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(54) Title: THYROTROPIN RELEASING HORMONE RECEPTOR-OREXIN RECEPTOR HETERO-DIMERS/-OLIGOMERS

(57) Abstract: A hetero-dimeric or hetero-oligomeric receptor, comprising at least one thyrotropin releasing hormone receptor subunit associated with at least one orexin receptor subunit.

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THYROTROPIN RELEASING HORMONE RECEPTOR-OREXIN RECEPTOR HETERO-DIMERS/-OLIGOMERS

Field of the Invention

The present invention relates to a hetero-dimeric or hetero-oligomeric receptor,
5 comprising at least one thyrotropin releasing hormone receptor subunit associated
with at least one orexin receptor subunit.

Background Art

Proteins do not act in isolation in a cell, but in stable or transitory complexes, with
protein-protein interactions being key determinants of protein function (Auerbach
10 *et al.*, (2002), *Proteomics*, 2, 611-623). Furthermore, proteins and protein
complexes interact with other cellular components like DNA, RNA and small
molecules. Understanding both the individual proteins involved in these
interactions and their interactions are important for a better understanding of
biological processes.

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The functions of thyrotropin-releasing hormone (TRH) in the central nervous
system (CNS) are reported by Gary (Gary, Keith A., et al., The Thyrotropin-
Releasing Hormone (TRH) Hypothesis of Homeostatic Regulation: Implications for
TRH-Based Therapeutics, JPET 305:410–416, 2003) as four anatomically distinct
20 components that together comprise a general TRH homeostatic system, being 1)
the hypothalamic-hypophysiotropic neuroendocrine system, 2) the
brainstem/midbrain/spinal cord system, 3) the limbic/cortical system, and 4) the
chronobiological system.

25 Gary further notes that “an appreciation of the global function of TRH to modulate
and normalize CNS activity, along with an appreciation of the inherent limitations
of TRH itself as a therapeutic agent, leads to rational expectations of therapeutic
benefit from metabolically stable TRH-mimetic drugs in a remarkably broad
spectrum of clinical situations, both as monotherapy and as an adjunct to other
30 therapeutic agents”.

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Narcolepsy with cataplexy is associated with low or undetectable levels of cerebrospinal fluid (CSF) orexin A levels in about 90% of patients (Baumann and Bassetti (2005) *Sleep Medicine Reviews* 9, 253-268). Mutations of the orexin receptor 2 gene lead to familial canine narcolepsy and a loss of orexin neurons and low CSF orexin A were observed with sporadic canine narcolepsy. Neurological disorders arising from acute traumatic brain injury, Guillain-Barre syndrome and advanced Parkinson's syndrome may also be linked with low or undetectable levels of CSF orexin A levels in some instances. Sakurai has postulated a role for the orexin system in feeding and energy homeostasis as the activity of orexin neurons is inhibited by glucose and leptin, and stimulated by ghrelin, a stomach-derived peptide which promotes feeding. This may have implications for the treatment of obesity (Sakurai (2005) *Sleep Medicine Reviews* 9, 231-241).

The preceding discussion is intended only to facilitate an understanding of the invention. It should not be construed as in any way limiting the scope or application of the following description of the invention, nor should it be construed as an admission that any of the information discussed was within the common general knowledge of the person skilled in the appropriate art at the priority date.

Disclosure of the Invention

The inventors have discovered that the orexin receptor and the thyrotropin releasing hormone receptor associate. This has important implications regarding therapies for ailments associated with either receptor.

Recent studies have shown that GPCRs may not only act as monomers but also as homo- and hetero-dimers which causes altered ligand binding, signalling and endocytosis (Rios *et al.* (2000) *Pharmacol. Ther.* 92, 71-87). The effect of drugs acting as agonists or antagonists of a specific receptor may therefore depend on the binding partners of this receptor. It may be desirable to limit the effect of a drug to a cellular response mediated by a specific receptor dimer. As Milligan (Milligan, (2006), *Drug Discovery Today*, 11, 541-549) observes, while homo-dimerisation and -oligomerisation have limited implications for the drug discovery

industry, “differential pharmacology, function and regulation of GCPR hetero-dimers and –oligomers suggest means to selectively target GPCRs in different tissues and hint that the mechanism of function of several pharmacological agents might be different *in vivo* than anticipated from simple ligand screening programmes that rely on heterologous expression of a single GPCR”.

The phrase “thyrotropin releasing hormone receptor” or “TRHR” is to be understood to at least include the G protein-coupled receptor analogous to that activated by the thyrotropin releasing hormone (TRH) in the thyrotrope cells of the anterior pituitary gland, as well as a number of structures in the central nervous system (Riehl *et al.* (2000) *Neuropsychopharmacology* 23, 34-45), that has, among other roles, a major regulatory role in stimulating the synthesis and secretion of thyrotropin (thyroid-stimulating hormone; TSH) and is synonymous with thyrotropin releasing hormone receptor 1 (TRHR1) (Gershengorn (2003) Thyrotropin-releasing hormone receptor signaling, in *Encyclopedia of hormones*. Eds Henry HL and Norman AW. Academic Press. Vol 3; 502-510). The phrase “thyrotropin releasing hormone receptor” or “TRHR” is also to be understood to mean thyrotropin releasing hormone receptor 2 or TRHR2, a second subtype of thyrotropin releasing hormone receptor known to be expressed at least in the rat and mouse and whose function is yet to be clearly elucidated (Gershengorn (2003) Thyrotropin-releasing hormone receptor signaling, in *Encyclopedia of hormones*. Eds Henry HL and Norman AW. Academic Press. Vol 3; 502-510). The phrase “thyrotropin releasing hormone receptor” or “TRHR” is to be further understood to include newly discovered TRHR family members. Throughout the examples, thyrotropin releasing hormone receptor and the acronym TRHR refers to TRHR1.

The phrase “orexin receptor” or “OxR” is to be understood to mean either orexin receptor 1 (OxR1; OXR1; OX₁R; hypocretin-1-receptor; hcrt1) or orexin receptor 2 (OxR2; OXR2; OX₂R; hypocretin-2-receptor; hcrt 2) being G protein-coupled receptors analogous to those described by Sakurai *et al.* to be activated by orexin A (OxA; hypocretin-1; Hcrt-1) and orexin B (OxB; hypocretin-2; Hcrt-2) (Sakurai *et al.* (1998) *Cell* 92, 573-585). “Orexin receptor” or “OxR” is to be further

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understood to include newly discovered orexin receptor family members.

In a first aspect of the invention, there is provided a hetero-dimeric or hetero-oligomeric receptor, comprising at least one thyrotropin releasing hormone
5 receptor subunit associated with at least one orexin receptor subunit.

In a second aspect of the invention, there is provided a method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor
10 agonist, inverse agonist or antagonist.

In one embodiment, the thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist may be co-administered with an orexin receptor agonist, inverse agonist or antagonist.

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In a third aspect of the invention, there is provided method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by administering a therapeutically effective amount of an orexin receptor agonist, inverse agonist or antagonist.

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In one embodiment, the orexin receptor agonist, inverse agonist or antagonist may be co-administered with a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

25 In a fourth aspect of the invention, there is provided a method for the manufacture of a medicament for the treatment of a patient suffering from an orexin-related ailment comprising use of a therapeutically effective amount of a thyrotropin releasing hormone receptor agonist, inverse agonist or antagonist.

30 In one embodiment, the medicament may contain an orexin receptor agonist, inverse agonist or antagonist.

In a fifth aspect of the invention, there is provided a method for the manufacture of

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a medicament for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment comprising use of a therapeutically effective amount of an orexin receptor agonist, inverse agonist or antagonist.

- 5 In one embodiment, the medicament may contain a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

In a sixth aspect of the invention, there is provided a method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin releasing hormone-selective binding agent, or
10 fragment thereof.

In one embodiment, the thyrotropin releasing hormone-selective binding agent is an antibody, including a humanised antibody, a polyclonal antibody, a monoclonal
15 antibody, a chimeric antibody, a CDR-grafted antibody and/or an anti-idiotypic antibody.

In a seventh aspect of the invention, there is provided a method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by administering a therapeutically effective amount of an orexin-selective binding
20 agent, or fragment thereof.

In one embodiment, the orexin-selective binding agent is an antibody, including a humanised antibody, a polyclonal antibody, a monoclonal antibody, a chimeric
25 antibody, a CDR-grafted antibody and/or an anti-idiotypic antibody.

In an eighth aspect of the invention, there is provided a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective activity, the method comprising the steps of:

- 30 a) determining whether, and/or the extent to which, the test compound interacts with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor; and
b) if the test compound interacts with the orexin receptor while the orexin

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receptor is associated with the thyrotropin releasing hormone receptor, determining whether, or the extent to which the test compound interacts with the orexin receptor in the absence of the thyrotropin releasing hormone receptor;

- 5 such that a test compound that exhibits greater affinity and/or potency and/or efficacy when interacting with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.
- 10 In a ninth aspect of the invention, there is provided a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective activity, the method comprising the steps of:
- a) determining whether, and/or the extent to which, the test compound interacts with the thyrotropin releasing hormone receptor while the
 - 15 thyrotropin releasing hormone receptor is associated with the orexin receptor; and
 - b) if the test compound interacts with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor, determining whether, or the extent to which the
 - 20 test compound interacts with the thyrotropin releasing hormone receptor in the absence of the orexin receptor;
- such that a test compound that exhibits greater affinity and/or potency and/or efficacy when interacting with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor
- 25 is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.

