sup>6</sup>NH, N=N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and J'' is (CR<sup>7</sup>R<sup>7</sup>)<sub>n</sub> and n=0-3, where Y is not a bond;

[0146] W is CR<sup>7</sup>R<sup>7</sup>CR<sup>7</sup>R<sup>7</sup>CR<sup>7</sup>R<sup>7</sup>-C=O, NR<sup>9</sup>-CR<sup>7</sup>R<sup>7</sup>, N=CR<sup>8</sup>, N=N, NR<sup>9</sup>-NR<sup>9</sup>, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein, when W is not NR<sup>9</sup>-CR<sup>7</sup>R<sup>7</sup>, N=CR<sup>8</sup>, N=N, NR<sup>9</sup>-NR<sup>9</sup>, or heterocyclo or substituted heterocyclo, then J' must be O, S, S=O, SO<sub>2</sub>, NH, NR<sup>7</sup>, OP=OOR<sup>2</sup>, OP=ONHR<sup>2</sup>, OSO<sub>2</sub>, N=NH, N=NR<sup>6</sup>, or N=N;

[0147] Q<sub>1</sub> is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, R<sup>1</sup>OC=O, R<sup>4</sup>C=O, R<sup>5</sup>R<sup>6</sup>NC=O, HO-CR<sup>7</sup>R<sup>7</sup>, nitro, R<sup>1</sup>OCH<sub>2</sub>, R<sup>1</sup>O, NH<sub>2</sub>, or NR<sup>4</sup>R<sup>5</sup>;

[0148] Q<sub>2</sub> is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, R<sup>1</sup>OC=O, R<sup>4</sup>C=O, R<sup>5</sup>R<sup>6</sup>NC=O, HO-CR<sup>7</sup>R<sup>7</sup>, nitro, R<sup>1</sup>OCH<sub>2</sub>, R<sup>1</sup>O, NH<sub>2</sub>, or NR<sup>4</sup>R<sup>5</sup>;

[0149] L is a bond, (CR<sup>7</sup>R<sup>7</sup>)<sub>n</sub>, NH, NR<sup>5</sup> or NR<sup>5</sup>(CR<sup>7</sup>R<sup>7</sup>)<sub>n</sub>, where n=0-3;

[0150] R<sup>1</sup> and R<sup>1'</sup> are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

[0151] R<sup>2</sup> is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

[0152] R<sup>3</sup> and R<sup>3'</sup> are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, hydroxylamine, hydroxamide, alkoxy or substituted alkoxy, amino, NR<sup>1</sup>R<sup>2</sup>, thiol, alkylthio or substituted alkylthio;

[0153]  $R^4$  is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl,  $R^1C=O$ ,  $R^1NHC=O$ , or  $SO_2NR^1R^1$ ;

[0154]  $R^5$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl,  $R^1C=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ , or  $SO_2NR^1R^1$ ;

[0155]  $R^6$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH,  $OR^1$ ,  $R^1C=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ , or  $SO_2NR^1R^1$ ;

[0156]  $R^7$  and  $R^7$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN,  $OR^4$ , nitro, hydroxylamine, hydroxylamide, amino,  $NHR^4$ ,  $NR^2R^5$ ,  $NOR^1$ , thiol, alkylthio or substituted alkylthio,  $R^1C=O$ ,  $R^1(C=O)O$ ,  $R^1OC=O$ ,  $R^1NHC=O$ ,  $SOR^1$ ,  $PO_3R^1R^1$ ,  $R^1R^1NC=O$ ,  $C=OSR^1$ ,  $SO_2R^1$ , or  $SO_2NR^1R^1$ ;

[0157]  $R^8$  and  $R^8$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, nitro, halo, CN,  $OR^1$ , amino,  $NHR^4$ ,  $NR^2R^5$ ,  $NOR^1$ , alkylthio or substituted alkylthio,  $C=OSR^1$ ,  $R^1OC=O$ ,  $R^1C=O$ ,  $R^1NHC=O$ ,  $R^1R^1NC=O$ ,  $S=OR^1$ ,  $SO_2R^1$ ,  $PO_3R^1R^1$ , or  $SO_2NR^1R^1$ ;

[0158]  $R^9$  and  $R^9$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH,  $OR^1$ ,  $R^1C=O$ ,  $R^1OC=O$ ,  $R^1NHC=O$ , or  $SO_2NR^1R^1$ ;

especially where the groups W and Y of this preferred subgenus are also within the definitions of W' and Y' of formula

1a, with the provisos (1) to (14) of said formula 1a where appropriate to this subgenus, and most preferably where (i) when Y' is  $-O-$  and W' is  $CR^7R^7-CR^7R^7$ ,  $A_1$  and  $A_2$  are not simultaneously CH; and (ii) when L is a bond, G is not an unsubstituted phenyl group.

[0159] Another, more preferred subgenus of the compounds of the invention includes compounds of the formula I or salts thereof wherein one or more, preferably all, of the following substituents are as defined below:

[0160] G is an aryl or heterocyclo (e.g., heteroaryl) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with hydrogen, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, halo, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo, arylalkyl or substituted arylalkyl, heterocycloalkyl or substituted heterocycloalkyl, CN,  $R^1C=O$ ,  $R^1HNC=O$ ,  $R^1R^2NC=O$ ,  $HO-CR^3R^3$ , nitro,  $R^1OCH_2$ ,  $R^1O$ ,  $NH_2$ ,  $NR^4R^5$ ,  $SO_2R^1$ , or  $SO_2NR^1R^1$ ;

[0161]  $Z_1$  is O;

[0162]  $Z_2$  is O;

[0163]  $A_1$  is  $CR^7$ ;

[0164]  $A_2$  is  $CR^7$ ;

[0165] Y is J-J'-J'' where J is  $(CR^7R^7)_n$  and  $n=0-3$ , J' is a bond or O, S,  $S=O$ ,  $SO_2$ , NH,  $NR^7$ ,  $CR^7R^7$ ,  $R^2P=O$ ,  $R^2P=S$ ,  $R^2OP=O$ ,  $R^2NHP=O$ ,  $OP=OOR^2$ ,  $OP=ONHR^2$ ,  $OP=OR^2$ ,  $OSO_2$ ,  $NHNH$ ,  $NHNR^6$ ,  $NR^6NH$ ,  $N=N$ , cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and J'' is  $(CR^7R^7)_n$  and  $n=0-3$ , where Y is not a bond;

[0166] W is  $CR^7R^7-CR^7R^7$ ,  $CR^7R^7-C=O$ ,  $NR^9-CR^7R^7$ ,  $N=CR^3$ ,  $N=N$ ,  $NR^9-NR^9$ , cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein, when W is not  $NR^9-CR^7R^7$ ,  $N=CR^3$ ,  $N=N$ ,  $NR^9-NR^9$ , or heterocyclo or substituted heterocyclo, then J' must be O, S,  $S=O$ ,  $SO_2$ , NH,  $NR^7$ ,  $OP=OOR^2$ ,  $OP=ONHR^2$ ,  $OSO_2$ ,  $NHNH$ ,  $NHNR^6$ ,  $NR^6NH$ , or  $N=N$ ;

[0167]  $Q_1$  is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN,  $R^4C=O$ ,  $R^5R^6NC=O$ ,  $HO-CR^7R^7$ , nitro,  $R^1OCH_2$ ,  $R^1O$ ,  $NH_2$ , or  $NR^4R^5$ ;

[0168]  $Q_2$  is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN,  $R^4C=O$ ,  $R^5R^6NC=O$ ,  $HO-CR^7R^7$ , nitro,  $R^1OCH_2$ ,  $R^1O$ ,  $NH_2$ , or  $NR^4R^5$ ;

[0169] L is a bond;

[0170]  $R^1$  and  $R^1$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or

substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

[0171]  $R^2$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

[0172]  $R^3$  and  $R^{3'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, alkoxy or substituted alkoxy, amino,  $NR^1R^2$ , alkylthio or substituted alkylthio;

[0173]  $R^4$  is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl,  $R^1C=O$ ,  $R^1NHC=O$ , or  $SO_2NR^1R^1$ ;

[0174]  $R^5$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl,  $R^1C=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ , or  $SO_2NR^1R^1$ ;

[0175]  $R^6$  is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH,  $OR^1$ ,  $R^1C=O$ ,  $R^1NHC=O$ ,  $SO_2R^1$  or  $SO_2NR^1R^1$ ;

[0176]  $R^7$  and  $R^{7'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN,  $OR^4$ , nitro, amino,  $NHR^4$ ,  $NR^2R^5$ , alkylthio or substituted alkylthio,  $R^1C=O$ ,  $R^1(C=O)O$ ,  $R^1NHC=O$ ,  $SO_2R^1$ ,  $R^1R^1NC=O$ , or  $SO_2NR^1R^1$ ;

[0177]  $R^8$  and  $R^8$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl,

cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, nitro, halo, CN,  $OR^1$ , amino,  $NHR^4$ ,  $NR^2R^5$ , alkylthio or substituted alkylthio,  $R^1C=O$ ,  $R^1NHC=O$ ,  $R^1R^1NC=O$ ,  $SO_2R^1$ , or  $SO_2NR^1R^1$ ; and

[0178]  $R^9$  and  $R^{9'}$  are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH,  $OR^1$ ,  $R^1C=O$ ,  $R^1NHC=O$ , or  $SO_2NR^1R^1$ ;

especially where the groups W and Y of this preferred subgenus are also within the definitions of W' and Y' of formula Ia, with the provisos (1) to (14) of said formula Ia where appropriate to this subgenus, and most preferably where (i) when Y' is  $-O-$  and W' is  $CR^7R^{7'}-CR^7R^{7'}$ ,  $A_1$  and  $A_2$  are not simultaneously CH; and (ii) when L is a bond, G is not an unsubstituted phenyl group.

[0179] A particularly preferred subgenus of the compounds of the invention includes compounds of the formula I or salts thereof wherein one or more, preferably all, of the substituents are as defined below:

[0180] G is an aryl (especially, phenyl or naphthyl) or heterocyclo (especially those heterocyclo groups G of the compounds of the Examples herein) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with substituents as exemplified in any of the compounds of the Examples herein;

[0181] L is a bond,  $(CR^7R^{7'})_n$  (where n is 1 and  $R^7$  and  $R^{7'}$  are each independently H, alkyl or substituted alkyl), or  $-CH_2-NH-$ ;

[0182]  $A_1$  and  $A_2$  are each independently  $CR^7$  where  $R^7$  (i) is hydrogen, alkyl or substituted alkyl, arylalkyl or substituted arylalkyl, alkenyl or substituted alkenyl (for example, alkenyl substituted with aryl (especially, phenyl or naphthyl) or substituted aryl, or alkenyl substituted with heterocyclo or substituted heterocyclo), aryl or substituted aryl, heterocyclo or substituted heterocyclo, heterocycloalkyl or substituted heterocycloalkyl, where, for each, preferred substituents are one or more groups selected from  $V^1$  (especially  $A_1$  and  $A_2$  groups of the formula  $CR^7$  where  $R^7$  for each of  $A_1$  and/or  $A_2$  is independently selected from unsubstituted  $C_{1-4}$  alkyl, or  $C_{1-4}$  alkyl which alkyl is substituted by one or more groups  $V^1$ ), or (ii) forms, together with  $R^7$  of a group W (especially where W is  $CR^7R^{7'}-CR^7R^{7'}$ ), a heterocyclic ring;

[0183]  $V^1$  is OH, CN, halo,  $-O$ -aryl,  $-O$ -substituted aryl,  $-O$ -heterocyclo (e.g.,  $-O$ -(optionally substituted pyridinyl) or  $-O$ -(optionally substituted pyrimidinyl)),  $-O$ -substituted heterocyclo,  $-O-CO$ -alkyl,  $-O-CO$ -substituted alkyl,  $-O$ -(alkylsilyl),  $-O$ -arylalkyl,  $-O$ -substituted arylalkyl,  $-O-CO$ -alkyl,  $-O-CO$ -substituted alkyl,  $-O-CO$ -arylalkyl,  $-O-CO$ -substituted arylalkyl,  $-O-CO$ -aryl,  $-O-CO$ -substituted aryl,  $-O-CO$ -heterocyclo,  $-O-CO$ -substituted heterocyclo,  $-S$ -(optionally substituted aryl)- $NH-CO$ -(op-

tionally substituted alkyl), —SO-(optionally substituted aryl)-NH—CO-(optionally substituted alkyl), —SO<sub>2</sub>-(optionally substituted aryl)-NH—CO-(optionally substituted alkyl), —NH—SO<sub>2</sub>-aryl, —NH—SO<sub>2</sub>-substituted aryl, —NH—CO—O-(optionally substituted arylalkyl), —NH—CO—O-alkyl, —NH—CO—O-substituted alkyl, —NH—CO-alkyl, —NH—CO-substituted alkyl, —NH—CO-aryl, —NH—CO-substituted aryl, —NH—CO-(optionally substituted arylalkyl), —NH—CO-(optionally substituted alkyl)-O-(optionally substituted aryl), —N(optionally substituted alkyl)(optionally substituted aryl), —N(optionally substituted alkyl)(optionally substituted arylalkyl), —COH, —COOH, —CO—O-alkyl, —CO—O-substituted alkyl, —CO—O-optionally substituted arylalkyl, —CO-aryl, —CO-substituted aryl, —O—CO—NH-aryl, —O—CO—NH-substituted aryl, —CO—NH-aryl, —CO—NH-substituted aryl, —CO—NH-arylalkyl, —CO—NH-substituted arylalkyl, —O-(optionally substituted aryl)-NH—CO-(optionally substituted alkyl);

[0184] Y is —O—, —SO—, —N(V<sup>2</sup>)—, —CH<sub>2</sub>—N(V<sup>2</sup>)—, —CO—N(alkyl)—, —CH<sub>2</sub>—S—, —CH<sub>2</sub>—SO<sub>2</sub>—;

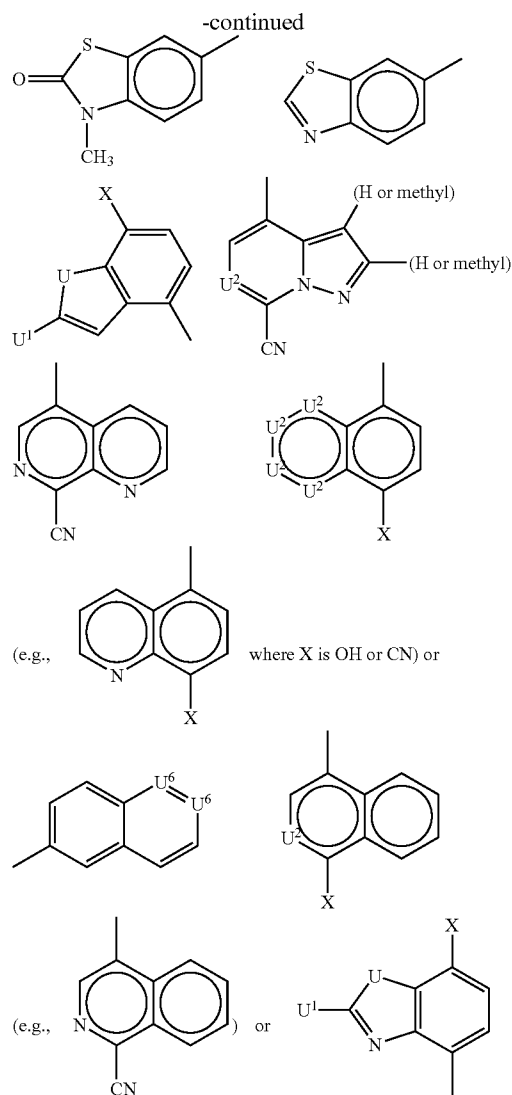
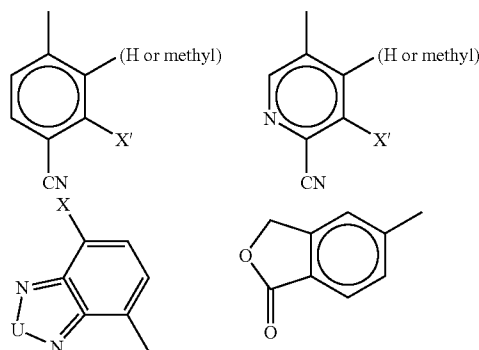
[0185] V<sup>2</sup> is hydrogen, alkyl, arylalkyl, —CO-alkyl, —CO—O-aryl, —CO—O-arylalkyl;

[0186] W is CR<sup>7</sup>R<sup>7'</sup>—CR<sup>7</sup>R<sup>7'</sup> (where R<sup>7</sup> and R<sup>7'</sup> are each independently selected from H, OH, alkyl or substituted alkyl (such as hydroxyalkyl), or where R<sup>7</sup> forms a heterocyclic ring together with R<sup>7'</sup> of A<sub>1</sub> or A<sub>2</sub>), CR<sup>8</sup>=CR<sup>8'</sup> (where R<sup>8</sup> and R<sup>8'</sup> are each independently selected from H, alkyl or substituted alkyl (such as hydroxyalkyl)), CR<sup>7</sup>R<sup>7'</sup>—C=O (where R<sup>7</sup> and R<sup>7'</sup> are each hydrogen, or where R<sup>7</sup> forms a heterocyclic ring together with R<sup>7'</sup> of A<sub>1</sub> or A<sub>2</sub>), N=CR<sup>8</sup> (where R<sup>8</sup> is alkyl), cycloalkyl or substituted cycloalkyl, or heterocyclo or substituted heterocyclo;

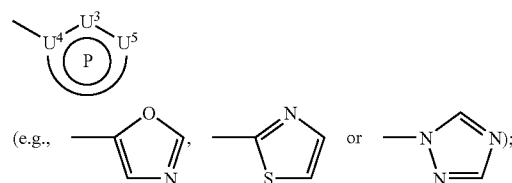
[0187] Z<sub>1</sub> and Z<sub>2</sub> are 0; and

[0188] Q<sub>1</sub> and Q<sub>2</sub> are H.

[0189] Preferred G-L groups are optionally substituted phenyl, optionally substituted naphthyl and optionally substituted fused bicyclic heterocyclic groups such as optionally substituted benzo-fused heterocyclic groups (e.g., bonded to the remainder of the molecule through the benzene portion), especially such groups wherein the heterocyclic ring bonded to benzene has 5 members exemplified by benzoxazole, benzothiazole, benzothiadiazole, benzoxadiazole or benzothiophene, for example:



where  
X=halo (especially F), OH, CN, NO<sub>2</sub> or



X'=halo (especially Cl, F, or J), CH<sub>3</sub>, CF<sub>3</sub>, CN or OCH<sub>3</sub>;  
U is O or S (where S can optionally be oxygenated, e.g., to SO);

U<sup>1</sup> is CH<sub>3</sub> or CF<sub>3</sub>;

[0190] each U<sup>2</sup> is independently N, CH or CF;

U<sup>3</sup> is N, O or S;

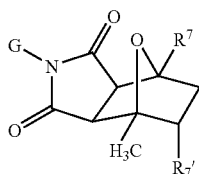
[0191] U<sup>4</sup> and U<sup>5</sup>, together with the atoms to which they are bonded, form an optionally substituted 5-membered hetero-

cyclic ring which can be partially unsaturated or aromatic and which contains 1 to 3 ring heteroatoms;  
each U<sup>6</sup> is independently CH or N; and



denotes optional double bond(s) within the ring formed by U<sup>3</sup>, U<sup>4</sup> and U<sup>5</sup>.

[0192] An especially preferred subgenus includes compounds of the formula I having the following structure, or salts thereof:



where G is an optionally substituted phenyl, naphthyl or benzo-fused bicyclic heterocyclic group, R<sup>7</sup> is CH<sub>3</sub> or C<sub>1-4</sub>alkyl substituted by V<sup>1</sup>, and one R<sup>7</sup> is H or hydroxyl and the other is H.

[0193] Preferred compounds of the invention include:

[0194] [3aS-(3α,4β,6β,7β,7α)]-4-(Octahydro-6-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0195] [3aR-(3α,4β,6β,7β,7α)]-4-(Octahydro-6-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0196] [3aS-(3α,4β,5β,6α,7β,7α)]-4-(Octahydro-5,6-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0197] [3aS-(3α,4β,5β,7β,7α)]-5-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-methyl-2-pyridinecarbonitrile;

[0198] [3aS-(3α,4β,5β,7β,7α)]-2-(2,1,3-Benzoxadiazol-5-yl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3 (2H)-dione;

[0199] [3aS-(3α,4β,5β,7β,7α)]-5-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3,4-dimethyl-2-pyridinecarbonitrile;

[0200] [3aR-(3α,4β,5β,7β,7α)]-5-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3,4-dimethyl-2-pyridinecarbonitrile;

[0201] [3aS-(3α,4β,5β,7β,7α)]-2-(6-Benzothiazolyl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione;

[0202] [3aR-(3α,4β,5β,7β,7α)]-2-(7-Chloro-2,1,3-benzoxadiazol-4-yl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3 (2H)-dione;

[0203] [3aR-(3α,4β,5α,7β,7α)]-5-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-(trifluoromethyl)-2-pyridinecarbonitrile;

[0204] [3aR-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile;

[0205] (3α,4β,5α,6β,7β,7α)-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-6-cyano-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid, methyl ester;

[0206] (3α,4β,7β,7α)-4-(Octahydro-4,7,8-trimethyl-1,3-dioxo-4,7-imino-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0207] [3aR-(3α,4β,5β,6α,7β,7α)]-4-(Octahydro-5,6-dichloro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0208] [3aS-(3α,4β,5β,6α,7β,7α)]-4-(Octahydro-5,6-dichloro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0209] (3α,4β,7β,7α)-4-(Octahydro-4,7-dimethyl-1,3,5-trioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile;

[0210] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[difluoromethyl]oxy]methyl]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0211] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[phenylmethoxycarbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0212] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[propyloxy-carbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0213] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[cyclopropylmethoxy]carbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0214] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[methoxycarbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0215] [3aR-(3α,4β,5β,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0216] [3aR-(3α,4β,4α,5α,6β,7α)]-4-(Octahydro-4a-hydroxy-4,6-dimethyl-1,3-dioxo-4,6-epoxycycloprop[*f*]isoindol-2(1H)-yl)-2-(trifluoromethyl)benzonitrile;

[0217] (3α,4β,5α,7β,7α)-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-N,N-dimethyl-1H-isoindole-5-carboxamide;

[0218] [3aR-(3α,4β,5β,6β,7β,7α)]-4-(Octahydro-5-chloro-6-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0219] [3aS-(3α,4β,5β,6β,7β,7α)]-4-(Octahydro-5-chloro-6-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0220] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[6-(trifluoromethyl)-4-pyrimidinyl]oxy]methyl]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0221] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[5-chloro-2-pyridinyl]oxy]methyl]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0222] [3aR-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-[[[phenylamino]carbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0223] [3aR-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-[[[1-methylethoxy]carbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

[0224] (3α,4β,5α,7β,7α)-4-(Octahydro-5-[[[5-fluoro-4-pyrimidinyl]oxy]methyl]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;

- [0225] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[ethyloxycarbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0226] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid, 4-pyridinylmethyl ester;
- [0227] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[4-pyridinylmethoxycarbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0228] 3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ ]-4-(Octahydro-5-[[2-methyl-5-(trifluoromethyl)-2H-pyrazol-3-yl]oxy]methyl]-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0229] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid, methyl ester;
- [0230] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-cyclopropylmethoxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0231] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-[[((phenylmethyl)amino)carbonyl]oxy]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile; and
- [0232] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-cyclopropyloxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- or pharmaceutically acceptable salts, solvates, prodrugs or stereoisomers thereof.
- [0233] More preferred compounds of the invention include:
- [0234] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-5-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-(trifluoromethyl)-2-pyridinecarbonitrile;
- [0235] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-5-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-(trifluoromethyl)-2-pyridinecarbonitrile;
- [0236] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Chloro-3-iodophenyl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3 (2H)-dione;
- [0237] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,6 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5,6-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0238] [3aR-(3 $\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-4,7-dimethyl-1,3,5-trioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0239] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0240] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0241] [3aS-(3 $\alpha$ ,4 $\beta$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-6-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0242] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Chloro-3-(trifluoromethyl)phenyl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione;
- [0243] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile;
- [0244] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile;
- [0245] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Chloro-3-(trifluoromethyl)pyridinyl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione;
- [0246] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-methyl-2-(trifluoromethyl)benzonitrile;
- [0247] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-methoxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0248] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-5-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-(trifluoromethyl)-2-pyridinecarbonitrile;
- [0249] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-5-(Octahydro-5-methoxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0250] [3aS-(3 $\alpha$ ,4 $\beta$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-6-fluoro-5,5-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0251] [3aR-(3 $\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-5-(Octahydro-4,7-dimethyl-1,3,5-trioxo-4,7-epoxy-2H-isoindol-2-yl)-3-(trifluoromethyl)-2-pyridinecarbonitrile;
- [0252] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Chloro-2-methyl-3-(trifluoromethyl)phenyl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3 (2H)-dione;
- [0253] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Chloro-2-methyl-3-(trifluoromethyl)phenyl)hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3 (2H)-dione;
- [0254] (3 $\alpha$ ,4 $\beta$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-6-fluoro-5,5-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile;
- [0255] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid, methyl ester;
- [0256] [3aS-(3 $\alpha$ ,4 $\beta$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-6-fluoro-5,5-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile;
- [0257] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-ethylsulfonamido-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0258] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[4-fluorophenylamino]carbonyl]oxy]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0259] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[1-methylethylamino]carbonyl]oxy]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0260] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[1-methylethoxy]carbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0261] [3aR-(3 $\alpha$ ,4 $\beta$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-6-fluoro-5,5-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile;
- [0262] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-N-methyl-N-phenyl-1H-isoindole-5-carboxamide;
- [0263] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-chloro-3-methylbenzonitrile;

- [0264] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[ethoxycarbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0265] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-[[[2-methyl-5-(trifluoromethyl)-2H-pyrazol-3-yl]oxy]methyl]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0266] [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Cyano-3-(trifluoromethyl)phenyl)-hexahydro-6-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid, methyl ester;
- [0267] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-[[[6-(trifluoromethyl)-4-pyrimidinyl]oxy]methyl]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0268] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-methoxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0269] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-2-(4-Cyano-3-(trifluoromethyl)phenyl)-hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carbonitrile;
- [0270] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,6 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5,6-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-methyl-2-(trifluoromethyl)benzonitrile;
- [0271] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[[cyclopropylmethyl]amino]carbonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0272] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[[dimethylamino]sulfonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0273] (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-benzene-sulfonamido-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0274] [3aR-(3 $\alpha$ ,4 $\beta$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-6-fluoro-5,5-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile;
- [0275] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Cyano-3-(trifluoromethyl)phenyl)-hexahydro-6-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid, methyl ester; and
- [0276] [3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-[[[dimethylamino]sulfonyl]amino]-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile; or pharmaceutically acceptable salts, solvates, prodrugs or stereoisomers thereof. Compounds where R<sup>7</sup> is hydroxyl can provide enhanced water solubility and metabolic stability, relative to the corresponding compounds where R<sup>7</sup> is H, in addition to having good permeability and high systemic blood levels. These hydroxyl-bearing compounds can be obtained in vivo by metabolism of the corresponding compound where R<sup>7</sup> is H, as well as by synthetic preparative methods such as those described herein.

