

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 January 2006 (05.01.2006)

PCT

(10) International Publication Number
WO 2006/002102 A2

(51) International Patent Classification:
A61K 31/4747 (2006.01) **C07D 471/10** (2006.01)

SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,
VN, YU, ZA, ZM, ZW.

(21) International Application Number:
PCT/US2005/021823

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(22) International Filing Date: 21 June 2005 (21.06.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/581,490 21 June 2004 (21.06.2004) US

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations* AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL,

(54) Title: SPIROCYCLIC COMPOUNDS USEFUL AS MODULATORS OF NUCLEAR HORMONE RECEPTOR FUNCTION

(57) Abstract: Spirocyclic compounds, methods of using such spirocyclic compounds in the treatment of nuclear hormone receptor-associated conditions such as cancer and immune disorders, and pharmaceutical compositions containing such compounds are disclosed.

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SPIROCYCLIC COMPOUNDS USEFUL AS MODULATORS OF NUCLEAR HORMONE RECEPTOR FUNCTION

FIELD OF THE INVENTION

5 **[0001]** The present invention relates to spirocyclic 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.

10 BACKGROUND OF THE INVENTION

[0002] 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
15 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,
20 testosterone, vitamine D3, 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)
25 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
30 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.

[0003] 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.

5 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

10 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

15 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),

20 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.

[0005] 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.

25 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

30 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).

[0006] 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 tissues 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 a non-natural 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.

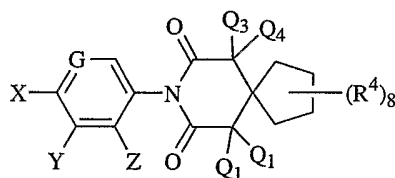
[0007] As mentioned above, non-natural ligands can be synthetically engineered to serve as modulators of the function of NHR's. In the case of SB-NHR's, engineering of a non-natural ligand can include the identification of a core structure which mimics the natural steroid core system. This can be achieved 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; US 5696133; US 5696130; US 5696127; US 5693647; US 5693646; US 5688810; US 5688808 and WO 9619458, all incorporated herein by reference.

[0008] The spirocyclic 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 herein.

SUMMARY OF THE INVENTION

[0009] The present invention provides spirocyclic compounds of the following formula (I) and salts thereof:



(I)

wherein the symbols have the following meanings, and are, for each occurrence, independently selected:

G is CH or N;

X and Y are independently selected from H, CN, NO₂, F, Cl, Br, I, CF₃, CF₂CF₃, R¹, OR¹, COR¹, CONR¹R^{1'}, and/or NR¹R^{1'};

Z is selected from H, CN, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, or CF₂CF₃;

where zero or one of X, Y, or Z is H;

either X and Y, or Y and Z can be fused to form a ring of 5 or 6 atoms that can be aryl, substituted aryl, heterocyclic, or heterocyclic substituted with one or more methyl or ethyl groups;

Q₁, Q₂, Q₃, and Q₄ are independently selected from H, alkyl, substituted alkyl,

cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, arylalkyl, substituted arylalkyl, and/or OR²;

R¹ and R^{1'} are independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, and/or substituted arylalkyl;

R² is selected from alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, R¹C=O, R¹OC=O, -N(R¹)HC=O, -C=ONR¹R^{1'}, -SO₂R¹, -SO₂OR¹, or -SO₂NR¹R^{1'}; and

each R⁴ is independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, R¹C=O, R¹OC=O, -N(R¹)HC=O, -SO₂R¹, -SOR¹, -C=ONR¹R^{1'}, -SO₂OR¹, and/or -SO₂NR¹R^{1'};

with the following provisos:

(1) when G is CH and Z is H, then X and Y cannot be fused to form an unsubstituted aryl ring;

(2) when G is CH and X is H, then Y and Z cannot be fused to form an unsubstituted aryl ring;

(3) when G is CH, Y is CH₃, and Z is H, then X is not Br; and

(4) when G is CH, Y is H, and Z is F, then X is not Cl.

[0010] In one aspect of the present invention, there is provided a pharmaceutical composition comprising at least one of the spirocyclic compounds of formula (I), a pharmaceutically acceptable salt, solvate, clathrate, hydrate, or prodrug thereof; and a pharmaceutically acceptable carrier.

[0011] According to another aspect of the present invention, a method of modulating the function of a nuclear hormone receptor comprising administering to a subject in need of treatment thereof, an effective amount of a compound of formula (I), or a pharmaceutically acceptable salt, solvate, clathrate, hydrate, or prodrug thereof.

[0012] In still another aspect of the present invention, a method of treating a condition or disorder comprising administering to a mammalian species in need thereof a therapeutically effective amount of a compound of formula (I), a pharmaceutically acceptable salt, solvate, clathrate, hydrate, or prodrug thereof.

- 5 [0013] These as well as other aspects of the invention will become more apparent from the following detailed description.

FURTHER DESCRIPTION OF THE INVENTION

[0014] The following are definitions of terms used in the present specification.

- 10 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.

[0015] 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
15 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, dodecyl, 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.

- 20 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 Cl₃ or CF₃), alkoxy, alkylthio, hydroxy, carboxy (i.e., -COOH), alkoxycarbonyl, alkylcarbonyloxy, CN, amino (i.e., -NH₂), alkylamino, dialkylamino, carbamoyl or
25 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)₂-aryl, -S(O)₂-heterocycle, -NHS(O)₂-aryl, -NHS(O)₂-heterocycle, -NHS(O)₂NH-heterocycle,
30 -NHS(O)₂NH-aryl, -P(O)₂-aryl, -P(O)₂-heterocycle, -NHP(O)₂-aryl, -NHP(O)₂-heterocycle, -NHP(O)₂NH-aryl, -NHP(O)₂NH-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-alkyl, -OC=OO-aryl, -OC=OO-heterocycle, -OC=ONH-aryl, -OC=ONH-heterocycle, -NHC=OO-aryl, -NHC=OO-heterocycle, -NHC=OO-alkyl, -C=ONH-aryl, -C=ONH-heterocycle, -C=OO-aryl, -C=OO-heterocycle, -N(alkyl)S(O)₂-aryl, -N(alkyl)S(O)₂-heterocycle, -N(alkyl)S(O)₂NH-aryl, -N(alkyl)S(O)₂NH-heterocycle, -N(alkyl)P(O)₂-aryl, -N(alkyl)P(O)₂-heterocycle, -N(alkyl)P(O)₂NH-aryl, -N(alkyl)P(O)₂NH-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)₂N(alkyl)-aryl, -NHS(O)₂N(alkyl)-heterocycle, -NHP(O)₂N(alkyl)-aryl, -NHP(O)₂N(alkyl)-heterocycle, -NHC=ON(alkyl)-aryl, -NHC=ON(alkyl)-heterocycle, -N(alkyl)S(O)₂N(alkyl)-aryl, -N(alkyl)S(O)₂N(alkyl)-heterocycle, -N(alkyl)P(O)₂N(alkyl)-aryl, -N(alkyl)P(O)₂N(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.
- [0016]** 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.

[0017] The term "cycloalkyl" refers to a fully saturated or partially saturated cyclic hydrocarbon group containing from 1 to 4 rings and 3 to 8 carbons per ring. Exemplary fully saturated cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. Exemplary partially saturated cycloalkyl groups include cyclobutenyl, cyclopentenyl, and cyclohexenyl. "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. Exemplary substituents also include spiro-attached or fused cyclic substituents, especially cycloalkenyl or substituted cycloalkenyl.

[0018] The terms "alkoxy" or "alkylthio" refer to an alkyl group as described above bonded through an oxygen linkage (-O-) or a sulfur linkage (-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.

[0019] The term "alkoxycarbonyl" refers to an alkoxy group bonded through a carbonyl group.

[0020] 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.

[0021] 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."

[0022] 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, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, cyano, alkyl-S(O)_m- (m=0, 1 or 2), alkyl, and substituted alkyl, as well as those groups recited above as exemplary alkyl substituents. Exemplary substituents also include
5 fused cyclic substituents, such as heterocyclo, cycloalkenyl, substituted heterocyclo, and cycloalkenyl groups (e.g., thereby forming a fluoroenyl, tetrahydronaphthalenyl, or dihydroindenyl group).