- In a tenth aspect of the invention, there is provided a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-
- 30 dimer/-oligomer selective antagonism or partial agonism, the method comprising the steps of:

- a) determining whether, and/or the extent to which, the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone

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receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:

- i). a first agent, comprising the orexin receptor coupled to a first reporter component;
- 5 ii). a second agent, comprising an interacting group coupled to a second reporter component;
- iii). a third agent, comprising the thyrotropin releasing hormone receptor;
- 10 iv). an agonist of the orexin receptor, the thyrotropin releasing hormone receptor and/or the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;

wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the thyrotropin releasing hormone receptor;

- 15 b) detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 20 c) if the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, determining whether, or the extent to which the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and
- 25 the orexin receptor in the absence of the thyrotropin releasing hormone receptor; such that a test compound that exhibits greater antagonistic or partial agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.
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In an eleventh aspect of the invention, there is provided a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective antagonism or partial agonism, the method

35 comprising the steps of:

- a) determining whether, and/or the extent to which, the test compound is an antagonist or partial agonist of the thyrotropin releasing

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hormone receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:

- i). a first agent, comprising the thyrotropin releasing hormone receptor coupled to a first reporter component;
- 5 ii). a second agent, comprising an interacting group coupled to a second reporter component;
- iii). a third agent, comprising the orexin receptor;
- iv). an agonist of the orexin receptor, the thyrotropin releasing hormone receptor and/or the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 10 wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the orexin receptor;
- b). detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 15 c) if the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, determining whether, or the extent to which the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and the orexin receptor in the absence of the thyrotropin releasing hormone receptor; such that a test compound that exhibits greater antagonistic or partial agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.
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30 In a twelfth aspect of the invention, there is provided a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective inverse agonism, the method comprising the steps of:

- a) determining whether, and/or the extent to which, the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:
- 35

- 5 i). a first agent, comprising the orexin receptor coupled to a first reporter component;
- ii). a second agent, comprising an interacting group coupled to a second reporter component;
- iii). a third agent, comprising a constitutively active thyrotropin releasing hormone receptor;
- 10 wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the thyrotropin releasing hormone receptor;
- 15 b) detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- c) if the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, determining whether, or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and the orexin receptor in the absence of the thyrotropin releasing hormone receptor; such that a test compound that exhibits greater inverse agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.
- 25

In a thirteenth aspect of the invention, there is provided a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer inverse agonism, the method comprising the steps of:

- 30 a) determining whether, and/or the extent to which, the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:
- i). a first agent, comprising the thyrotropin-releasing hormone receptor coupled to a first reporter component;
- 35 ii). a second agent, comprising an interacting group coupled to a second reporter component;

- 10 -

- iii). a third agent, comprising a constitutively active orexin receptor; wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the orexin receptor;
- 5 b) detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 10 c) if the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, determining whether, or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and the orexin receptor in the absence of the thyrotropin releasing hormone receptor; such that a test compound that
- 15 exhibits greater inverse agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.
- 20 In the methods of the eighth, ninth, tenth, eleventh, twelfth and thirteenth aspects of the invention, the step of determining whether, and/or the extent to which, the test compound interacts with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor; and/or the step of determining whether, and/or the extent to which, the test
- 25 compound interacts with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor may be performed by way of one or more of the methods described in the applicant's co-pending international patent application "Detection System and Uses Therefor", which derives priority from the same Australian provisional patent application
- 30 2006906292.

In a fourteenth aspect of the invention, there are provided selective agonists and/or antagonists and/or inverse agonists of the thyrotropin releasing hormone receptor/orexin receptor hetero-dimer/-oligomer.

Brief Description of the Drawings

Figures 1 to 3 are illustrative of the technique by which the association of thyrotropin releasing hormone receptor and the orexin receptor was detected.

- 5 Figure 1 shows the composition of the agents forming the basis of the system for detecting molecular associations: A first agent comprises a first interacting group coupled to a first reporter component; a second agent comprises a second interacting group coupled to a second reporter component; and a third agent comprises a third interacting group.

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Figure 2 shows how the administration of the modulator modulates the association of the second interacting group with the third interacting group, preferably by interacting with the third interacting group, either alone, or simultaneously with the first interacting group.

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Figure 3 shows that if the first and third interacting groups are associated, modulation of the association of the second and third interacting groups consequently modulates the proximity of the first and second reporter components thereby modulating the signal that is able to be detected by the detector.

- 20 Therefore monitoring the signal generated by proximity of the first and second reporter components by the detector constitutes monitoring the association of the first and third agents. If the first and third interacting groups are not associated, the first and second reporter components will remain spatially separated and generation of a detectable signal is unlikely.

25

- Figure 4 shows the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and a range of different GPCRs as IG3. eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc and barr2/Venus with either pcDNA3, orexin receptor 2 (OxR2), CXC chemokine receptor 2 (CXCR2), hemagglutinin epitope-tagged melanocortin receptor 3 or 4 (HA-MC3R or HA-MC4R), or dopamine D2 receptor long form (D2LR) or short form (D2SR) following the treatment of each with their respective ligands. The different ligand treatment (10^{-6} M) for each
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receptor was thyrotropin releasing hormone (TRH) for TRHR/Rluc (with pcDNA3); orexin A (OxA) for OXR2; interleukin-8 (IL-8) for CXCR2; alpha-melanocyte-stimulating hormone (α -MSH) for HA-MC3R, HA-MC4R; and bromocriptine (BROM) for D2LR and D2SR.

5

Figure 5 shows the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc as RC1, either beta-arrestin 1 (barr1) or beta-arrestin 2 (barr2) as IG2, EGFP as RC2 and OxR2 as IG3. eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc and EGFP/barr1 or
10 EGFP/barr2 with either pcDNA3 or OXR2. Ligand treatments were either OxA or TRH only or both OxA and TRH combined. Phosphate-buffered saline (PBS) was used as a vehicle control.

Figure 6 shows the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and OxR1 or OxR2 as IG3. eBRET measurements were carried out at 37C on HEK293 cells transiently co-expressing TRHR/Rluc and barr2/Venus with either pcDNA3, OxR1 or OxR2 following pretreatment with 10^{-6} M OxR1-selective antagonist, SB-334867-A, for approximately 40 minutes and then 10^{-6} M OxA (IG3 ligand; modulator) or 10^{-6} M
20 TRH (IG1 ligand), or both, was added. Where antagonist was not preincubated, cells were treated with PBS instead for the same amount of time.

Figure 7 shows the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc as RC1, beta-arrestin 1 (barr1) or beta-arrestin 2 (barr2) as IG2, EGFP as RC2
25 and hemagglutinin epitope-tagged OxR2 (HA-OxR2) as IG3. eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc and EGFP/barr1 or EGFP/barr2 with either pcDNA3 or HA-OxR2. Ligand treatments were either OxA or TRH only. Phosphate-buffered saline (PBS) was used as a vehicle control.

30

Figure 8 shows the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc as RC1, beta-arrestin 1 (barr1) or beta-arrestin 1 phosphorylation-independent mutant R169E (barr1R169E) as IG2, EGFP as RC2 and OxR2 as IG3. eBRET

measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc and EGFP/barr1 or EGFP/barr1R169E with either pcDNA3 or OxR2. Ligand treatments were either OxA or TRH only. Phosphate-buffered saline (PBS) was used as a vehicle control.

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Figure 9 shows the thyrotropin releasing hormone receptor truncated at amino acid 335 (TRHR335) as IG1, Rluc as RC1, beta-arrestin 1 (barr1) as IG2, EGFP as RC2 and OxR2 or TRHR as IG3. eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR335/Rluc and EGFP/barr1
10 with either OxR2 or TRHR. Ligand treatments were either OxA or TRH only.

Figure 10 shows a dose-response curve for the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and in the absence of IG3. BRET measurements at 37C were carried out on
15 HEK293 cells transiently co-expressing TRHR/Rluc, barr2/Venus and pcDNA3 with increasing doses of TRH. Sigmoidal dose response curves were plotted using Prism (GraphPad), either assuming a Hill slope of 1 or allowing for variable slope. The EC₅₀ and Hill slope values for the variable slope curve are included in a table in the graph.

20

Figure 11 shows a dose-response curve for OxR2 as IG1, Rluc as RC1, barr2 as IG2, Venus as RC2 and in the absence of IG3. BRET measurements at 37C were carried out on HEK293 cells transiently co-expressing OxR2/Rluc, barr2/Venus and pcDNA3 with increasing doses of OxA. Sigmoidal dose response curves were
25 plotted using Prism (GraphPad), either assuming a Hill slope of 1 or allowing for variable slope. The EC₅₀ and Hill slope values for the variable slope curve are included in a table in the graph.

Figure 12 shows dose-response curves for the thyrotropin releasing hormone
30 receptor (TRHR) as IG1, Rluc as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and OxR2 as IG3. BRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc, barr2/Venus and OxR2 with increasing doses of OxA. Sigmoidal dose response curves were plotted using Prism

(GraphPad), either assuming a Hill slope of 1 or allowing for variable slope. The EC_{50} and Hill slope values for the variable slope curves are included in a table in the graph. Curves generated using coelenterazine h and EnduRen as two forms of Rluc substrate (reporter component initiator) are shown.