#### USE AND UTILITY

[0277] The compounds of the present invention modulate the function of nuclear hormone receptors (NHR), and include compounds which are, for example, agonists, partial agonists, antagonists or partial antagonists of the androgen receptor (AR), the estrogen receptor (ER), the progesterone receptor (PR), the glucocorticoid receptor (GR), the mineralocorticoid receptor (MR), the steroid and xenobiotic receptor (SXR), other steroid binding NHR's, the Orphan receptors

or other NHR's. Selective modulation of one such NHR relative to others within the NHR family is preferred. "Modulation" includes, for example, activation (e.g., agonist activity such as selective androgen receptor agonist activity) or inhibition (e.g., antagonist activity).

[0278] The present compounds are thus useful in the treatment of NHR-associated conditions. A "NHR-associated condition", as used herein, denotes a condition or disorder which can be treated by modulating the function of a NHR in a subject, wherein treatment comprises prevention (e.g., prophylactic treatment), partial alleviation or cure of the condition or disorder. Modulation may occur locally, for example, within certain tissues of the subject, or more extensively throughout a subject being treated for such a condition disorder.

[0279] The compounds of the present invention are useful for the treatment of a variety of conditions and disorders including, but not limited to, those described following:

[0280] Compounds of formula I can be applied as agonists, partial agonists, antagonists, or partial antagonists of the estrogen receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the estrogen receptor pathway. Applications of said compounds include but are not limited to: osteoporosis, hot flushes, vaginal dryness, prostate cancer, breast cancer, endometrial cancer, cancers expressing the estrogen receptor such as the aforementioned cancers and others, contraception, pregnancy termination, menopause, amenorrhea, and dysmenorrhea.

[0281] Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the progesterone receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the progesterone receptor pathway. Applications of said compounds include but are not limited to: breast cancer, other cancers containing the progesterone receptor, endometriosis, cachexia, contraception, menopause, cyclesynchrony, meningioma, dysmenorrhea, fibroids, pregnancy termination, labor induction and osteoporosis. Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the glucocorticoid receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the glucocorticoid receptor pathway. Applications of said compounds include but are not limited to: inflammatory diseases, autoimmune diseases, prostate cancer, breast cancer, Alzheimer's disease, psychotic disorders, drug dependence, non-insulin dependent Diabetes Mellitus, and as dopamine receptor blocking agents or otherwise as agents for the treatment of dopamine receptor mediated disorders.

[0282] Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the mineralocorticoid receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the mineralocorticoid receptor pathway. Applications of said compounds include but are not limited to: drug withdrawal syndrome and inflammatory diseases.

[0283] Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the aldosterone receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the aldosterone receptor pathway. One application of said compounds includes but is not limited to: congestive heart failure.

**[0284]** Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the androgen receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the androgen receptor pathway. Applications of said compounds include but are not limited to: hirsutism, acne, seborrhea, Alzheimer's disease, androgenic alopecia, hypogonadism, hyperpilosity, benign prostate hypertrophy, adenomas and neoplasies of the prostate (such as advanced metastatic prostate cancer), treatment of benign or malignant tumor cells containing the androgen receptor such as is the case for breast, brain, skin, ovarian, bladder, lymphatic, liver and kidney cancers, pancreatic cancers modulation of VEGF expression and applications therein for the treatment of heart disease, inflammation and immune modulations, modulation of VEGF expression and the applications therein for use as antiangiogenic agents, osteoporosis, suppressing spermatogenesis, libido, cachexia, endometriosis, polycystic ovary syndrome, anorexia, androgen supplement for age related decreased testosterone levels in men, male menopause, male hormone replacement, male and female sexual dysfunction, and inhibition of muscular atrophy in ambulatory patients. For example, pan AR modulation is contemplated, with prostate selective AR modulation ("SARM") being particularly preferred, such as for the treatment of early stage prostate cancers.

**[0285]** Compounds of formula I can be applied as (preferably, selective) antagonists of the mutated androgen receptor, for example, found in many tumor lines. Examples of such mutants are those found in representative prostate tumor cell lines such as LNCap, (T877A mutation, *Biophys. Acta*, 187, 1052 (1990)), PCa2b, (L701H & T877A mutations, *J. Urol.*, 162, 2192 (1999)) and CWR22, (H874Y mutation, *Mol. Endo.*, 11, 450 (1997)). Applications of said compounds include but are not limited to: adenomas and neoplasies of the prostate, breast cancer and endometrial cancer.

**[0286]** Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the steroid and xenobiotic receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the steroid and xenobiotic receptor pathway. Applications of said compounds include but are not limited to: treatment of dysregulation of cholesterol homeostasis, attenuation of metabolism of pharmaceutical agents by co-administration of an agent (compound of the present invention) which modulates the P450 regulator effects of SXR.

**[0287]** Along with the aforementioned NHR, there also exist a number of NHR for which the activating or deactivating ligands may not be characterized. These proteins are classified as NHR due to strong sequence homology to other NHR, and are known as the Orphan receptors. Because the Orphan receptors demonstrate strong sequence homology to other NHR, compounds of formula I include those which serve as modulators of the function of the Orphan NHR. Orphan receptors which are modulated by NHR modulators such as compounds within the scope of formula I are exemplified, but not limited to, those listed in Table 1. Exemplary therapeutic applications of modulators of said Orphan receptors are also listed in Table 1, but are not limited to the examples therein.

**[0288]** Exemplary Orphan nuclear hormone receptors, form (M=monomeric, D=heterodimeric, H=homodimeric), tissue expression and target therapeutic applications. (CNS=central nervous system)

TABLE 1

Receptor	Form	Tissue Expression	Target Therapeutic Application
NURR1	M/D	Dopaminergic Neurons	Parkinson's Disease
RZR $\beta$	M	Brain (Pituitary), Muscle	Sleep Disorders
ROR $\alpha$	M	Cerebellum, Purkinje Cells	Arthritis, Cerebellar Ataxia
NOR-1	M	Brain, Muscle, Heart, Adrenal, Thymus	CNS Disorders, Cancer
NGFI-B $\beta$	M/D	Brain	CNS Disorders
COUP-Tf $\alpha$	H	Brain	CNS Disorders
COUP-TF $\beta$	H	Brain	CNS Disorders
COUP-TF $\gamma$	H	Brain	CNS Disorders
Nur77	H	Brain, Thymus, Adrenals	CNS Disorders
Rev-ErbA $\alpha$	H	Muscle, Brain (Ubiquitous)	Obesity
HNF4 $\alpha$	H	Liver, Kidney, Intestine	Diabetes
SF-1	M	Gonads, Pituitary	Metabolic Disorders
LXR $\alpha$ , $\beta$	D	Kidney (Ubiquitous)	Metabolic Disorders
GDNF	M/H	Testes, Ovary	Infertility
ERR $\alpha$ , $\beta$	M	Placenta, Bone	Infertility, Osteoporosis
FXR	D	Liver, Kidney	Metabolic Disorders
CAR $\alpha$	H	Liver, Kidney	Metabolic Disorders
PXR	H	Liver, Intestine	Metabolic Disorders
COUP-TF2 (ARP1)	D	Testis	Oncology/angiogenesis
RORbeta	M	CNS, retina, pineal gland	Metabolic Disorders

**[0289]** The present invention thus provides methods for the treatment of NHR-associated conditions, comprising the step of administering to a subject in need thereof at least one compound of formula I in an amount effective therefor. Other therapeutic agents such as those described below may be employed with the inventive compounds in the present methods (for example, separately, or formulated together as a fixed dose). In the methods of the present invention, such other therapeutic agent(s) can be administered prior to, simultaneously with or following the administration of the compound(s) of the present invention.

**[0290]** The present invention also provides pharmaceutical compositions comprising at least one of the compounds of the formula I capable of treating a NHR-associated condition in an amount effective therefor, and a pharmaceutically acceptable carrier (vehicle or diluent). The compositions of the present invention can contain other therapeutic agents as described below, and can be formulated, for example, by employing conventional solid or liquid vehicles or diluents, as well as pharmaceutical additives of a type appropriate to the mode of desired administration (for example, excipients, binders, preservatives, stabilizers, flavors, etc.) according to techniques such as those well known in the art of pharmaceutical formulation.

**[0291]** It should be noted that the compounds of the present invention are, without limitation as to their mechanism of action, useful in treating any of the conditions or disorders listed or described herein such as inflammatory diseases or cancers, or other proliferate diseases, and in compositions for treating such conditions or disorders. Such conditions and disorders include, without limitation, any of those described previously, as well as those described following such as: maintenance of muscle strength and function (e.g., in the elderly); reversal or prevention of frailty or age-related functional decline ("ARFD") in the elderly (e.g., sarcopenia); treatment of catabolic side effects of glucocorticoids; prevention and/or treatment of reduced bone mass, density or growth (e.g., osteoporosis and osteopenia); treatment of chronic



fatigue syndrome (CFS); chronic malalgia; treatment of acute fatigue syndrome and muscle loss following elective surgery (e.g., post-surgical rehabilitation); acceleration of wound healing; accelerating bone fracture repair (such as accelerating the recovery of hip fracture patients); accelerating healing of complicated fractures, e.g. distraction osteogenesis; in joint replacement; prevention of post-surgical adhesion formation; acceleration of tooth repair or growth; maintenance of sensory function (e.g., hearing, sight, olfaction and taste); treatment of periodontal disease; treatment of wasting secondary to fractures and wasting in connection with chronic obstructive pulmonary disease (COPD), chronic liver disease, AIDS, weightlessness, cancer cachexia, burn and trauma recovery, chronic catabolic state (e.g., coma), eating disorders (e.g., anorexia) and chemotherapy; treatment of cardiomyopathy; treatment of thrombocytopenia; treatment of growth retardation in connection with Crohn's disease; treatment of short bowel syndrome; treatment of irritable bowel syndrome; treatment of inflammatory bowel disease; treatment of Crohn's disease and ulcerative colitis; treatment of complications associated with transplantation; treatment of physiological short stature including growth hormone deficient children and short stature associated with chronic illness; treatment of obesity and growth retardation associated with obesity; treatment of anorexia (e.g., associated with cachexia or aging); treatment of hypercortisolism and Cushing's syndrome; Paget's disease; treatment of osteoarthritis; induction of pulsatile growth hormone release; treatment of osteochondrodysplasias; treatment of depression, nervousness, irritability and stress; treatment of reduced mental energy and low self-esteem (e.g., motivation/assertiveness); improvement of cognitive function (e.g., the treatment of dementia, including Alzheimer's disease and short term memory loss); treatment of catabolism in connection with pulmonary dysfunction and ventilator dependency; treatment of cardiac dysfunction (e.g., associated with valvular disease, myocardial infarction, cardiac hypertrophy or congestive heart failure); lowering blood pressure; protection against ventricular dysfunction or prevention of reperfusion events; treatment of adults in chronic dialysis; reversal or slowing of the catabolic state of aging; attenuation or reversal of protein catabolic responses following trauma (e.g., reversal of the catabolic state associated with surgery, congestive heart failure, cardiac myopathy, burns, cancer, COPD etc.); reducing cachexia and protein loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis; treatment of immunosuppressed patients; treatment of wasting in connection with multiple sclerosis or other neurodegenerative disorders; promotion of myelin repair; maintenance of skin thickness; treatment of metabolic homeostasis and renal homeostasis (e.g., in the frail elderly); stimulation of osteoblasts, bone remodeling and cartilage growth; regulation of food intake; treatment of insulin resistance, including NIDDM, in mammals (e.g., humans); treatment of insulin resistance in the heart; improvement of sleep quality and correction of the relative hyposomatotropism of senescence due to high increase in REM sleep and a decrease in REM latency; treatment of hypothermia; treatment of congestive heart failure; treatment of lipodystrophy (e.g., in patients taking HIV or AIDS therapies such as protease inhibitors); treatment of muscular atrophy (e.g., due to physical inactivity, bed rest or reduced weight-bearing conditions); treatment of musculoskeletal impairment (e.g., in the elderly); improvement of the overall pulmonary function; treat-

ment of sleep disorders; and the treatment of the catabolic state of prolonged critical illness; treatment of hirsutism, acne, seborrhea, androgenic alopecia, anemia, hyperpilosity, benign prostate hypertrophy, adenomas and neoplasias of the prostate (e.g., advanced metastatic prostate cancer) and malignant tumor cells containing the androgen receptor, such as is the case for breast, brain, skin, ovarian, bladder, lymphatic, liver and kidney cancers; cancers of the skin, pancreas, endometrium, lung and colon; osteosarcoma; hypercalcemia of malignancy; metastatic bone disease; treatment of spermatogenesis, endometriosis and polycystic ovary syndrome; contracting preeclampsia, eclampsia of pregnancy and preterm labor; treatment of premenstrual syndrome; treatment of vaginal dryness; age related decreased testosterone levels in men, male menopause, hypogonadism, male hormone replacement, male and female sexual dysfunction (e.g., erectile dysfunction, decreased sex drive, sexual well-being, decreased libido), male and female contraception, hair loss, Reaven's Syndrome and the enhancement of bone and muscle performance/strength; and the conditions, diseases, and maladies collectively referenced to as "Syndrome X" or Metabolic Syndrome as detailed in Johansson J. Clin. Endocrinol. Metab., 82, 727-34 (1997).

**[0292]** The present compounds have therapeutic utility in the modulation of immune cell activation/proliferation, e.g., as competitive inhibitors of intercellular ligand/receptor binding reactions involving CAMs (Cellular Adhesion Molecules) and Leukointegrins. For example, the present compounds modulate LFA-ICAM 1, and are particularly useful as LFA-ICAM 1 antagonists, and in the treatment of all conditions associated with LFA-ICAM 1 such as immunological disorders. Preferred utilities for the present compounds include, but are not limited to: inflammatory conditions such as those resulting from a response of the non-specific immune system in a mammal (e.g., adult respiratory distress syndrome, shock, oxygen toxicity, multiple organ injury syndrome secondary to septicemia, multiple organ injury syndrome secondary to trauma, reperfusion injury of tissue due to cardiopulmonary bypass, myocardial infarction or use with thrombolysis agents, acute glomerulonephritis, vasculitis, reactive arthritis, dermatosis with acute inflammatory components, stroke, thermal injury, hemodialysis, leukapheresis, ulcerative colitis, necrotizing enterocolitis and granulocyte transfusion associated syndrome) and conditions resulting from a response of the specific immune system in a mammal (e.g., psoriasis, organ/tissue transplant rejection, graft vs. host reactions and autoimmune diseases including Raynaud's syndrome, autoimmune thyroiditis, dermatitis, multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes mellitus, uveitis, inflammatory bowel disease including Crohn's disease and ulcerative colitis, and systemic lupus erythematosus). The present compounds can be used in treating asthma or as an adjunct to minimize toxicity with cytokine therapy in the treatment of cancers. The present compounds can be employed in the treatment of all diseases currently treatable through steroid therapy. The present compounds may be employed for the treatment of these and other disorders alone or with other immunosuppressive or antiinflammatory agents. In accordance with the invention, a compound of the formula I can be administered prior to the onset of inflammation (so as to suppress an anticipated inflammation) or after the initiation of inflammation. When provided prophylactically, the immunosuppressive compound(s) are preferably provided in advance of any inflammatory response or symptom

(for example, prior to, at, or shortly after the time of an organ or tissue transplant but in advance of any symptoms or organ rejection). The prophylactic administration of a compound of the formula I prevents or attenuates any subsequent inflammatory response (such as, for example, rejection of a transplanted organ or tissue, etc.) Administration of a compound of the formula I attenuates any actual inflammation (such as, for example, the rejection of a transplanted organ or tissue).

**[0293]** The compounds of the formula I can be administered for any of the uses described herein by any suitable means, for example, orally, such as in the form of tablets, capsules, granules or powders; sublingually; buccally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally, including administration to the nasal membranes, such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents. The present compounds can, for example, be administered in a form suitable for immediate release or extended release. Immediate release or extended release can be achieved by the use of suitable pharmaceutical compositions comprising the present compounds, or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps. The present compounds can also be administered liposomally.

**[0294]** Exemplary compositions for oral administration include suspensions which can contain, for example, microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners or flavoring agents such as those known in the art; and immediate release tablets which can contain, for example, microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and/or lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants such as those known in the art. The compounds of formula I can also be delivered through the oral cavity by sublingual and/or buccal administration. Molded tablets, compressed tablets or freeze-dried tablets are exemplary forms which may be used. Exemplary compositions include those formulating the present compound(s) with fast dissolving diluents such as mannitol, lactose, sucrose and/or cyclodextrins. Also included in such formulations may be high molecular weight excipients such as celluloses (avicel) or polyethylene glycols (PEG). Such formulations can also include an excipient to aid mucosal adhesion such as hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (SCMC), maleic anhydride copolymer (e.g., Gantrez), and agents to control release such as polyacrylic copolymer (e.g. Carbopol 934). Lubricants, glidants, flavors, coloring agents and stabilizers may also be added for ease of fabrication and use.

**[0295]** Exemplary compositions for nasal aerosol or inhalation administration include solutions in saline which can contain, for example, benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other solubilizing or dispersing agents such as those known in the art.

**[0296]** Exemplary compositions for parenteral administration include injectable solutions or suspensions which can contain, for example, suitable non-toxic, parenterally acceptable diluents or solvents, such as mannitol, 1,3-butanediol,

water, Ringer's solution, an isotonic sodium chloride solution, or other suitable dispersing or wetting and suspending agents, including synthetic mono- or diglycerides, and fatty acids, including oleic acid, or Cremaphor.

**[0297]** Exemplary compositions for rectal administration include suppositories which can contain, for example, a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

**[0298]** Exemplary compositions for topical administration include a topical carrier such as Plastibase (mineral oil gelled with polyethylene).

**[0299]** The effective amount of a compound of the present invention can be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for an adult human of from about 1 to 100 (for example, 15 or lower, especially 1 to 3 or less) mg/kg of body weight of active compound per day, which can be administered in a single dose or in the form of individual divided doses, such as from 1 to 4 times per day. It will be understood that the specific dose level and frequency of dosage for any particular subject can be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats and the like, subject to NHR-associated conditions.

**[0300]** As mentioned above, the compounds of the present invention can be employed alone or in combination with each other and/or other suitable therapeutic agents useful in the treatment of NHR-associated conditions, e.g., an antibiotic or other pharmaceutically active material.

**[0301]** For example, the compounds of the present invention can be combined with growth promoting agents, such as, but not limited to, TRH, diethylstilbesterol, theophylline, enkephalins, E series prostaglandins, compounds disclosed in U.S. Pat. No. 3,239,345, e.g., zanolol, and compounds disclosed in U.S. Pat. No. 4,036,979, e.g., sulbenox or peptides disclosed in U.S. Pat. No. 4,411,890.

**[0302]** The compounds of the invention can also be used in combination with growth hormone secretagogues such as GHRP-6, GHRP-1 (as described in U.S. Pat. No. 4,411,890 and publications WO 89/07110 and WO 89/07111), GHRP-2 (as described in WO 93/04081), NN703 (Novo Nordisk), LY444711 (Lilly), MK-677 (Merck), CP424391 (Pfizer) and B-HT920, or with growth hormone releasing factor and its analogs or growth hormone and its analogs or somatomedins including IGF-1 and IGF-2, or with alpha-adrenergic agonists, such as clonidine or serotonin 5-HT<sub>2D</sub> agonists, such as sumatriptan, or agents which inhibit somatostatin or its release, such as physostigmine and pyridostigmine. A still further use of the disclosed compounds of the invention is in combination with parathyroid hormone, PTH(1-34) or bisphosphonates, such as MK-217 (alendronate).

**[0303]** A still further use of the compounds of the invention is in combination with estrogen, testosterone, a selective estrogen receptor modulator, such as tamoxifen or raloxifene, or other androgen receptor modulators, such as those dis-

closed in Edwards, J. P. et al., *Bio. Med. Chem. Let.*, 9, 1003-1008 (1999) and Hamann, L. G. et al., *J. Med. Chem.*, 42, 210-212 (1999).

**[0304]** A further use of the compounds of this invention is in combination with progesterone receptor agonists ("PRA"), such as levonorgestrel, medroxyprogesterone acetate (MPA).

**[0305]** The compounds of the present invention can be employed alone or in combination with each other and/or other modulators of nuclear hormone receptors or other suitable therapeutic agents useful in the treatment of the aforementioned disorders including: anti-diabetic agents; anti-osteoporosis agents; anti-obesity agents; anti-inflammatory agents; anti-anxiety agents; anti-depressants; anti-hypertensive agents; anti-platelet agents; anti-thrombotic and thrombolytic agents; cardiac glycosides; cholesterol/lipid lowering agents; mineralocorticoid receptor antagonists; phosphodiesterase inhibitors; protein tyrosine kinase inhibitors; thyroid mimetics (including thyroid receptor agonists); anabolic agents; HIV or AIDS therapies; therapies useful in the treatment of Alzheimer's disease and other cognitive disorders; therapies useful in the treatment of sleeping disorders; anti-proliferative agents; and anti-tumor agents.

**[0306]** Examples of suitable anti-diabetic agents for use in combination with the compounds of the present invention include biguanides (e.g., metformin), glucosidase inhibitors (e.g., acarbose), insulins (including insulin secretagogues or insulin sensitizers), meglitinides (e.g., repaglinide), sulfonylureas (e.g., glimepiride, glyburide and glipizide), biguanide/glyburide combinations (e.g., Glucovance®), thiazolidinediones (e.g., troglitazone, rosiglitazone and pioglitazone), PPAR-alpha agonists, PPAR-gamma agonists, PPAR alpha/gamma dual agonists, SGLT2 inhibitors, glycogen phosphorylase inhibitors, inhibitors of fatty acid binding protein (aP2) such as those disclosed in U.S. Ser. No. 09/519,079 filed Mar. 6, 2000, glucagon-like peptide-1 (GLP-1), and dipeptidyl peptidase IV (DP4) inhibitors.

**[0307]** Examples of suitable anti-osteoporosis agents for use in combination with the compounds of the present invention include alendronate, risedronate, PTH, PTH fragment, raloxifene, calcitonin, steroidal or non-steroidal progesterone receptor agonists, RANK ligand antagonists, calcium sensing receptor antagonists, TRAP inhibitors, selective estrogen receptor modulators (SERM), estrogen and AP-1 inhibitors.

**[0308]** Examples of suitable anti-obesity agents for use in combination with the compounds of the present invention include aP2 inhibitors, such as those disclosed in U.S. Ser. No. 09/519,079 filed Mar. 6, 2000, PPAR gamma antagonists, PPAR delta agonists, beta 3 adrenergic agonists, such as AJ9677 (Takeda/Dainippon), L750355 (Merck), or CP331648 (Pfizer) or other known beta 3 agonists as disclosed in U.S. Pat. Nos. 5,541,204, 5,770,615, 5,491,134, 5,776,983 and 5,488,064, a lipase inhibitor, such as orlistat or ATL-962 (Alizyme), a serotonin (and dopamine) reuptake inhibitor, such as sibutramine, topiramate (Johnson & Johnson) or axokine (Regeneron), a thyroid receptor beta drug, such as a thyroid receptor ligand as disclosed in WO 97/21993 (U. Cal SF), WO 99/00353 (KaroBio) and GB98/284425 (KaroBio), and/or an anorectic agent, such as dexamphetamine, phentermine, phenylpropanolamine or mazindol.

**[0309]** Examples of suitable anti-inflammatory agents for use in combination with the compounds of the present invention include prednisone, dexamethasone, Enbrel®, cyclooxygenase inhibitors (i.e., COX-1 and/or COX-2 inhibitors such as NSAIDs, aspirin, indomethacin, ibuprofen, piroxicam,

Naproxen®, Celebrex®, Vioxx®), CTLA4-Ig agonists/antagonists, CD40 ligand antagonists, IMPDH inhibitors, such as mycophenolate (CellCept®) integrin antagonists, alpha-4 beta-7 integrin antagonists, cell adhesion inhibitors, interferon gamma antagonists, ICAM-1, tumor necrosis factor (TNF) antagonists (e.g., infliximab, OR1384), prostaglandin synthesis inhibitors, budesonide, clobazimine, CNI-1493, CD4 antagonists (e.g., priliximab), p38 mitogen-activated protein kinase inhibitors, protein tyrosine kinase (PTK) inhibitors, IKK inhibitors, and therapies for the treatment of irritable bowel syndrome (e.g., Zelmec® and Maxi-K® openers such as those disclosed in U.S. Pat. No. 6,184,231 B1).

**[0310]** Example of suitable anti-anxiety agents for use in combination with the compounds of the present invention include diazepam, lorazepam, buspirone, oxazepam, and hydroxyzine pamoate.

**[0311]** Examples of suitable anti-depressants for use in combination with the compounds of the present invention include citalopram, fluoxetine, nefazodone, sertraline, and paroxetine.

**[0312]** Examples of suitable anti-hypertensive agents for use in combination with the compounds of the present invention include beta adrenergic blockers, calcium channel blockers (L-type and T-type; e.g. diltiazem, verapamil, nifedipine, amlodipine and mybefradil), diuretics (e.g., chlorothiazide, hydrochlorothiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methylchlorothiazide, trichloromethiazide, polythiazide, benzthiazide, ethacrynic acid, tricyclanide, chlorthalidone, furosemide, musolimine, bumetanide, triamtrenene, amiloride, spironolactone), renin inhibitors, ACE inhibitors (e.g., captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazapril, delapril, pentopril, quinapril, ramipril, lisinopril), AT-1 receptor antagonists (e.g., losartan, irbesartan, valsartan), ET receptor antagonists (e.g., sitaxsentan, atrisentan and compounds disclosed in U.S. Pat. Nos. 5,612,359 and 6,043,265), Dual ET/AII antagonist (e.g., compounds disclosed in WO 00/01389), neutral endopeptidase (NEP) inhibitors, vasopeptidase inhibitors (dual NEP-ACE inhibitors) (e.g., omapatrilat and gemopatrilat), and nitrates.

**[0313]** Examples of suitable anti-platelet agents for use in combination with the compounds of the present invention include GPIIb/IIIa blockers (e.g., abciximab, eptifibatide, tirofiban), P2Y<sub>12</sub> antagonists (e.g., clopidogrel, ticlopidine, CS-747), thromboxane receptor antagonists (e.g., ifetroban), aspirin, and PDE-III inhibitors (e.g., dipyridamole) with or without aspirin.