[0023] "Carbamoyl" refers to the group -CONH- which is bonded on one end to the remainder of the molecule and on the other to hydrogen or an organic moiety
10 (such as alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, alkylcarbonyl, hydroxyl, and substituted nitrogen). "Carbamate" refers to the group -O-CO-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 -NH-CO-NH- which is bonded on one end to the remainder of the molecule and
15 on the other to hydrogen or an organic moiety (such as those listed above). "Amidinyl" refers to the group -C(=NH)(NH₂). "Substituted carbamoyl," "substituted carbamate," "substituted urea", and "substituted amidinyl" refer to carbamoyl, carbamate, urea., and amidinyl groups, respectively, as described above in which one more of the hydrogen groups are replaced by an organic moiety (such as
20 those listed above).

[0024] 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
25 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 "heteroarylium" refers to a heteroaryl group bearing a quaternary nitrogen atom and
30 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. Exemplary monocyclic heterocyclic groups include ethylene oxide, azetidiny, pyrrolidinyl,

pyrrolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodiny, 2-oxoazepinyl, 5 azepinyl, hexahydrodiazepinyl, 4-piperidonyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, triazolyl, tetrazolyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane, tetrahydro-1,1-dioxothienyl, and the like. Exemplary bicyclic heterocyclic groups include indolyl, isoindolyl, benzothiazolyl, benzodioxolyl, benzoxazolyl, 10 benzoxadiazolyl, benzothienyl, quinuclidinyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, 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, 15 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.

[0025] “Substituted heterocycle,” “substituted heterocyclic,” and “substituted heterocyclo” (such as “substituted heteroaryl”) refer to heterocycle, heterocyclic, or 20 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, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, nitro; oxo (i.e., = O), cyano, alkyl-S(O)_m- (m = 0, 1 or 2), alkyl, and 25 substituted alkyl, as well as those groups recited above as exemplary alkyl substituents.

[0026] 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 or N-methylpyridinium), the 30 positively charged nitrogen in protonated ammonium species (e.g., trimethylhydroammonium or N-hydropyridinium), the positively charged nitrogen in amine N-

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

[0027] The terms "halogen" or "halo" refer to chlorine, bromine, fluorine, and iodine.

5 [0028] The terms "hydroxylamine" and "hydroxylamide" refer to the groups OH-NH- and OH-NH-CO-, respectively.

[0029] 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
10 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).

[0030] Unless otherwise indicated, any heteroatom with unsatisfied valences is assumed to have hydrogen atoms sufficient to satisfy the valences.

[0031] Carboxylate anion refers to a negatively charged group -COO^- .

15 [0032] The spirocyclic compounds of formula I may form salts which are also within the scope of this invention. Reference to a spirocyclic 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
20 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 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
25 which may be employed during preparation. Salts of the spirocyclic compounds of the formula I may be formed, for example, by reacting a spirocyclic compound of formula 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.

30 [0033] The spirocyclic compounds of formula I may 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, 5 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- 10 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.

[0034] The spirocyclic compounds of formula I may contain an acidic moiety, 15 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- 20 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 25 chain halides (e.g. decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides), aralkyl halides (e.g. benzyl and phenethyl bromides), and others.

[0035] Prodrugs and solvates of the spirocyclic 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 30 metabolic or chemical processes to yield a compound of the formula I, or a salt and/or solvate thereof. Solvates of the spirocyclic compounds of formula I include, for example, hydrates.

[0036] 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 spirocyclic 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.

[0037] All configurational isomers of the spirocyclic compounds of the present invention are contemplated, either in admixture, or in pure or substantially pure form. 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.

[0038] Throughout the specifications, groups and substituents thereof may be chosen to provide stable moieties and compounds.

[0039] Embodiments indicated herein as exemplary or preferred are intended to be illustrative and not limiting.

[0040] In one non-limiting embodiment, the spirocyclic compound of formula (I) has a structure in which:

X and Y are independently selected from H, CN, NO₂, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, and/or CF₂CF₃;

Z is selected from H, CN, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, or CF₂CF₃;

G, Q₁, Q₂, Q₃, Q₄, R¹, R^{1'}, R², and R⁴ are defined hereinabove, and provided that:

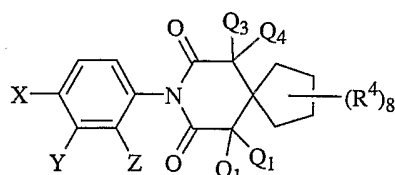
- (1) when G is CH and Z is H, then X and Y cannot be fused to form an unsubstituted aryl ring;
- (2) when G is CH and X is H, then Y and Z cannot be fused to form an

unsubstituted aryl ring;

(3) when G is CH, Y is CH₃, and Z is H, then X is not Br; and

(4) when G is CH, Y is H, and Z is F, then X is not Cl.

[0041] In non-limiting embodiment II, the spirocyclic compound of formula (I)
 5 has a structure in which G is CH. The spirocyclic compound of this embodiment has the formula (II):



(II)

wherein X, Y, Z, Q₁, Q₂, Q₃, Q₄, R¹, R^{1'}, R², and R⁴ are defined hereinabove and
 10 provided that:

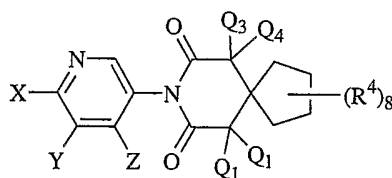
(1) when Z is H, then X and Y cannot be fused to form an unsubstituted aryl ring;

(2) when X is H, then Y and Z cannot be fused to form an unsubstituted aryl ring;

(3) when Y is CH₃ and Z is H, then X is not Br; and

(4) when Y is H and Z is F, then X is not Cl.

[0042] In non-limiting embodiment III, the spirocyclic compound of formula (I)
 15 has a structure in which G is N. The spirocyclic compound of this embodiment has the formula (III):



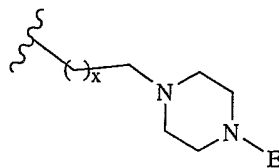
(III)

20 wherein X, Y, Z, Q₁, Q₂, Q₃, Q₄, R¹, R^{1'}, R², and R⁴ are defined hereinabove.

[0043] In one non-limiting embodiment, the spirocyclic compound of formula I has a structure in which Q₁, Q₂, Q₃, and Q₄ are independently selected from H, methyl, ethyl, propyl, and/or CH₂CF₃.

[0044] In a still different non-limiting embodiment, the spirocyclic compound of
 25 formula I has at least one R⁴ substituent comprising a piperazinyl group or a substituted piperazinyl group. Examples of suitable R⁴ substituents include, but are

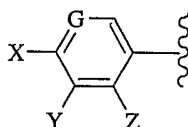
not limited to, the piperazinyl groups and the substituted piperazinyl groups represented by formula (IV):



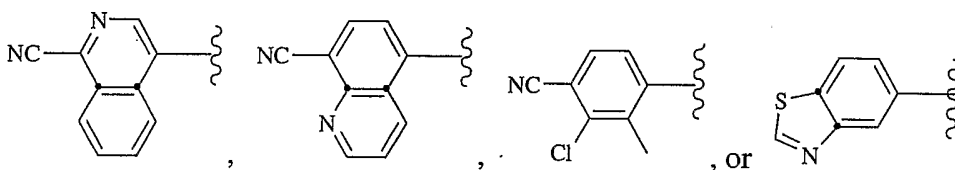
(IV)

- 5 wherein x is an integer in the range of from 1 to 8; and E is H, $R^1C=O$, $R^1OC=O$, $-C=OR^1NH$, $-SO_2R^1$, or $-SO_2NHR^1$.