5

Figure 13 shows dose-response curves for TRHR as IG1, Rluc as RC1, barr1 as IG2, EGFP as RC2 in the presence or absence of OxR2 as IG3. BRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc and EGFP/barr1 in the absence of OxR2 with increasing doses of TRH, as well as HEK293 cells transiently co-expressing TRHR/Rluc and EGFP/barr1 with OxR2 with increasing doses of OxA with and without 10^{-6} M TRH. A curve mathematically generated by addition of the ligand-induced signal generated with 10^{-6} M TRH (from the TRH: TRHR/Rluc + EGFP/barr1 curve) to each of the points generated for the OxA: TRHR/Rluc + EGFP/barr1 + OxR2 curve is also plotted (TRHR/Rluc + EGFP/barr1 + OxR2: TRH (10^{-6} M) + OxA: Data calculated).

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Figure 14 shows dose-response curves for TRHR as IG1, Rluc as RC1, barr1 as IG2, EGFP as RC2 in the presence or absence of OxR2 as IG3. BRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc and EGFP/barr1 in the absence of OxR2 with increasing doses of TRH, as well as HEK293 cells transiently co-expressing TRHR/Rluc and EGFP/barr1 with OxR2 with increasing doses of OxA, or increasing doses of TRH with 10^{-6} M OxA. A curve mathematically generated by addition of the ligand-induced signal generated with 10^{-6} M OxA (from the OxA: TRHR/Rluc + EGFP/barr1 + OxR2 curve) to each of the points generated for the TRH: TRHR/Rluc + EGFP/barr1 curve is also plotted (TRHR/Rluc + EGFP/barr1 + OxR2: TRH + OxA (10^{-6} M): Data calculated).

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Figure 15 shows dose response curves for TRHR335 as IG1, Rluc as RC1, barr2 as IG2, Venus as RC2 and OxR2 as IG3. BRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR335/Rluc, barr2/Venus and OxR2 with increasing doses of TRH and OxA alone or in

30

combination.

Figure 16 shows cumulative eBRET reads over time for each combination of receptors (IG1 and IG3; data captured over 83mins). TRHR is IG1, Rluc is RC1, barr1 is IG2, EGFP is RC2 and OxR2 is IG3. The same amount of EGFP/barr1 (IG2-RC2) is transfected for each experiment. TRHR/Rluc (IG1-RC1) is transfected at a constant amount (0.1µg DNA/well) while OxR2 (IG3) is transfected at varying amounts of DNA (0, 0.01, 0.05, 0.1, 0.5, 0.7µg DNA /well). eBRET measurements at 37C were carried out on HEK293 cells following addition of 10⁻⁶M OxA (modulator) to each well. The signal is only detected when OxR2 (IG3) is expressed (no signal was recorded at 0µg OxR2).

Figure 17 shows dose response curves for TRHR as IG1, Rluc as RC1, barr2 as IG2, Venus as RC2 and OxR2 as IG3. BRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc, barr2/Venus and OxR2 with increasing doses of OxA in either 96-well or 384-well microplates.

Figure 18 shows OxR2 as IG1, Rluc8 as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and hemagglutinin epitope-tagged TRHR (HA-TRHR) as IG3. eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing OxR2/Rluc8 and barr2/Venus with either pcDNA3 or HA-TRHR. Ligand treatments were either OxA or TRH only. Phosphate-buffered saline (PBS) was used as a vehicle control. Data presented as ligand-induced BRET ratios.

Figure 19 shows the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc8 as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and hemagglutinin epitope-tagged OxR2 (HA-OxR2) as IG3. eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc8 and barr2/Venus with HA-OxR2 aliquoted into all wells of a 96-well plate. Phosphate-buffered saline (PBS) was added to the first two rows and the last two rows of the 96-well plate (48 wells in total) as a vehicle control. Data presented as fluorescence/luminescence.

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Figure 20 shows the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc8 as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and hemagglutinin epitope-tagged OxR2 (HA-OxR2) as IG3. eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc8 and barr2/Venus with HA-OxR2 aliquoted into all wells of a 96-well plate. OxA was added to the middle four rows of the 96-well plate (48 wells in total). Data presented as fluorescence/luminescence.

Figure 21 shows z-factor data for the thyrotropin releasing hormone receptor (TRHR) as IG1, Rluc8 as RC1, beta-arrestin 2 (barr2) as IG2, Venus as RC2 and hemagglutinin epitope-tagged OxR2 (HA-OxR2) as IG3. As shown in figures 19 and 20, eBRET measurements at 37C were carried out on HEK293 cells transiently co-expressing TRHR/Rluc8 and barr2/Venus with HA-OxR2 aliquoted into all wells of a 96-well plate. Phosphate-buffered saline (PBS) was added to the first two rows and the last two rows of the 96-well plate (48 wells in total) as a vehicle control. OxA was added to the middle four rows of the 96-well plate (48 wells in total). Data presented as fluorescence/luminescence.

ABBREVIATIONS

20	a-MSH	alpha-melanocyte-stimulating hormone.
	barr	beta-arrestin.
	BRET	Bioluminescence resonance energy transfer.
	BROM	Bromocriptine.
25	CB	Cannabinoid receptor.
	CCR	CC chemokine receptor.
	CCR5(5)TYFP	CCR5 linked to TYFP via a 5 amino acid linker region.
	CSF	Cerebrospinal fluid.
30	CXCR	CXC chemokine receptor.
	D2LR	Dopamine D2 receptor (long-form).
	D2SR	Dopamine D2 receptor (short-form).
	DOP	Delta opioid.

eBRET	extended BRET: BRET monitored over extended time periods.
ECFP	<u>E</u> nhanced <u>C</u> yan <u>F</u> luorescent <u>P</u> rotein, which is a variant of the <i>Aequorea victoria</i> green fluorescent protein gene (GFP).
5 EGFP	<u>E</u> nhanced <u>G</u> reen <u>F</u> luorescent <u>P</u> rotein is a red-shifted variant of wild-type GFP.
EYFP	<u>E</u> nhanced <u>Y</u> ellow <u>F</u> luorescent <u>P</u> rotein.
FRET	Fluorescence resonance energy transfer.
10 GPCRs	G-protein coupled receptors.
HA	Hemagglutinin epitope-tag.
His(6)	Histidine tag consisting of 6 consecutive histidine residues.
IG	Interacting group.
15 IL-8	Interleukin-8.
KOP	Kappa opioid.
MCP1	Monocyte chemoattractant protein 1 (CCR2 selective ligand).
MCR	Melanocortin receptor.
MIP1b	Macrophage inflammatory protein 1b (CCR5 selective ligand).
20 mRFP1	Monomeric red fluorescent protein.
OR	Opioid receptor.
OxA	Orexin A.
OxB	Orexin B.
OxR	Orexin receptor.
25 PBS	Phosphate-buffered saline.
pcDNA3	Eukaryotic expression vector.
RC	Reporter component.
REM	Rapid eye movement.
RET	Resonance energy transfer.
30 Rluc	Renilla luciferase.
Rluc8	An improved Renilla luciferase.
SWS	Slow wave sleep.
TRH	Thyrotropin releasing hormone.

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TRHR	Thyrotropin releasing hormone receptor.
TYFP	Topaz Yellow Fluorescent Protein.
Venus	An improved Yellow Fluorescent Protein.
wt	Wild type.

5 Best Mode(s) for Carrying Out the Invention

General

All publications, including patents and patent applications, cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety. However, publications mentioned herein are cited for the purpose of describing and disclosing the protocols, reagents and vectors that are reported in the publications and which may be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Furthermore, the practice of the present invention employs, unless otherwise indicated, conventional molecular biology, chemistry and fluorescence techniques, within the skill of the art. Such techniques are well known to the skilled worker, and are explained fully in the literature. See, eg., Coligan, Dunn, Ploegh, Speicher and Wingfield "Current protocols in Protein Science" (1999) Volume I and II (John Wiley & Sons Inc.); and Bailey, J.E. and Ollis, D.F., Biochemical Engineering Fundamentals, McGraw-Hill Book Company, NY, 1986; Lakowicz, J. R. Principles of Fluorescence Spectroscopy, New York : Plenum Press (1983) for fluorescence techniques.

As used herein and in the appended claims, the singular forms "a," "an," and "the" include the plural unless the context clearly dictates otherwise. Thus, for example, a reference to "a protein" includes a plurality of such proteins, and a reference to "an analyte" is a reference to one or more analytes, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to

which this invention belongs. Although any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

- 5 The invention described herein may include one or more ranges of values (e.g. size, concentration etc). A range of values will be understood to include all values within the range, including the values defining the range, and values adjacent to the range that lead to the same or substantially the same outcome as the values immediately adjacent to that value which defines the boundary to the range.

10

Throughout this specification, unless the context requires otherwise, the word "comprise" or variations, such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer, or group of integers, but not the exclusion of any other integers or group of integers.

15

Specific

- As is apparent from the preceding summary of the invention, the invention relates, *inter alia*, to hetero-dimeric or hetero-oligomeric receptor, comprising at least one
20 thyrotropin releasing hormone receptor subunit associated with at least one orexin receptor subunit. The terms "hetero-dimer" and "hetero-oligomer", and variations such as "hetero-dimeric" and "hetero-oligomeric", as used herein, refer to an entity within which at least one thyrotropin releasing hormone receptor is associated with at least one orexin receptor.