**[0314]** Examples of suitable cardiac glycosides for use in combination with the compounds of the present invention include digitalis and ouabain.

**[0315]** Examples of suitable cholesterol/lipid lowering agents for use in combination with the compounds of the present invention include HMG-CoA reductase inhibitors (e.g., pravastatin, lovastatin, atorvastatin, simvastatin, NK-104 (a.k.a. itavastatin, or nisvastatin or nisbastatin) and ZD-4522 (a.k.a. rosuvastatin, or atavastatin or visastatin)), squalene synthetase inhibitors, fibrates, bile acid sequestrants, ACAT inhibitors, MTP inhibitors, lipooxygenase inhibitors, cholesterol absorption inhibitors, and cholesterol ester transfer protein inhibitors (e.g., CP-529414).

**[0316]** Examples of suitable mineralocorticoid receptor antagonists for use in combination with the compounds of the present invention include spironolactone and eplerenone.

[0317] Examples of suitable phosphodiesterase inhibitors for use in combination with the compounds of the present invention include PDEIII inhibitors such as cilostazol, and PDE V inhibitors such as sildenafil.

[0318] Examples of suitable thyroid mimetics for use in combination with the compounds of the present invention include thyrotropin, polythyroid, KB-130015, and dronedarone.

[0319] Examples of suitable anabolic agents for use in combination with the compounds of the present invention include testosterone, TRH diethylstilbesterol, estrogens, O-agonists, theophylline, anabolic steroids, dehydroepiandrosterone, enkephalins, E-series prostagladins, retinoic acid and compounds as disclosed in U.S. Pat. No. 3,239,345, e.g., Zeranol®; U.S. Pat. No. 4,036,979, e.g., Sulbenox® or peptides as disclosed in U.S. Pat. No. 4,411,890.

[0320] Examples of suitable HIV or AIDS therapies for use in combination with the compounds of the present invention include indinavir sulfate, saquinavir, saquinavir mesylate, ritonavir, lamivudine, zidovudine, lamivudine/zidovudine combinations, zalcitabine, didanosine, stavudine, and megestrol acetate.

[0321] Examples of suitable therapies for treatment of Alzheimer's disease and cognitive disorders for use in combination with the compounds of the present invention include donepezil, tacrine, revastigmine, 5HT<sub>6</sub>, gamma secretase inhibitors, beta secretase inhibitors, SK channel blockers, Maxi-K blockers, and KCNQs blockers.

[0322] Examples of suitable therapies for treatment of sleeping disorders for use in combination with the compounds of the present invention include melatonin analogs, melatonin receptor antagonists, ML1B agonists, and GABA/NMDA receptor antagonists.

[0323] Examples of suitable anti-proliferative agents for use in combination with the compounds of the present invention include cyclosporin A, paclitaxel, FK 506, and adriamycin.

[0324] Examples of suitable anti-tumor agents for use in combination with the compounds of the present invention include paclitaxel, adriamycin, epothilones, cisplatin and carboplatin.

[0325] Compounds of the present invention can further be used in combination with nutritional supplements such as those described in U.S. Pat. No. 5,179,080, especially in combination with whey protein or casin, amino acids (such as leucine, branched amino acids and hydroxymethylbutyrate), triglycerides, vitamins (e.g., A, B6, B12, folate, C, D and E), minerals (e.g., selenium, magnesium, zinc, chromium, calcium and potassium), carnitine, lipoic acid, creatine, and coenzyme Q-10. In addition, compounds of the present invention can be used in combination with therapeutic agents used in the treatment of sexual dysfunction, including but not limited to PDE5 inhibitors, such as sildenafil or IC-351; with an antiresorptive agent, hormone replacement therapies, vitamin D analogues, calcitonins, elemental calcium and calcium supplements, cathepsin K inhibitors, MMP inhibitors, vitronectin receptor antagonists, Src SH<sub>2</sub> antagonists, vacular —H<sup>+</sup>-ATPase inhibitors, progesterone receptor agonists, ipriflavone, fluoride, RANK antagonists, PTH and its analogues and fragments, Tibolone, HMG-CoA reductase inhibitors, SERM's, p38 inhibitors, prostanoids, 17-beta hydroxysteroid dehydrogenase inhibitors and Src kinase inhibitors.

[0326] Compounds of the present invention can be used in combination with male contraceptives, such as nonoxynol 9 or therapeutic agents for the treatment of hair loss, such as minoxidil and finasteride or chemotherapeutic agents, such as with LHRH agonists.

[0327] For their preferred anticancer or antiangiogenic use, the compounds of the present invention can be administered either alone or in combination with other anti-cancer and cytotoxic agents and treatments useful in the treatment of cancer or other proliferative diseases, for example, where the second drug has the same or different mechanism of action than the present compounds of formula I. Examples of classes of anti-cancer and cytotoxic agents useful in combination with the present compounds include but are not limited to: alkylating agents such as nitrogen mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazenes; EGFR inhibitors such as small molecule EGFR inhibitors, EGFR antibodies such as C225 (Erbix®); antimetabolites such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes such as L-asparaginase; farnesyl-protein transferase inhibitors; 5 $\alpha$  reductase inhibitors; inhibitors of 17 $\beta$ -hydroxy steroid dehydrogenase type 3 or type 1; hormonal agents such as glucocorticoids, estrogens/antiestrogens, androgens/antiandrogens, progestins, and luteinizing hormone-releasing hormone antagonists, octreotide acetate; microtubule-disruptor agents, such as ecteinascidins or their analogs and derivatives; microtubule-stabilizing agents such as taxanes, for example, paclitaxel (Taxoli®), docetaxel (Taxotere®), and their analogs, and epothilones, such as epothilones A-F and their analogs; plant-derived products, such as vinca alkaloids, epipodophyllotoxins, taxanes; and topoisomerase inhibitors; prenyl-protein transferase inhibitors; and miscellaneous agents such as hydroxyurea, procarbazine, mitotane, hexamethylmelamine, platinum coordination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators and monoclonal antibodies. The compounds of the invention may also be used in conjunction with radiation therapy.

[0328] Representative examples of these classes of anti-cancer and cytotoxic agents include but are not limited to mechlorethamine hydrochloride, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustine, lomustine, semustine, streptozocin, thiotepa, dacarbazine, methotrexate, thioguanine, mercaptopurine, fludarabine, pentastatin, cladribin, cytarabine, fluorouracil, doxorubicin hydrochloride, daunorubicin, idarubicin, bleomycin sulfate, mitomycin C, actinomycin D, safracins, saframycins, quinocarcins, discodermolides, vincristine, vinblastine, vinorelbine tartrate, etoposide, etoposide phosphate, teniposide, paclitaxel, tamoxifen, estramustine, estramustine phosphate sodium, flutamide, buserelin, leuprolide, pteridines, diynes, levamisole, aflacon, interferon, interleukins, aldesleukin, filgrastim, sargramostim, rituximab, BCG, tretinoin, irinotecan hydrochloride, betamethosone, gemcitabine hydrochloride, altretamine, and topoteca and any analogs or derivatives thereof.

[0329] Preferred member of these classes include, but are not limited to, paclitaxel, cisplatin, carboplatin, doxorubicin, caminomycin, daunorubicin, aminopterin, methotrexate, methopterin, mitomycin C, ecteinascidin 743, or porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine

arabinoside, podophyllotoxin or podophyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosine, vindesine and leurosine.

**[0330]** Examples of anticancer and other cytotoxic agents include the following: epothilone derivatives as found in German Patent No. 4138042.8; WO 97/19086, WO 98/22461, WO 98/25929, WO 98/38192, WO 99/01124, WO 99/02224, WO 99/02514, WO 99/03848, WO 99/07692, WO 99/27890, WO 99/28324, WO 99/43653, WO 99/54330, WO 99/54318, WO 99/54319, WO 99/65913, WO 99/67252, WO 99/67253 and WO 00/00485; cyclin dependent kinase inhibitors as found in WO 99/24416 (see also U.S. Pat. No. 6,040,321); and prenyl-protein transferase inhibitors as found in WO 97/30992 and WO 98/54966; and agents such as those described generically and specifically in U.S. Pat. No. 6,011,029 (the compounds of which U.S. patent can be employed together with any NHR modulators (including, but not limited to, those of present invention) such as AR modulators, ER modulators, with LHRH modulators, or with surgical castration, especially in the treatment of cancer).

**[0331]** The combinations of the present invention can also be formulated or co-administered with other therapeutic agents that are selected for their particular usefulness in administering therapies associated with the aforementioned conditions. For example, the compounds of the invention may be formulated with agents to prevent nausea, hypersensitivity and gastric irritation, such as antiemetics, and H<sub>1</sub> and H<sub>2</sub> antihistaminics.

**[0332]** As it pertains to the treatment of cancer, the compounds of this invention are most preferably used alone or in combination with anti-cancer treatments such as radiation therapy and/or with cytostatic and/or cytotoxic agents, such as, but not limited to, DNA interactive agents, such as cisplatin or doxorubicin; inhibitors of farnesyl protein transferase, such as those described in U.S. Pat. No. 6,011,029; topoisomerase II inhibitors, such as etoposide; topoisomerase I inhibitors, such as CPT-11 or topotecan; tubulin stabilizing agents, such as paclitaxel, docetaxel, other taxanes, or epothilones; hormonal agents, such as tamoxifen; thymidilate synthase inhibitors, such as 5-fluorouracil; antimetabolites, such as methotrexate; antiangiogenic agents, such as angiostatin, ZD6474, ZD6126 and comberstatin A2; kinase inhibitors, such as her2 specific antibodies, Iressa and CDK inhibitors; histone deacetylase inhibitors, such as CI-994 and MS-27-275. Such compounds may also be combined with agents which suppress the production of circulating testosterone such as LHRH agonists or antagonists or with surgical castration. Exemplary combination therapies (e.g., for the treatment of prostate cancer) for use with a compound of the present invention include an LHRH modulator or prednisone.

**[0333]** The present invention also contemplates kits, for example, for the treatment of prostate cancer, comprising a first container (such as a vial) containing a pharmaceutical formulation comprising a compound of the present invention, said compound optionally in a pharmaceutically acceptable carrier, and a second container (such as a vial) containing a pharmaceutical formulation comprising one or more agents (such as an LHRH modulator) to be used in combination with said compound of the present invention, said agent(s) optionally in a pharmaceutically acceptable carrier.

**[0334]** For example, known therapies for advanced metastatic prostate cancer include "complete androgen ablation therapy" wherein tumor growth is inhibited by controlling the

supply of androgen to the prostate tissues via chemical castration (castration serves to inhibit the production of circulating testosterone (T) and dihydrotestosterone (DHT)) followed by the administration of androgen receptor (AR) antagonists (which inhibit the function T/DHT derived from the conversion of circulating androgen precursors to T/DHT by the prostate tissue). The compounds of the present invention can be employed as AR antagonists in complete ablation therapy, alone or in combination with other AR antagonists such as Flutamide, Casodex, Nilutamide, or Cyproterone acetate.

**[0335]** The present invention provides compounds which can be used to treat patients suffering from prostate cancer resistant to androgen receptor antagonists which are not within formula I of the invention (or salts thereof), such as bicalutimide. The invention thus further contemplates a method of treating prostate cancer resistant to an androgen receptor antagonist other than those of formula I or salts thereof, comprising the step of administering to a patient in need thereof a compound capable of reducing the growth rate of the tumor mass of said cancer in an amount effective therefor. The term "reducing the growth rate of said tumor mass" denotes reduction in the growth rate (including, of course, stabilization or reduction in size) of said tumor mass upon treatment relative to the growth rate upon treatment with said androgen receptor antagonist other than those of formula I or salts thereof. Compounds of the formula I and pharmaceutically acceptable salts thereof of the present invention are preferred such compounds.

**[0336]** The present invention also contemplates use of an antiestrogen and/or aromatase inhibitor in combination with a compound of the present invention, for example, to assist in mitigating side effects associated with antiandrogen therapy such as gynecomastia. Exemplary antiestrogen and/or aromatase inhibitors include anastrozole (Arimidex), tamoxifen citrate (Nolvadex), exemestane (Aromasin), toremifene citrate (Fareston), letrozole (Femara), raloxifene hydrochloride (Evista), Faslodex, or 923 (Wyeth Ayerst).

**[0337]** The compounds of the present invention may be employed adjuvant to surgery.

**[0338]** Another application of the present compounds is in combination with antibody therapy such as but not limited to antibody therapy against PSCA. An additional application is in concert with vaccine/immune modulating agents for the treatment of cancer.

**[0339]** Compounds of the present invention can be employed in accordance with the methods described in U.S. Provisional Patent Application Ser. No. 60/284,438, entitled "Selective Androgen Receptor Modulators and Methods for Their Identification, Design and Use" filed Apr. 18, 2001 by Mark E. Salvati et al. (Attorney Docket No. LD0250(PSP)), which Provisional patent application is incorporated herein by reference in its entirety (including, but not limited to, reference to all specific compounds within formula I of the present invention), and U.S. patent application Ser. No. 09/885,827, entitled "Selective Androgen Receptor Modulators and Methods for Their Identification, Design and Use" filed Jun. 20, 2001 by Mark E. Salvati et al. (Attorney Docket No. LD0250(NP)), which patent application is incorporated herein by reference in its entirety (including, but not limited to, reference to all specific compounds within formula I of the present invention).

**[0340]** For racemates of compounds of the present invention, one enantiomer can, for example be a full AR antagonist

while the other can be an AR antagonist in tumor tissue while having no activity or agonist activity in nontumor tissue containing the androgen receptor.

**[0341]** The above other therapeutic agents, when employed in combination with the compounds of the present invention, can be used, for example, in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

**[0342]** The following assays can be employed in ascertaining the activity of a compound as a NHR modulator. Preferred are those compounds with an activity greater than 20  $\mu$ m for binding or transactivation in any of these assays. Various compounds of the present invention were determined to have AR modulator activity utilizing the transactivation assay, and standard AR binding assays as described following.

#### Transactivation Assays:

##### AR Specific Assay:

**[0343]** Compounds of the present invention were tested in transactivation assays of a transfected reporter construct and using the endogenous androgen receptor of the host cells. The transactivation assay provides a method for identifying functional agonists and partial agonists that mimic, or antagonists that inhibit, the effect of native hormones, in this case, dihydrotestosterone (DHT). This assay can be used to predict in vivo activity as there is a good correlation in both series of data. See, e.g. T. Berger et al., *J. Steroid Biochem. Molec. Biol.* 773 (1992), the disclosure of which is herein incorporated by reference.

**[0344]** For the transactivation assay a reporter plasmid is introduced by transfection (a procedure to induce cells to take foreign genes) into the respective cells. This reporter plasmid, comprising the cDNA for a reporter protein, such as secreted alkaline phosphatase (SEAP), controlled by prostate specific antigen (PSA) upstream sequences containing androgen response elements (AREs). This reporter plasmid functions as a reporter for the transcription-modulating activity of the AR. Thus, the reporter acts as a surrogate for the products (mRNA then protein) normally expressed by a gene under control of the AR and its native hormone. In order to detect antagonists, the transactivation assay is carried out in the presence of constant concentration of the natural AR hormone (DHT) known to induce a defined reporter signal. Increasing concentrations of a suspected antagonist will decrease the reporter signal (e.g., SEAP production). On the other hand, exposing the transfected cells to increasing concentrations of a suspected agonist will increase the production of the reporter signal.

**[0345]** For this assay, LNCaP and MDA 453 cells were obtained from the American Type Culture Collection (Rockville, Md.), and maintained in RPMI 1640 or DMEM medium supplemented with 10% fetal bovine serum (FBS; Gibco) respectively. The respective cells were transiently transfected by electroporation according to the optimized procedure described by Heiser, 130 *Methods Mol. Biol.*, 117 (2000), with the pSEAP2/PSA540/Enhancer reporter plasmid. The reporter plasmid, was constructed as follows: commercial human placental genomic DNA was used to generate by Polymerase Cycle Reaction (PCR) a fragment containing the BglIII site (position 5284) and the Hind III site at position 5831 of the human prostate specific antigen promoter (Accession # U37672), Schuur, et al., *J. Biol. Chem.*, 271 (12): 7043-51 (1996). This fragment was subcloned into the

pSEAP2/basic (Clontech) previously digested with BglIII and HindIII to generate the pSEAP2/PSA540 construct. Then a fragment bearing the fragment of human PSA upstream sequence between positions -5322 and -3873 was amplified by PCR from human placental genomic DNA. A XhoI and a BglIII sites were introduced with the primers. The resulting fragment was subcloned into pSEAP2/PSA540 digested with XhoI and BglIII respectively, to generate the pSEAP2/PSA540/Enhancer construct. LNCaP and MDA 453 cells were collected in media containing 10% charcoal stripped FBS. Each cell suspension was distributed into two Gene Pulser Cuvetts (Bio-Rad) which then received 8  $\mu$ g of the reporter construct, and electroporated using a Bio-Rad Gene Pulser at 210 volts and 960  $\mu$ Faraday. Following the transfections the cells were washed and incubated with media containing charcoal stripped fetal bovine serum in the absence (blank) or presence (control) of 1 nM dihydrotestosterone (DHT; Sigma Chemical) and in the presence or absence of the standard anti-androgen bicalutamide or compounds of the present invention in concentrations ranging from 10<sup>-10</sup> to 10<sup>-5</sup> M (sample). Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory workstation.

**[0346]** After 48 hours, a fraction of the supernatant was assayed for SEAP activity using the Phospha-Light Chemiluminescent Reporter Gene Assay System (Tropix, Inc). Viability of the remaining cells was determined using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (MTS Assay, Promega). Briefly, a mix of a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine methosulfate; PMS) are added to the cells. MTS (Owen's reagent) is bioreduced by cells into a formazan that is soluble in tissue culture medium, and therefore its absorbance at 490 nm can be measured directly from 96 well assay plates without additional processing. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture. For each replicate the SEAP reading was normalized by the Abs<sub>490</sub> value derived from the MTS assay. For the antagonist mode, the % Inhibition was calculated as:

$$\% \text{ Inhibition} = 100 \times (1 - [\text{average control} - \text{average blank} / \text{average sample} - \text{average blank}])$$

**[0347]** Data was plotted and the concentration of compound that inhibited 50% of the normalized SEAP was quantified (IC<sub>50</sub>).

**[0348]** For the agonist mode % Control was referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and was calculated as:

$$\% \text{ Control} = 100 \times \text{average sample} - \text{average blank} / \text{average control} - \text{average blank}$$

**[0349]** Data was plotted and the concentration of compound that activates to levels 50% of the normalized SEAP for the control was quantified (EC<sub>50</sub>).

#### GR Specificity Assay:

**[0350]** The reporter plasmid utilized was comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein was controlled by the mouse mammary tumor virus long terminal repeat (MMTV LTR) sequences that con-

tains three hormone response elements (HREs) that can be regulated by both GR and PR see, e.g. G. Chalepakis et al., *Cell*, 53(3), 371 (1988). This plasmid was transfected into A549 cells, which expresses endogenous GR, to obtain a GR specific transactivation assay. A549 cells were obtained from the American Type Culture Collection (Rockville, Md.), and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco). Determination of the GR specific antagonist activity of the compounds of the present invention was identical to that described for the AR specific transactivation assay, except that the DHT was replaced with 5 nM dexamethasone (Sigma Chemicals), a specific agonist for GR. Determination of the GR specific agonist activity of the compounds of the present invention was performed as described for the AR transactivation assay, wherein one measures the activation of the GR specific reporter system by the addition of a test compound, in the absence of a known GR specific agonists ligand.

#### PR Specific Assay:

**[0351]** The reporter plasmid utilized was comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein was controlled by the mouse mammary tumor virus long terminal repeat (MMTV LTR) sequences that contains three hormone response elements (HREs) that can be regulated by both GR and PR. This plasmid was transfected into T47D, which expresses endogenous PR, to obtain a PR specific transactivation assay. T47D cells were obtained from the American Type Culture Collection (Rockville, Md.), and maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS; Gibco). Determination of the PR specific antagonist activity of the compounds of the present invention was identical to that described for the AR specific transactivation assay, except that the DHT was replaced with 1 nM Promegestone (NEN), a specific agonist for PR. Determination of the PR specific agonist activity of the compounds of the present invention was performed as described for the AR transactivation assay, wherein one measures the activation of the PR specific reporter system by the addition of a test compound, in the absence of a known PR specific agonists ligand.

#### AR Binding Assay:

**[0352]** For the whole cell binding assay, human LNCaP cells (T877A mutant AR) or MDA 453 (wild type AR) in 96-well microtiter plates containing RPMI 1640 or DMEM supplemented with 10% charcoal stripped CA-FBS (Cocalco Biologicals) respectively, were incubated at 37° C. to remove any endogenous ligand that might be complexed with the receptor in the cells. After 48 hours, either a saturation analysis to determine the  $K_d$  for tritiated dihydrotestosterone, [ $^3$ H]-DHT, or a competitive binding assay to evaluate the ability of test compounds to compete with [ $^3$ H]-DHT were performed. For the saturation analysis, media (RPMI 1640 or DMEM—0.2% CA-FBS) containing [ $^3$ H]-DHT (in concentrations ranging from 0.1 nM to 16 nM) in the absence (total binding) or presence (non-specific binding) of a 500-fold molar excess of unlabeled DHT were added to the cells. After 4 hours at 37° C., an aliquot of the total binding media at each concentration of [ $^3$ H]-DHT was removed to estimate the amount of free [ $^3$ H]-DHT. The remaining media was removed, cells were washed three times with PBS and har-

vested onto UniFilter GF/B plates (Packard), Microscint (Packard) was added and plates counted in a Top-Counter (Packard) to evaluate the amount of bound [ $^3$ H]-DHT.

**[0353]** For the saturation analysis, the difference between the total binding and the non-specific binding, was defined as specific binding. The specific binding was evaluated by Scatchard analysis to determine the  $K_d$  for [ $^3$ H]-DHT. See e.g. D. Rodbard, *Mathematics and statistics of ligand assays: an illustrated guide*. In: J. Langon and J. J. Clapp, eds., *Ligand Assay*, Masson Publishing U.S.A., Inc., New York, pp. 45-99, (1981), the disclosure of which is herein incorporated by reference.

**[0354]** For the competition studies, media containing 1 nM [ $^3$ H]-DHT and compounds of the invention ("test compounds") in concentrations ranging from  $10^{-10}$  to  $10^{-5}$  M were added to the cells. Two replicates were used for each sample. After 4 hours at 37° C., cells were washed, harvested and counted as described above. The data was plotted as the amount of [ $^3$ H]-DHT (% of control in the absence of test compound) remaining over the range of the dose response curve for a given compound. The concentration of test compound that inhibited 50% of the amount of [ $^3$ H]-DHT bound in the absence of competing ligand was quantified ( $IC_{50}$ ) after log-logit transformation. The  $K_i$  values were determined by application of the Cheng-Prusoff equation to the  $IC_{50}$  values, where:

$$K_i = \frac{IC_{50}}{(1 + ([^3H-DHT]/K_d \text{ for } ^3H-DHT))}$$

**[0355]** After correcting for non-specific binding,  $IC_{50}$  values were determined. The  $IC_{50}$  is defined as the concentration of competing ligand needed to reduce specific binding by 50%. The  $K_{i,s}$  for [ $^3$ H]-DHT for MDA 453 and LNCaP were 0.7 and 0.2 nM respectively.

#### Human Prostate Cell Proliferation Assay:

**[0356]** Compounds of the present invention were tested ("test compounds") on the proliferation of human prostate cancer cell lines. For that, MDA PCa2b cells, a cell line derived from the metastasis of a patient that failed castration, Navone et al., *Clin. Cancer Res.*, 3, 2493-500 (1997), were incubated with or without the test compounds for 72 hours and the amount of [ $^3$ H]-thymidine incorporated into DNA was quantified as a way to assess number of cells and therefore proliferation. The MDA PCa2b cell line was maintained in BRFF-HPC1 media (Biological Research Faculty & Facility Inc., MD) supplemented with 10% FBS. For the assay, cells were plated in Biocoated 96-well microplates and incubated at 37° C. in 10% FBS (charcoal-stripped)/BRFF-BMZ-ERO (without androgens). After 24 hours, the cells were treated in the absence (blank) or presence of 1 nM DHT (control) or with test compounds (sample) of the present invention in concentrations ranging from  $10^{-10}$  to  $10^{-5}$  M. Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory work station. Seventy two hours later 0.44 uCi. of [ $^3$ H]-Thymidine (Amersham) was added per well and incubated for another 24 h followed by trypsinization, harvesting of the cells onto GF/B filters. Micro-scint PS were added to the filters before counting them on a Beckman TopCount.



[0357] The % Inhibition was calculated as:

$$\% \text{ Inhibition} = 100 \times (1 - [\text{average}_{\text{control}} - \text{average}_{\text{blank}}] / [\text{average}_{\text{sample}} - \text{average}_{\text{blank}}])$$

[0358] Data was plotted and the concentration of compound that inhibited 50% of the [<sup>3</sup>H]-Thymidine incorporation was quantified (IC<sub>50</sub>).

#### C2C12 Mouse Myoblast Transactivation Assay:

[0359] Two functional transactivation assays were developed to assess the efficacy of androgen agonists in a muscle cell background using a luciferase reporter. The first assay (ARTA Stable 1) uses a cell line, Stable 1 (clone #72), which stably expresses the full length rat androgen receptor but requires the transient transfection of an enhancer/reporter. This cell line was derived from C2C12 mouse myoblast cells. The second assay (ARTA Stable 2) uses a cell line, Stable 2 (clone #133), derived from Stable 1 which stably expresses both rAR and the enhancer/luciferase reporter.

[0360] The enhancer/reporter construct used in this system is pGL3/2XDR-1/luciferase. 2XDR-1 was reported to be an AR specific response element in CV-1 cells, Brown et. al. *The Journal of Biological Chemistry* 272, 8227-8235, (1997). It was developed by random mutagenesis of an AR/GR consensus enhancer sequence.

#### ARTA Stable 1:

[0361] 1. Stable 1 cells are plated in 96 well format at 6,000 cells/well in high glucose DMEM without phenol red (Gibco BRL, Cat. No.: 21063-029) containing 10% charcoal and dextran treated FBS (HyClone Cat. No.: SH30068.02), 50 mM HEPES Buffer (Gibco BRL, Cat. No.: 15630-080), 1x MEM Na Pyruvate (Gibco BRL, Cat. No.: 11360-070), 0.5x Antibiotic-Antimycotic, and 800 µg/ml Geneticin (Gibco BRL, Cat. No.: 10131-035).