[0045] In a still further non-limiting embodiment, the X, Y, and Z substituents are selected to provide the moiety



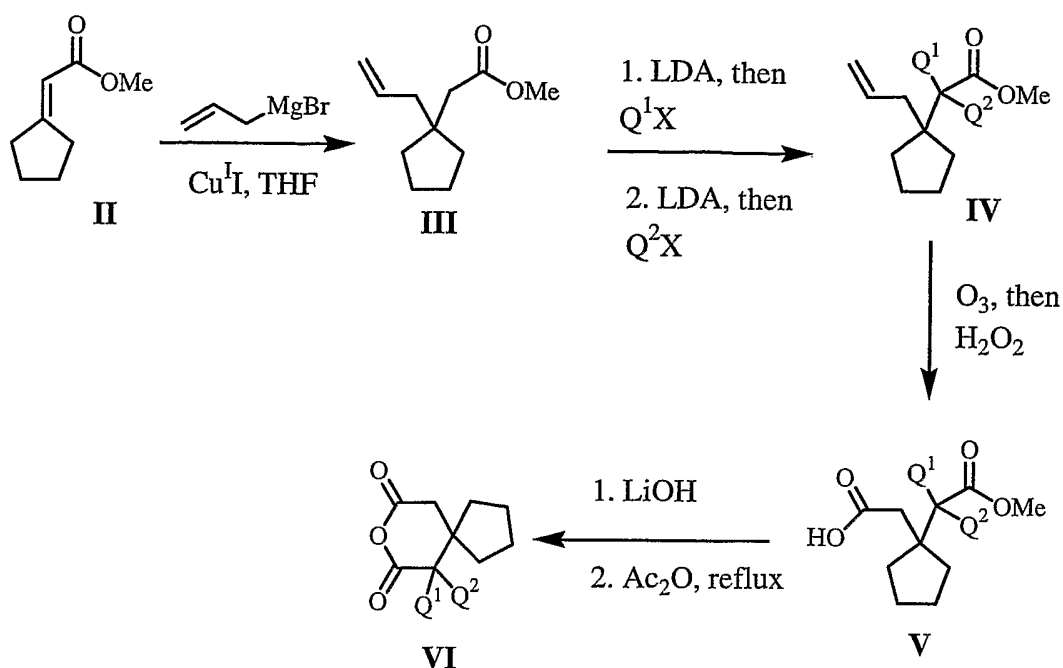
- 10 of the spirocyclic compound of formula (I) with one of the following structures:



METHODS OF PREPARATION

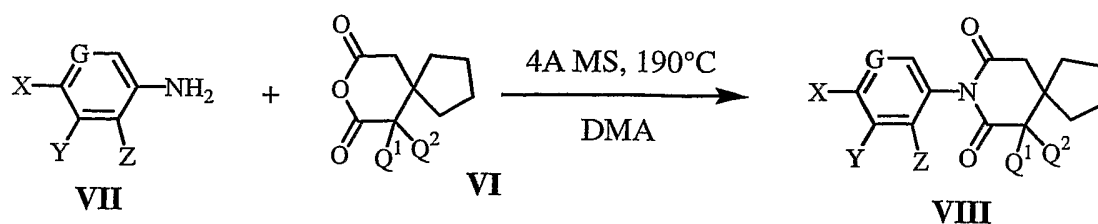
- 15 [0046] The spirocyclic compounds of the present invention may be prepared by methods such as those illustrated in the following Schemes I and II. Solvents, temperatures, pressures, and other reaction conditions may readily be selected by one of ordinary skill in the art. Starting materials are commercially available or readily prepared by one skilled in the art. Combinatorial techniques may be employed in the
- 20 preparation of compounds, for example, where the intermediates possess groups suitable for these techniques.

Scheme I



[0047] Scheme I demonstrates a possible synthesis of substituted spirocyclic anhydrides VI. The methyl enolate II can be treated with an allyl organometallic such as allyl magnesiumbromide in the presence of copper (I) iodide to give the product III via 1,4-conjugate addition (*Journal of Organic Chemistry*, 51(10), 1745-53, 1986). The ester can then be alkylated in the alpha position by successive treatment with a base such as LDA and an alkylating agent such as methyl iodide. The alkylating agent can vary widely as known by one skilled in the art. This process can then be repeated to give compounds of general formula IV. Ozonolysis of the primary olefin under conditions known to one skilled in the art will give the acid of general formula V (*Organic Process Research and Development*, 5(5), 531-534, 2001 or *Bioorganic and Medicinal Chemistry Letters*, 11(4), 475-477, 2001). Conversion to the spirocyclic anhydride VI can occur by treatment with acetic anhydride as presented in (M. G. Zeid; F. A. El-Telbany; M. Khalifa *Pharmazie*, 35, H11(1980)) or by other methods known to one skilled in the art.

Scheme 2



[0048] The spirocyclic anhydride of formula VI can be coupled with the amine of formula VII under a variety of dehydrating conditions to give a compound of formula VIII which is a compound of formula I, as exemplified in U.S. Patent Publication 2002/0058290 and 2003/0181728. The conditions shown have been successful for compounds presented herein.

USE AND UTILITY

[0049] The spirocyclic compounds of the present invention are useful for modulating 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) or inhibition (e.g., antagonist activity).

[0050] The present spirocyclic 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, 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.

[0051] The spirocyclic 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:

[0052] The spirocyclic 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
5 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.

[0053] The spirocyclic compounds of formula I can be applied as agonists, partial
10 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,
15 meningioma, dysmenorrhea, fibroids, pregnancy termination, labor induction, and osteoporosis.

[0054] The spirocyclic 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
20 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.
25 Glucocorticoid receptor AP-1 ("GR AP-1") inhibitors (which compounds can, for example, avoid side effects connected with GR agonists) can be used as anti-inflammatory and immunosuppressive agents, for example, to treat a wide variety of inflammatory and autoimmune diseases. These diseases include without limitation: rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, asthma, chronic
30 obstructive pulmonary disease, prevention of transplant rejection, multiple sclerosis, and psoriasis, among others. GR AP-1 inhibitors of the present invention can be

employed together with known GR AP-1 inhibitors, such as the steroid prednisone (which is used to treat the above diseases).

[0055] The spirocyclic 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.

[0056] The spirocyclic 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.

[0057] The spirocyclic 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 VCAM 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.

[0058] The spirocyclic 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)),
5 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.

[0059] The spirocyclic compounds of formula I can be applied as agonists, partial
10 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-
15 administration of an agent (compound of the present invention) which modulates the P450 regulator effects of SXR.

[0060] 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
20 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 that 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
25 applications of modulators of said Orphan receptors are also listed in Table 1, but are not limited to the examples therein.

Table 1.

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

<u>Receptor</u>	<u>Form</u>	<u>Tissue Expression</u>	<u>Target Therapeutic Application</u>
NURR1	M/D	Dopaminergic Neurons	Parkinson's Disease
RZR β	M	Brain (Pituitary), Muscle	Sleep Disorders
ROR α	M	Cerebellum, Purkinje Cells	Arthritis, Cerebellar Ataxia
NOR-1	M	Brain, Muscle, Heart,	CNS Disorders, Cancer Adrenal, Thymus
NGFI-B β	M/D	Brain	CNS Disorders
COUP-Tf α	H	Brain	CNS Disorders
COUP-TF β	H	Brain	CNS Disorders
COUP-TF γ	H	Brain	CNS Disorders
Nur77	H	Brain, Thymus, Adrenals	CNS Disorders
Rev-ErbA α	H	Muscle, Brain (Ubiquitous)	Obesity
HNF4 α	H	Liver, Kidney, Intestine	Diabetes
SF-1	M	Gonads, Pituitary	Metabolic Disorders
LXR α,β	D	Kidney (Ubiquitous)	Metabolic Disorders
GCNF	M/H	Testes, Ovary	Infertility
ERR α,β	M	Placenta, Bone	Infertility, Osteoporosis
FXR	D	Liver, Kidney	Metabolic Disorders
CAR α	H	Liver, Kidney	Metabolic Disorders
PXR	H	Liver, Intestine	Metabolic Disorders

[0061] 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 spirocyclic 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 spirocyclic compound(s) of the present invention.

10 [0062] The present invention also provides pharmaceutical compositions comprising at least one of the spirocyclic 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
5 desired administration (for example, excipients, binders, preservatives, stabilizers, flavors, etc.) according to techniques such as those well known in the art of pharmaceutical formulation.

[0063] It should be noted that the spirocyclic compounds of the present invention are, without limitation as to their mechanism of action, useful in treating any of the
10 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.

[0064] The present compounds have therapeutic utility in the modulation of immune cell activation/proliferation, e.g., as competitive inhibitors of intercellular
15 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
20 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
25 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
30 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 spirocyclic compound of the formula I attenuates any actual inflammation (such as, for example, the rejection of a transplanted organ or tissue).

[0065] The spirocyclic 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.

[0066] 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
5 excipients, binders, extenders, disintegrants, diluents and lubricants such as those known in the art. The spirocyclic 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
10 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),
15 maleic anhydride copolymer (e.g., Gantrez), and agents to control release such as polyacrylic copolymer (e.g. Carbopol™ 934 polymer, B.F. Goodrich Co., NY). Lubricants, glidants, flavors, coloring agents and stabilizers may also be added for ease of fabrication and use.

[0067] Exemplary compositions for nasal aerosol or inhalation administration
20 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.

[0068] Exemplary compositions for parenteral administration include injectable solutions or suspensions which can contain, for example, suitable non-toxic,
25 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.

[0069] Exemplary compositions for rectal administration include suppositories
30 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 liquefy and/or dissolve in the rectal cavity to release the drug.