25

- The phrase "associated with", as used herein, refers to combination via any known direct or indirect stabilising atomic or molecular level interaction or any combination thereof, where the interactions include, without limitation, bonding interactions such as covalent bonding, ionic bonding, hydrogen bonding, co-
30 ordinate bonding, or any other molecular bonding interaction, electrostatic interactions, polar or hydrophobic interactions, or any other classical or quantum mechanical stabilising atomic or molecular interaction.

- 20 -

Instances of different tissues having different repertoires of hetero-dimers have been reported. For example, 6'guanidinoaltrindole, an analogue of a well-known KOP receptor ligand, has been identified as a DOP-KOP hetero-dimer selective agonist, with efficacy as a spinally selective analgesic, leading to the conclusion
5 that DOP-KOP heterodimers are expressed in the spinal cord, but not in the brain (Waldhoer, M. *et al.* (2005) A hetero-dimer selective agonist shows *in vivo* relevance of G-protein coupled receptor dimers. *Proc. Natl. Acad. Sci. USA* 102, 9050-9055). Accordingly, the hetero-dimeric or hetero-oligomeric receptor, comprising at least one thyrotropin releasing hormone receptor subunit associated
10 with at least one orexin receptor subunit represents a novel drug target.

As is the case with 6'guanidinoaltrindole, known ligands may exhibit differing abilities to trigger a hetero-dimeric receptor, which may uncover new applications for pre-existing molecules:

- 15 - Hilaiet *et al.* 2003 (*J. Biol. Chem.* 278, 23731-23737) have recently shown that CB1 antagonists suppress appetite by acting through a CB1/OxR1 hetero-dimer pair.
- It has been shown that somatostatin SSTR5 receptor will hetero-dimerise with a dopamine D2 receptor (Rocheville *et al.* (2000) *Science*
20 288, 154-157).
- An angiotensin AT1 receptor/bradykinin B2 receptor hetero-dimer is believed to be responsible for pre-eclampsia in pregnant women. Evidence suggests that the hetero-dimer is more sensitive to Angiotensin II (AbdAlla *et al.* (2001) *Nat. Med.* 7, 1003-1009).

25

As will be apparent from the following examples, the inventors herein have identified and characterised the molecular association of the thyrotropin releasing hormone receptor with the orexin receptor.

30 It will be apparent to a person skilled in the art that association of the thyrotropin releasing hormone receptor with orexin receptor enables the use of ligands of one receptor (be they agonists, inverse agonists or antagonists) in the treatment of ailments related to the other receptor.

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Thus, the present invention encompasses a method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

The thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist may be co-administered with an orexin receptor agonist, inverse agonist or antagonist.

10

The present invention further encompasses a method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by administering a therapeutically effective amount of an orexin receptor agonist, inverse agonist or antagonist.

15

The present invention further encompasses a method for the manufacture of a medicament for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin releasing hormone receptor agonist, inverse agonist or antagonist.

20

The medicament may further contain an orexin receptor agonist, inverse agonist or antagonist.

The present invention further encompasses a method for the manufacture of a medicament for the treatment of a patient suffering from a thyrotropin-releasing hormone -related ailment by administering a therapeutically effective amount of an orexin receptor agonist, inverse agonist or antagonist.

25

The medicament may further contain a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

30

Thus, the present invention encompasses a method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically

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effective amount of a thyrotropin-releasing hormone-selective binding agent, or fragment thereof.

The thyrotropin-releasing hormone-selective binding agent may be an antibody,
5 including a humanised antibody, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody and/or an anti-idiotypic antibody.

The present invention further encompasses a method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by
10 administering a therapeutically effective amount of an orexin-selective binding agent, or fragment thereof.

The orexin-selective binding agent may be an antibody, including a humanised antibody, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a
15 CDR-grafted antibody and/or an anti-idiotypic antibody.

The present invention further encompasses a method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment or an orexin-related ailment by administering a therapeutically effective amount of a
20 thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective agonist, inverse agonist or antagonist.

The present invention further encompasses the use of a therapeutically effective amount of a thyrotropin releasing hormone receptor / orexin receptor hetero-
25 dimer/-oligomer selective agonist, inverse agonist or antagonist for the manufacture of a medicament for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment or an orexin-related ailment.

The present invention further encompasses a method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by
30 administering a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist.

The selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist may be co-administered with a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

5

The selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist may be co-administered with an orexin receptor agonist, inverse agonist or antagonist.

10 The present invention further encompasses a method for the treatment of a patient suffering from a orexin-related ailment by administering a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist.

15 The selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist may be co-administered with a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

20 The selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist may be co-administered with an orexin receptor agonist, inverse agonist or antagonist.

25 The present invention further encompasses a method for the manufacture of a medicament for the treatment of a patient suffering from an thyrotropin-releasing hormone -related ailment comprising use of a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist.

30 The medicament may contain an orexin receptor agonist, inverse agonist or antagonist.

The medicament may contain an thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

35

The present invention further encompasses a method for the manufacture of a medicament for the treatment of a patient suffering from an orexin-related ailment comprising use of a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse

agonist or antagonist.

The medicament may contain an orexin receptor agonist, inverse agonist or antagonist.

5

The medicament may contain an thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

Thyrotropin releasing hormone-related ailments include ailments that are related
10 to increased or decreased production of thyrotropin releasing hormone, and/or
increased or decreased responsiveness of cells to thyrotropin releasing hormone.
The following list (Gary, Keith A., et al., The Thyrotropin-Releasing Hormone
(TRH) Hypothesis of Homeostatic Regulation: Implications for TRH-Based
Therapeutics, JPET 305:410–416, 2003) provides some examples of TRH-related
15 ailments:

- Depression, especially accompanied by hypersomnolence;
- Chronic fatigue syndromes;
- Excessive daytime sleepiness (including narcolepsy), neurasthenia, and
lethargy;
- 20 Sedation secondary to drugs, chemotherapy, or radiation therapy;
- Sedative intoxication/respiratory distress (ER setting);
- Recovery from general anesthesia;
- Attention deficit/hyperactive disorder;
- Disturbances of circadian rhythm (e.g. jet lag);
- 25 Bipolar affective disorder as a mood stabilizer*;
- Anxiety disorders*;
- Alzheimer's disease and other dementias with cognition deficits*;
- Seizure disorders*; and
- Motor neuron disorders*.

30 * May be particularly effective as adjunctive therapy

However, it should be understood that the phrase thyrotropin releasing hormone-related ailment is not limited thereto.

- 25 -

Orexin-related ailments include ailments that are related to increased or decreased production of orexin, and/or increased or decreased responsiveness of cells to orexin. A major example of an orexin-related ailment is narcolepsy with cataplexy. This is associated with low or undetectable levels of cerebrospinal fluid (CSF) orexin A levels in about 90% of patients (Baumann and Bassetti (2005) *Sleep Medicine Reviews* 9, 253-268). Mutations of the orexin receptor 2 gene lead to familial canine narcolepsy and a loss of orexin neurons and low CSF orexin A were observed with sporadic canine narcolepsy. Neurological disorders arising from acute traumatic brain injury, Guillain-Barre syndrome and advanced Parkinson's syndrome may also be linked with low or undetectable levels of CSF orexin A levels in some instances. Sakurai has postulated a role for the orexin system in feeding and energy homeostasis as the activity of orexin neurons is inhibited by glucose and leptin, and stimulated by ghrelin, a stomach-derived peptide which promotes feeding. This may have implications for the treatment of obesity (Sakurai (2005) *Sleep Medicine Reviews* 9, 231-241).

However, it should be understood that the phrase orexin-related ailment is not limited thereto.

Known orexin receptor modulators include orexin A (OxA; hypocretin-1; Hcrt-1), orexin B (OxB; hypocretin-2; Hcrt-2) and fragments thereof (Lang *et al.* (2004) *J Med Chem* 47, 1153-1160).

Known antagonists for both OxR1 and OxR2 include 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogues (Hirose M *et al.* (2003) *Bioorg. Med. Chem. Lett.* 13, 4497-4499), Almorexant ((2R)-2-((1S)-6,7-dimethoxy-1-[2-(4-trifluoromethylphenyl)-ethyl]-3,4-dihydro-1H-isoquinolin-2-yl)-N-methyl-2-phenylacetamide; ACT-078573; Actelion Pharmaceuticals Ltd., Allschwil, Switzerland; Brisbare-Roch *et al.* (2007) *Nature Medicine* 13, 150-155).

30

Known OxR1 antagonists include SB-334867-A (1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl urea hydrochloride), SB-674042 (1-(5-(2-fluoro-phenyl)-2-methyl-thiazol-4-yl)-1-((S)-2-(5-phenyl-(1,3,4)oxadiazol-2-ylmethyl)-pyrrolidin-1-yl)-

methanone), SB-408124 (1-(6,8-difluoro-2-methyl-quinolin-4-yl)-3-(4-dimethylamino-phenyl)-urea) and SB-410220 (1-(5,8-difluoro-quinolin-4-yl)-3-(4-dimethylamino-phenyl)-urea) (Haynes *et al.* (2000) *Regulatory Peptides* 96, 45-51; Langmead *et al.* (2004) *British Journal of Pharmacology* 141, 340-346).