[0362] 2. 48 hours later, cells are transfected with pGL3/2XDR-1/luciferase using LipofectAMINE Plus™ Reagent (Gibco BRL, Cat. No.: 10964-013). Specifically, 5 ng/well pGL3/2XDR-1/luciferase DNA and 50 ng/well Salmon Sperm DNA (as carrier) are diluted with 5 µl/well Opti-MEM media (Gibco BRL, Cat. No.: 31985-070). To this, 0.5 µl/well Plus reagent is added. This mixture is incubated for 15 minutes at room temperature. In a separate vessel, 0.385 µl/well LipofectAMINE reagent is diluted with 5 µl/well Opti-MEM. The DNA mixture is then combined with the LipofectAMINE mixture and incubated for an additional 15 minutes at room temperature. During this time, the media from the cells is removed and replaced with 60 µl/well of Opti-MEM. To this is added 10 µl/well of the DNA/LipofectAMINE transfection mixture. The cells are incubated for 4 hours.

[0363] 3. The transfection mixture is removed from the cells and replaced with 90 µl of media as in #1 above.

[0364] 4. 10 µl/well of appropriate drug dilution is placed in each well.

[0365] 5. 24 hours later, the Steady-Glo™ Luciferase Assay System is used to detect activity according to the manufacturer's instructions (Promega, Cat. No.: E2520).

#### ARTA Stable 2

[0366] 1. Stable 2 cells are plated in 96 well format at 6,000 cells/well in high glucose DMEM without phenol red (Gibco BRL, Cat. No.: 21063-029) containing 10% charcoal and

dextran treated FBS (HyClone Cat. No.: SH30068.02), 50 mM HEPES Buffer (Gibco BRL, Cat. No.: 15630-080), 1xMEM Na Pyruvate (Gibco BRL, Cat. No.: 11360-070), 0.5xAntibiotic-Antimycotic, 800 µg/ml Geneticin (Gibco BRL, Cat. No.: 10131-035) and 800 µg/ml Hygromycin P (Gibco BRL, Cat. No.: 10687-010).

[0367] 2. 48 hours later, the media on the cells is removed and replaced with 90 µl fresh. 10 µl/well of appropriate drug dilution is placed in each well.

[0368] 3. 24 hours later, the Steady-Glo™ Luciferase Assay System is used to detect activity according to the manufacturer's instructions (Promega, Cat. No.: E2520).

[0369] See U.S. patent application Ser. No. 09/885,831, entitled "Cell Lines and Cell-Based Assays for Identification of Androgen Receptor Modulators" filed Jun. 20, 2001 by Jacek Ostrowski et al. (Attorney Docket No. D0177), which patent application is incorporated herein by reference in its entirety.

#### Proliferation Assays

##### Murine Breast Cell Proliferation Assay:

[0370] The ability of compounds of the present invention ("test compounds") to modulate the function of the AR was determined by testing said compounds in a proliferation assay using the androgen responsive murine breast cell line derived from the Shionogi tumor, Hiraoka et al., *Cancer Res.*, 47, 6560-6564 (1987). Stable AR dependent clones of the parental Shionogi line were established by passing tumor fragments under the general procedures originally described in Tetuo, et. al., *Cancer Research* 25, 1168-1175 (1965). From the above procedure, one stable line, SC114, was isolated, characterized and utilized for the testing of example compounds. SC114 cells were incubated with or without the test compounds for 72 hours and the amount of [<sup>3</sup>H]-thymidine incorporated into DNA was quantified as a surrogate endpoint to assess the number of cells and therefore the proliferation rate as described in Suzuki et. al., *J. Steroid Biochem. Mol. Biol.* 37, 559-567 (1990). The SC114 cell line was maintained in MEM containing 10<sup>-8</sup> M testosterone and 2% DCC-treated FCS. For the assay, cells were plated in 96-well microplates in the maintenance media and incubated at 37° C. On the following day, the medium was changed to serum free medium [Ham's F-12:MEM (1:1, v/v) containing 0.1% BSA] with (antagonist mode) or without (agonist mode) 10<sup>-8</sup> M testosterone and the test compounds of the present invention in concentrations ranging from 10<sup>-10</sup> to 10<sup>-5</sup> M. Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory work station. Seventy two hours later 0.44 uCi of [<sup>3</sup>H]-Thymidine (Amersham) was added per well and incubated for another 2 hr followed by trypsinization, and harvesting of the cells onto GF/B filters. Micro-scint PS were added to the filters before counting them on a Beckman TopCount.

[0371] For the antagonist mode, the % Inhibition was calculated as:

$$\% \text{ Inhibition} = 100 \times (1 - [\text{average}_{\text{sample}} - \text{average}_{\text{blank}}] / [\text{average}_{\text{control}} - \text{average}_{\text{blank}}])$$

[0372] Data was plotted and the concentration of compound that inhibited 50% of the [<sup>3</sup>H]-Thymidine incorporation was quantified (IC<sub>50</sub>).

[0373] For the agonist mode % Control was referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and was calculated as:



$$\% \text{ Control} = 100 \times (\text{average}_{\text{sample}} - \text{average}_{\text{blank}}) / (\text{average}_{\text{control}} - \text{average}_{\text{blank}})$$

**[0374]** Data was plotted and the concentration of compound that inhibited 50% of the [<sup>3</sup>H]-Thymidine incorporation was quantified (EC<sub>50</sub>).

#### In Vitro Assay to Measure GR Induced AP-1 Transrepression:

**[0375]** The AP-1 assay is a cell based luciferase reporter assay. A549 cells, which contain endogenous glucocorticoid receptor, were stably transfected with an AP-1 DNA binding site attached to the luciferase gene. Cells are then grown in RPMI+10% fetal calf serum (charcoal-treated)+Penicillin/Streptomycin with 0.5 mg/ml geneticin. Cells are plated the day before the assay at approximately 40000 cells/well. On assay day, the media is removed by aspiration and 20 µl assay buffer (RPMI without phenol red+10% FCS (charcoal-treated)+Pen/Strep) is added to each well. At this point either 20 µl assay buffer (control experiments), the compounds of the present invention ("test compounds") (dissolved in DMSO and added at varying concentrations) or dexamethasone (100 nM in DMSO, positive control) are added to each well. The plates are then pre-incubated for 15 minutes at 37° C., followed by stimulation of the cells with 10 ng/ml PMA. The plates are then incubated for 7 hrs at 37° C. after which 40 µl luciferase substrate reagent is added to each well. Activity is measured by analysis in a luminometer as compared to control experiments treated with buffer or dexamethasone. Activity is designated as % inhibition of the reporter system as compared to the buffer control with 10 ng/ml PMA alone. The control, dexamethasone, at a concentration of ≤10 µM typically suppresses activity by 65%. Test compounds which demonstrate an inhibition of PMA induction of 50% or greater at a concentration of test compound of ≤10 µM are deemed active.

#### Wet Prostate Weight Assay AR Antagonist Assay:

**[0376]** The activity of compounds of the present invention as AR antagonists was investigated in an immature male rat model, a standard, recognized test of antiandrogen activity of a given compound, as described in L. G. Hershberger et al., *Proc. Soc. Expt. Biol. Med.*, 83, 175 (1953); P. C. Walsh and R. F. Gittes, "Inhibition of extratesticular stimuli to prostate growth in the castrated rat by antiandrogens", *Endocrinology*, 86, 624 (1970); and B. J. Furr et al., "ICI 176,334: A novel non-steroid, peripherally selective antiandrogen", *J. Endocrinol.*, 113, R7-9 (1987), the disclosures of which are herein incorporated by reference.

**[0377]** The basis of this assay is the fact that male sexual accessory organs, such as the prostate and seminal vesicles, play an important role in reproductive function. These glands are stimulated to grow and are maintained in size and secretory function by the continued presence of serum testosterone (T), which is the major serum androgen (>95%) produced by the Leydig cells in the testis under the control of the pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH). Testosterone is converted to the more active form, dihydrotestosterone (DHT), within the prostate by 5α-reductase. Adrenal androgens also contribute about 20% of total DHT in the rat prostate, compared to 40% of that in 65-year-old men. F. Labrie et al. *Clin. Invest. Med.*, 16, 475-492 (1993). However, this is not a major pathway, since in both animals and humans, castration leads to almost complete involution of the prostate and seminal vesicles without con-

comitant adrenalectomy. Therefore, under normal conditions, the adrenals do not support significant growth of prostate tissues. M. C. Luke and D. S. Coffey, "*The Physiology of Reproduction*" ed. By E. Knobil and J. D. Neill, 1, 1435-1487 (1994). Since the male sex organs are the tissues most responsive to modulation of the androgen activity, this model is used to determine the androgen dependent growth of the sex accessory organs in immature castrated rats.

**[0378]** Male immature rats (19-20 days old Sprague-Dawley, Harlan Sprague-Dawley) were castrated under metofane anesthesia. Five days after surgery these castrated rats (60-70 g, 23-25 day-old) were dosed for 3 days. Animals were dosed sub-cutaneously (s.c.) 1 mg/kg with Testosterone Propionate (TP) in arachis oil vehicle and anti-androgen test compounds (compounds of the present invention) were dosed orally by gavage (p.o.) in dissolved/suspensions of 80% PEG 400 and 20% Tween 80 (PEGTW). Animals were dosed (v/w) at 0.5 ml of vehicle/100 g body weight. Experimental groups were as follows:

1. Control vehicle
2. Testosterone Propionate (TP) (3 mg/rat/day, subcutaneous)
3. TP plus Casodex (administered p.o. in PEGTW, QD), a recognized antiandrogen, as a reference compound.
4. To demonstrate antagonist activity, a compound of the present invention ("test compound") was administered (p.o. in PEGTW, QD) with TP (s.c. as administered in group 2) in a range of doses.
5. To demonstrate agonist activity a compound of the present invention ("test compound") was administered alone (p.o. in PEGTW, QD) in a range of doses.

**[0379]** At the end of the 3-day treatment, the animals were sacrificed, and the ventral prostate weighed. To compare data from different experiments, the sexual organs weights were first standardized as mg per 100 g of body weight, and the increase in organ weight induced by TP was considered as the maximum increase (100%). ANOVA followed by one-tailed Student or Fischer's exact test was used for statistical analysis.

**[0380]** The gain and loss of sexual organ weight reflect the changes of the cell number (DNA content) and cell mass (protein content), depending upon the serum androgen concentration. See Y. Okuda et al., *J. Urol.*, 145, 188-191 (1991), the disclosure of which is herein incorporated by reference. Therefore, measurement of organ wet weight is sufficient to indicate the bioactivity of androgens and androgen antagonist. In immature castrated rats, replacement of exogenous androgens increases seminal vesicles (SV) and the ventral prostate (VP) in a dose dependent manner.

**[0381]** The maximum increase in organ weight was 4 to 5-fold when dosing 3 mg/rat/day of testosterone (T) or 1 mg/rat/day of testosterone propionate (TP) for 3 days. The EC<sub>50</sub> of T and TP were about 1 mg and 0.03 mg, respectively. The increase in the weight of the VP and SV also correlated with the increase in the serum T and DHT concentration. Although administration of T showed 5-times higher serum concentrations of T and DHT at 2 hours after subcutaneous injection than that of TP, thereafter, these high levels declined very rapidly. In contrast, the serum concentrations of T and DHT in TP-treated animals were fairly consistent during the 24 hours, and therefore, TP showed about 10-30-fold higher potency than free T.

**[0382]** In this immature castrated rat model, a known AR antagonist (Casodex) was also administered simultaneously with 0.1 mg of TP (ED<sub>80</sub>), inhibiting the testosterone-medi-

ated increase in the weights of the VP and SV in a dose dependent manner. The antagonist effects were similar when dosing orally or subcutaneously. Compounds of the invention also exhibited AR antagonist activity by suppressing the testosterone-mediated increase in the weights of VP and SV.

#### Levator Ani & Wet Prostate Weight Assay AR Agonist Assay:

**[0383]** The activity of compounds of the present invention as AR agonists was investigated in an immature male rat model, a recognized test of anabolic effects in muscle and sustaining effects in sex organs for a given compound, as described in L. G. Hershberger et al., *Proc. Soc. Expt. Biol. Med.*, 83, 175 (1953); B. L. Beyler et al., "Methods for evaluating anabolic and catabolic agents in laboratory animals", *J. Amer. Med. Women's Ass.*, 23, 708 (1968); H. Fukuda et al., "Investigations of the levator ani muscle as an anabolic steroid assay", *Nago Dai. Yak. Ken. Nem.* 14, 84 (1966) the disclosures of which are herein incorporated by reference.

**[0384]** The basis of this assay lies in the well-defined action of androgenic agents on the maintenance and growth of muscle tissues and sexual accessory organs in animals and man. Androgenic steroids, such as testosterone (T), have been well characterized for their ability to maintain muscle mass. Treatment of animals or humans after castrations with an exogenous source of T results in a reversal of muscular atrophy. The effects of T on muscular atrophy in the rat levator ani muscle have been well characterized. M. Masuoka et al., "Constant cell population in normal, testosterone deprived and testosterone stimulated levator ani muscles" *Am. J. Anat.* 119, 263 (1966); Z. Gori et al., "Testosterone hypertrophy of levator ani muscle of castrated rats. I. Quantitative data" *Boll.-Soc. Ital. Biol. Sper.* 42, 1596 (1966); Z. Gori et al., "Testosterone hypertrophy of levator ani muscle of castrated rats. II. Electron-microscopic observations" *Boll.-Soc. Ital. Biol. Sper.* 42, 1600 (1966); A. Boris et al., *Steroids* 15, 61 (1970). As described above, the effects of androgens on maintenance of male sexual accessory organs, such as the prostate and seminal vesicles, is well described. Castration results in rapid involution and atrophy of the prostate and seminal vesicles. This effect can be reversed by exogenous addition of androgens. Since both the levator ani muscle and the male sex organs are the tissues most responsive to the effects of androgenic agents, this model is used to determine the androgen dependent reversal of atrophy in the levator ani muscle and the sex accessory organs in immature castrated rats. Sexually mature rats (200-250 g, 6-8 weeks-old, Sprague-Dawley, Harlan) were acquired castrated from the vendor (Taconic). The rats were divided into groups and treated daily for 7 to 14 days with one of the following:

1. Control vehicle
2. Testosterone Propionate (TP) (3 mg/rat/day, subcutaneous)
3. TP plus Casodex (administered p.o. in PEGTW, QD), a recognized antiandrogen, as a reference compound.
4. To demonstrate antagonist activity, a compound of the present invention ("test compound") was administered (p.o. in PEGTW, QD) with TP (s.c. as administered in group 2) in a range of doses.
5. To demonstrate agonist activity a compound of the present invention ("test compound") was administered alone (p.o. in PEGTW, QD) in a range of doses.

**[0385]** At the end of the 7-14-day treatment, the animals were sacrificed by carbon dioxide, and the levator ani, seminal vesicle and ventral prostate weighed. To compare data from different experiments, the levator ani muscle and sexual

organ weights were first standardized as mg per 100 g of body weight, and the increase in organ weight induced by TP was considered as the maximum increase (100%). Super-anova (one factor) was used for statistical analysis.

**[0386]** The gain and loss of sexual organ weight reflect the changes of the cell number (DNA content) and cell mass (protein content), depending upon the serum androgen concentration. See Y. Okuda et al., *J. Urol.*, 145, 188-191 (1991), the disclosure of which is herein incorporated by reference. Therefore, measurement of organ wet weight is sufficient to indicate the bioactivity of androgens and androgen antagonist. In immature castrated rats, replacement of exogenous androgens increases levator ani, seminal vesicles (SV) and prostate in a dose dependent manner.

**[0387]** The maximum increase in organ weight was 4 to 5-fold when dosing 3 mg/rat/day of testosterone (T) or 1 mg/rat/day of testosterone propionate (TP) for 3 days. The EC<sub>50</sub> of T and TP were about 1 mg and 0.03 mg, respectively. The increase in the weight of the VP and SV also correlated with the increase in the serum T and DHT concentration. Although administration of T showed 5-times higher serum concentrations of T and DHT at 2 hours after subcutaneous injection than that of TP, thereafter, these high levels declined very rapidly. In contrast, the serum concentrations of T and DHT in TP-treated animals were fairly consistent during the 24 hours, and therefore, TP showed about 10-30-fold higher potency than free T.

#### MDA PCa2b Human Prostate Xenograft Assay:

**[0388]** In Vivo Antitumor Testing: MDA-PCa-2b human prostate tumors were maintained in Balb/c nu/nu nude mice. Tumors were propagated as subcutaneous transplants in adult male nude mice (4-6 weeks old) using tumor fragments obtained from donor mice. Tumor passage occurred every 5-6 weeks.

**[0389]** For antitumor efficacy trial, the required number of animals needed to detect a meaningful response were pooled at the start of the experiment and each was given a subcutaneous implant of a tumor fragment (~50 mg) with a 13-gauge trocar. Tumors were allowed to grow to approx. 100-200 mg (tumors outside the range were excluded) and animals were evenly distributed to various treatment and control groups. Treatment of each animal was based on individual body weight. Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provides a measure of treatment-related toxicity.

**[0390]** Tumor response was determined by measurement of tumors with a caliper twice a week, until the tumors reach a predetermined "target" size of 0.5 gm. Tumor weights (mg) were estimated from the formula: Tumor weight=(length×width<sup>2</sup>)÷2

**[0391]** Tumor response end-point was expressed in terms of tumor growth inhibition (% T/C), defined as the ratio of median tumor weights of the treated tumors (T) to that of the control group (C).

**[0392]** To estimate tumor cell kill, the tumor volume doubling time was first calculated with the formula:

$$TVDT = \frac{\text{Median time (days) for control tumors to reach target size} - \text{Median time (days) for control tumors to reach half the target size}}{\ln 2}$$

$$\text{And, Log cell kill} = (T - C) \div (3.32 \times TVDT)$$

Statistical evaluations of data were performed using Gehan's generalized Wilcoxon test.

## Dunning Prostate Tumor:

[0393] Dunning R3327H prostate tumor is a spontaneously derived, well differentiated androgen responsive adenocarcinoma of the prostate (Smolev J K, Heston W D, Scott W W, and Coffey D S, *Cancer Treat Rep.* 61, 273-287 (1977)). The growth of the R3327H subline has been selected for its highly androgen-dependent and reproducible growth in intact male rats. Therefore, this model and other sublines of this tumor have been widely used to evaluate in vivo antitumor activities of antiandrogens such as flutamide and bicalutamide/Casodex (Maucher A., and von Angerer, *J. Cancer Res. Clin. Oncol.*, 119, 669-674 (1993), Furr B. J. A. *Euro. URL.* 18 (suppl. 3), 2-9 (1990), Shain S. A. and Huot R I. *J. Steriod Biochem.* 31, 711-718 (1988)).

[0394] At the beginning of the study, the Dunning tumor pieces (about 4×4 mm) are transplanted subcutaneously to the flank of mature male Copenhagen rats (6-7 weeks old, Harlan-Sprague Dawley, Indianapolis, Md.). About 6 weeks after the implantation, the animals with tumors of measurable size (about 80-120 mm<sup>2</sup>) are randomized into treatment groups (8-10 rats/group) and the treatments are initiated. One group of the rats are castrated to serve as the negative control of tumor growth. Animals are treated daily with compounds of the current invention, standard antiandrogens such as bicalutamide or vehicle (control) for an average of 10 to 14 weeks. Test compounds are dissolved in a vehicle of (2.5 ml/kg of body weight) 10% polyethylene glycol and 0.05% Tween-80 in 1% carboxymethyl cellulose, PEG/CMC, (Sigma, St Louis, Mo.). Typical therapeutic experiments would include three groups of three escalating doses for each standard or test compound (in a range of 300-3 mg/kg).

[0395] Tumors in the vehicle (control) group reach a size of 1500 to 2500 mm<sup>3</sup>, whereas the castrated animal group typically shows tumor stasis over the 14 weeks of observation. Animals treated orally with 20 mg/kg of bicalutamide or flutamide would be expected to show a 40% reduction in tumor volumes compared to control after 14 weeks of treatment. The size of tumors are measured weekly by vernier caliper (Froboz, Switzerland), taking perpendicular measurements of length and width. Tumor volumes are measured in mm using the formula: Length×Width×Height=Volume. Statistical differences between treatment groups and control are evaluated using multiple ANOVA analysis followed by one tail non-parametric Student t test.

## Mature Rat Prostate Weight Assay:

[0396] The activity of compounds of the present invention were investigated in a mature male rat model, which is a variation of the Levator ani & wet prostate weight assay described above. The above in vivo assays are recognized assays for determining the anabolic effects in muscle and sustaining effects in sex organs for a given compound, as described in L. G. Hersherberger et al., 83 *Proc. Soc. Expt. Biol. Med.*, 175 (1953); B. L. Beyler et al., "Methods for evaluating anabolic and catabolic agents in laboratory animals", 23 *J. Amer. Med. Women's Ass.*, 708 (1968); H. Fukuda et al., "Investigations of the levator ani muscle as an anabolic steroid assay", 14 *Nago Dai. Yak. Ken. Nem.* 84 (1966) the disclosures of which are herein incorporated by reference. The basis of this assay lies in the well-defined action of androgenic agents on the maintenance and growth of muscle tissues and sexual accessory organs in animals and man.

[0397] The male sexual accessory organs, such as the prostate and seminal vesicles, play an important role in reproductive function. These glands are stimulated to grow and are maintained in size and secretory function by the continued presence of serum testosterone (T), which is the major serum androgen (>95%) produced by the Leydig cells in the testis under the control of the pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH). Testosterone is converted to the more active form, dihydrotestosterone (DHT), within the prostate by 5 $\alpha$ -reductase. Adrenal androgens also contribute about 20% of total DHT in the rat prostate, compared to 40% of that in 65-year-old men. F. Labrie et. al. 16 *Clin. Invest. Med.*, 475-492 (1993). However, this is not a major pathway, since in both animals and humans, castration leads to almost complete involution of the prostate and seminal vesicles without concomitant adrenalectomy. Therefore, under normal conditions, the adrenals do not support significant growth of prostate tissues, M. C. Luke and D. S. Coffey, "The Physiology of Reproduction" ed. By E. Knobil and J. D. Neill, 1, 1435-1487 (1994). Since the male sex organs and the levator ani are the tissues most responsive to modulation of the androgen activity, this model is used to determine the activity of compounds that modulate the androgen receptor pathway in mature rats.

[0398] Along with its mitogenic activity on tissues such as prostate, seminal vesicle and muscle, testosterone also serves as a negative regulator for its own biosynthesis. Testosterone production in the Leydig cells of the testis is controlled by the level of circulating LH released from the pituitary gland. LH levels are themselves controlled by the level of LHRH produced in the hypothalamic region. Testosterone levels in the blood serve to inhibit the secretion of LHRH and subsequently reduce levels of LH and ultimately the levels of circulating testosterone levels. By measuring blood levels of LH as they are effected by compounds of the present invention ("test compounds"), it is possible to determine the level of agonist or antagonist activity of said compounds at the hypothalamic axis of this endocrine cycle.

[0399] Matched sets of Harlan Sprague-Dawley rats (40-42 days old, 180-220 g), were dosed orally by gavage (p.o.) with the test compounds in dissolved/suspensions of 80% PEG 400 and 20% Tween 20 (PEGTW) for 14 days. Two control groups, one intact and one castrated were dose orally only with the PEGTW vehicle. Animals were dosed (v/w) at 0.5 ml of vehicle/100 g body weight. Experimental groups were as follows:

1. Intact vehicle (p.o., PEGTW, QD)
2. Control vehicle (p.o., PEGTW, QD)
3. Bicalutamide (Casodex, a recognized antiandrogen, as a reference compound) or a compound of the present invention, p.o. in PEGTW QD. (in a range of doses).

[0400] At the end of the 14-day treatment, the animals were sacrificed, and the ventral prostate, the seminal vesicles, and the levator ani were removed surgically and weighed. To compare data from different experiments, the organs weights were first standardized as mg per 100 g of body weight, and expressed as a percentage of the value of the respective organ in the intact group.

[0401] Rat luteinizing hormone (rLH) is quantitatively determined with the Biotrak [125 I] kit (Amersham Pharmacia Biotech), following the manufacturer directions. The assay is based on the competition by the LH present in the serum of the binding of [<sup>125</sup>I] rLH to an Amerlex-M bead/antibody suspension. The radioactivity that remains after incubation

with the serum and subsequent washes is extrapolated into a standard curve to obtain a reading in ng/ml.

**[0402]** The gain and loss of sexual organ and levator ani weight reflect the changes of the cell number (DNA content) and cell mass (protein content), depending upon the serum androgen concentration, see Y. Okuda et al., *J. Urol.*, 145, 188-191 (1991), the disclosure of which is herein incorporated by reference. Therefore, measurement of organ wet weight is sufficient to indicate the bioactivity of androgens and androgen antagonist. In the mature rats assay, active agonist agents will have no effect or will increase the weight of one or more of the androgen responsive organs (levator ani, prostate, seminal vesicle) and will have no effect or a suppressive effect on LH secretion. Compounds with antagonist activity will decrease the weight of one or more of the androgen responsive organs (levator ani, prostate, seminal vesicle) and will have no effect or a reduced suppressive effect on LH secretion.

#### CWR22 Human Prostate Xenograft Assay:

**[0403]** In Vivo Antitumor Testing: CWR22 human prostate tumors were maintained in Balb/c nu/nu nude mice. Tumors were propagated as subcutaneous transplants in adult male nude mice (4-6 weeks old) using tumor fragments obtained from donor mice. Tumor passage occurred every 5-6 weeks.

**[0404]** For antitumor efficacy trial, the required number of animals needed to detect a meaningful response were pooled at the start of the experiment and each was given a subcutaneous implant of a tumor fragment (~50 mg) with a 13-gauge trocar. Tumors were allowed to grow to approx. 100-200 mg (tumors outside the range were excluded) and animals were evenly distributed to various treatment and control groups. Treatment of each animal was based on individual body weight. Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provides a measure of treatment-related toxicity.

**[0405]** Tumor response was determined by measurement of tumors with a caliper twice a week, until the tumors reach a predetermined "target" size of 0.5 gm. Tumor weights (mg) were estimated from the formula: Tumor weight=(length $\times$ width $\times$ 2).

**[0406]** Tumor response end-point was expressed in terms of tumor growth inhibition (% T/C), defined as the ratio of median tumor weights of the treated tumors (T) to that of the control group (C).

**[0407]** To estimate tumor cell kill, the tumor volume doubling time was first calculated with the formula:

$$TVDT = \frac{\text{Median time (days) for control tumors to reach target size} - \text{Median time (days) for control tumors to reach half the target size}}{\ln 2}$$

$$\text{And, Log cell kill} = (T - C) \div (3.32 \times TVDT)$$

Statistical evaluations of data were performed using Gehan's generalized Wilcoxon test.