[0070] Exemplary compositions for topical administration include a topical carrier such as Plastibase (mineral oil gelled with polyethylene).

[0071] The effective amount of the spirocyclic 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) 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.

[0072] As mentioned above, the spirocyclic 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.

[0073] For their preferred anticancer or antiangiogenic use, the spirocyclic 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 triazines; 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; 5α reductase inhibitors; inhibitors of 17β -hydroxy steroid dehydrogenase type 3; 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 (Taxol®), 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 spirocyclic compounds of the invention may also be used in conjunction with radiation therapy.

[0074] Representative examples of these classes of anti-cancer and cytotoxic agents include but are not limited to mechlorethamine hydrochloride, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustin, 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 topotecan and any analogs or derivatives thereof.

[0075] Preferred member of these classes include, but are not limited to, paclitaxel, cisplatin, carboplatin, doxorubicin, carminomycin, 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.

[0076] 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. Patent 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. Patent No. 6,011,029 (the spirocyclic 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).

[0077] The spirocyclic compounds 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 spirocyclic compounds of the invention may be formulated with agents to prevent nausea, hypersensitivity and gastric irritation, such as antiemetics, and H₁ and H₂ antihistaminics.

[0078] As it pertains to the treatment of cancer, the spirocyclic 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. Patent 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, antagonists, or with surgical castration.

[0079] For example, known therapies for advanced metastatic prostate cancer include "complete androgen ablation therapy" wherein tumor growth is inhibited by
5 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 spirocyclic
10 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.

[0080] The spirocyclic compounds of the present invention may be employed adjuvant to surgery.

15 [0081] 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.

[0082] The above other therapeutic agents, when employed in combination with
20 the spirocyclic 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.

[0083] The following assays can be employed in ascertaining the activity of the spirocyclic compounds of formula I as a NHR modulator. Preferred are those
25 compounds with an activity greater than 20 μ m for binding or transactivation in any of these assays. The AR modulator activity of various spirocyclic compounds of the present invention can be determined by utilizing the transactivation assay and standard AR binding assays as described following.

TRANSACTIVATION ASSAYS

AR Specific Assay:

[0084] The spirocyclic compounds of the present invention are tested in
5 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
10 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.

[0085] 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
15 secreted alkaline phosphatase (SEAP), is 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
20 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
25 of the reporter signal.

[0086] For this assay, LNCaP and MDA 453 cells are 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 are transiently transfected by electroporation
30 according to the optimized procedure described by Heiser, 130 *Methods Mol. Biol.*,
117 (2000), with the pSEAP2/PSA540/Enhancer reporter plasmid. The reporter
plasmid, is constructed as follows: commercial human placental genomic DNA is

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 is subcloned into the pSEAP2/basic (Clontech) previously digested with BglII and HindIII to generate the pSEAP2/PSA540 construct. Then a fragment bearing the fragment of human PSA upstream sequence between positions -5322 and -3873 is amplified by PCR from human placental genomic DNA. A XhoI and a BglII sites are introduced with the primers. The resulting fragment is subcloned into pSEAP2/PSA540 digested with XhoI and BglII respectively, to generate the pSEAP2/PSA540/Enhancer construct. LNCaP and MDA 453 cells are collected in media containing 10% charcoal stripped FBS. Each cell suspension is distributed into two Gene Pulser Cuvetts (Bio-Rad) which then receive 8 µg of the reporter construct, and is electoporated using a Bio-Rad Gene Pulser at 210 volts and 960 µFaraday. Following the transfections, the cells are 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^{-10} to 10^{-5} M (sample). Duplicates are used for each sample. The spirocyclic compound dilutions are performed on a Biomek 2000 laboratory workstation.

[0087] After 48 hours, a fraction of the supernatant is assayed for SEAP activity using the Phospha-Light Chemiluminescent Reporter Gene Assay System (Tropix, Inc). Viability of the remaining cells is 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 bio-reduced by cells into a formazan that is soluble in tissue culture medium, and therefore its absorbance at 490nm can be measured directly from 96 well assay plates without additional processing. The quantity of formazan product as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture. For each replicate the SEAP reading is normalized by the Abs490 value

derived from the MTS assay. For the antagonist mode, the % Inhibition is calculated as:

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

Data is plotted and the concentration of the spirocyclic compound that inhibited 50% of the normalized SEAP is quantified (IC_{50}).

- 10 [0088] For the agonist mode, % Control is referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and is calculated as:

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

Data is plotted and the concentration of the spirocyclic compound that activates to levels 50% of the normalized SEAP for the control is quantified (EC_{50}).

20 **GR Specificity Assay:**

- [0089] The reporter plasmid utilized is comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein is 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 see, e.g. G. Chalepakidis et al., *Cell*, 53(3), 371 (1988). This plasmid is transfected into A549 cells, which expresses endogenous GR, to obtain a GR specific transactivation assay. A549 cells are obtained from the American Type Culture Collection (Rockville, MD), and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco).
- 30 Determination of the GR specific antagonist activity of the spirocyclic compounds of the present invention is identical to that described for the AR specific transactivation assay, except that the DHT is replaced with 5 nM dexamethasone (Sigma Chemicals),

a specific agonist for GR. Determination of the GR specific agonist activity of the spirocyclic compounds of the present invention is 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:

[0090] The reporter plasmid utilized is comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein is 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 is transfected into T47D, which expresses endogenous PR, to obtain a PR specific transactivation assay. T47D cells are 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 spirocyclic compounds of the present invention is identical to that described for the AR specific transactivation assay, except that the DHT is replaced with 1 nM Promegastone (NEN), a specific agonist for PR. Determination of the PR specific agonist activity of the spirocyclic compounds of the present invention is 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:

[0091] 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 (Cocaleco Biologicals) respectively, are 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, [^3H]-DHT, or a competitive binding assay to evaluate the ability of test compounds to compete with [^3H]-DHT is

performed. For the saturation analysis, media (RPMI 1640 or DMEM – 0.2% CA-FBS) containing [³H]-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 are added to the cells. After 4 hours at 37°C, an aliquot of the total
5 binding media at each concentration of [³H]-DHT is removed to estimate the amount of free [³H]-DHT. The remaining media is removed, cells are washed three times with PBS and harvested onto UniFilter GF/B plates (Packard), Microscint (Packard) is added and plates counted in a Top-Counter (Packard) to evaluate the amount of bound [³H]-DHT.

10 [0092] For the saturation analysis, the difference between the total binding and the non-specific binding, is defined as specific binding. The specific binding is evaluated by Scatchard analysis to determine the K_d for [³H]-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-
15 99, (1981), the disclosure of which is herein incorporated by reference.

[0093] For the competition studies, media containing 1 nM [³H]-DHT and compounds of the invention ("test compounds") in concentrations ranging from 10⁻¹⁰ to 10⁻⁵ M are added to the cells. Two replicates are used for each sample. After 4 hours at 37°C, cells are washed, harvested, and counted as described above. The data
20 is plotted as the amount of [³H]-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 [³H]-DHT bound in the absence of competing ligand is quantified (IC₅₀) after log-logit transformation. The K_i values are determined by application of the Cheng-Prusoff equation to the IC₅₀
25 values, where:

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

[0094] After correcting for non-specific binding, IC₅₀ values are determined. The
30 IC₅₀ is defined as the concentration of competing ligand needed to reduce specific binding by 50%. The K_d values for [³H]-DHT for MDA 453 and LNCaP are 0.7 and 0.2 nM respectively.

Human Prostate Cell Proliferation Assay:

[0095] Compounds of the present invention are 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), are incubated with or without the test compounds for 72 hours and the amount of [³H]-thymidine incorporated into DNA is quantified as a way to assess number of cells and therefore proliferation. The MDA PCa2b cell line is maintained in BRFF-HPC1 media (Biological Research Faculty & Facility Inc., MD) supplemented with 10% FBS. For the assay, cells are plated in Biocoated 96-well microplates and incubated at 37°C in 10% FBS (charcoal-stripped)/BRFF-BMZERO (without androgens). After 24 hours, the cells are 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 are used for each sample. The compound dilutions are performed on a Biomek 2000 laboratory work station. Seventy two hours later 0.44 uCi. of [³H]-Thymidine (Amersham) is added per well and incubated for another 24 h followed by trypsinization, harvesting of the cells onto GF/B filters. Micro-scint PS are added to the filters before counting them on a Beckman TopCount.

The % Inhibition was calculated as:

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

Data is plotted and the concentration of compound that inhibited 50% of the [³H]-Thymidine incorporation is quantified (IC₅₀).