5

Known OxR2 antagonists include *N*-Arylmethyl *tert*-leucyl 6,7-dimethoxy-1,2,3,4-tetrahydroiso-quinoline analogues and *N*-acyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogues (Hirose M *et al.* (2003) *Bioorg. Med. Chem. Lett.* 13, 4497-4499), and substituted 4-phenyl-[1,3]dioxanes, particularly 1-(2,4-dibromo-phenyl)-3-((4*S*,5*S*)-2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea (McAtee LC *et al.* (2004) *Bioorg. Med. Chem. Lett.* 14, 4225-4229).

Known modulators of the thyrotropin releasing hormone receptor include thyrotropin releasing hormone (TRH; thyroliberin; TRF; pGlu-His-Pro-NH₂), [Glu2]TRH, [Glu2]TRH with the amino-terminal pyroglutamyl residue replaced with a pyridinium moiety (Prokai-Tatrai *et al.* (2005) *Med. Chem.* 1, 141-152), methyl-TRH, (3-methyl-His2)TRH, montirelin ((3*R*,6*R*)-6-methyl-5-oxo-3-thiomorpholinyl carbonyl-L-histidyl-L-prolinamide tetrahydrate; CG-3703; Grunenthal GmbH, Aachen, Germany), CNK-602A (N-[(6-methyl-5-oxo-3-thiomorpholinyl) carbonyl]-L-histidyl-L-prolinamide; Renming *et al.* (1992) *Eur. J. Pharmacol.* 223, 185-192), taltirelin ((-)-N-[(*S*)-hexahydro-1-methyl-2,6-dioxo-4-pyrimidinylcarbonyl]-L-histidyl-L-prolinamide tetrahydrate; Ceredist; TA-0910; Tanabe Seiyaku Co., Ltd., Osaka, Japan), JTP-2942 (N^{alpha}-[(1*S*,2*R*)-2-methyl-4-oxocyclopentylcarbonyl]-L-histidyl-L-prolinamide monohydrate; Japan Tobacco, Inc., Tokyo, Japan), azetirelin (YM-14673; Yamanouchi Pharmaceutical Co., Ltd, Tokyo, Japan), DN-1417 (Gamma-butyrolactone-gamma-carbonyl-histidyl-prolinamide citrate; Miyamoto M *et al.* (1981) *Life Sci.* 28, 861-869), RX-77368 (pGlu-His-(3,3'-dimethyl)-Pro-NH₂; Ferring Pharmaceuticals, Feltham, Middlesex, UK), CG-3509 (Grunenthal GmbH, Stolberg, Germany), MK-771 (1-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide; Merck, Rahway, NJ), posatirelin (RGH 2202; L-6-ketopiperidine-2-carbonyl-L-leucyl-L-proline amide; Gedeon Richter Pharmaceuticals, Budapest, Hungary), Ro 24-9975 (1*S*,3*R*,5(2*S*),5*S*)-5-[(5-oxo-1-phenylmethyl)-2-pyrrolidinyl]-methyl-5-[(1*H*-imidazol-5-yl)methyl]-cyclohexaneacetamide; Hoffman-La Roche,

Basel, Switzerland), protirelin (5-oxo-L-prolyl-L-histidyl-L-proline amide; Thyrel® TRH; Ferring Pharmaceuticals, Tarrytown, NY), midazolam, diazepam and chlordiazepoxide (inverse agonists; Jinsi-Parimoo A and Gershengorn MC (1997) *Endocrinology* 138, 1471-1475).

5

A strong association between the orexin system and narcolepsy with cataplexy has been established (Sakurai (2005) *Sleep Medicine Reviews* 9, 231-241). Furthermore, Nishino *et al.* suggest that TRH analogs may be useful for the treatment of excessive daytime sleepiness in narcolepsy (Nishino *et al.* (1997) *The Journal of Neuroscience* 17, 6401-6408). The TRH analogs CG-3703 and TA-0910 significantly reduced slow wave sleep (SWS) and rapid eye movement (REM) sleep in a dose- and time-dependent manner. Furthermore, the TRH analogs completely suppressed cataplexy in most of the animals studied. Serum T₃ and T₄ did not change significantly "suggesting that the anticataplectic and alerting effects of TRH and analogs of TRH are mediated by neuromodulatory CNS properties and not by indirect effects on the thyroid axis." (Nishino *et al.* (1997) *The journal of neuroscience* 17, 6401-6408). These observations were supported by a further study in 2000 (Riehl *et al.* (2000) *Neuropsychopharmacology* 23, 34-45). The mode of action of TRH and orexins (and analogs thereof) in the pathophysiology of narcolepsy remains to be elucidated, however, the hetero-dimer/-oligomer interaction identified in this invention contributes to the integration of these receptor systems. Riehl *et al.* comment, "The mechanism underlying the involvement of the hypocretin system in the pathophysiology of narcolepsy remains unclear. It is interesting to note, however, that hypocretin [orexin]-containing neurons are exclusively localized in the lateral hypothalamus (Sakurai et al. 1998 [*Cell*, 92, 573-585]; Peyron et al. 1998 [*J. Neurosc.* 18, 9996-10015]), an area that is rich in TRH neurons (Kreider et al. 1985 [*Peptides* 6, 997-1000]). In addition, both hypocretin [orexin] and TRH receptors are G-protein coupled receptors for neuropeptides, and that the TRH receptor exhibits the second highest (25%) homology (with the Y2 neuropeptide Y receptor having the highest homology) to the hypocretin [orexin] receptors (Sakurai et al. 1998 [*Cell*, 92, 573-585]), suggesting that TRH may play an important role in the pathophysiology of narcolepsy through an unknown specific

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interaction with the hypocretin [orexin] system.” (Riehl *et al.* (2000) *Neuropsychopharmacology* 23, 34-45). The authors have identified the likelihood of TRH and orexin system integration without identifying that such integration could occur as a result of the receptor hetero-dimerization/-oligomerization
5 identified in this invention.

In addition to narcolepsy, the TRH and orexin receptor systems may integrate with regard to the control of feeding and metabolic homeostasis. Thyroid hormone secretion is suppressed during starvation, whereas preprohypocretin (the
10 precursor of orexin peptides) mRNA is upregulated in the lateral hypothalamus. Such observations led Kok *et al.* to investigate the integration of the TRH and orexin systems as, “although the topography of hypocretin- [orexin-] and thyrotrope neural circuits suggests that TRH neuronal activity is governed by hypocretin [orexin] input, the nature of the signal (i.e. excitatory or inhibitory)
15 remains unclear” (Kok *et al.* (2005) *AJP – Endocrinology and Metabolism* 288, 892-899). This study demonstrated significantly lower average plasma TSH concentrations in orexin-deficient narcoleptic humans compared to controls. It is important to note that, as well as feedforward signalling, complex feedback pathways involving autocrine and paracrine feedback via receptors expressed on
20 or in the locality of hormone-/neurotransmitter-secreting neurons are likely to be common in such systems and may play a physiological or pathophysiological role in system integration where these receptors form hetero-dimers/-oligomers.

In one embodiment, the present invention provides a method for the treatment of
25 a patient suffering from an orexin-related ailment other than narcolepsy by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

In one embodiment, the present invention provides a method for the treatment of
30 a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist other than TA0910 (Ceredist).

In one embodiment, the present invention provides a method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist other than TA0910 (Ceredist), CG3703 and
5 CG3509.

In one embodiment, the present invention provides a method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor
10 agonist, inverse agonist or antagonist selected from the group: thyrotropin releasing hormone (TRH; thyroliberin; TRF; pGlu-His-Pro-NH₂), [Glu2]TRH, [Glu2]TRH with the amino-terminal pyroglutamyl residue replaced with a pyridinium moiety (Prokai-Tatrai *et al.* (2005) *Med. Chem.* 1, 141-152), methyl-TRH, (3-methyl-His2)TRH, montirelin ((3R,6R)-6-methyl-5-oxo-3-thiomorpholinyl
15 carbonyl-L-histidyl-L-prolinamide tetrahydrate; CG-3703; Grunenthal GmbH, Aachen, Germany), CNK-602A (N-[(6-methyl-5-oxo-3-thiomorpholinyl) carbonyl]-L-histidyl-L-prolinamide; Renming *et al.* (1992) *Eur. J. Pharmacol.* 223, 185-192), JTP-2942 (N^{alpha}-[(1S,2R)-2-methyl-4-oxocyclopentylcarbonyl]-L-histidyl-L-prolinamide monohydrate; Japan Tobacco, Inc., Tokyo, Japan), azetirelin (YM-
20 14673; Yamanouchi Pharmaceutical Co., Ltd, Tokyo, Japan), DN-1417 (Gamma-butyrolactone-gamma-carbonyl-histidyl-prolinamide citrate; Miyamoto M *et al.* (1981) *Life Sci.* 28, 861-869), RX-77368 (pGlu-His-(3,3'-dimethyl)-Pro-NH₂; Ferring Pharmaceuticals, Feltham, Middlesex, UK), CG-3509 (Grunenthal GmbH, Stolberg, Germany), MK-771 (1-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-
25 carboxamide; Merck, Rahway, NJ), posatirelin (RGH 2202; L-6-ketopiperidine-2-carbonyl-L-leucyl-L-proline amide; Gedeon Richter Pharmaceuticals, Budapest, Hungary), Ro 24-9975 (1S,3R,5(2S),5S)-5-[(5-oxo-1-phenylmethyl)-2-pyrrolidinyl]-methyl]-5-[(1H-imidazol-5-yl)methyl]-cyclohexaneacetamide; Hoffman-La Roche, Basel, Switzerland), protirelin (5-oxo-L-prolyl-L-histidyl-L-proline amide; Thyrel®
30 TRH; Ferring Pharmaceuticals, Tarrytown, NY), midazolam, diazepam and chlordiazepoxide (inverse agonists; Jinsi-Parimoo A and Gershengorn MC (1997) *Endocrinology* 138, 1471-1475).