**[0408]** The following Examples illustrate embodiments of the present invention, and are not intended to limit the scope of the claims. Within certain Examples, one compound of the formula I is prepared and then employed to further prepare one or more additional compounds of the formula I or salts thereof. Methods employed to prepare one compound of the

formula I or salt thereof as described herein can be employed as appropriate to prepare other compounds of the invention.

#### ABBREVIATIONS

**[0409]** The following abbreviations are used herein:

DBU=1,8-diazabicyclo[5.4.0]undec-7-ene

4-DMAP=4-dimethylaminopyridine

ee=enantiomeric excess

DMF=dimethylformamide

EtOAc=ethyl acetate

LDA=lithium diisopropylamide

Hünig's Base=N,N-diisopropylethylamine

Me=methyl

RT=retention time

TFA=trifluoroacetic acid

THF=tetrahydrofuran

TLC=thin layer chromatography

TMS=trimethylsilyl

pTSA=para-toluenesulfonic acid

⊕=heat

t-Bu=tert-butyl

PhCH<sub>3</sub>=toluene

Pd/C=palladium on activated charcoal

TsCl=tosyl chloride

TBSOTf=tert-butyldimethylsilyl trifluoromethane sulfonate

TBS=tert-butyldimethylsilane

MeI=methyl iodide

(BOC)<sub>2</sub>O=di-tert-butyl dicarbonate

TEA=triethylamine

n-BuLi=n-butyllithium

rt=room temperature

LC=liquid chromatography

Ts=tosyl

Ph=phenyl

EtOH=ethanol

DCE=dichloroethane

DMSO=dimethylsulfoxide

Ra—Ni=Raney Nickel

**[0410]** MS=molecular sieves

MS(ES)=Electro-Spray Mass Spectrometry

**[0411]** mCPBA=m-chloroperoxybenzoic acid

sat=saturated

AcOH=acetic acid

MeOH=methanol

Et<sub>2</sub>O=diethyl ether

Ac=acetyl

DEAD=diethyl azodicarboxylate

h=hours

Et=ethyl

WSDCC=water soluble dicarbonyl diimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

TBAF=tetrabutylammonium fluoride

DBAD=di-terbutylazodicarboxylate

DCC=Dicyclohexylcarbodiimide

**[0412]** Wilkinson's catalyst=RhCl(PPh<sub>3</sub>)<sub>3</sub>

ADDP=1,1-[azodicarbonyl]dipiperidine

DMA=dimethylacetamide

DME=1,2-dimethoxyethane

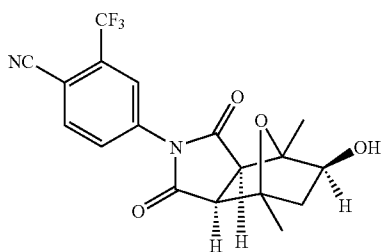
BOP=benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate

HRMS=high resolution mass spectrometry  
 TBME=MTBE=methyl tert-butyl ether (i.e., 2-methoxy-2-methyl-propane)  
 $\text{TiCl}_2 \cdot \text{Cp}_2$ =bis(cyclopentadienyl)titanium dichloride  
 DPPA=diphenylphosphoryl azide  
 HMPA=hexamethylphosphoryl amide  
 V %=volume percent  
 $\text{BH}_3 \cdot \text{DMS}$ =borane dimethylsulfate  
 vvm=volume gas per volume liquid per minute

## EXAMPLE 221

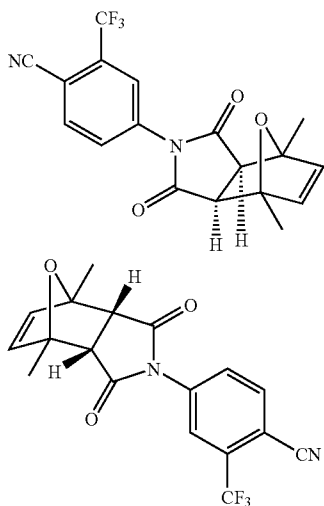
(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221B)

[0413]



A. (3 $\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-4-(hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221Ai) & (3 $\alpha$ ,4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ )-4-(hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221Aii)

[0414]



[0415] A solution of 2,5-dimethylfuran (0.800 mL, 7.51 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (synthesized as described in Example 1B, using 4-cyano-3-trifluoromethylaniline in place of 4-bromo-3-methylaniline) (1.00 g, 3.75 mmol) in benzene

(4 mL) was heated at 60° C. overnight. The reaction mixture was concentrated under reduced pressure and placed on a high vacuum pump until the oil solidified to give a 3:1 mixture (determined by LC and NMR) of compounds 221Ai & 221Aii, respectively, as a brown solid, which was used directly in the next step without further purification.

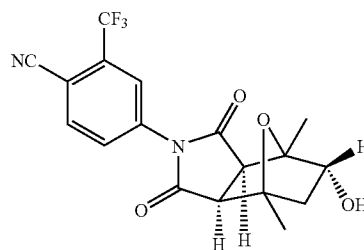
B. (3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221B)

[0416]  $\text{BH}_3 \cdot \text{THF}$  (3.75 mL, 3.75 mmol, 1M in THF) was added to a solution of crude compounds 221Ai & 221Aii (3.75 mmol) in THF (12.5 mL) at 0° C. After the starting material was consumed the reaction mixture was concentrated under reduced pressure. The resulting residue was then dissolved in toluene (12.5 mL),  $\text{Me}_3\text{NO}$  (845 mg, 11.2 mmol) was added and the mixture was heated to reflux overnight. The reaction mixture was then cooled to rt, added to  $\text{H}_2\text{O}$  and extracted with EtOAc (3 $\times$ ). The combined organic layers were dried over  $\text{MgSO}_4$  and concentrated under reduced pressure. Purification by flash chromatography on  $\text{SiO}_2$  eluting with 75% EtOAc/hexanes gave 0.354 g (25%) of compound 221B as a tan powder. HPLC: 90% at 2.45 min (retention time) (YMC S5 ODS column 4.6 $\times$ 50 mm eluting with 10-90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES):  $m/z$  381.11  $[\text{M}+\text{H}]^+$ .

## EXAMPLE 222

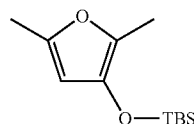
(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (222D)

[0417]



A. 3-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-2,5-dimethylfuran (222A)

[0418]

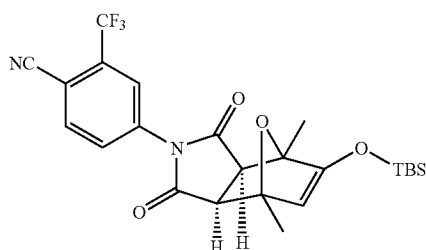


[0419] 2,5-Dimethyl-3(3H)-furanone (2.00 g, 17.8 mmol) was dissolved in methylene chloride (180 mL). TEA (7.43 mL, 53.5 mmol) was added followed by TBSOTf (4.92 mL, 21.4 mmol) at 25° C. After 1 h, the reaction was concentrated in vacuo and the resulting slurry was run through a silica gel

column conditioned with 3% TEA in hexanes. The product was eluted with 3% TEA/hexanes to give 3.6 g (89%) of compound 222A as an orange oil which was used directly in subsequent reactions.

B. (3 $\alpha$ ,4 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-4-[5-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1,3,3a,4,7,7a-hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (222B)

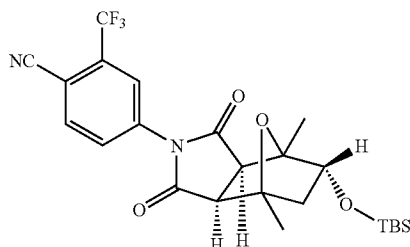
[0420]



[0421] 4-(2,5-Dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (1.00 g, 3.85 mmol) was dissolved in benzene (5.0 mL) and the compound 222A (1.30 g, 5.77 mmol) was added. The reaction mixture was warmed to 60° C. for 2 h and then cooled to 25° C. The solution was then concentrated in vacuo to give compound 222B as a yellow oil which was carried on to the next reaction without purification. HPLC: 60% at 4.013 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-[5-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (222C)

[0422]



[0423] Crude compound 222B (3.85 mmol) was dissolved in ethyl acetate (75 mL) and 10% Pd/C (1.20 g) was added. Hydrogen was then introduced via a balloon. After 24 h, the reaction was filtered through Celite rinsing with ethyl acetate and concentrated in vacuo to give a yellow oil. The crude product was purified by flash chromatography on silica gel eluting with methylene chloride/acetone (0%-1%-2% acetone) to give 0.710 g (35%) compound 222C as a yellow solid. HPLC: 100% at 4.160 min (retention time) (YMC S5

ODS column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): *m/z* 517.6 [M+Na]<sup>+</sup>.

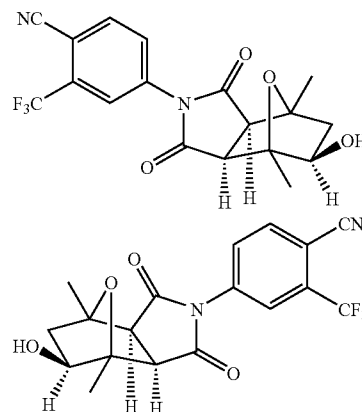
D. (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (222D)

[0424] Compound 222C (0.040 g, 0.081 mmol) was dissolved in THF (1.0 mL) and HF·Pyridine (0.5 mL) was added. After 2 h, the reaction was carefully poured into cold saturated aq. NaHCO<sub>3</sub>. The mixture was then extracted with methylene chloride (3×10 mL). The combined organics were washed with 1 N HCl (1×10 mL) and dried over anhydrous sodium sulfate. Concentration in vacuo gave 0.031 g (10%) compound 222D as a yellow solid. NOE experiments confirmed the assigned isomer. HPLC: 98% at 2.777 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): *m/z* 403.06 [M+Na]<sup>+</sup>.

#### EXAMPLE 254

[3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile, (254ii & 254ii)

[0425]

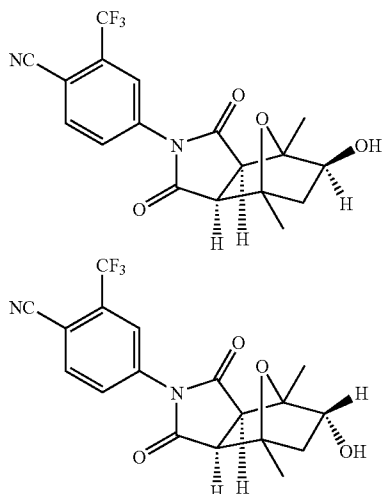


[0426] Racemic compound 221B was separated into its enantiomers by normal phase preparative chiral HPLC (CHIRALPAK AD 5×50 cm column; eluting with 20% MeOH/EtOH (1:1) in heptane (isocratic) at 50 mL/min) to give the faster eluting compound 254i (Chiral HPLC: 10.02 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min) and the slower eluting 254ii (Chiral HPLC: 14.74 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). (Names of title compounds based on absolute stereochemistry determination).

## EXAMPLE 458

(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221B & 222D)

[0427]



[0428] Compound 20B was converted to compounds 221B and 222D (also synthesized as compounds 221B and 222D) by biotransformation.

[0429] Compound 20B was hydroxylated by *Amycolatopsis orientalis* (ATCC 43491). A 1 mL culture from a frozen vial was used to inoculate 100 mL medium in a 500 mL portion Erlenmeyer flask and the flask was incubated at 28° C., at 200 rpm for 3 days. A 10 mL portion of this culture was used to inoculate 100 mL medium in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C., at 200 rpm for 1 day. 10 mL portions of the 1-day culture were distributed to each of three 50 mL flasks. Compound 20B (3 mg in 0.1 mL methanol) was added to each culture and the incubations were continued for 3 days. The culture broth in each flask was extracted with 20 mL ethyl acetate, and the pooled ethyl acetate extracts were evaporated to dryness at 40° C. under a nitrogen stream. The residue was dissolved in 1.2 mL methanol and analyzed by HPLC, LC/MS and LC/NMR. The solution contained 2.5 mg of remaining Compound 20B, 1.6 mg of compound 221B, and 1.3 mg of compound 222D. MS and NMR analyses were in agreement with the structures shown above.

[0430] Medium: 0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K<sub>2</sub>HPO<sub>4</sub>, 0.5% NaCl, adjusted to pH 7 with HCl (R. V. Smith and J. P. Rosazza, Arch. Biochem. Biophys., 161, 551-558 (1974))

## HPLC Analysis

Column: Phenomenex Luna C18, 150×2 mm, 5 $\mu$

[0431] mobile phase: solvent A: 95% 20 mM ammonium acetate pH 5.1, 5% acetonitrile

[0432] solvent B: 95% acetonitrile, 5% 20 mM ammonium acetate pH 5.1 linear gradient going from 100% solvent A to 5% solvent A in 25 minutes followed by equilibration at 100% solvent A for 8 minutes.

temperature: 40° C.

detection: 250 nm injection volume: 1  $\mu$ L

retention times: compound 20B, 20.8 min; compound 221B, 16.5 min; compound 222D, 17.8 min

## HPLC Conditions

[0433] Chiral HPLC conditions were employed for the separation of enantiomers and achiral HPLC conditions were employed for the separation of diastereomers of the hydroxylated analogs of compound 20B (i.e., compounds 221B and 222D and compounds 254I and 254II)

[0434] Two methods were used under chiral HPLC conditions, reverse phase (RP) for chiral analysis of biotransformation products in biological samples and normal phase (NP) for non-biological samples.

## Chiral RP-HPLC Condition

[0435]

Column:	CHIRALPAK AD-R 4.6 × 250 mm, 10 $\mu$
Temperature:	40° C.
Injection Volume:	5 or 20 $\mu$ L
Mobile Phase:	A: MeCN B: H <sub>2</sub> O Isocratic, 30% of A, 18 min.
Flow Rate:	1 mL/min.
UV Detection:	242 nm

## Chiral NP-HPLC Condition

[0436]

Column:	CHIRALPAK AD 4.6 × 250 mm, 10 $\mu$
Temperature:	25° C.
Injection Volume:	5 or 20 $\mu$ L
Mobile Phase:	A: Heptane B: MeOH/Ethanol (1:1) Isocratic, 80% of A, 20 min.
Flow Rate:	1 mL/min.
UV Detection:	242 nm

[0437] Under these conditions compounds 254i and 254ii had retention times of 8.5 minutes and 9.85 minutes, respectively.

[0438] Reverse phase HPLC was employed for the separation of the diastereomeric compounds 221B and 222D:

## Mobile Phase:

[0439]

Solvent A:	95% 20 mM ammonium acetate pH 5.1, 5% acetonitrile
Solvent B:	95% acetonitrile, 5% 20 mM ammonium acetate pH 5.1

Gradient: Linear gradient going from 100% solvent A to 5% solvent A in 25 minutes followed by equilibration at 100% solvent A for 8 minutes. Total run time of 36 minutes.

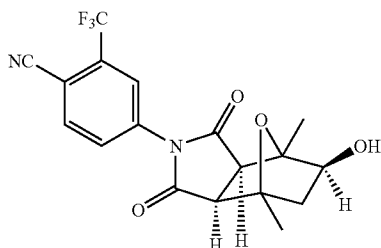
Flow Rate:	0.2 mL/min
Column:	Phenomenex Luna 5 micron C <sub>18</sub> 150 × 2.0 mm id
Detection:	UV detection at 242 nm

[0440] Under these conditions, compounds 221B and 222D had retention times of 18.983 min and 20.362 min, respectively.

## EXAMPLE 471

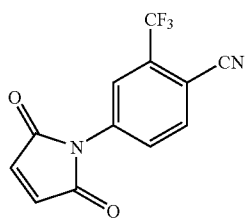
[3aR-(3a $\beta$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7a $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471Di) & [3aS-(3a $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7a $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471Dii)

[0441]



A. 4-(2,5Dioxo-2,5-dihydro-pyrrol-1-yl)-2-trifluoromethyl-benzonitrile (471A)

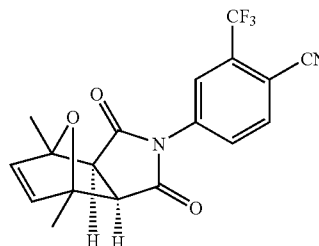
[0442]



[0443] A mixture of 3-trifluoromethyl-4-cyano-aniline (24.0 g, 129 mmol) and maleic anhydride (14.0 g, 143 mmol) in 50 mL of acetic acid was heated at 115° C. overnight. A precipitate was obtained during the heating period. The reaction was allowed to stand at rt for an additional overnight period. The solid was removed by filtration, the filter cake was washed with diethyl ether and dried to give 21 g (79 mmol, 61%) of compound 471A as an off white solid. HPLC: 100% at 2.11 min (retention time) (YMC S5 ODS column, 4.6x50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3a $\alpha$ ,4 $\beta$ ,7 $\beta$ ,7a $\alpha$ )-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471B)

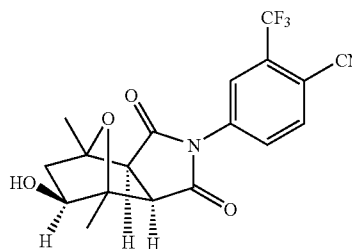
[0444]



[0445] A suspension of compound 471A (13.0 g, 48.8 mmol) and 2,5-dimethylfuran (10.5 mL, 98.6 mmol) in 50 mL of toluene was heated at 60° C., under argon. A solution was obtained on initial heating and a precipitate was observed after approximately 1 h. Heating was continued overnight. After cooling to rt, the suspension was allowed to stand at 4° C. overnight. The resulting solid was filtered and the filter cake was washed with cold toluene followed by air drying to give 13.2 g of pure compound 471B as a white solid. The filtrate volume was reduced in vacuo by one half and the resulting solution was treated as above to yield an additional 2.8 g of pure compound 471B (total 16.0 g, 90%). HPLC: 90% at 3.65 min (retention time) (YMC S5 ODS column, 4.6x50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3a $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7a $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471C)

[0446]



[0447] A solution of compound 471B (25 g, 69 mmol) in 125 mL of THF, in a dry flask under nitrogen, was cooled to 10° C. with an ice bath. To this solution was added neat borane-dimethylsulfide complex (13.0 mL, 138 mmol) dropwise over 10 min, while maintaining a reaction temperature of <15° C. The reaction mixture was stirred for 30 min at rt and then in an ice bath cooled to 10° C. To the cool solution was slowly added 480 mL of pH 7 phosphate buffer, which resulted in a strong exothermic reaction and vigorous gas evolution. The solution was maintained at <21° C. throughout the addition by means of an ice bath. To the resulting solution was added 240 mL of ethanol and the resulting mixture was cooled to 5° C. with an ice bath. To the cooled solution was



added 50 mL of 30% hydrogen peroxide and the resulting mixture was stirred at 10-20° C. for 1.5 h. The mixture was extracted with ethyl acetate (2×1 L) and the combined organic layers were washed with 10% sodium sulfite (1×500 mL) and brine (2×300 mL) and dried over MgSO<sub>4</sub>—Concentration in vacuo afforded 29 g of crude product as a white solid. This material was subjected to flash chromatography on a 1.2 L column of silica gel equilibrated with 100% CH<sub>2</sub>Cl<sub>2</sub>. The material was applied to the column as a solution consisting of 100 mL EtOAc (warm) and 400 mL CH<sub>2</sub>Cl<sub>2</sub>. Initial elution with CH<sub>2</sub>Cl<sub>2</sub> (3 L), followed by 25% EtOAc/75% CH<sub>2</sub>Cl<sub>2</sub> (3 L) and finally 50% EtOAc/50% CH<sub>2</sub>Cl<sub>2</sub> (6 L) gave 11.8 g (45%) of compound 471C which is a racemic mixture.

[0448] Alternatively compound 471C can be made by the following approach: A dry flask containing compound 471B (8.90 g, 24.6 mmol) and Wilkinson's catalyst (0.57 mg, 0.62 mmol) was degassed 4× with vacuum/argon. THF (40 mL) was added to the flask and the mixture was stirred until a clear brown solution was obtained. Catecholborane (49 mL, 49 mmol, 1 M in THF) was then added dropwise over 20 min and a slight exotherm was observed. Stirring was continued for 45 min followed by cooling of the reaction mixture with an ice bath. pH 7 phosphate buffer (175 mL) was slowly added, followed by the consecutive addition of ethanol (87 mL) and 30% hydrogen peroxide (18 mL). Stirring was continued with cooling and the reaction progress was monitored by HPLC for 4 h. The reaction was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×250 mL). The combined extracts were washed with 1:1 1N NaOH: 15% sodium sulfite (300 mL) and brine, dried over MgSO<sub>4</sub>, and the solvent was removed in vacuo to afford 8.5 g of a tan solid. The crude product was subjected to flash chromatography on a 500 cm<sup>3</sup> silica gel column eluting with a gradient of 25-50% EtOAc/CH<sub>2</sub>Cl<sub>2</sub> to give 6.00 g compound 471C (15.8 mmol, 64%) as a white solid. HPLC: 90% at 2.45 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 [M+H]<sup>+</sup>.

D. [3aR-(3α,4β,5β,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471Di) & [3aS-(3α,4β,5β,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471Dii)

[0449] The individual antipodes of compound 471C were separated by normal phase preparative chiral HPLC (CHIRALPAK AD, 5×50 cm column). A 2.5 g portion of 471C was dissolved into 25 mL of warm acetone and diluted to 50-75 mL with hexane for injection. Isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 50 mL/min gave the faster eluting compound 471Di (Chiral HPLC: 10.02 min; CHIRALPAK AD 4.6×250 mm column; isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min) and the slower eluting compound 471Dii (Chiral HPLC: 14.74 min; CHIRALPAK AD 4.6×250 mm column; isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). Compounds 471Di & 471Dii: HPLC: 90% at 2.45 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 [M+H]<sup>+</sup>. The absolute stereochemistry of compounds

471Di & 471Dii was determined by single crystal X-ray diffraction studies and is as described by the designated nomenclature.

[0450] The resulting HPLC purified fractions of compounds 471Di & 471Dii were further purified by crystallization using any one of the procedures described below.

#### 1. From Ethyl Acetate

[0451] A 700 mg portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in ethyl acetate (10 mL) at rt. The solution was diluted with small portions of hexane (20 mL) until cloudiness was observed. The solution was allowed to stand overnight at rt. The resulting white solid was filtered and air dried to afford 430 mg of compound 471Di as a white powder. This sample was further dried at 60° C. (3 h, 0.5 Torr), then at 70° C., (12 h, 0.5 Torr).

#### 2. From Acetone

[0452] A 500 mg portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in a minimal amount of acetone (3 mL) and slowly diluted with hexane (1 mL). The clear colorless solution was allowed to stand overnight at rt. The resulting white solid was filtered and air dried to afford 440 mg of compound 471Di as a white powder. This sample was further dried at 60° C., (3 h, 0.5 Torr) then at 70° C., (12 h, 0.5 Torr).

#### 3. From Methanol

[0453] A 500 mg portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in 5 mL of hot (steam bath) methanol. The clear colorless solution was allowed to stand at rt for 2 h, then at 4° C. overnight. The resulting solid was filtered, washed with minimal cold methanol and air dried for to afford 360 mg of compound 471Di as a white powder. This sample was further dried at 70° C., (12 h, 0.5 Torr).

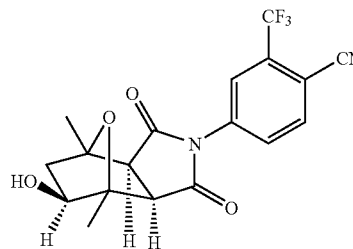
#### 4. From CH<sub>2</sub>Cl<sub>2</sub>

[0454] A 7.00 g portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in 75 mL of CH<sub>2</sub>Cl<sub>2</sub> at rt. The clear and colorless solution was slowly diluted with hexane (48 mL) until crystallization was observed. The solution was allowed to stand at rt for 1 h, then at 4° C. overnight. The resulting crystalline material was filtered and then washed with a minimal amount of cold 2:1 CH<sub>2</sub>Cl<sub>2</sub>:hexane. The large crystals were ground to a fine powder and dried at 50° C. (12 h, 0.5 Torr) to yield 5.96 g of compound 471Di as a white powder.

#### EXAMPLE 508

(3α,4β,5β,7β,7α)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (508)

[0455]

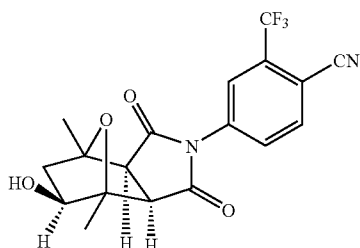


[0456] To THF (275 mL) that had been cooled to  $-3^{\circ}\text{C}$ , was added compound 507 (55.0 g, 152 mmol), which resulted in a slurry. To the slurry was added borane methylsulfide (14.4 mL, 152 mmol), at a rate such that the temperature did not exceed  $0^{\circ}\text{C}$ . The reaction mixture was slowly warmed to  $20^{\circ}\text{C}$  over 2.5 h. The temperature of the reaction mixture was then returned to  $0^{\circ}\text{C}$ , where upon phosphate buffer (1056 mL, pH 7) was carefully added at a rate to control the hydrogen gas evolution and maintain a temperature  $\leq 20^{\circ}\text{C}$ . The resulting suspension was dissolved by adding ethanol (528 mL, 190 proof). At  $15^{\circ}\text{C}$ , hydrogen peroxide (55 mL, 30 wt %) was added at a rate to maintain the temperature  $\leq 20^{\circ}\text{C}$ . The homogeneous solution was left stirring for 12 h at  $20^{\circ}\text{C}$  and pH 7.8, whereby crystallization occurred. The resulting slurry was collected by filtration and washed with water (4x100 mL) and methyl-tert-butyl ether (2x100 mL). Drying in vacuo afforded 37.3 g (64.6%) of compound 508. The aqueous mother liquor was extracted with ethyl acetate (3x500 mL). The combined rich organics were washed with 10 wt % aqueous sodium sulfite (1x100 mL), and 25 wt % aqueous sodium chloride (1x100 mL). The organics were dried over sodium sulfate, filtered, and concentrated to recover 8.9 g (15.4%) of compound 508, and a third 11.5 g (20%) fraction of compound 508 was recovered from the methyl-tert-butyl ether cake wash. The above three solid samples of crude material were separately recrystallized from 190 proof ethanol (1 g/10 mL) to afford a total of 35.6 g (61.7%) of compound 508 having a purity level of 98.7% as determined by HPLC analysis (conditions as below). A second crop of compound 508 was isolated from the mother liquor to afford 9.3 g (16.1%) of solid having a purity level of 98.4% as determined by HPLC analysis. The remaining mother liquor was purified by silica gel chromatography using 200 g of  $\text{SiO}_2$  and eluting with 4 L of 50V % ethyl acetate and 50V % heptane to yield 5.6 g (9.7%) of compound 508, having a purity level of 94.0% as determined by HPLC analysis. HPLC conditions: 9.74 minute retention time on a YMC S50DS-AQ column (4.6x150 mm) using a gradient elution from 100% solvent A to 100% solvent B over 15 minutes at 1.0 mL/min. Solvent A=95 V % water (0.01 M  $\text{NH}_4\text{OAc}$ ); 5 V % acetonitrile. Solvent B=5 V % water (0.01 M  $\text{NH}_4\text{OAc}$ ); 95 V % acetonitrile. Dual wavelength detector set at 210 and 245 nm. MS (ES):  $m/z$  381.11  $[\text{M}+\text{H}]^+$ .