C2C12 Mouse Myoblast Transactivation Assay:

[0096] Two functional transactivation assays are 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 is 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.

- [0097] 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:

- [0098] 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).
- [0099] 2. 48 hours later, the 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.
- [00100] 3. The transfection mixture is removed from the cells and replaced with 90 µl of media as in #1 above.
- [00101] 4. 10 µl/well of appropriate drug dilution is placed in each well. 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:

[00102] 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), 1X MEM Na Pyruvate (Gibco BRL, Cat. No.: 11360-070), 0.5X Antibiotic-Antimycotic, 800 µg/ml Geneticin (Gibco BRL, Cat. No.: 10131-035) and 800 µg/ml Hygromycin β (Gibco BRL, Cat. No.: 10687-010).

[00103] 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.
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).

PROLIFERATION ASSAYS

15

Murine Breast Cell Proliferation Assay:

[00104] The ability of the spirocyclic compounds of the present invention ("test compounds") to modulate the function of the AR is 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 are established by passing tumor fragments under the general procedures originally described in Tetuo, *et. al.*, *Cancer Res.*, **25**, 1168-1175 (1965). From the above procedure, one stable line, SC114, is isolated, characterized, and utilized for the testing of example compounds. SC114 cells are incubated with or without the test compounds for 72 hours and the amount of [³H]-thymidine incorporated into DNA is 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 is maintained in MEM containing 10⁻⁸ M testosterone and 2% DCC-treated FCS. For the assay, cells are plated in 96-well microplates in the maintenance media and incubated at 37°C. On the following day, the medium is 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^{-8} M testosterone and the test compounds of the present invention in concentrations ranging from 10^{-10} to 10^{-5} M. Duplicates are used for each sample. The spirocyclic compound dilutions are performed on a Biomek 2000 laboratory work station. Seventy two hours later 0.44 μ Ci of [3 H]-Thymidine (Amersham) is added per well and incubated for another 2 hr followed by tripsinization, and harvesting of the cells onto GF/B filters. Micro-scint PS is added to the filters before counting them on a Beckman TopCount. For the antagonist mode, the % Inhibition is calculated as:

10
$$\% \text{ Inhibition} = 100 \times (1 - [(average_{\text{sample}} - average_{\text{blank}})/(average_{\text{control}} - average_{\text{blank}})])$$

Data is plotted and the concentration of compound that inhibited 50% of the [3 H]-Thymidine incorporation is quantified (IC_{50}).

15 **[00105]** For the agonist mode % Control is referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and is calculated as:

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

20

Data is plotted and the concentration of compound that inhibited 50% of the [3 H]-Thymidine incorporation is quantified (EC_{50}).

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

25 **[00106]** The AP-1 assay is a cell based luciferase reporter assay. A549 cells, which contain endogenous glucocorticoid receptor, are 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 0.5mg/ml geneticin. Cells are plated the day before the assay at approximately 40000 cells/well.

30 On assay day, the media is removed by aspiration and 20 μ l assay buffer (RPMI without phenol red + 10% FCS (charcoal-treated) + Pen/Strep) is added to each well. At this point either 20 μ l assay buffer (control experiments), the spirocyclic

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 that 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:

[00107] The activity of compounds of the present invention as AR antagonists is 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.

[00108] 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 5 α -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 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 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.

5 [00109] Male immature rats (19-20 days old Sprague-Dawley, Harlan Sprague-Dawley) are castrated under metofane anesthesia. Five days after surgery these castrated rats (60-70g, 23-25 day-old) are dosed for 3 days. Animals are dosed subcutaneously (s.c.) 1mg/kg with Testosterone Propionate (TP) in arachis oil vehicle and anti-androgen test compounds (compounds of the present invention) are dosed orally by gavage (p.o.) in dissolved/suspensions of 80% PEG 400 and 20% Tween 80 surfactant(PEGTW). Animals are dosed (v/w) at 0.5 ml of vehicle /100g body weight. Experimental groups are 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.
- 20 4. To demonstrate antagonist activity, a compound of the present invention ("test compound") is 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") is administered alone (p.o. in PEGTW, QD) in a range of doses.

25

[00110] At the end of the 3-day treatment, the animals are sacrificed, and the ventral prostate weighed. To compare data from different experiments, the sexual organs weights are 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%).

30 ANOVA followed by one-tailed Student or Fischer's exact test is used for statistical analysis.

[00111] 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.

[00112] The maximum increase in organ weight is 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 EC₅₀ of T and TP are about 1 mg and 0.03 mg, respectively. The increase in the weight of the VP and SV also correlates with the increase in the serum T and DHT concentration. Although administration of T shows 5-times higher serum concentrations of T and DHT at 2 hours after subcutaneous injection than that of TP, thereafter, these high levels decline very rapidly. In contrast, the serum concentrations of T and DHT in TP-treated animals are fairly consistent during the 24 hours, and therefore, TP showed about 10-30-fold higher potency than free T.

[00113] In this immature castrated rat model, a known AR antagonist (Casodex) is also administered simultaneously with 0.1 mg of TP (ED₈₀), inhibiting the testosterone-mediated increase in the weights of the VP and SV in a dose dependent manner. The antagonist effects are similar when dosing orally or subcutaneously. Compounds of the invention also exhibit 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:

[00114] The activity of compounds of the present invention as AR agonists is 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. Hersherberger 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.

[00115] 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 resulted in a reversal of muscular atrophy. The effects of T on muscular atrophy in the rat levator ani muscle have been 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, was 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) are acquired castrated from the vendor (Taconic). The rats are 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") is 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") is administered alone (p.o. in PEGTW, QD) in a range of doses.

[00116] At the end of the 7-14-day treatment, the animals are 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 are first standardized as mg per 100 g of body weight, and the increase in organ weight induced by TP is considered as the maximum increase (100%). Super-anova (one factor) is used for statistical analysis.

[00117] 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 increase levator ani, seminal vesicles (SV) and prostate in a dose dependent manner.

[00118] The maximum increase in organ weight is 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 EC₅₀ of T and TP are about 1 mg and 0.03 mg, respectively. The increase in the weight of the VP and SV also correlates with the increase in the serum T and DHT concentration. Although administration of T shows 5-times higher serum concentrations of T and DHT at 2 hours after subcutaneous injection than that of TP, thereafter, these high levels decline very rapidly. In contrast, the serum concentrations of T and DHT in TP-treated animals are fairly consistent during the 24 hours, and therefore, TP shows about 10-30-fold higher potency than free T.

MDA PCa2b Human Prostate Xenograft Assay:

[00119] *In Vivo* Antitumor Testing: MDA-PCa-2b human prostate tumors are maintained in Balb/c nu/nu nude mice. Tumors are propagated as subcutaneous transplants in adult male nude mice (4-6 weeks old) using tumor fragments obtained from donor mice. Tumor passage occurs every 5-6 weeks.

[00120] For antitumor efficacy trial, the required number of animals needed to detect a meaningful response are pooled at the start of the experiment and each is given a subcutaneous implant of a tumor fragment (~50 mg) with a 13-gauge trocar. Tumors are allowed to grow to approximately 100-200 mg (tumors outside the range

were excluded) and animals are evenly distributed to various treatment and control groups. Treatment of each animal is based on individual body weight. Treated animals are checked daily for treatment related toxicity/mortality. Each group of animals is weighed before the initiation of treatment (W_{t1}) and then again following the last treatment dose (W_{t2}). The difference in body weight ($W_{t2}-W_{t1}$) provides a measure of treatment-related toxicity.

- [00121] Tumor response is 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) are estimated from the formula: Tumor weight = $(\text{length} \times \text{width}^2) \div 2$.

10 [00122] Tumor response end-point is 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).

[00123] To estimate tumor cell kill, the tumor volume doubling time is first calculated with the formula:

15 TVDT = (Median time (days) for control tumors to reach target size) –
(Median time (days) for control tumors to reach half the target sizes)
And, Log cell kill = $(T-C) \div (3.32 \times \text{TVDT})$.

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

20

Dunning Prostate Tumor:

[00124] Dunning R3327H prostate tumor is a spontaneously derived, well differentiated androgen responsive adenocarcinoma of the prostate (Smolev JK, Heston WD, Scott WW, and Coffey DS, *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)).

25
30

[00125] At the beginning of the study, the Dunning tumor pieces (about 4 x 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 mm²) are randomized into treatment groups (8-10 rats/group) and the treatments are initiated. One group of the rats is 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 surfactant 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).