- 30 -

In one embodiment, the present invention provides a method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist selected from the group: thyrotropin releasing hormone (TRH; thyroliberin; TRF; pGlu-His-Pro-NH₂), [Glu2]TRH, [Glu2]TRH with the amino-terminal pyroglutamyl residue replaced with a pyridinium moiety (Prokai-Tatrai *et al.* (2005) *Med. Chem.* 1, 141-152), methyl-TRH, (3-methyl-His2)TRH, CNK-602A (N-[(6-methyl-5-oxo-3-thiomorpholinyl) carbonyl]-L-histidyl-L-prolinamide; Renming *et al.* (1992) *Eur. J. Pharmacol.* 223, 185-192), JTP-2942 (N^{alpha}-[(1S,2R)-2-methyl-4-oxocyclopentylcarbonyl]-L-histidyl-L-prolinamide monohydrate; Japan Tobacco, Inc., Tokyo, Japan), azetirelin (YM-14673; Yamanouchi Pharmaceutical Co., Ltd, Tokyo, Japan), DN-1417 (Gamma-butyrolactone-gamma-carbonyl-histidyl-prolinamide citrate; Miyamoto M *et al.* (1981) *Life Sci.* 28, 861-869), RX-77368 (pGlu-His-(3,3'-dimethyl)-Pro-NH₂; Ferring Pharmaceuticals, Feltham, Middlesex, UK), MK-771 (1-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide; Merck, Rahway, NJ), posatirelin (RGH 2202; L-6-ketopiperidine-2-carbonyl-L-leucyl-L-proline amide; Gedeon Richter Pharmaceuticals, Budapest, Hungary), Ro .24-9975 (1S,3R,5(2S),5S)-5-[(5-oxo-1-phenylmethyl)-2-pyrrolidinyl]-methyl]-5-[(1H-imidazol-5-yl)methyl]-cyclohexaneacetamide; Hoffman-La Roche, Basel, Switzerland), protirelin (5-oxo-L-prolyl-L-histidyl-L-proline amide; Thyrel® TRH; Ferring Pharmaceuticals, Tarrytown, NY), midazolam, diazepam and chlordiazepoxide (inverse agonists; Jinsi-Parimoo A and Gershengorn MC (1997) *Endocrinology* 138, 1471-1475).

25

The present invention comprises a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective activity, the method comprising the steps of:

- a) determining whether, and/or the extent to which, the test compound interacts with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor; and
- b) if the test compound interacts with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor,

determining whether, or the extent to which the test compound interacts with the orexin receptor in the absence of the thyrotropin releasing hormone receptor;

5 such that a test compound that exhibits greater affinity and/or potency and/or efficacy when interacting with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.

10 The present invention comprises a method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective activity, the method comprising the steps of:

- 15 a) determining whether, and/or the extent to which, the test compound interacts with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor; and
- b) if the test compound interacts with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor, determining whether, or the extent to which the test compound interacts with the thyrotropin releasing hormone receptor
- 20 in the absence of the orexin receptor;

such that a test compound that exhibits greater affinity and/or potency and/or efficacy when interacting with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor is selective for the thyrotropin releasing hormone receptor / orexin receptor

25 hetero-dimer/-oligomer.

In a preferred embodiment of the invention, the step of determining whether, and/or the extent to which, the test compound interacts with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is

30 associated with the orexin receptor; and/or the step of determining whether, and/or the extent to which, the test compound interacts with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor are performed by way of the methods described in the applicant's co-

pending international patent application "Detection System and Uses Therefor", which derives priority from the same Australian provisional patent application 2006906292.

- 5 The present invention includes selective agonists and/or antagonists and/or inverse agonists of the thyrotropin releasing hormone receptor/orexin receptor hetero-dimer/-oligomer.

As used herein the term "patient" refers to any animal that may be suffering from
10 one or more of orexin- or thyrotropin releasing hormone-related ailments. Most preferably the animal is a mammal. The term will be understood to include for example human, farm animals (i.e., cattle, horses, goats, sheep and pigs), household pets (i.e., cats and dogs) and the like.

- 15 The phrase "therapeutically effective amount" as used herein refers to an amount sufficient to modulate a biological activity associated with the interaction of orexin receptor agonist, inverse agonist or antagonist with the orexin receptor or thyrotropin releasing hormone receptor agonist, inverse agonist or antagonist with the thyrotropin-releasing hormone receptor or of orexin receptor/thyrotropin-releasing hormone receptor hetero-dimer/oligomer-specific agonist, inverse agonist
20 or antagonist with an orexin receptor/thyrotropin-releasing hormone receptor hetero-dimer/oligomer. In the context of aspects of the invention where both a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist and a orexin receptor agonist, inverse agonist or antagonist are administered in
25 combination, a therapeutically effective amount of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist or a therapeutically effective amount of an orexin receptor agonist, inverse agonist or antagonist in combination may be lower than therapeutically effective amounts of thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist or orexin
30 receptor agonist, inverse agonist or antagonist when administered alone. That is, the administration of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist and a orexin receptor agonist, inverse agonist or antagonist

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in combination may generate a therapeutic effect at what would otherwise be sub-therapeutic doses of either.

Medicaments of the invention may be administered by injection, or prepared for oral,
5 pulmonary, nasal or for any other form of administration. Preferably the medicaments are administered, for example, intravenously, subcutaneously, intramuscularly, intraorbitally, ophthalmically, intraventricularly, intracranially, intracapsularly, intraspinally, intracisternally, intraperitoneally, buccal, rectally, vaginally, intranasally or by aerosol administration.

10

The mode of administration must, however, be at least suitable for the form in which the medicament has been prepared. The mode of administration for the most effective response may need to be determined empirically and the means of administration described below are given as examples, and do not limit the
15 method of delivery of the composition of the present invention in any way. All the above formulations are commonly used in the pharmaceutical industry and are commonly known to suitably qualified practitioners.

In addition to the agonist(s) and/or inverse agonist(s) and/or antagonist(s), the
20 medicaments of the invention may include pharmaceutically acceptable nontoxic excipients and carriers and administered by any parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections. In addition the formulations may optionally contain one or more adjuvants.

25 The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water-soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Alternatively, the compounds of the invention may be encapsulated in liposomes and delivered in injectable solutions to assist their transport across cell membrane.
30 Alternatively or in addition such preparations may contain constituents of self-assembling pore structures to facilitate transport across the cellular membrane. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene

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glycol, and the like), suitable mixtures thereof, and vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of the injectable compositions can be brought
5 about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions may be prepared by incorporating the active compounds in the required amount in an appropriate solvent with various of the
10 other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle that contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile
15 powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying techniques that yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Contemplated for use herein are oral solid dosage forms, which are described
20 generally in *Martin, Remington's Pharmaceutical Sciences*, 18th Ed. (1990 Mack Publishing Co. Easton PA 18042) at Chapter 89, which is herein incorporated by reference. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres
25 reported in U.S. Patent No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatised with various polymers (*E.g.*, U.S. Patent No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given by Marshall, in *Modern Pharmaceutics*, Chapter 10, Banker and Rhodes ed., (1979), herein incorporated by reference. In general, the formulation will include the
30 compounds described as part of the invention (or a chemically modified form thereof), and inert ingredients which allow for protection against the stomach environment, and release of the biologically active material in the intestine.

For the agonists, antagonists and inverse agonists of the invention the location of release may be the stomach, the small intestine (the duodenum, the jejunum, or the ileum), or the large intestine. One skilled in the art has available formulations that will not dissolve in the stomach, yet will release the material in the duodenum or
5 elsewhere in the intestine. Preferably, the release will avoid the deleterious effects of the stomach environment, either by protection of the composition or by release of the compounds beyond the stomach environment, such as in the intestine.

To ensure full gastric resistance, a coating impermeable to at least pH 5.0 is
10 essential. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These coatings may be used as mixed films.

15 A coating or mixture of coatings can also be used on tablets, which are not intended for protection against the stomach. This can include sugar coatings, or coatings that make the tablet easier to swallow. Capsules may consist of a hard shell (such as gelatin) for delivery of dry therapeutic *i.e.* powder; for liquid forms, a soft gelatin shell
20 may be used. The shell material of cachets could be thick starch or other edible paper. For pills, lozenges, moulded tablets or tablet triturates, moist massing techniques can be used.

The therapeutic can be included in the formulation as fine multiparticulates in the
25 form of granules or pellets of particle size about 1mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

Colourants and flavouring agents may all be included. For example, compounds
30 may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavouring agents.