## EXAMPLE 509

(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (509)

[0457]



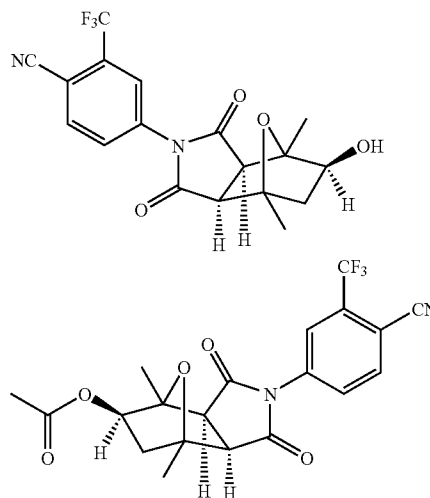
[0458] Compound 471A (0.37 g, 1.4 mmol) and 2,5-dimethylfuran (0.73 mL, 6.9 mmol) were combined to form a

slurry which was heated to  $60^{\circ}\text{C}$  for 1 h. The reaction mixture was cooled to  $-10^{\circ}\text{C}$  and THF (1.0 mL) was added followed by the addition of borane tetrahydrofuran (2.1 mL, 1 M). The reaction mixture was stirred for 30 minutes at  $0^{\circ}\text{C}$  and 30 minutes at  $+10^{\circ}\text{C}$ . To the reaction mixture was added acetone (3.0 mL), and the resulting mixture was warmed to  $20^{\circ}\text{C}$  and maintained at  $20^{\circ}\text{C}$  for 1 h. To this solution was added sodium bicarbonate (1.5 mL, pH 9, 8 wt %) and the mixture was then cooled to  $5^{\circ}\text{C}$  before adding hydrogen peroxide (0.3 mL, 30 wt %). Addition of hydrogen peroxide was exothermic bringing the temperature to  $20^{\circ}\text{C}$ . A solution of sodium sulfite (4.0 mL, 10 wt %) was added at  $20^{\circ}\text{C}$  resulting in an exotherm to  $30^{\circ}\text{C}$ . The biphasic mixture was allowed to stand at  $25^{\circ}\text{C}$  for 12 h. After the phases were separated, the aqueous waste was back extracted twice with ethyl acetate (5 mL) and the combined organic layers were washed with water (2 mL) followed by sodium chloride (2 mL, 25 wt %). The organic layers were concentrated in vacuo to yield a yellow oil which rapidly crystallized. To the crude product was added 190 proof ethanol (5.0 mL) and the mixture was heated to  $60^{\circ}\text{C}$  to afford complete dissolution. Cooling to  $20^{\circ}\text{C}$  for 17 h resulted in crystallization. The crystal slurry was collected by filtration, washed with heptane (5 mL), and dried at  $60^{\circ}\text{C}$  under vacuum (30 in/Hg) to afford 0.23 g (44%) of compound 509 having 93.1 HPLC Area %. HPLC conditions: 9.74 minute retention time on a YMC S50DS-AQ column (4.6x150 mm) using a gradient elution from 100% solvent A to 100% solvent B over 15 minutes at 1.0 mL/min. Solvent A=95 V % water (0.01 M  $\text{NH}_4\text{OAc}$ ); 5 V % acetonitrile. Solvent B=5 V % water (0.01 M  $\text{NH}_4\text{OAc}$ ); 95 V % acetonitrile. Detector set at 245 nm. MS (ES):  $m/z$  381.11  $[\text{M}+\text{H}]^+$ .

## EXAMPLE 510

[3A $\alpha$ -(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (510i) & [3A $\alpha$ -(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-[5-(Acetyloxy)octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (510ii)

[0459]



[0460] Compound 509 (4 mg), vinyl acetate (0.1 mL) and toluene (2 mL) were combined and 20 mg of each of the enzymes shown in Table 12 were added. The mixture was stirred with a magnetic stirring bar at rt in a 16x100 mm capped tube for the time period listed in Table 12. The enantioselective acetylation of the racemic mixture resulted in the formation of compound 510i and the acetylated compound 510ii. The enantiomeric purity of compound 510i was determined by chiral HPLC (method below) and the results for each enzyme are as shown in Table 12. The resulting information was used to prepare a large scale batch of compounds 510i & 510ii as described below.

TABLE 12

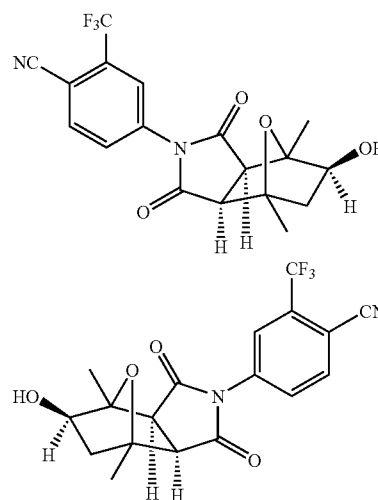
Enzyme	Supplier	Source	Time H	Comp.	Comp.	Comp.	Comp.
				510i mg/mL	510i % yield	510i % ee	510ii mg/mL
AK-20	Amano	<i>Pseudomonas fluorescens</i>	15	0.74	39	100	1.02
AP-12	Amano	<i>Aspergillus niger</i>	144	1.10	58	55.4	0.74
PS-30	Amano	<i>Pseudomonas cepacia</i>	15	0.46	24	100	1.24
Acylase 30000	Amano	<i>Aspergillus</i>	15	0.51	27	12.2	1.26
Chirazyme L3	Boehringer	<i>Candida rugosa</i>	144	0.81	42	87.2	1.04
Lipase type VII	Sigma	<i>Candida rugosa</i>	144	0.74	39	100	1.06

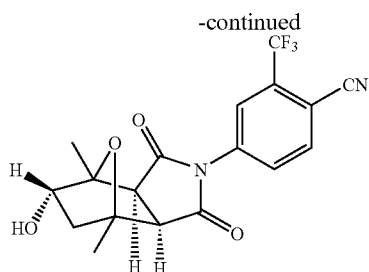
[0461] To a 500 mL jacketed flask were added Amano lipase AK20 from *Pseudomonas fluorescens* (25 g), compound 509 (25 g), methyl-isobutyl-ketone (475 mL) and vinyl acetate (25 mL). The flask was maintained at 25° C. with a circulating water bath and stirred with a magnetic stir bar. The incubation was continued for 42 h, at which point the enantiomeric excess of compound 510i reached 100%. The solution was filtered through Whatman 4 filter paper to remove enzyme and the filter cake was washed with 50 mL methyl isobutyl ketone. The filtrate was concentrated in vacuo and the resulting residue was dissolved in EtOAc (50 mL) followed by the addition of heptane (50 mL). This solution was loaded onto a Phenomenex cartridge column (silica 800 g) in a Biotage 75L system and the column was eluted with 75% EtOAc/heptane at flow rate 110 mL/min. Fractions were collected (500 mL) which contained compound 510ii and then the eluting solvent was changed to 100% EtOAc to elute off compound 510i. The desired fractions were pooled and the solvent was removed in vacuo to yield 11.0 g of compound 510i, (44%, 100% ee) and 12.10 g of compound 510ii (44%). Compound 510i was re-crystallized from 95% EtOH (5 mL/g) in two crops to afford 9.61 g (38%) of compound 510i as a white crystalline solid. Compounds 510i: Chiral HPLC: 10.02 min (retention time) (CHIRALPAK AD 4.6x250 mm column; isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). HPLC: 99% at 2.45 min (retention time) (YMC S5 ODS column, 4.6x50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 [M+H]<sup>+</sup>.

## EXAMPLE 513

[3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (513i) & [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (513ii) & (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (513iii)

[0462]





**[0463]** A series of microbial biotransformation reactions were set up to generate compounds 513i, 513ii & 513iii. The details of the reactions for several microorganisms are shown in Table 15 and a general procedure is described below. One thawed vial of the microbe (1 mL culture) was inoculated into sterile soybean-glucose media (10 mL) in a 50 mL flask. The microbes were grown by shaking at 200 rpm at 28° C. for 40 h. A solution of compound 472 (10 mg in 100  $\mu$ L DMSO) was added to each flask and the flasks were shaken at 200 rpm at 28° C. At 24 and 48 h, 5 mL of the reaction mixtures were extracted by EtOAc (10 mL). A portion of EtOAc extract (1 mL) was evaporated, redissolved in acetonitrile (1 mL) and analyzed by reversed phase HPLC (C-18, acetonitrile:water 20:80 to 90:10 in 12 min) to determine the area ratio of compound 472 (RT=11.2 min) and the product compounds 513i (RT=8.9 min), 513ii (RT=8.9 min) & 513iii (RT=9.6 min). A second portion of the EtOAc extract (4 mL) was evaporated, redissolved in isopropyl alcohol-heptane (1:1, 1 mL) and analyzed by chiral HPLC (Chiralpak AD, Heptane: Ethanol 85:15, 0.5 mL/min) to determine the % ee of compounds 513i (RT=32.2 min) compound 513ii (RT=34.8 min) in this system.

## EXAMPLE 514

[3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (514i) & [3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\beta$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (514ii) & (3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (514iii)

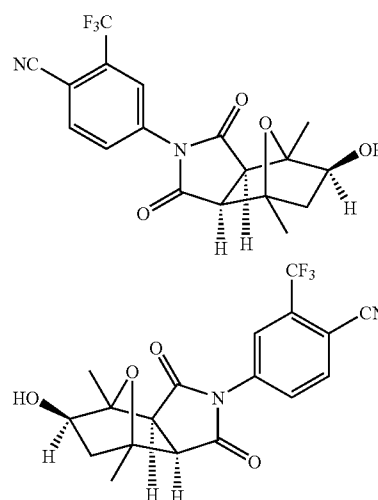
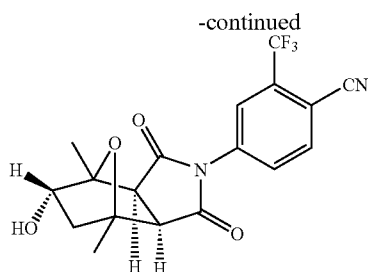
**[0464]**

TABLE 15

Microorganism	ID	Time Hrs	Analysis by Reversed Phase HPLC (Area Ratio)			Analysis for % ee by HPLC		% ee of 513i
			Comp 513i	Comp 513ii	Comp 472	Comp 513i	Comp 513ii	
<i>Streptomyces</i> sp	SC1754	24	7%	0%	93%	96.9%	3.1%	93.9%
		48	12%	0%	88%	96.7%	3.3%	93.3%
<i>Streptomyces</i> sp	SC3740	24	1%	0%	99%	88.0%	12.0%	76.0%
		48	2%	0%	98%	85.8%	14.2%	71.7%
<i>Nocardia</i>	ATCC	24	5%	0%	95%	93.2%	6.8%	86.3%
<i>interforma</i>	21072	48	8%	0%	92%	92.6%	7.4%	85.1%
<i>Streptomyces</i>	ATCC	24	11%	1%	88%	95.7%	4.3%	91.4%
<i>antibioticus</i>	14890	48	48%	13%	39%	94.1%	5.9%	88.2%
<i>Streptomyces</i>	ATCC	24	1%	0%	99%	82.0%	18.0%	64.1%
<i>mediocidicus</i>	13278	48	7%	0%	93%	79.4%	20.6%	58.8%
<i>Streptomyces</i>	NRRL	24	23%	0%	77%	85.0%	15.0%	70.0%
<i>griseus</i>	B8090	48	28%	0%	72%	85.9%	14.1%	71.8%
<i>Amycolatopsis</i>	ATCC	24	12%	1%	86%	81.3%	18.7%	62.5%
<i>orientalis</i>	43490	48	25%	4%	71%	77.4%	22.6%	54.9%



**[0465]** A series of microbial biotransformation reactions were set up to generate compounds 514i, 514ii & 514iii. The details of the reactions for several microorganisms are shown in Table 16 and a general procedure is described below. A 1 mL culture of *Streptomyces griseus* SC13971 from a frozen vial was used to inoculate 100 mL of medium (0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K<sub>2</sub>HPO<sub>4</sub>, 0.5% NaCl, adjusted to pH 7 with 1N HCl (R. V. Smith and J. P. Rosazza, Arch. Biochem. Biophys., 161, 551-558 (1974)) in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C. at 200 rpm for 3 days. 10 mL of this culture was used to inoculate 100 mL of medium (as above) in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C. at 200 rpm for 1 day. For the filamentous fungi *Mucor rouxii* and *Cunninghamella echinulata*, 1 mL of spore suspension, prepared by washing a slant with 10 mL water, was used to inoculate 100 mL of medium (as above) in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C. at 200 rpm for 1 day. Compound 472 (30 mg in 1 mL methanol) was added to each culture and the incubations were continued for 6 to 10 days. Samples of 10 mL of the culture broth in each flask were removed and extracted with ethyl acetate (20 mL). Samples of 10 mL of the organic layers were each individually evaporated to dryness at 40° C. under a nitrogen stream. The residues were dissolved in 1.2 mL isopropanol and analyzed by reversed phase HPLC (YMC Pak ODS 150×6 mm, 3μ C-18, acetonitrile: water 20:80 to 90:10 in 12 min, 1 mL/min, 40° C.) to determine the concentration of compound 472 (RT=11.2 min) and product compounds 514i (RT=8.9 min), 514ii (RT=8.9 min) & 514iii (RT=9.6 min). The same samples were analyzed by chiral HPLC (Chiralpak AD, heptane:ethanol 85:15, 0.5 mL/min) to determine the % ee of compounds 514i (RT=32.2 min) compound 514ii (RT=34.8 min) in this system.

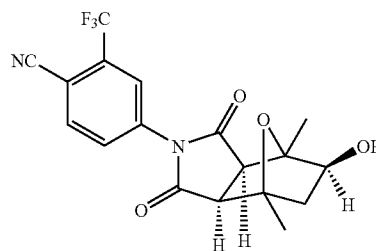
TABLE 16

strain	SC	ATCC	time days	Comp.	Comp.	Comp	Comp
				472 mg/ml	514i mg/ml	514i ee %	514iii mg/ml
1. <i>Mucor rouxii</i>	13920	24905	3	0.30	0.01	100.00	0.000
			6	0.27	0.01	100.00	0.000
2. <i>Streptomyces griseus</i>	13971	13273	3	0.29	0.01	100.00	0.000
			6	0.30	0.01	100.00	0.000
3. <i>Cunninghamella echinulata</i>	16027	9244	3	0.34	0.02	100.00	0.002
			6	0.06	0.02	100.00	0.001
			10	0.16	0.02	100.00	0.001

## EXAMPLE 515

[3aR-(3α,4β,5β,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (515)

[0466]



**[0467]** Microbial transformation of compound 472 to compound 515 was conducted on a 3 L scale in a 5 L fermentor, using *Cunninghamella echinulata* SC 16027 (ATCC 9244) and a medium consisting of the following: 0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K<sub>2</sub>HPO<sub>4</sub>, 0.5% NaCl, adjusted to pH 7 with 1N HCl (R. V. Smith and J. P. Rosazza, Arch. Biochem. Biophys., 161, 551-558 (1974)). The fermentor was batched with 0.05% SAG antifoam before sterilization. Spore inoculum was prepared by washing the spores from a 10 day slant culture of *Cunninghamella echinulata* SC 16027 (ATCC 9244) with 0.9% saline/0.1% Tween 80. The inoculum stage was prepared by adding 1 mL of spore inoculum into 100 mL medium in a 500 mL flask, then the cultures were grown at 28° C. at 200 rpm for 1 day. 10% inoculum from the flask was blended in a sterile Waring blender and used to inoculate the sterile fermentor, containing 3 L of sterile media. The fermentor was run at 28° C. at 600 rpm and 1 vvm aeration. A sterile solution of three antibiotics was added to the fermentor after inoculation; 12 mg of tetracycline chloride, 12 mg of kanamycin sulfate, and 60 mg of cephalixin hydrate in 10 mL of deionized water. After 22 hours of growth in the fermentor, a sterile substrate solution was added containing 0.75 g of compound 472 dissolved in 30 mL of methanol, this step was repeated two hours later for a total of 0.5 g/L of compound 472 in the bioconversion. The fermentation conditions were maintained at 28° C. at 600 rpm and 1 vvm aeration. pH 6.5 was maintained by the automatic addition of 10% H<sub>2</sub>SO<sub>4</sub> or 10% NaOH. Periodically, 10 mL

aseptic samples were taken and extracted with two 10 mL portions of ethyl acetate. The ethyl acetate layer was isolated, dried under a nitrogen stream at 40° C., and the residue was dissolved in 2.0 mL of isopropyl alcohol. The samples were analyzed by reverse phase HPLC (method below) to determine the ratio of compound 472 and the product compound 515. In addition, each sample was analyzed by chiral HPLC (method below) in order to determine the % ee of compound 515. During the bioconversion process, a sterile solution of 30% cerelese and 1.5% yeast extract was fed into the reaction at -5 mL/hour. After 114 h from the time of substrate addition, reverse phase HPLC analysis indicated the production of a 78% yield of compound 515. Chiral HPLC analysis measured the % e.e. of compound 515 at 94.9%. The above process was repeated in another 3 L bioconversion, and the reaction was conducted at 28° C. at 600 rpm, 1 vvm aeration, with 0.5 g/L of compound 472 input. After 44 h, this reaction gave a 80% yield of compound 515 with 95% e.e. The broth was flittered through a pad of HyFlo™ to provide a clarified fermentation broth. The broth was flittered through a pad of HyFlo™ to provide a clarified fermentation broth. Compound 515 was completely adsorbed onto 55 g of XAD-16 and extracted back into a 1:1 mixture of EtOAc and acetone (3x100 mL) or methyl-tert-butyl ether (3x100 mL). The solvent was removed in vacuo and the resulting residue was purified by silica pad (5 g), eluting with EtOAc. The desired fractions were collected and treated with activated carbon (0.5 g) to decolorize the solution and the solvent was removed in vacuo to yield 1.27 g of compound 515. Re-crystallization of this material from EtOAc/heptane (10 mL/20 mL) resulted 950 mg of crystalline compound 515 having 97% purity by reverse phase HPLC and 95% ee by chiral HPLC. Reverse Phase HPLC: YMC Pak ODS-A C18 column, 4.6x50 mm, eluting with a gradient of: 0 min 20% acetonitrile/80% 0.1% TFA in water, 12 min 90% acetonitrile/10% 0.1% TFA in water, 12.01-15 min 20% acetonitrile/80% 0.1% TFA in water, monitoring at 250 nm, 40° C., 5 µL injection volume). Compound 515: RT=8.86 min. Chiral HPLC: CHIRALPAK OD 25x0.46 cm column; isocratic elution with 15% ethanol/85% heptane at 0.5 mL/min, 18° C., monitoring at 220 nm, injection volume: 20 µL. Compound 515: RT=36.5 min.

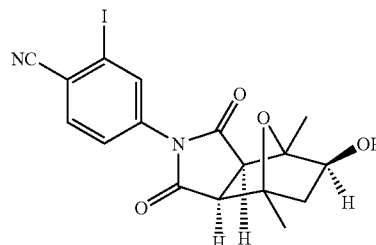
[0468] In an alternate recovery process, the fermentation broth (1L) from the above biohydroxylation reaction was filtered and the cake of cells was washed with 100 mL of water. Clear broth was extracted with ethyl acetate (2x600 mL) and the cake of cells was extracted with 400 mL of ethyl acetate. The combined ethyl acetate layers were concentrated and the resulting residue was dissolved in 5 mL of 1:1 heptane/ethyl acetate and loaded on to silica gel pad (70 g in 250 mL fritted glass filter). The silica gel pad was eluted with a gradient of 80 to 90% EtOAc/heptane. Fractions were collected and the fractions containing compound 515 were pooled. The solvent was removed in vacuo and resulting product was crystallized from EtOAc/heptane to give a 90% yield of compound 515 with 98% purity by reverse phase HPLC and 95% ee by chiral HPLC. Reverse Phase HPLC: YMC Pak ODS-A C18 column, 4.6x50 mm, eluting with a gradient of: 0 min 20% acetonitrile/80% 0.1% TFA in water, 12 min 90% acetonitrile/10% 0.1% TFA in water, 12.01-15 min 20% acetonitrile/80% 0.1% TFA in water, monitoring at 250 nm, 40° C., 5 µL injection volume). Compound 472: RT=11.12 min. Compound 515: RT=8.86 min. Chiral HPLC: CHIRALPAK OD 25x0.46 cm column; isocratic elution with 15% ethanol/85% heptane at 0.5 mL/min, 18° C., monitoring

at 220 nm, injection volume: 20 µL. Compound 472: RT=27.4 min. Compound 515: RT=36.5 min. Compound (514iii) RT=39.1 min.

## EXAMPLE 753

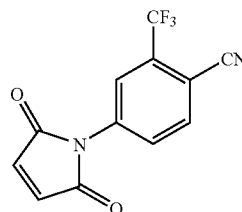
[3aR-(3α,4β,5β,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (753G)

[0469]



A. 4-(2,5Dioxo-2,5-dihydro-pyrrol-1-yl)-2-trifluoromethyl-benzonitrile (453A)

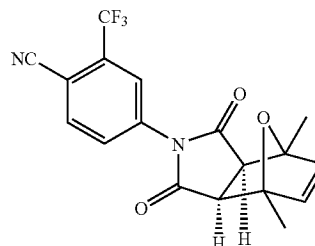
[0470]



[0471] A mixture of 3-trifluoromethyl-4-cyano-aniline (24.0 g, 129 mmol) and maleic anhydride (14.0 g, 143 mmol) in 50 mL of acetic acid was heated at 115° C. overnight. A precipitate was obtained during the heating period. The reaction was allowed to stand at rt for an additional overnight period. The solid was removed by filtration, the filter cake was washed with diethyl ether and dried to give 21 g (79 mmol, 61%) of compound 753A as an off white solid. HPLC: 100% at 2.11 min (retention time) (YMC S5 ODS column, 4.6x50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3α,4β,7β,7α)-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (753B)

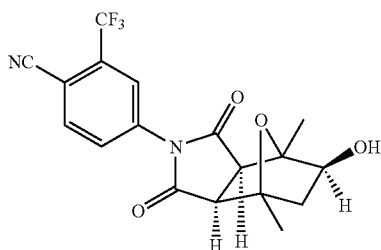
[0472]



[0473] A slurry of 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethyl-benzonitrile (15.0 g, 56.35 mmol) and 2,5-dimethylfuran (32.5 g, 338 mmol) was stirred at 60° C. for 3 h, followed by continued stirring for another 14 h at 23° C. The mixture was diluted with cold (−5° C.) toluene (15 mL) and the resulting off white solid was collected by filtration while cold. The filter cake was washed with cold toluene (20 mL) and dried at 23° C. in vacuum oven for 24 h to provide compound 753B (18.6 g, 91% yield) with a purity of 98.7% as judged by HPLC.

C. [3 aR-(3α,4β,5β,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isindol-2-yl)-2-(trifluoromethyl)benzonitrile (753C)

[0474]

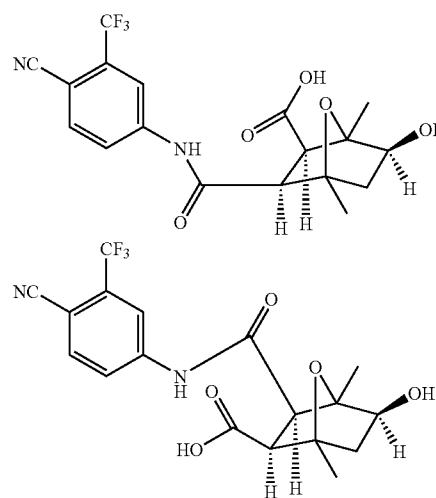


[0475] A slurry of compound 753B (5.0 g, 13.81 mmol), allylpalladium chloride dimer (10 mg, 0.027 mmol) and (R)-2-methoxy-2'-diphenylphosphino-1,1'-binaphthyl (R-MOP, 26 mg, 0.055 mmol) in THF (14 mL) was purged with nitrogen and cooled to 10° C. To the above mixture, was added trichlorosilane (3.7 g, 27.62 mmol) slowly over a period of 10 min and the resulting heterogeneous mixture was stirred for 24 h at 10° C. The mixture was diluted with THF (85 mL) and further cooled to −20° C. To the above mixture, a solution of triethylamine (9.76 g, 96.67 mmol) in ethanol (6.35 g, 138.1 mmol) was added slowly keeping the temperature <25° C. and stirred for another 2 h at rt. The white solid was filtered and the filter cake was washed with THF (50 mL) and the filtrate was evaporated under the reduced pressure. The oily residue was dissolved in ethyl acetate (100 mL) and stirred at 23° C. in the presence of trithiocyanuric acid (TMT, 500 mg) and charcoal (500 mg) for 5 h. The slurry was filtered through a pad of celite and silica gel and the filtrate was evaporated under the reduced pressure. The residue was dissolved in a mixture of THF (175 mL) and methanol (125 mL), and to this mixture was added anhydrous potassium fluoride (2.0 g, 34.5 mmol), potassium hydrogen carbonate (6.9 g, 69 mmol), followed by urea-hydrogen peroxide adduct (6.5 g, 69 mmol). This suspension was stirred at rt for 14 h and an additional amount of potassium fluoride (800 mg, 13.81 mmol), potassium hydrogen carbonate (1.38 g, 13.81 mmol) and urea-hydrogen peroxide adduct (2.59 g, 27.62 mmol) was added. Stirring continued for another 12 h and the slurry was filtered and the filter cake was washed with ethyl acetate (100 mL). The filtrate was washed consecutively with 10% aqueous sodium hydrogen sulfite (50 mL×2), water (50 mL) and brine (50 mL). The solvent was evaporated under the reduced pressure to provide a light brown foam. Column chromatography of this foam using silica gel and EtOAc-heptane (2:3) afforded compound 753C (4.72 g, 90%, >99% pure by HPLC) as an off white solid. This material has an enantio-

meric purity of >96% ee by chiral HPLC and LC-MS: m/z 379 (M+H<sup>+</sup>). The absolute stereochemistry of compound 753C was established by comparison to existing material of known stereochemistry as described in Example 483.