[00126] Tumors in the vehicle (control) group reach a size of 1500 to 2500 mm³, 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 mm³ using the formula: Length x Width x Height = Volume. Statistical differences between treatment groups and control are evaluated using multiple ANOVA analysis followed by one tail non-parametric Student t test.

25 **Mature Rat Prostate Weight Assay:**

[00127] The activity of compounds of the present invention are 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. Hershberger *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.

[00128] 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 α -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.

[00129] 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.

[00130] Matched sets of Harlan Sprague-Dawely rats (40-42 days old, 180-220 g), are dosed orally by gavage (p.o.) with the test compounds in dissolved/suspensions of 80% PEG 400 and 20% Tween 20 surfactant (PEGTW) for 14 days. Two control groups, one intact and one castrated are doses orally only with the PEGTW vehicle. Animals are dosed (v/w) at 0.5 ml of vehicle /100g body weight. Experimental groups are 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).

[00131] At the end of the 14-day treatment, the animals are sacrificed, and the ventral prostate, the seminal vesicles, and the levator ani are removed surgically and weighed. To compare data from different experiments, the organs weights are 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.

[00132] Rat luteinizing hormone (rLH) is quantitatively determined with the Biotrak [¹²⁵I] kit (Amersham Pharmacia Biotek), following the manufacturer directions. The assay is based on the competition by the LH present in the serum of the binding of [¹²⁵I] 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.

[00133] 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.

5 **CWR22 Human Prostate Xenograft Assay:**

[00134] *In Vivo* Antitumor Testing: CWR22 human prostate tumors are maintained in Balb/c nu/nu nude mice. Tumors are propagated as subcutaneous transplants in adult male nude mice (4-6 weeks old) using tumor fragments obtained from donor mice. Tumor passage occurs every 5-6 weeks.

10 [00135] For antitumor efficacy trial, the required number of animals needed to detect a meaningful response are pooled at the start of the experiment and each is given a subcutaneous implant of a tumor fragment (~50 mg) with a 13-gauge trocar. Tumors are allowed to grow to approx. 100-200 mg (tumors outside the range were excluded) and animals are evenly distributed to various treatment and control groups.

15 Treatment of each animal is based on individual body weight. Treated animals are checked daily for treatment related toxicity/mortality. Each group of animals is weighed before the initiation of treatment (W_{t1}) and then again following the last treatment dose (W_{t2}). The difference in body weight ($W_{t2}-W_{t1}$) provides a measure of treatment-related toxicity.

20 [00136] Tumor response is 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) are estimated from the formula: Tumor weight = $(\text{length} \times \text{width}^2) \div 2$.

[00137] Tumor response end-point is expressed in terms of tumor growth inhibition (%T/C), defined as the ratio of median tumor weights of the treated tumors (T) to that
25 of the control group (C).

[00138] To estimate tumor cell kill, the tumor volume doubling time is first calculated with the formula:

$$\text{TVDT} = [(\text{Median time (days) for control tumors to reach target size}) - (\text{Median time (days) for control tumors to reach half the target size})].$$

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

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

[00139] In general, preferred compounds of the present invention, such as particular compounds disclosed in the following examples, have been identified to modulate the activity of one or more of nuclear hormone receptors.

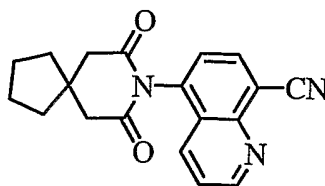
5 [00140] The spirocyclic compounds of the present invention that are described in the examples herein, have been tested in the one or more assay(s) described above and have been identified to modulate the activity of one or more nuclear hormone receptors. Potencies can be calculated and expressed as either inhibition constants (K_i values) or as IC_{50} (inhibitory concentration 50%) values, and refer to activity measured employing the *in vitro* assay systems described herein. Exemplary values
10 for compounds that inhibit activity in one or more assays include concentrations equivalent to or more potent than 20 μ M, preferably 10 μ M, and more preferably 1 μ M.

[00141] The following Examples illustrate embodiments of the present invention, and are not intended to limit the scope of the claims.

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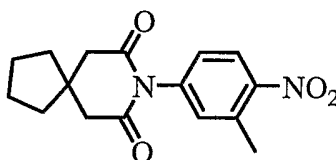
ABBREVIATIONS

The following abbreviations are used herein:
EtOAc = ethyl acetate
g = gram
h = hours
HPLC = high pressure liquid chromatography
Me = methyl
mL = milliliter
mmol = millimole
MS (ES) = Electro-Spray Mass Spectrometry

Example 1**5-(7,9-Dioxo-8-aza-spiro[4.5]dec-8-yl)-quinoline-8-carbonitrile**

[00142] 5-Amino-8-cyanoquinoline (0.125 g, 0.74 mmol) and 3,3-
5 tetramethyleneglutaric anhydride (0.250 g, 1.49 mmol) were suspended in 1,4-
dioxane (1.0 mL) in a high pressure tube. TEA (0.5 mL) and MgSO_4 (0.100 g) were
then added and the reaction was heated to 150°C for 12 h. After 12 h, the mixture
was cooled to 22°C and filtered rinsing with EtOAc (10 mL). The resulting solution
was concentrated in vacuo to give a brown oil. The crude product was purified by
10 flash chromatography on SiO_2 using gradient elution (0 – 10 – 20% acetone in
chloroform) to give 0.134 g of Example 1 as a yellow solid. HPLC: 100% at 2.513
min (retention time) (YMC S5 ODS column 4.6 x 50 mm, 10-90% aqueous methanol
over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm),
MS (ES): m/z 320.23 $[\text{M}+\text{H}]^+$.

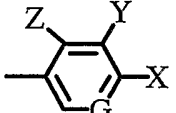
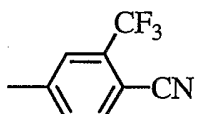
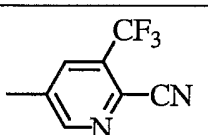
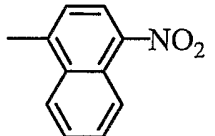
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Example 2**8-(3-Methyl-4-nitro-phenyl)-8-aza-spiro[4.5]decane-7,9-dione**

[00143] 4-Nitro-3-methylaniline (0.0152 g, 0.100 mmol) and 3,3-
20 tetramethyleneglutaric anhydride (0.025 g, 0.150 mmol) were dissolved in AcOH (0.5
mL) and heated at 110°C for 10 h. The mixture was then cooled to 22°C and
quenched with cold saturate aqueous K_2CO_3 resulting in precipitation of the
intermediate acid/amide. The intermediate acid/amide was filtered, rinsed with water,
and dried in a vacuum oven for 14 h. The intermediate acid/amide was dissolved in
25 acetic anhydride (0.5 mL) and heated at 135°C for 3 h. The reaction was cooled to
22°C and poured into cold saturated aqueous K_2CO_3 and stirred vigorously. After 10
min, the solution was filtered rinsing with water. The crude product was dried in a

vacuum oven for 14 h. The crude material was purified by preparative thin-layer chromatography eluting with 100% methylene chloride to give 0.021 g of Example 2 as a tan solid. HPLC: 100% at 2.840 min (retention time) (YMC S5 ODS column 4.6 x 50 mm, 10-90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm), MS (ES): m/z 303.50 [M+H]⁺.