One may dilute or increase the volume of the therapeutic with an inert material. These diluents could include carbohydrates, especially mannitol, alpha-lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate,
5 magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrants include but are not limited to starch
10 including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as
15 binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used to hold the therapeutic compounds together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch
20 and gelatin. Others include methylcellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

25 An antifrictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to: stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be
30 used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, and Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the compound during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

- 5 To aid dissolution of the therapeutic into the aqueous environment, a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethonium chloride. The list of potential nonionic detergents that
10 could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the compounds either alone or as a mixture in different ratios.

15

Additives which potentially enhance uptake of the compounds are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

- Controlled release formulation may be desirable. The compounds could be
20 incorporated into an inert matrix that permits release by either diffusion or leaching mechanisms *i.e.*, gums. Slowly degenerating matrices may also be incorporated into the formulation. Another form of a controlled release of this therapeutic is by a method based on the Oros therapeutic system (Alza Corp.), *i.e.* the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out
25 through a single small opening due to osmotic effects. Some enteric coatings also have a delayed release effect.

30

A mix of materials might be used to provide the optimum film coating. Film coating may be carried out in a pan coater or in a fluidized bed or by compression coating.

Also contemplated herein is pulmonary delivery of the compounds. The compounds may be delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream.

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered-dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Missouri; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colorado; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Massachusetts.

All such devices require the use of formulations suitable for the dispensing of the compounds. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants and/or carriers useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise the compounds suspended in water. The formulation may also include a buffer and a simple sugar (e.g., for protein stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the compounds caused by atomization of the solution in forming the aerosol.

Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the compounds suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or

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combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing the compound and may also include a bulking agent, such as lactose, sorbitol, sucrose, or mannitol in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation. The compounds (or derivative) should most advantageously be prepared in particulate form with an average particle size of less than 10 microns, most preferably 0.5 to 5 microns, for most effective delivery to the distal lung.

Nasal delivery of the compounds is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

It will be appreciated that the medicaments of the invention may be given as a single dose schedule, or preferably, in a multiple dose schedule. A multiple dose schedule is one in which a primary course of delivery may be with 1 to 10 separate doses, followed by other doses given at subsequent time intervals required to maintain or reinforce the treatment. The dosage regimen will also, at least in part, be determined by the need of the individual and the judgement of the practitioner.

The invention will now be further described by way of reference only to the following non-limiting examples. It should be understood, however, that the examples following are illustrative only, and should not be taken in any way as a restriction on the generality of the invention described above.

EXAMPLES

General methods

- 40 -

Briefly, referring to Figures 1 to 3, the IGs are provided in the form of the two receptors (TRHR and OxR). One of the two is attached to an RC (IG1-RC1, IG3). A second IG (IG2-RC2) is derived from a molecule that interacts with the receptors upon ligand binding (e.g. beta-arrestin, or a mutant thereof). The
5 detection system not only detects the formation of the TRHR-OxR heterodimer but can distinguish whether a ligand or drug acts as an agonist, partial agonist, antagonist, inverse agonist or partial inverse agonist at the receptor hetero-dimer.

Cells were seeded in 6-well plates at a density of approximately 630,000 cells/well
10 (HEK293FT) or approximately 150,000 cells/well (COS-7) and maintained at 37 °C, 5% CO₂ in Complete Media (DMEM containing 0.3 mg/ml glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco)) supplemented with 10% fetal calf serum (FCS; Gibco). Transient transfections were carried out 24 h after seeding using GeneJuice (Novagen) or Metafectene (Biontex) according to
15 manufacturer instructions. 24 h post-transfection, cells were washed with PBS, detached using 0.05% trypsin/0.53 mM EDTA, resuspended in HEPES-buffered phenol red free Complete Media containing 5% FCS and added to a poly-L-lysine-coated white microplate (Nunc). 48 h post-transfection, eBRET assays were carried out following pre-incubation of cells with EnduRenTM (Promega) at a final
20 concentration of 30-40 µM, at 37 °C, 5% CO₂ for 2 h. For original BRET studies, the HEPES-buffered phenol red free Complete Media was replaced with PBS and coelenterazine h (Molecular Probes) added to a final concentration of 5 µM immediately prior to commencing the assay. BRET measurements were taken at
25 37 °C using the Victor Light plate reader with Wallac 1420 software (Perkin-Elmer). Filtered light emissions were sequentially measured for 3-5 s in each of the 'donor wavelength window' (400-475 nm) and 'acceptor wavelength window' (>500 nm for EGFP or 520-540 nm for EYFP, Topaz (TYFP) or Venus). The BRET signal observed between interacting proteins is normalized by subtracting the background BRET ratio. This can be done in one of two ways (see Pflieger *et al.* (2006) *Cell Signal* 18, 1664-1670; Pflieger *et al.* (2006) *Nat Protoc* 1, 336-344):
30 1) the ratio of light emission through the acceptor wavelength window over the 400-475 nm emission for a cell sample containing only the donor construct is subtracted from the same ratio for a sample containing the interacting acceptor

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and donor fusion proteins; 2) the ratio of light emission through the 'acceptor wavelength window' over the 400-475 nm emission for a cell sample treated with vehicle is subtracted from the same ratio for a second aliquot of the same cell sample treated with ligand. In the following examples, the first calculation will be used, unless the signal is described as the 'ligand-induced BRET ratio'. Alternatively, and particularly when illustrating z-factor data (Zhang *et al.* (1999) *J Biomol Screen* 4, 67-73), the BRET signal observed between interacting proteins can be shown in conjunction with (as oppose to being subtracted by) the background BRET ratio to evaluate error associated with the BRET signal observed between interacting proteins and the error associated with the background BRET ratio independently. In this case, data are shown as 'fluorescence/luminescence' being the ratio of light emission through the 'acceptor wavelength window' over the 400-475 nm emission for a particular cell sample. Unless otherwise stated, BRET signals were measured in 96-well microplates.

15

EXAMPLE 1 MEASUREMENT OF A DETECTABLE SIGNAL INDICATIVE OF THE MOLECULAR ASSOCIATION OF THE THYROTROPIN RELEASING HORMONE RECEPTOR WITH THE OREXIN RECEPTOR

20 Referring now to Figure 4, eBRET signals were measured from cells transiently co-expressing TRHR/Rluc and barr2/Venus with either pcDNA3, OxR2, CXCR2, HA-MC3R, HA-MC4R, D2LR or D2SR following the treatment of each with their respective ligands.

25 Prior to ligand treatment (added at 0 minutes), a baseline eBRET signal was recorded for each of the receptor combinations. Within the first minute, TRH treatment of cells co-expressing TRHR/Rluc and barr2/Venus with pcDNA3, resulted in the eBRET signal rapidly reaching a peak of greater than 0.17 and this signal remained high for the entire recording period (nearly 2 hours). A signal was also observed following OxA treatment of cells co-expressing TRHR/Rluc, 30 barr2/Venus and OxR2. This signal however took up to 30 minutes to reach approximately 0.07-0.08. No ligand-induced eBRET signals were observed for any of the other receptor combinations.

This example demonstrates that a signal resulting from the proximity of RC1 and RC2 is detected specifically for the combination where the thyrotropin releasing hormone receptor (TRHR) is IG1, Rluc is RC1, beta-arrestin 2 (barr2) is IG2, Venus is RC2 and OxR2 is IG3, and when the modulator, in this case OxA, modulates the association of IG2 and IG3 as a result of interacting specifically with IG3. A signal is not detected when IG3 is CXCR2, HA-MC3R, HA-MC4R, D2LR or D2SR and agonists specific for these IG3s modulate the association of IG2 and IG3, demonstrating the specificity of the signal for the combination with OxR2 as IG3.

This example demonstrates that the inventors have identified the molecular association of the thyrotropin releasing hormone receptor with the orexin receptor.

This example further demonstrates that the kinetic profile observed for the signal resulting from RC1 and RC2 proximity due to modulation of the association of IG2 and IG3 is distinct from the kinetic profile observed for an eBRET signal resulting from RC1 and RC2 proximity due to association of IG1 and IG2 when this IG1-IG2 association is modulated by ligand, in this case TRH, interacting specifically with IG1. The former profile is substantially delayed compared to the latter profile.

EXAMPLE 2 MEASUREMENT OF ADDITIVE DETECTABLE SIGNALS INDICATIVE OF THE MOLECULAR ASSOCIATION OF THE THYROTROPIN RELEASING HORMONE RECEPTOR WITH THE OREXIN RECEPTOR

25

Referring now to Figure 5, eBRET signals were measured from cells transiently co-expressing TRHR/Rluc and EGFP/barr1 or EGFP/barr2 with either pcDNA3 or OXR2. Ligand treatments were either OxA or TRH only or both OxA and TRH combined.