D. [1S-(1β,2α,3α,4β,6β)]-3-[[4-Cyano-3-(trifluoromethyl)phenyl]amino]carbonyl]-6-hydroxy-1,4-dimethyl-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid & [1R-(1β,2α,3α,4β,5β)]-3-[[4-Cyano-3-(trifluoromethyl)phenyl]amino]carbonyl]-5-hydroxy-1,4-dimethyl-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (753Di & 753Dii)

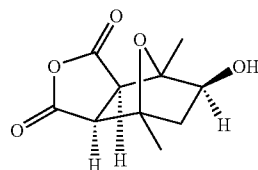
[0476]



[0477] Compound 753C (25.0 g, 65.7 mmol) was dissolved in THF (100 mL) at 22° C. and 1 N NaOH (100 mL) was added. After 1 h, THF (100 mL), 1 N HCl (110 mL) and brine (100 mL) were added. The mixture was then extracted once with EtOAc (200 mL) and twice with 1:1 THF/EtOAc (200 mL). The combined organics were dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated in vacuo to give compounds 753Di and 753Dii (1:1 by HPLC) as a white solid. No purification was necessary. HPLC: 100% at 2.217 and 2.413 min (retention time) (YMC S50DS-A column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

E. [3 aR-(3α,4β,7β,7α)]-Hexahydro-5-hydroxy-4,7-dimethyl-4,7-epoxyisobenzofuran-1,3-dione (753E)

[0478]

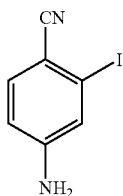


[0479] Compounds 753Di and 753Dii were suspended in a mixture of THF (500 mL) and AcOH (200 mL) and heated to

60° C. for 16 h. The reaction became homogenous after 4 h. The reaction was cooled to 22° C. and concentrated in vacuo. Toluene (200 mL) was then added and the mixture was heated to 90° C. for 4 h until all product was dissolved. The mixture was then cooled to 22° C. and left standing for 20 h. Compound 753E precipitates from solution over the 20 h period. The resulting solid was filtered and rinsed with toluene followed by drying in vacuo. A yield of 11.93 g of crude compound 753E was obtained as an off-white solid. Crude compound 753E (10 g) was dissolved in 350 mL of warm EtOAc (the solution was turbid), and then 5 g of decolorizing carbon was added and the mixture was stirred at rt for 40 mins. After filtering through celite, the filter cake was washed with hot EtOAc (2×50 mL). Concentration of the filtrate afforded an off-white solid that was dissolved in 40 mL of hot acetonitrile. After carefully adding 200 mL of ethyl ether, 300 mL of hexane was added and the flask was allowed to stand 18 hrs at rt. Filtration and drying afforded 8.32 g of compound 753E as a colorless crystalline solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ=5.11 (d, 1H, J=6.0 Hz), 3.78 (dd, 1H, J=7.2, 30.6 Hz), 3.27 (d, 1H, J=7.2 Hz), 3.20 (d, 1H, J=7.3 Hz), 2.27 (dd, 1H, J=7.3, 13.1 Hz), 1.44 (s, 3H), 1.33 (s, 3H) and 1.32 ppm (m, 1H).

#### F. 4-Amino-2-iodo-benzonitrile (753F)

##### [0480]



[0481] 2-Iodo-4-nitro-benzonitrile (1.00 g, 3.65 mmol, was prepared by the method given in *J. Med. Chem.* 2000, 43, 3344-47) was dissolved in THF (20 mL) at 60° C. with mechanical stirring. EtOH (25 mL) was then added followed by aq NH<sub>4</sub>Cl (0.293 g, 5.48 mmol in 20 mL H<sub>2</sub>O). Iron powder (325 mesh, 0.815 g, 14.6 mmol) was then added with vigorous stirring. After 2 h, reaction was complete. Cooled to 22° C. and filtered through celite rinsing with EtOAc. The mixture was then concentrated to ~20 mL and then diluted with EtOAc (200 mL) and washed once with 1 N NaOH (50 mL), once with brine (50 mL) and dried over anhydrous MgSO<sub>4</sub>, to give, compound 753F (0.710 g) as a light yellow solid. No additional purification was needed. HPLC: 99% at 2.147 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

G. [3 aR-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (753G)

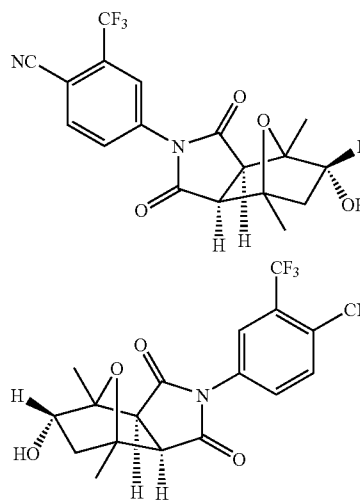
[0482] A mixture of compound 753E (1.60 g, 7.57 mmol), compound 753F (1.32 g, 5.41 mmol), 4 Å molecular sieves (2.0 g) in dimethylacetamide (8 mL) was stirred in a 175° C. oil bath for 4 h. The resulting dark mixture was cooled, diluted with EtOAc (50 mL) then filtered to remove sieves and rinsed with EtOAc. The filtrate was partitioned between EtOAc (50 mL) and water (75 mL). The organic phase was separated, washed with water (2×75 mL), brine (50 mL), then diluted with tetrahydrofuran (20 mL), dried over sodium sulfate and concentrated in vacuo to give a solid foam. The crude

material was triturated with diethyl ether (100 mL) then stirred for 1.5 h. The solid precipitate was collected on a Buchner funnel then suspended in diethyl ether (50 mL) and stirred for 16 h. The resulting solid was collected on a Buchner funnel, rinsed with diethyl ether and dried under vacuum (90° C.) to afford compound 753G (67%) as an off-white solid. HPLC: 96% at 3.00 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 438.90 [M+H]<sup>+</sup>. The absolute stereochemistry of compound 753G is established by the known stereochemistry of the intermediate compound 753C and the retention of configuration therein. The absolute stereochemistry is as drawn in the above figure and rendered by the nomenclature.

#### EXAMPLE 774

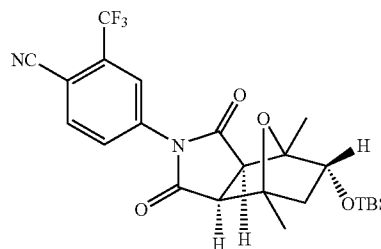
[3aR-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & [3aS-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (774Bi and 774Bii)

##### [0483]

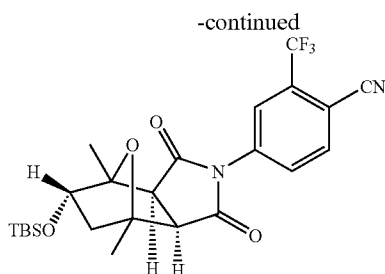


[3aR-(3α,4β,5α,7β,7α)]-4-[5-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile & [3 aS-(3α,4β,5α,7β,7α)]-4-[5-[[[(1,1Dimethylethyl)dimethylsilyl]oxy]octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (774Ai & 774Aii)

##### [0484]







**[0485]** Racemic compound 222C was separated into its enantiomers by normal phase preparative chiral HPLC (CHIRALPAK AD 5×50 cm column; eluting with 7% EtOH in hexanes (isocratic) at 50 mL/min) to give the faster eluting compound 774Ai (18 mins) and the slower eluting compound 774Aii.

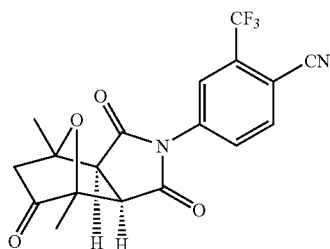
[3aR-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isindol-2-yl)-2-(trifluoromethyl)benzonitrile & [3aS-(3α,4β,5α,7β,7α)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isindol-2-yl)-2-(trifluoromethyl)benzonitrile (774Bi & 774Bii)

**[0486]** 774Ai and 774Aii (0.12 g, 0.25 mmol) were each dissolved in 2 mL of THF and 2 mL of 10% HCl/MeOH was added and the reactions stirred overnight at rt. Saturated sodium bicarbonate and EtOAc were added and the organic layer was separated, washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo to give compound 774Bi (0.084 mg, 88%) and compound 774Bii (0.076 g, 82%) as white solids. Compound 774Bi: HPLC: 100% at 2.89 min (YMC S5 ODS column) eluting with 10-90% aqueous methanol containing 0.2% phosphoric acid over a 4 min gradient monitoring at 220 nm. MS (ES): m/z 381.19 [M+H]<sup>+</sup>. Compound 774Bii: HPLC: 100% at 2.89 min (YMC S5 ODS column) eluting with 10-90% aqueous methanol containing 0.2% phosphoric acid over a 4 min gradient monitoring at 220 nm. MS (ES): m/z 381.16 [M+H]<sup>+</sup>. The absolute stereochemistry of compounds 774Bi & 774Bii has not been established. Although each compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

#### EXAMPLE 796

[3aR-(3α,4β,7β,7α)]-4-(Octahydro-4,7-dimethyl-1,3,5-trioxo-4,7-epoxy-2H-isindol-2-yl)-2-(trifluoromethyl)benzonitrile (796)

**[0487]**

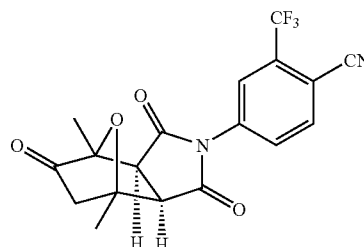


**[0488]** Compound 471Di (0.10 g, 0.263 mmol) was dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) and THF (2.0 mL) at 22° C. Dess-Martin periodinane (0.279 g, 0.658 mmol) was added with stirring. After 3 h, the reaction was quenched with a 1:1 mixture of sat aq NaHCO<sub>3</sub> and sat aq NaHSO<sub>3</sub> (5 mL). After stirring for 15 min, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×10 mL) and the combined organics were dried over Na<sub>2</sub>SO<sub>4</sub>. The crude material was purified by silica gel flash chromatography eluting with 5-10-20% acetone in chloroform to give compound 796 (0.090 g) as a white solid. HPLC: 100% at 3.170 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm), MS (ES): m/z 379.11 [M+H]<sup>+</sup>. Chiral analytical HPLC using a Chiracel OD column, 4.6×250 mm, eluting with 20% (1:1) EtOH/MeOH in hexanes at 2.0 mL/min and monitoring at 220 nm gave a retention time of 9.097 mins. The absolute stereochemistry of compound 796 is established by the known stereochemistry of the intermediate compound 741Di and the retention of configuration therein. The absolute stereochemistry is as drawn in the above figure and rendered by the nomenclature.

#### EXAMPLE 797

[3aS-(3α,4β,7β,7α)]-4-(Octahydro-4,7-dimethyl-1,3,5-trioxo-4,7-epoxy-2H-isindol-2-yl)-2-(trifluoromethyl)benzonitrile (797)

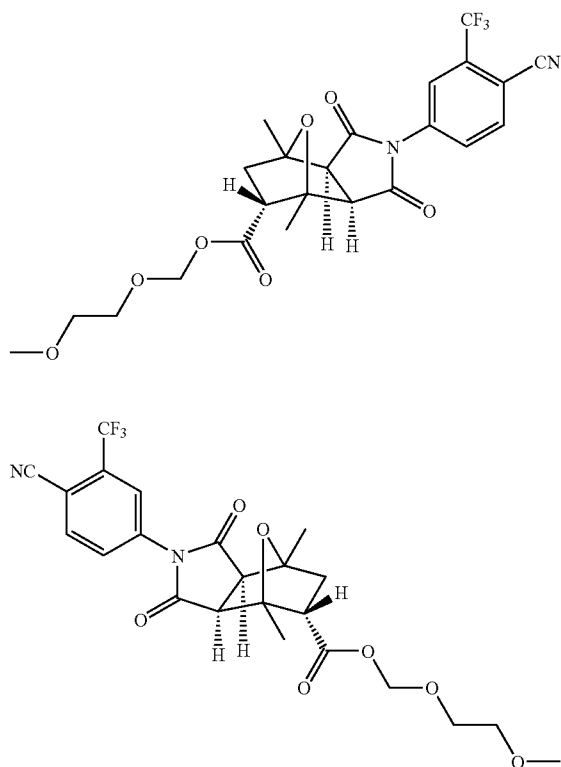
**[0489]**



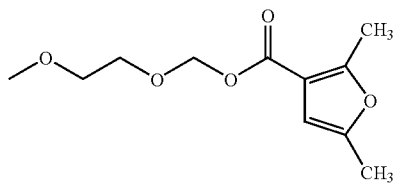
**[0490]** Compound 471Dii (0.10 g, 0.263 mmol) was dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) and THF (2.0 mL) at 22° C. Dess-Martin periodinane (0.279 g, 0.658 mmol) was added with stirring. After 3 h, the reaction was quenched with a 1:1 mixture of sat aq NaHCO<sub>3</sub> and sat aq NaHSO<sub>3</sub> (5 mL). After stirring for 15 min, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×10 mL) and the combined organics were dried over Na<sub>2</sub>SO<sub>4</sub>. The crude material was purified by silica gel flash chromatography on SiO<sub>2</sub> eluting with 5-10-20% acetone in chloroform to give compound 797 (0.094 g) as a white solid. HPLC: 100% at 3.170 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm), MS (ES): m/z 379.11 [M+H]<sup>+</sup>. Chiral analytical HPLC using a Chiracel OD column, 4.6×250 mm, eluting with 20% (1:1) EtOH/MeOH in hexanes at 2.0 mL/min and monitoring at 220 nm gave a retention time of 5.710 mins. The absolute stereochemistry of compound 797 is established by the known stereochemistry of the intermediate compound 471Dii and the retention of configuration therein. The absolute stereochemistry is as drawn in the above figure and rendered by the nomenclature.

## EXAMPLE 803

[0491]

A. 2,5Dimethylfuran-3-carboxylic Acid  
2-Methoxyethoxymethyl Ester (803A)

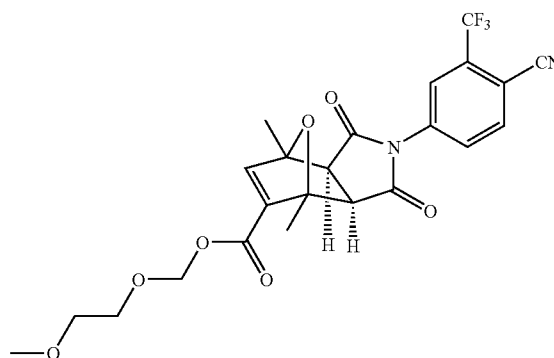
[0492]



[0493] A 2 L round-bottomed flask equipped with a mechanical stirrer was charged with 2,5-dimethyl-3-furoic acid (81.0 g, 0.58 mol) and potassium carbonate (95.9 g, 0.69 mol) in DMF (500 mL). An ice bath was used to cool the reaction as 2-methoxyethoxymethyl chloride (72.2 g, 0.58 mol) was added portionwise. The reaction was stirred for 5 h at rt. The mixture was then diluted with water (1.5 L) and extracted with EtOAc (2×600 mL). The combined organics were washed with water (2×900 mL) and saturated sodium chloride solution (1 L), dried over  $\text{MgSO}_4$ , filtered and concentrated to afford compound 803A (103 g, 82%) as a yellow oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ =6.23 (s, 1H), 5.47 (s, 1H), 3.84 (m, 2H), 3.56 (m, 2H), 3.38 (s, 3H), 2.51 (s, 3H) and 2.20 ppm (s, 3H).

## B. (803B)

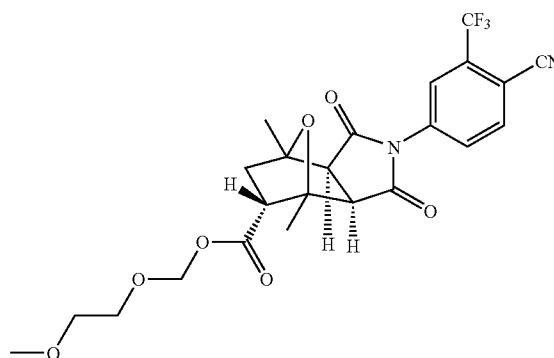
[0494]



[0495] A 250 mL round-bottomed flask was charged with 4-(2,5-dioxo-2,5-dihydropyrrol-1-yl)-2-trifluoromethylbenzonitrile (15.0 g, 0.056 mol) and 2,5-dimethylfuran-3-carboxylic acid 2-methoxyethoxymethyl ester (22.8 g, 0.10 mol). The mixture was heated to 125° C. for 1.5 h then stirred at rt overnight. The crude product was dissolved in EtOAc and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with 30% EtOAc in hexanes, afforded compound 803B (8.21 g, 30%) as a viscous oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ =7.95 (d,  $J$ =8.3 Hz, 1H), 7.86 (d,  $J$ =1.9 Hz, 1H), 7.75 (m, 1H), 7.13 (s, 1H), 5.44 (m, 2H), 3.83 (m, 2H), 3.57 (m, 2H), 3.39 (s, 3H), 3.19 (d,  $J$ =6.5 Hz, 1H), 3.09 (d,  $J$ =6.5 Hz, 1H), 2.04 (s, 3H) and 1.91 ppm (s, 3H).

## C. (803C)

[0496]



[0497] A 250 mL Parr hydrogenation bottle was charged with compound 803B (7.75 g, 0.015 mol), EtOAc (80 mL) and palladium on carbon (0.40 g, 10% Pd, 50% wet). The bottle was placed on a Parr hydrogenation apparatus, pressurized with hydrogen to 5 psi, and shook until the uptake of hydrogen ceased. Filtration of the contents over celite and concentration afforded a yellow oil. Purification by silica gel chromatography, eluting with 30% EtOAc in hexanes, afforded compound 803C (3.92 g, 50%) as a white solid. HPLC: 100% at 14.5 min (retention time) (Hypersil C18 BDS column, 250×4.6 mm, 5  $\mu\text{m}$ , a detection wavelength of 254 nm, and a flow rate of 1 mL/min. A linear gradient of 90% of

0.1% trifluoroacetic acid in water, 10% acetonitrile (start) to 100% acetonitrile over 15 min, then 100% acetonitrile for 5 min was used).  $R_f=0.61$  ( $\text{SiO}_2$ , 60% EtOAc in hexanes).  $\text{Mpt}>300^\circ\text{C}$ .  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta=7.94$  (d,  $J=8.3$  Hz, 1H), 7.84 (d,  $J=1.9$  Hz, 1H), 7.74 (m, 1H), 5.46 (d,  $J=6.1$  Hz, 1H), 5.34 (d,  $J=6.1$  Hz, 2H), 3.85 (m, 2H), 3.57 (t,  $J=4.5$  Hz, 2H), 3.38 (s, 3H), 3.33 (d,  $J=7.2$  Hz, 1H), 3.19 (d,  $J=7.2$  Hz, 1H), 3.04 (m, 1H), 2.28 (m, 1H), 2.05 (t,  $J=12.2$  Hz, 1H), 1.78 (s, 3H) and 1.64 ppm (s, 3H).  $m/z=496$   $[\text{M}+\text{H}]^+$ .

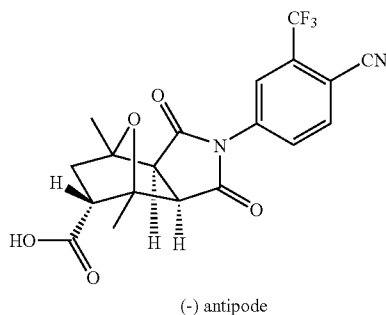
#### D. (803Di and 803Dii)

**[0498]** Racemic compound 803C was separated into its two enantiomers by preparative chiral HPLC using a Chiracel OD column (50x500 mm) eluting with 50% EtOH in Heptane at 100 mL/min and 290 nm detection. Compound 803Di had a retention time of 6.68 min and  $[\alpha]_{25}^D=+28.7^\circ$  ( $c=1.0$ , MeOH). Compound 803Dii had a retention time of 13.9 min and  $[\alpha]_{25}^D=-29.2^\circ$  ( $c=1.0$ , MeOH). The absolute stereochemistry of compounds 803Di & 803Dii has not been established. Although each compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

#### EXAMPLE 804

[3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid (804)

**[0499]**

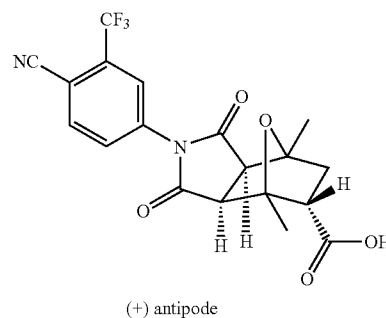


**[0500]** A 250 mL round-bottomed flask was charged with compound 803Di (9.85 g, 19.8 mmol) and THF (60 mL). A solution of 3N hydrochloric acid (50 mL) was added and the reaction mixture stirred for 16 h at rt. Water (60 mL) was then added and the aqueous layer extracted with EtOAc (3x120 mL). The combined organics were dried over sodium sulfate, filtered and concentrated to afford compound 804 (8.00 g, 98%) which was shown to be identical to compound 791. HPLC: 100% at 13.3 min (retention time) (Hypersil C18 BDS column, 250x4.6 mm, 5  $\mu\text{m}$ , a detection wavelength of 254 nm, and a flow rate of 1 mL/min. A linear gradient of 90% of 0.1% trifluoroacetic acid in water, 10% acetonitrile (start) to 100% acetonitrile over 15 min, then 100% acetonitrile for 5 min was used), MS (ES):  $m/z$  409  $[\text{M}+\text{H}]^+$ .  $R_f=0.41$  ( $\text{SiO}_2$ , 10% MeOH in  $\text{CH}_2\text{Cl}_2$ ).  $\text{Mpt}>300^\circ\text{C}$ .  $[\alpha]_{25}^D=-28.5^\circ$  ( $c=1.0$ , MeOH).  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta=8.12$  (d,  $J=8.3$  Hz, 1H), 7.92 (s, 1H), 7.82 (dd,  $J=8.3$  and 2.0 Hz, 1H), 3.39 (t,  $J=6.7$  Hz, 1H), 3.30 (d,  $J=6.7$  Hz, 1H), 3.00 (dd,  $J=12.0$  and 5.2 Hz, 1H), 2.23 (dd,  $J=12.0$  and 5.2 Hz, 1H), 2.01 (t,  $J=12.0$  Hz, 1H), 1.70 (s, 3H) and 1.57 ppm (s, 3H). The absolute stereochemistry of compound 804 has not been established. Although the compound represent a single antipode the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

#### EXAMPLE 805

[3S-(3a $\beta$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-2-(4-Cyano-3-(trifluoromethyl)phenyl)hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindole-5-carboxylic acid (805)

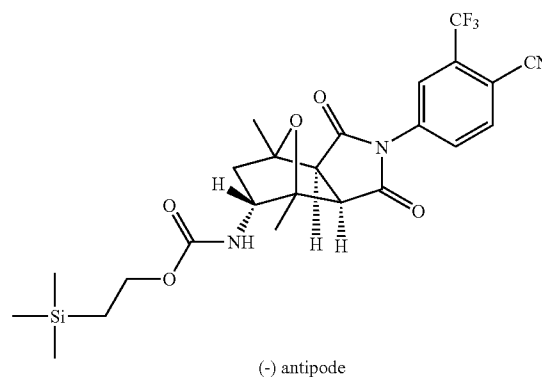
**[0501]**



**[0502]** Compound 805 was prepared in identical fashion as described in example 804 with the exception that compound 803Dii was the starting material in place of compound 803Di. Compound 805 was obtained in 98% yield.  $[\alpha]_{25}^D=+28.00$  ( $c=1.0$ , MeOH). The absolute stereochemistry of compound 805 has not been established. Although the compound represent a single antipode the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

#### EXAMPLE 806

**[0503]**

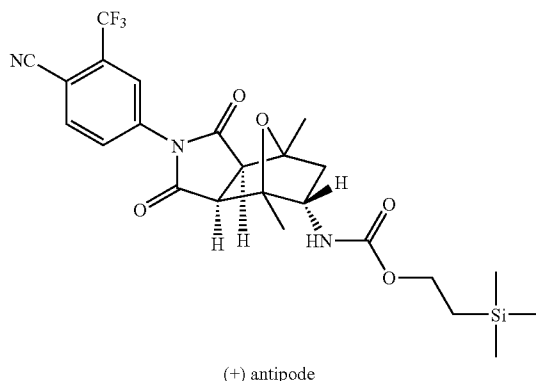


**[0504]** A 50 mL round-bottomed flask was charged with dioxane (6 mL), compound 804 (400 mg, 0.984 mmol), diphenylphosphoryl azide (334 mg, 1.21 mmol), triethylamine (123 mg, 1.22 mmol) and powdered 4 Å molecular sieves (400 mg). The resulting suspension was heated at 50° C. for 1.5 h, the temperature was then raised to 75° C. and 2-(trimethylsilyl)ethanol (590 mg, 5.02 mmol) was added. Heating was continued for an additional 1.5 h. The reaction mixture was then cooled, filtered through a celite pad and the filtrate concentrated under reduced pressure. Purification of the residue by silica gel chromatography, eluting with 20% then 40% EtOAc in hexanes, afforded compound 806 (400 mg, 78%) as a white solid. HPLC: 100% at 15.3 min (retention time) (Hypersil C18 BDS column, 250x4.6 mm, 5  $\mu\text{m}$ , a detection wavelength of 254 nm, and a flow rate of 1 mL/min. A linear gradient of 90% of 0.1% trifluoroacetic acid in water, 10% acetonitrile (start) to 100% acetonitrile over 15 min, then 100% acetonitrile for 5 min was used), MS (ES):  $m/z$  524

$[M+H]^+$ ,  $R_f=0.79$  ( $\text{SiO}_2$ , 50% EtOAc in hexanes),  $\text{Mpt}>300^\circ\text{C}$ .  $[\alpha]_{25}^D=-1.8^\circ$  ( $c=1.0$ , MeOH).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta=7.96$  (d,  $J=8.3$  Hz, 1H), 7.83 (s, 1H), 7.73 (dd,  $J=8.3$  and 2.0 Hz, 1H), 4.72 (bs, 1H), 4.19 (t,  $J=12.0$  Hz, 2H), 4.05 (m, 1H), 3.45 (d,  $J=8.3$  Hz, 1H), 3.12 (d,  $J=8.3$  Hz, 1H), 2.36 (t,  $J=12.0$  Hz, 1H), 1.61 (s, 6H), 1.04 (t,  $J=12.0$  Hz, 2H) and 0.05 ppm (s, 6H). The absolute stereochemistry of compound 806 has not been established. Although the compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