Table 2

Ex. No.		Compound Name	Retention Time Min.	Procedure of Example
3		4-(7,9-Dioxo-8-aza-spiro[4.5]dec-8-yl)-2-trifluoromethyl-benzonitrile	3.020 LC 335.19 [M-H] ⁺	1
4		5-(7,9-Dioxo-8-aza-spiro[4.5]dec-8-yl)-3-trifluoromethyl-pyridine-2-carbonitrile	3.157 LC 338.18 [M+H] ⁺	1
5		8-(4-Nitro-naphthalen-1-yl)-8-aza-spiro[4.5]decane-7,9-dione.	3.123 LC 339.24 [M+H] ⁺	2

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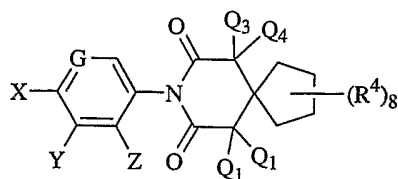
Table 3

Example #	¹ H NMR 400 MHz, CDCl ₃ (ppm)
1	9.15 (1H, dd, J = 1.7, 4.3 Hz) 8.20 (1H, d, J = 7.7 Hz) 7.90 (1H, dd, J = 1.7, 8.6 Hz) 7.57 (1H, dd, J = 4.3, 8.6 Hz) 7.39 (1H, d, J = 7.7 Hz) 2.90 (4 H, m) 1.9 (8H, m)
2	8.0 (1 H, d, J = 9.2 Hz) 7.01 (2 H, m) 2.71 (4 H, s) 2.54 (3 H, s) 1.72 (4 H, m) 1.59 (4 H, m)
3	7.85 (1 H, d, J = 8.2 Hz) 7.46 (1 H, d, J = 1.5 Hz)

Example #	¹ H NMR 400 MHz, CDCl ₃ (ppm)
	7.36 (1 H, dd, J = 1.5, 8.2 Hz) 2.73 (4 H, s) 1.73 (4 H, m) 1.59 (4 H, m)
4	8.64 (1 H, d, J = 2.2 Hz) 7.89 (1 H, d, J = 2.2 Hz) 2.83 (4 H, s) 1.80 (8 H, m)
5	8.57 (1 H, d, J = 8.8 Hz) 8.25 (1 H, d, J = 7.7 Hz) 7.74 (1 H, m) 7.63 (2 H, m) 7.32 (1 H, d, 8.2 Hz) 2.90 (4 H, m) 1.85 (6 H, m) 1.75 (2 H, m)

What is claimed is:

1. A compound having formula (I):



(I)

- 5 wherein the symbols have the following meanings, and are, for each occurrence, independently selected:
 - G is CH or N;
 - X and Y are independently selected from H, CN, NO₂, F, Cl, Br, I, CF₃, CF₂CF₃, R¹, OR¹, COR¹, CONR¹R^{1'}, and/or NR¹R^{1'};
 - 10 Z is selected from H, CN, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, or CF₂CF₃;
 - where zero or one of X, Y, or Z is H;
 - either X and Y, or Y and Z can be fused to form a ring of 5 or 6 atoms that can be aryl, substituted aryl, heterocyclic, or heterocyclic substituted with one or more methyl or ethyl groups;
 - 15 Q₁, Q₂, Q₃, and Q₄ are independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, arylalkyl, substituted arylalkyl, and/or OR²;
 - R¹ and R^{1'} are independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, and/or substituted arylalkyl;
 - R² is selected from alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, R¹C=O, R¹OC=O, -N(R¹)HC=O, -C=ONR¹R^{1'}, -SO₂R¹, -SO₂OR¹, or -SO₂NR¹R^{1'}; and
 - each R⁴ is independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, and/or substituted arylalkyl;
 - 20
 - 25
 - 30

substituted aryl, arylalkyl, substituted arylalkyl, $R^1C=O$, $R^1OC=O$, $-N(R^1)HC=O$, $-SO_2R^1$, $-SOR^1$, $-C=ONR^1R^{1'}$, $-SO_2OR^1$, and/or $-SO_2NR^1R^{1'}$;

with the following provisos:

(1) when G is CH and Z is H, then X and Y cannot be fused to form an unsubstituted aryl ring;

(2) when G is CH and X is H, then Y and Z cannot be fused to form an unsubstituted aryl ring;

(3) when G is CH, Y is CH_3 , and Z is H, then X is not Br; and

(4) when G is CH, Y is H, and Z is F, then X is not Cl.

10

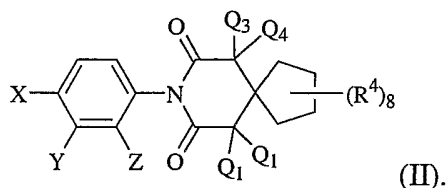
2. The spirocyclic compound according to claim 1 wherein:

X and Y are independently selected from H, CH, NO_2 , F, Cl, Br, I, CH_3 , CH_2CH_3 , CF_3 , and/or CF_2CF_3 ; and

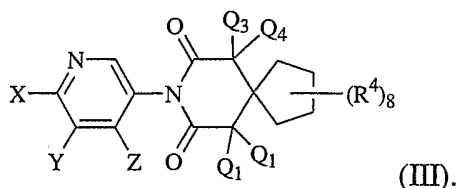
Z is H, CH, F, Cl, Br, I, CH_3 , CH_2CH_3 , CF_3 , or CF_2CF_3 .

15

3. The spirocyclic compound according to claim 1 having formula (II):



4. The spirocyclic compound according to claim 1 having formula (III):



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5. The spirocyclic compound according to claim 1 selected from:

5-(7,9-Dioxo-8-aza-spiro[4.5]dec-8-yl)-quinoline-8-carbonitrile;

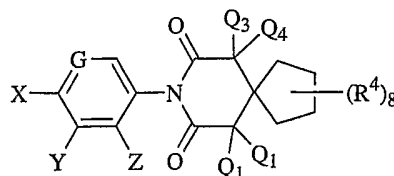
4-(7,9-Dioxo-8-aza-spiro[4.5]dec-8-yl)-2-trifluoromethyl-benzonitrile;

25 5-(7,9-Dioxo-8-aza-spiro[4.5]dec-8-yl)-3-trifluoromethyl-pyridine-2-carbonitrile;

8-(4-Nitro-naphthalen-1-yl)-8-aza-spiro[4.5]decane-7,9-dione; or

8-(3-Methyl-4-nitro-phenyl)-8-aza-spiro[4.5]decane-7,9-dione.

6. A pharmaceutical composition capable of treating a NHR-associated condition, comprising a compound of formula (I):



(I)

5 wherein the symbols have the following meanings, and are, for each occurrence, independently selected:

G is CH or N;

X and Y are independently selected from H, CN, NO₂, F, Cl, Br, I, CF₃, CF₂CF₃, R¹, OR¹, COR¹, CONR¹R^{1'}, and/or NR¹R^{1'};

10 Z is selected from H, CN, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, or CF₂CF₃;

where zero or one of X, Y, or Z is H;

either X and Y, or Y and Z can be fused to form a ring of 5 or 6 atoms that can be aryl, substituted aryl, heterocyclic, or heterocyclic substituted with one or more methyl or ethyl groups;

15 Q₁, Q₂, Q₃, and Q₄ are independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, arylalkyl, substituted arylalkyl, and/or OR²;

R¹ and R^{1'} are independently selected from alkyl, substituted alkyl, alkenyl, 20 substituted alkenyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, and/or substituted arylalkyl;

R² is selected from alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, 25 heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, R¹C=O, R¹OC=O, -N(R¹)HC=O, -C=ONR¹R^{1'}, -SO₂R¹, -SO₂OR¹, or -SO₂NR¹R^{1'}; and

each R⁴ is independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, 30 substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl,

substituted aryl, arylalkyl, substituted arylalkyl, $R^1C=O$, $R^1OC=O$, $-N(R^1)HC=O$, $-SO_2R^1$, $-SOR^1$, $-C=ONR^1R^{1'}$, $-SO_2OR^1$, and/or $-SO_2NR^1R^{1'}$;

with the following provisos:

- (1) when G is CH and Z is H, then X and Y cannot be fused to form an unsubstituted aryl ring;
 - (2) when G is CH and X is H, then Y and Z cannot be fused to form an unsubstituted aryl ring;
 - (3) when G is CH, Y is CH_3 , and Z is H, then X is not Br; and
 - (4) when G is CH, Y is H, and Z is F, then X is not Cl;
- or a prodrug, pharmaceutically acceptable salt, or solvate thereof; and a pharmaceutically acceptable carrier.

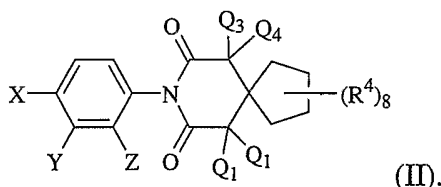
7. The pharmaceutical composition according to claim 6, wherein:

X and Y are independently selected from H, CH, NO_2 , F, Cl, Br, I, CH_3 , CH_2CH_3 , CF_3 , and/or CF_2CF_3 ; and

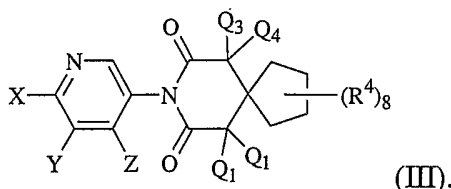
Z is H, CH, F, Cl, Br, I, CH_3 , CH_2CH_3 , CF_3 , or CF_2CF_3 .