## EXAMPLE 807

[0505]

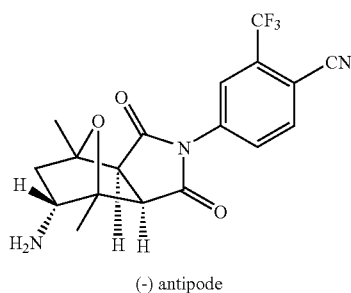


[0506] Compound 807 was prepared in identical fashion as described in example 806 with the exception that compound 805 was the starting material in place of compound 804. Compound 807 was obtained in 80% yield.  $[\alpha]_{25}^D=+1.1^\circ$  ( $C=1.0$ , MeOH). The absolute stereochemistry of compound 807 has not been established. Although the compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

## EXAMPLE 808

[3aR-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-amino-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (808)

[0507]



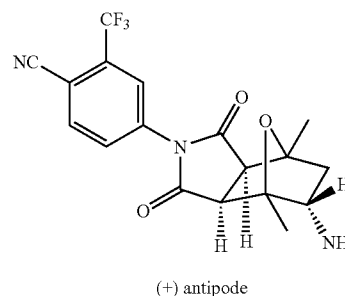
[0508] A solution of compound 806 (400 mg, 0.76 mmol) in methylene chloride (10 mL) was treated with trifluoroacetic acid (2 mL) and the mixture stirred at rt for 2 h. After this time the reaction was rendered basic (pH=9) by the addition of saturated aqueous sodium carbonate solution (20 mL) and solid potassium carbonate. The organic phase was then separated and the aqueous layer extracted with methylene chloride (3x40 mL). The combined organics were dried over sodium

sulfate, filtered, and concentrated to afford compound 808 (283 mg, 99%) as a white solid. HPLC: 100% at 10.5 min (retention time) (Hypersil C18 BDS column, 250x4.6 mm, 5  $\mu\text{m}$ , a detection wavelength of 254 nm, and a flow rate of 1 mL/min. A linear gradient of 90% of 0.1% trifluoroacetic acid in water, 10% acetonitrile (start) to 100% acetonitrile over 15 min, then 100% acetonitrile for 5 min was used), MS (ES):  $m/z$  380  $[M+H]^+$ .  $R_f=0.59$  ( $\text{SiO}_2$ , 5% MeOH in  $\text{CH}_2\text{Cl}_2$ ).  $[\alpha]_{25}^D=-26.70$  ( $c=1.0$ , MeOH).  $\text{Mpt}=150-152^\circ\text{C}$ .  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ ):  $\delta=8.12$  (d,  $J=8.3$  Hz, 1H), 7.94 (s, 1H), 7.83 (dd,  $J=8.3$  and 2.0 Hz, 1H), 3.66 (d,  $J=7.2$  Hz, 1H), 3.21 (m, 4H), 2.16 (t,  $J=12.0$  Hz, 1H), 1.51 (s, 3H), 1.49 (s, 3H) and 1.42 ppm (dd,  $J=12.0$  and 5.0 Hz, 1H). The absolute stereochemistry of compound 808 has not been established. Although the compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

## EXAMPLE 809

[3aS-(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )]-4-(Octahydro-5-amino-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (809)

[0509]



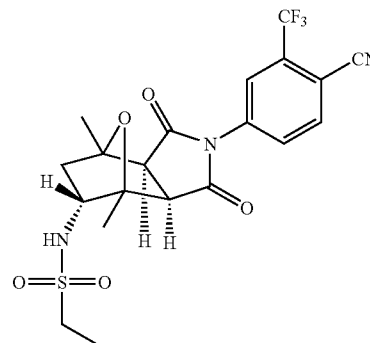
[0510] Compound 809 was prepared in identical fashion as described in example 808 with the exception that compound 807 was the starting material in place of compound 806. Compound 809 was isolated in 94% overall yield.  $[\alpha]_{25}^D=+27.3^\circ$  ( $c$

[0511]  $=1.0$ , MeOH). The absolute stereochemistry of compound 809 has not been established. Although the compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

## EXAMPLE 810

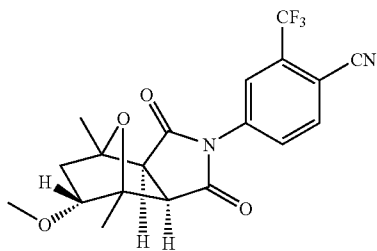
(3 $\alpha$ ,4 $\beta$ ,5 $\alpha$ ,7 $\beta$ ,7 $\alpha$ )-4-(Octahydro-5-ethylsulfonamido-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (810)

[0512]

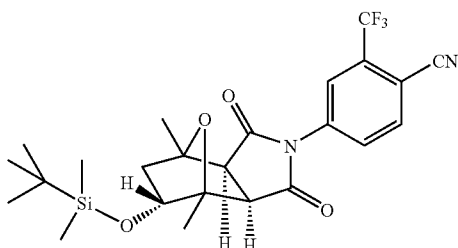


**[0513]** A 25 mL round-bottomed flask was charged with a racemic mixture of compounds 808 & 809 (102 mg, 0.27 mmol) and methylene chloride (10 mL). Ethanesulfonyl chloride (54.3 mg, 0.42 mmol) and triethylamine (43.6 mg, 0.43 mmol) were added and the resulting mixture stirred at rt overnight. Analysis of the reaction mixture by TLC (SiO<sub>2</sub>, EtOAc) indicated the presence of starting material so an additional equivalent of ethanesulfonyl chloride (54.3 mg, 0.42 mmol) was added and stirring continued at rt for 4 h. The reaction mixture was then diluted with CH<sub>2</sub>Cl<sub>2</sub> (25 mL) and washed with water (10 mL). Drying over magnesium sulfate, filtration, and concentration afforded a yellow oil. Purification of this oil by silica gel chromatography, eluting with 25% EtOAc in hexanes, then 50% EtOAc in hexanes and finally EtOAc, afforded compound 810 (55.9 mg, 44%) as a white solid. HPLC: 100% at 13.8 min (retention time) (Hypersil C18 BDS column, 250×4.6 mm, 5 μm, a detection wavelength of 254 nm, and a flow rate of 1 mL/min. A linear gradient of 90% of 0.1% trifluoroacetic acid in water, 10% acetonitrile (start) to 100% acetonitrile over 15 min, then 100% acetonitrile for 5 min was used), MS (ES): m/z 472 [M+H]<sup>+</sup>. R<sub>f</sub>=0.72 (EtOAc). Mpt=189-192° C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ=7.94 (d, J=8.3 Hz, 1H), 7.85 (s, 1H), 7.74 (dd, J=8.3 and 2.0 Hz, 1H), 5.72 (d, J=8.3 Hz, 1H), 3.71 (m, 1H), 3.51 (d, J=7.2 Hz, 1H), 3.18 (d, J=7.2 Hz, 1H), 3.11 (q, J=7.2 Hz, 2H), 2.37 (t, J=12.0 Hz, 1H), 1.67 (m, 1H), 1.62 (s, 3H), 1.61 (s, 3H) and 1.40 ppm (t, J=7.2 Hz, 3H). The absolute stereochemistry of compound 810 has not been established. Although the compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

## EXAMPLE 857

**[0514]**

A. (857A)



B. (857B)

**[0515]**

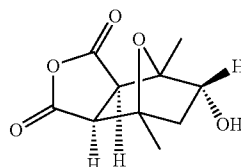
Compound 798i was converted to compound 857A by the procedure described in Example 774. HPLC: 95% at 4.307 min (retention time) (YMC S50DS-A column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

**[0517]** Compound 857A was converted to compound 857B by the procedure described in Example 774. HPLC: 100% at 2.89 min (retention time) (YMC S50DS-A column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.16 [M+H]<sup>+</sup>.

C. (857C)

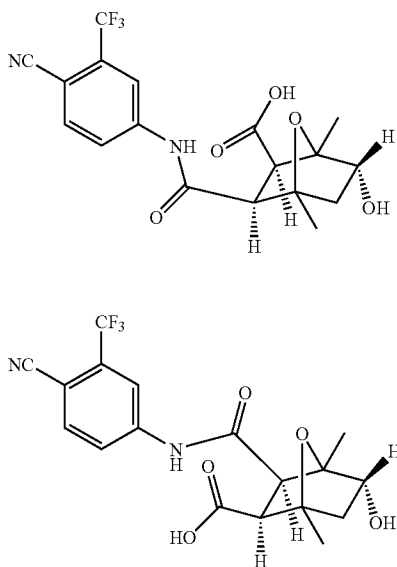
**[0518]** Compound 857B (0.100 g, 0.26 mmol), MeI (0.163 mL, 2.6 mmol), Ag<sub>2</sub>O (0.603 g, 2.60 mmol) and acetonitrile (2.5 mL) were added to a high pressure reaction vessel. The vessel was sealed and heated to 75° C. After 16 h, the reaction was cooled to rt, diluted with EtOAc and filtered through celite, rinsing with EtOAc. The crude material was purified by flash chromatography on silica eluting with 0-5-8% acetone in chloroform to give compound 857C as a white solid. HPLC: 90% at 2.887 min (retention time) (YMC S50DS-A column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 395.07 [M+H]<sup>+</sup>. The absolute stereochemistry of compound 857C has not been established. Although the compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

## EXAMPLE 858

**[0519]**

## A. (858Ai &amp; 858Aii)

[0520]



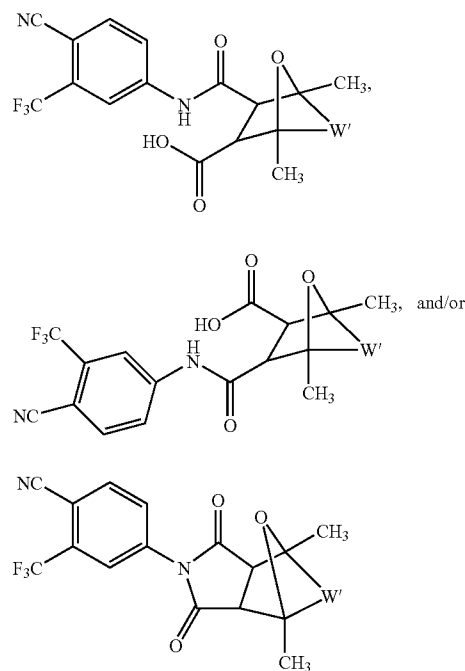
[0521] Compound 857B (2.50 g, 6.57 mmol) was dissolved in THF (10.0 mL) at 22° C. and 1 N NaOH (10.0 mL) was added. After 1 h, THF (10.0 mL), 1 N HCl (1.10 mL) and brine (10.0 mL) were added. The mixture was then extracted once with EtOAc (20.0 mL) and twice with 1:1 THF/EtOAc (20.0 mL). The combined organics were dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated in vacuo to give compounds 858Ai and 858Aii (1:1 by HPLC) as a white solid. No purification was necessary. HPLC: 100% at 2.383 and 2.573 min (retention time) (YMC S50DS-A column 4.6×50 mm eluting with 10-90% aqueous methanol over 4 min-containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

## B. (858B)

[0522] Compounds 858Ai and 858Aii were suspended in a mixture of THF (50.0 mL) and AcOH (20.0 mL) and heated to 60° C. for 16 h. The reaction became homogenous after 4 h. The reaction was cooled to 22° C. and concentrated in vacuo. Toluene (20.0 mL) was then added and mixture heated to 90° C. for 4 h until all product was dissolved. The mixture was then cooled to 22° C. and left standing for 20 h. Compound 858B precipitates from solution over the 20 h period. The product was filtered and rinsed with toluene followed by drying in vacuo. Compound 858B (1.10 g) was isolated as an off-white solid and used without further purification.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$ =4.91 (d, 1H,  $J$ =6.2 Hz), 3.97 (dd, 1H,  $J$ =3.9, 6.2 Hz), 3.82 (d, 1H,  $J$ =7.6 Hz), 3.31 (d, 1H,  $J$ =7.6 Hz), 2.27 (dd, 1H,  $J$ =10.2, 12.8 Hz), 1.77 (s, 3H), 1.49 (m, 1H) and 1.47 ppm (s, 3H). The absolute stereochemistry of compound 858B has not been established. Although the compound represents a single antipode, the nomenclature and structure shown does not reflect the absolute stereochemistry of the compound.

What is claimed is:

1. A composition comprising two or more compounds of the following formulae:



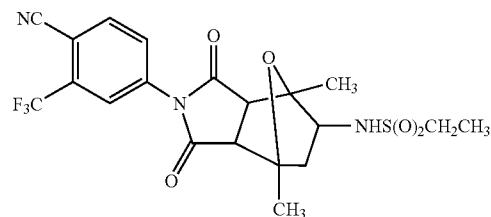
wherein:

$W'$  is  $-\text{CHR}^7-\text{CH}_2-$  or  $-\text{CH}_2-\text{C}(=\text{O})-$ ; and

$R^7$  is  $-\text{OH}$  or  $-\text{NHSO}_2\text{CH}_2\text{CH}_3$ ;

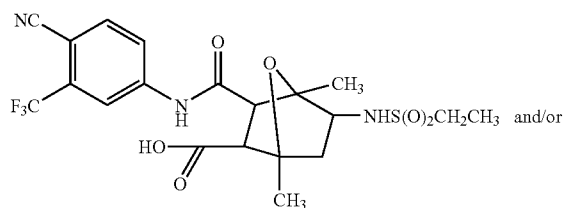
or pharmaceutically acceptable salts or stereoisomers thereof.

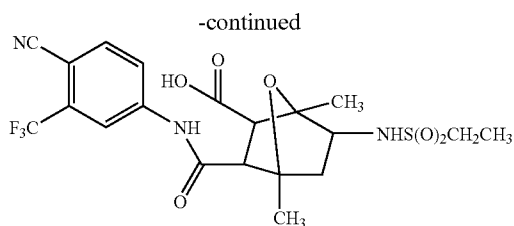
2. The composition according to claim 1, wherein said composition comprises:



or pharmaceutically acceptable salts or stereoisomers thereof.

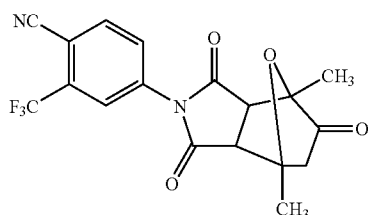
3. The composition according to claim 2, wherein said composition further comprises





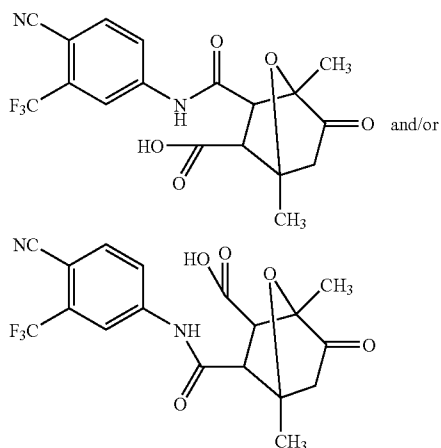
or pharmaceutically acceptable salts or stereoisomers thereof.

4. The composition according to claim 2, wherein said composition further comprises



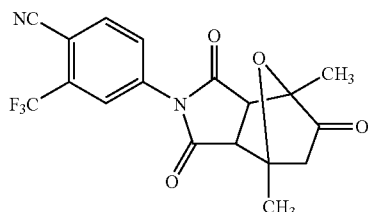
or pharmaceutically acceptable salts or stereoisomers thereof.

5. The composition according to claim 2, wherein said composition further comprises



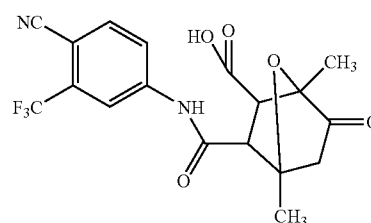
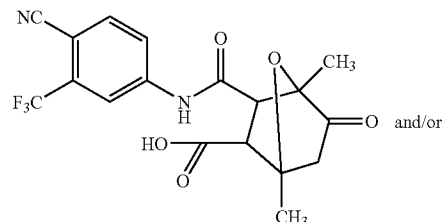
or pharmaceutically acceptable salts or stereoisomers thereof.

6. The composition according to claim 2, wherein said composition further comprises



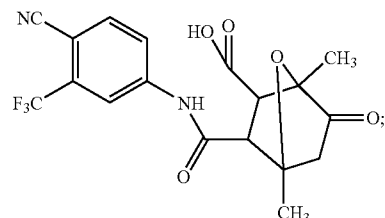
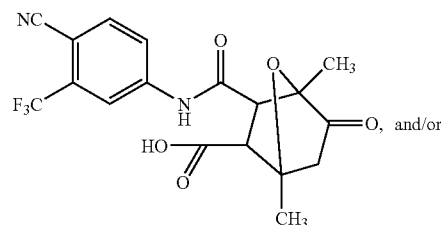
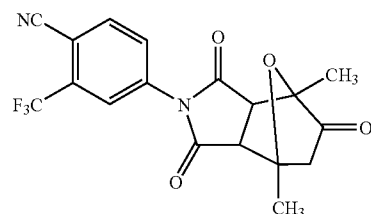
or pharmaceutically acceptable salts or stereoisomers thereof.

7. The composition according to claim 2, wherein said composition further comprises



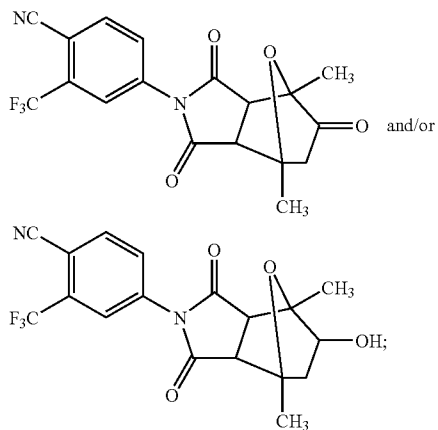
or pharmaceutically acceptable salts or stereoisomers thereof.

8. The composition according to claim 1, wherein said composition comprises:



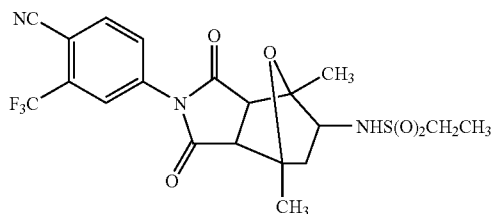
or pharmaceutically acceptable salts or stereoisomers thereof.

9. The composition according to claim 1, wherein said composition comprises:



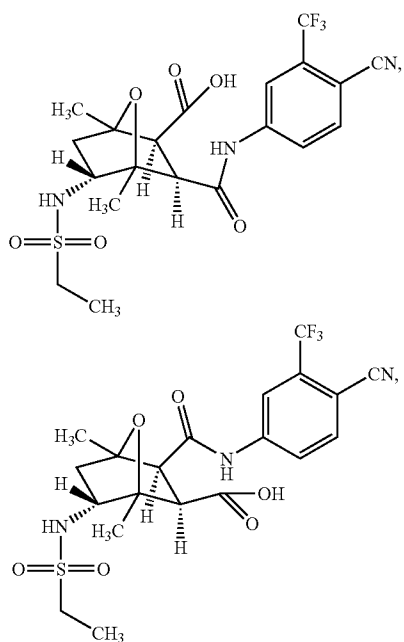
or pharmaceutically acceptable salts or stereoisomers thereof.

10. The composition according to claim 9, further comprising:

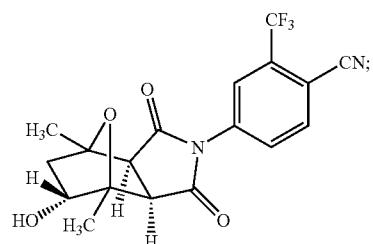
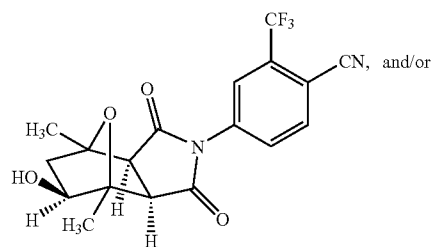
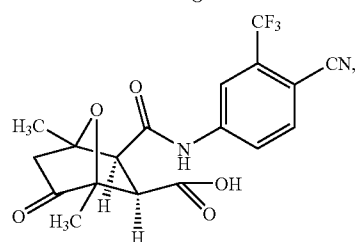
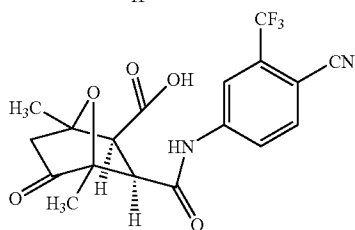
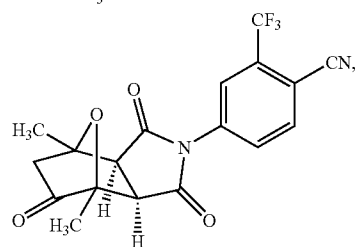
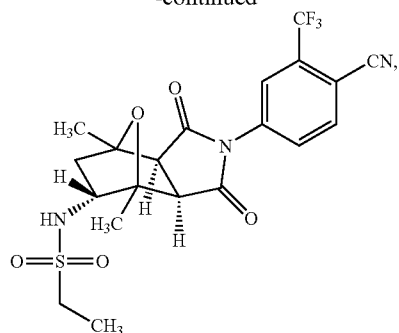


or pharmaceutically acceptable salts or stereoisomers thereof.

11. The composition according to claim 1, wherein said two or more compounds are:



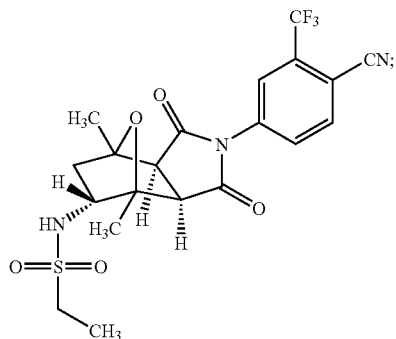
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or pharmaceutically acceptable salts or stereoisomers thereof.

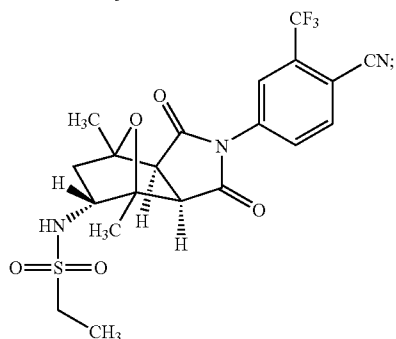
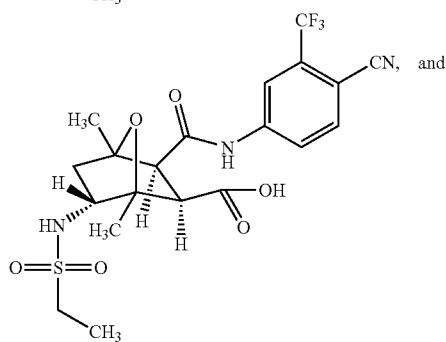
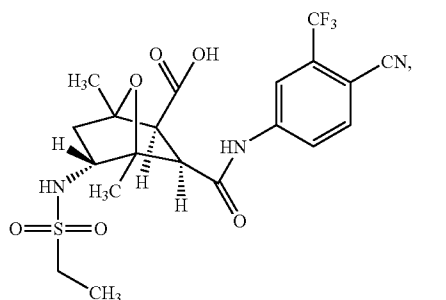


**12.** The composition according to claim 11, wherein said composition comprises:



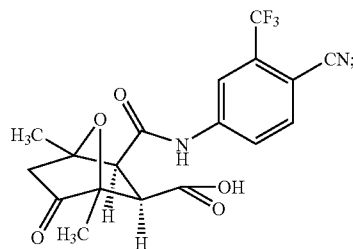
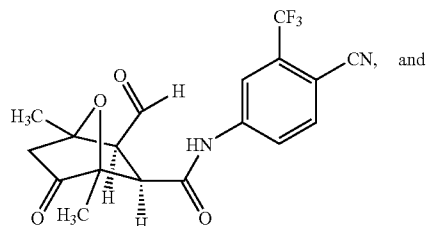
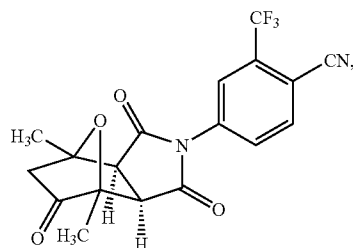
or pharmaceutically acceptable salts or stereoisomers thereof.

**13.** The composition according to claim 11, wherein said composition comprises:



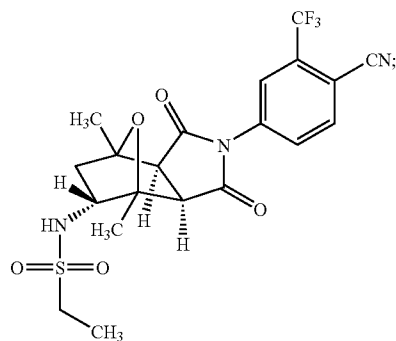
or pharmaceutically acceptable salts or stereoisomers thereof.

**14.** The composition according to claim 11, wherein said composition comprises:



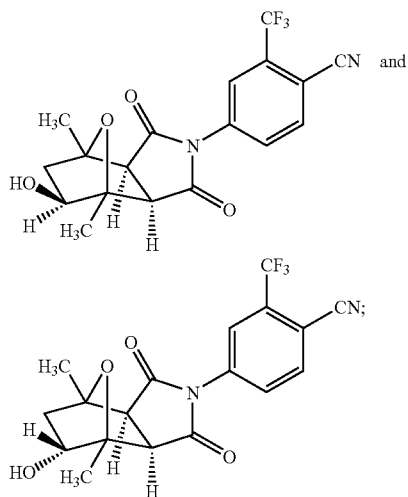
or pharmaceutically acceptable salts or stereoisomers thereof.

**15.** The composition according to claim 14, wherein said composition further comprises:



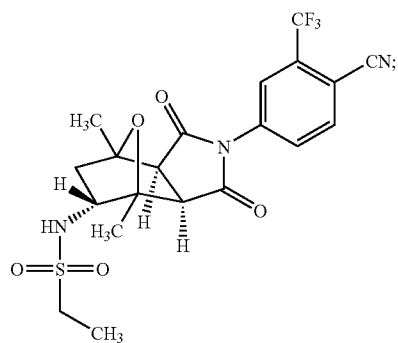
or pharmaceutically acceptable salts or stereoisomers thereof.

16. The composition according to claim 11, wherein said composition comprises:



or pharmaceutically acceptable salts or stereoisomers thereof.

17. The composition according to claim 16, wherein said composition further comprises:



or pharmaceutically acceptable salts or stereoisomers thereof.

\* \* \* \* \*