8. The pharmaceutical composition according to claim 6, wherein the

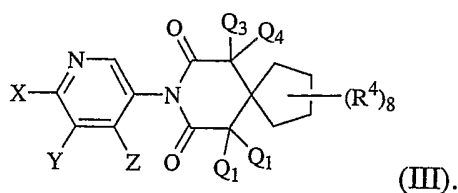
spirocyclic compound has formula (II):



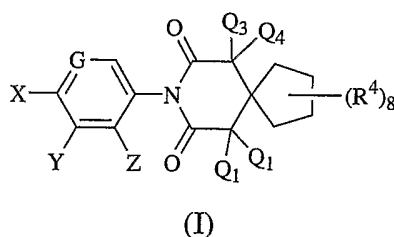
9. The spirocyclic compound according to claim 6 having formula (III):



10. The pharmaceutical composition according to claim 6, wherein the spirocyclic compound has formula (III):



11. A method of modulating the function of a nuclear hormone receptor which comprises administering to a mammalian species in need thereof an effective
5 nuclear hormone receptor modulating amount of a compound of the following formula (I):



- wherein the symbols have the following meanings, and are, for each occurrence,
10 independently selected:
- G is CH or N;
- X and Y are independently selected from H, CN, NO₂, F, Cl, Br, I, CF₃, CF₂CF₃, R¹, OR¹, COR¹, CONR¹R^{1'}, and/or NR¹R^{1'};
- Z is selected from H, CN, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, or CF₂CF₃;
- 15 where zero or one of X, Y, or Z is H;
- either X and Y, or Y and Z can be fused to form a ring of 5 or 6 atoms that can be aryl, substituted aryl, heterocyclic, or heterocyclic substituted with one or more methyl or ethyl groups;
- Q₁, Q₂, Q₃, and Q₄ are independently selected from H, alkyl, substituted alkyl,
20 cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, arylalkyl, substituted arylalkyl, and/or OR²;
- R¹ and R^{1'} are independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted
25 heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, and/or substituted arylalkyl;
- R² is selected from alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl,

heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, $R^1C=O$, $R^1OC=O$, $-N(R^1)HC=O$, $-C=ONR^1R^{1'}$, $-SO_2R^1$, $-SO_2OR^1$, or $-SO_2NR^1R^{1'}$; and

- 5 each R^4 is independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, $R^1C=O$, $R^1OC=O$, $-N(R^1)HC=O$, $-SO_2R^1$, $-SOR^1$, $-C=ONR^1R^{1'}$, $-SO_2OR^1$, and/or $-SO_2NR^1R^{1'}$;
- 10 with the following provisos:

(1) when G is CH and Z is H, then X and Y cannot be fused to form an unsubstituted aryl ring;

(2) when G is CH and X is H, then Y and Z cannot be fused to form an unsubstituted aryl ring;

- 15 (3) when G is CH, Y is CH_3 , and Z is H, then X is not Br; and

(4) when G is CH, Y is H, and Z is F, then X is not Cl;

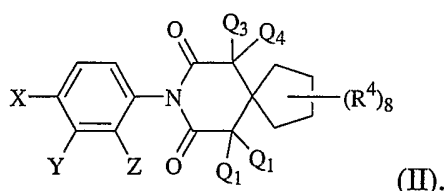
or a prodrug, pharmaceutically acceptable salt, or solvate thereof.

12. The method according to claim 11, wherein:

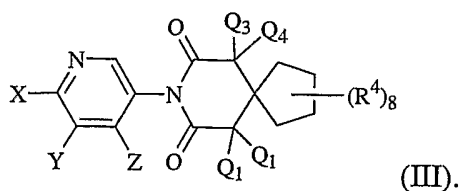
- 20 X and Y are independently selected from H, CH, NO_2 , F, Cl, Br, I, CH_3 , CH_2CH_3 , CF_3 , and/or CF_2CF_3 ; and

Z is H, CH, F, Cl, Br, I, CH_3 , CH_2CH_3 , CF_3 , or CF_2CF_3 .

13. The method according to claim 11, wherein the spirocyclic compound
- 25 has formula (II):



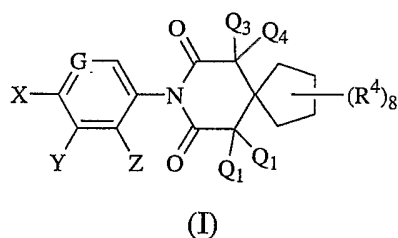
14. The method according to claim 11, wherein the spirocyclic compound has formula (III):



15. The method of claim 11 wherein the nuclear hormone receptor is selected from estrogen receptor, progesterone receptor, glucocorticoid receptor,
 5 mineralocorticoid receptor, aldosterone receptor, or steroid and xenobiotic receptor.

16. The method of claim 11 wherein said nuclear hormone receptor is androgen receptor or mutated androgen receptor.

10 17. A method for treating a condition or disorder comprising administering to a mammalian species in need thereof a therapeutically effective amount of a compound of the following formula (I):



15 wherein the symbols have the following meanings, and are, for each occurrence, independently selected:

G is CH or N;

X and Y are independently selected from H, CN, NO₂, F, Cl, Br, I, CF₃, CF₂CF₃, R¹, OR¹, COR¹, CONR¹R^{1'}, and/or NR¹R^{1'};

20 Z is selected from H, CN, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, or CF₂CF₃;

where zero or one of X, Y, or Z is H;

either X and Y, or Y and Z can be fused to form a ring of 5 or 6 atoms that can be aryl, substituted aryl, heterocyclic, or heterocyclic substituted with one or more methyl or ethyl groups;

25 Q₁, Q₂, Q₃, and Q₄ are independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, arylalkyl, substituted arylalkyl, and/or

OR²;

R¹ and R^{1'} are independently selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, and/or substituted arylalkyl;

R² is selected from alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, R¹C=O, R¹OC=O, -N(R¹)HC=O, -C=ONR¹R^{1'}, -SO₂R¹, -SO₂OR¹, or -SO₂NR¹R^{1'}; and

each R⁴ is independently selected from H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclo, substituted heterocyclo, cycloalkylalkyl, substituted cycloalkylalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, R¹C=O, R¹OC=O, -N(R¹)HC=O, -SO₂R¹, -SOR¹, -C=ONR¹R^{1'}, -SO₂OR¹, and/or -SO₂NR¹R^{1'};

with the following provisos:

(1) when G is CH and Z is H, then X and Y cannot be fused to form an unsubstituted aryl ring;

(2) when G is CH and X is H, then Y and Z cannot be fused to form an unsubstituted aryl ring;

(3) when G is CH, Y is CH₃, and Z is H, then X is not Br; and

(4) when G is CH, Y is H, and Z is F, then X is not Cl;

or a prodrug, pharmaceutically acceptable salt, or solvate thereof;

wherein said condition or disorder is selected from the group consisting of:

proliferate diseases, cancers, benign prostate hypertrophy, adenomas and neoplasies of the prostate, benign or malignant tumor cells containing an androgen receptor, heart disease, angiogenic conditions or disorders, hirsutism, acne, hyperpilosity, inflammation, immune modulation, seborrhea, endometriosis, polycystic ovary syndrome, androgenic alopecia, hypogonadism, osteoporosis, suppressing spermatogenesis, libido, cachexia, anorexia, inhibition of muscular atrophy in ambulatory patients, androgen supplementation for age related decreased testosterone levels in men, cancers expressing an estrogen receptor, prostate cancer, breast cancer,

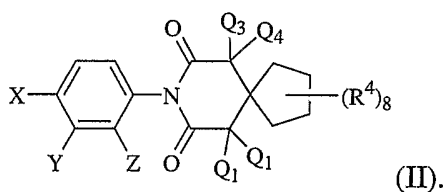
endometrial cancer, hot flushes, vaginal dryness, menopause, amenorrhea, dysmenorrhea, contraception, pregnancy termination, cancers containing a progesterone receptor, menopause, cyclesynchrony, meningioma, fibroids, labor induction, autoimmune disease, Alzheimer's disease, psychotic disorders, drug
 5 dependence, non-insulin dependent Diabetes Mellitus, dopamine receptor mediated disorders, congestive heart failure, dysregulation of cholesterol homeostasis, and attenuation of metabolism of a pharmaceutical agent.

18. The method according to claim 17, wherein:

10 X and Y are independently selected from H, CH, NO₂, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, and/or CF₂CF₃; and

Z is H, CH, F, Cl, Br, I, CH₃, CH₂CH₃, CF₃, or CF₂CF₃.

19. The method according to claim 17, wherein the spirocyclic compound
 15 has formula (II):



20. The method according to claim 17, wherein the spirocyclic compound has formula (III):