30

Prior to ligand treatment (added at 0 minutes), baseline eBRET signals were recorded for each of the receptor combinations. PBS treated cells expressing each of the combinations exhibited only baseline eBRET signals for the entire

recording period (70 minutes). Following treatment with OxA, cells expressing OxR2 and either EGFP/barr1 (crosses) or EGFP/barr2 (grey inverted triangles) exhibited an eBRET signal reaching a plateau after more than 10 minutes. In cells expressing TRHR/Rluc only (no OxR2) with either of the beta arrestins, TRH treatment resulted in a rapid stimulation of the eBRET signal. The signal with barr2 (black circles) was greater than that for barr1 (grey triangles) however there was no difference for either beta arrestin if OxA was present (barr2, black triangles; barr1, grey circles). In cells expressing both TRHR/Rluc and OxR2 (barr1, grey squares; barr2, black squares), the addition of both ligands showed an increased eBRET signal over and above that seen following addition of OxA or TRH alone.

This example demonstrates that a signal resulting from the proximity of RC1 and RC2 is detected for the combination where the thyrotropin releasing hormone receptor (TRHR) is IG1, Rluc is RC1, either beta-arrestin 1 (barr1) or beta-arrestin 2 (barr2) is IG2, EGFP is RC2 and OxR2 is IG3 when the modulator, OxA, modulates the association of IG2 and IG3 as a result of interacting specifically with IG3.

Therefore, this example demonstrates signal detection using an alternative combination from that shown in example 1, including use of a different IG2 and RC2.

As in example 1, this example demonstrates the delayed kinetic profile observed for the signal resulting from RC1 and RC2 proximity due to modulation of the association of IG2 and IG3, in this case by OxA, as distinct from the more rapid kinetic profile observed for an eBRET signal resulting from RC1 and RC2 proximity due to association of IG1 and IG2 when this IG1-IG2 association is modulated by ligand, in this case TRH, interacting specifically with IG1. However, in addition to that demonstrated in example 1, this example demonstrates the additive effect of combined treatment with IG1 ligand (TRH) and IG3 ligand (OxA; modulator).

Therefore, this example provides further and distinct evidence for the molecular association of the thyrotropin releasing hormone receptor with the orexin receptor, as this additive effect is indicative of RC1 and RC2 proximity as a result of IG1-IG2 association in addition to IG2-IG3-IG1 association. This provides evidence
5 against signals originating from non-specific IG1-IG2 association in the absence of an IG1-specific ligand. Without wishing to be bound by theory, this additive effect may also be partly due to IG1 ligand acting as a modulator to modulate the association of IG2 and IG3 via allosteric effects on IG3. Furthermore, this additive effect may also be partly due to an active IG conformation (one that is bound to
10 agonist) being more favourable for signal generation, perhaps enabling increased proximity of RC1 and RC2, or more favourable relative orientation of RC1 and RC2.

EXAMPLE 3 MEASUREMENT OF THE EFFECT ON SIGNAL GENERATION OF
15 **AN ANTAGONIST THAT COMPETES FOR MODULATOR BINDING**

Referring now to Figure 6, eBRET signals were measured from cells transiently co-expressing TRHR/Rluc and barr2/Venus with either pcDNA3, OxR1 or OxR2 following pretreatment with 10^{-6} M OxR1-selective antagonist, SB-334867-A, for
20 approximately 40 minutes prior to addition of 10^{-6} M OxA (IG3 ligand; modulator) or 10^{-6} M TRH (IG1 ligand), or both. Cells not pretreated with antagonist were pretreated with PBS instead for the same amount of time.

Prior to agonist treatment (added at 0 minutes), baseline eBRET signal was
25 recorded for each of the receptor combinations. A small eBRET signal was observed for OxA-treated TRHR/Rluc and barr2/Venus and OxR1 (grey diamonds). This signal was reduced in the presence of antagonist (open squares). The addition of both TRH and OxA to the OxR1-expressing cells resulted in a much larger signal (white triangles) and the size of this signal was
30 reduced in the presence of the antagonist (grey circles). An eBRET signal was observed following OxA treatment of cells co-expressing TRHR/Rluc, barr2/Venus and OxR2 (black diamonds). This signal was not affected by the pre-treatment of antagonist (white squares). The addition of both TRH and OxA to the OxR2-

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expressing cells resulted in a signal that did not differ in either the presence (black circles) or the absence (grey triangles) of antagonist.

5 This example shows a signal resulting from the proximity of RC1 and RC2 detected for the combination where the thyrotropin releasing hormone receptor (TRHR) is IG1, Rluc is RC1, beta-arrestin 2 (barr2) is IG2, Venus is RC2 and OxR1 or OxR2 is IG3 when the modulator, OxA, modulates the association of IG2 and IG3 as a result of interacting specifically with IG3.

10 This example demonstrates that specific antagonism of modulator binding, in this case the specific antagonism of OxA acting on OxR1 by the OxR1-selective antagonist SB-334867-A, can be detected as a result of its effect on the signal due to the proximity of RC1 and RC2 modulated by the modulator, in this case OxA.

15

EXAMPLE 4 USE OF A TAG ON IG3 THAT DOES NOT CONSTITUTE A REPORTER COMPONENT

Referring now to Figure 7, eBRET signals were measured from cells transiently co-expressing TRHR/Rluc and EGFP/barr1 or EGFP/barr2 with either pcDNA3 or HA-OxR2. Ligand treatments were either OxA or TRH only.

25 Prior to ligand treatment (added at 0 minutes), baseline eBRET signals were recorded for each of the receptor combinations. PBS treated cells expressing each of the combinations exhibited only baseline eBRET signals for the entire recording period (80 minutes) (data not shown). Following treatment with OxA, cells expressing HA-OxR2 and either of the EGFP/barrs exhibited an eBRET signal reaching a plateau after more than 10 minutes (EGFP/barr1, black diamonds and EGFP/barr2, black circles). In cells expressing TRHR/Rluc only (no HA-OxR2), TRH stimulated a rapid increase in eBRET signal reaching a peak in the first few minutes, the signal then drifted down slightly over the remainder of the recording period (grey squares). No increase in eBRET signal above baseline was observed following OxA addition to cells lacking HA-OxR2 (grey triangles).

This example shows a signal resulting from the proximity of RC1 and RC2 detected for the combination where the thyrotropin releasing hormone receptor (TRHR) is IG1, Rluc is RC1, beta-arrestin 1 (barr1) or beta-arrestin 2 (barr2) is IG2, EGFP is RC2 and hemagglutinin (HA) epitope-tagged OxR2 (HA-OxR2) is IG3 when the modulator, OxA, modulates the association of IG2 and IG3 as a result of interacting specifically with IG3.

This example demonstrates that IG3 can be tagged, such as by the addition of a hemagglutinin (HA) epitope-tag, however, this tag does not constitute a reporter component and does not interfere with and/or contribute to the signal generated by the proximity of RC1 and RC2. Such tagging enables additional information to be ascertained, such as the relative expression level of IG3.

EXAMPLE 5 USE OF A MUTANT BETA-ARRESTIN AS AN INTERACTING GROUP

Referring now to Figure 8, eBRET signals were measured from cells transiently co-expressing TRHR/Rluc and EGFP/barr1 or EGFP/barr1 phosphorylation-independent mutant R169E (EGFP/barr1R169E) with either pcDNA3 or OxR2. Ligand treatments were either OxA or TRH only.

Prior to ligand treatment (added at 0 minutes), baseline eBRET signals were recorded for each of the receptor combinations. PBS treated cells expressing each of the combinations exhibited only baseline eBRET signals for the entire recording period (100 minutes) (white squares, white diamonds and black diamonds). Following treatment with OxA, cells expressing OxR2 and either EGFP/barr1 (black circles) or EGFP/barr1R169E (black triangles) exhibited an eBRET signal with the EGFP/barr1 reaching a plateau after more than 10 minutes while the EGFP/barr1R169E showed a lower signal which reached a plateau by 20 minutes. In cells expressing TRHR/Rluc only (no OxR2) with either of the barrs, TRH stimulated a rapid increase in eBRET signal reaching a peak in the first few minutes, the signal then drifted down slightly over the remainder of the

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recording period. The signal for the EGFP/barr1R169E (white triangles) was lower than that for EGFP/barr1 (white circles), which may reflect lower expression levels of this protein.

- 5 This example shows a signal resulting from the proximity of RC1 and RC2 detected for the combination where the thyrotropin releasing hormone receptor (TRHR) is IG1, Rluc is RC1, barr1 or barr1R169E is IG2, EGFP is RC2 and OxR2 is IG3.
- 10 This example demonstrates that a detectable signal can be generated when using a mutant beta-arrestin, such as the beta-arrestin 1 phosphorylation-independent mutant R169E, as one of the interacting groups.

15 **EXAMPLE 6 MEASUREMENT OF A DETECTABLE SIGNAL INDICATIVE OF THE MOLECULAR ASSOCIATION OF THE C-TERMINALLY TRUNCATED THYROTROPIN RELEASING HORMONE RECEPTOR WITH THE OREXIN RECEPTOR**

Referring now to Figure 9, eBRET signals were measured from cells transiently co-expressing TRHR335/Rluc and EGFP/barr1 with either OxR2 or TRHR. Ligand treatments were either OxA or TRH only.

Prior to ligand treatment (added at 0 minutes), baseline eBRET signals were recorded for each of the receptor combinations. Following treatment with OxA, cells expressing OxR2 (black circles) exhibited an eBRET signal reaching a plateau after about 20 minutes. In contrast, no eBRET signal above baseline was observed from cells expressing TRHR when treated with OxA (white triangles), or from cells expressing OxR2 when treated with TRH (black squares).

- 30 This example shows a signal resulting from the proximity of RC1 and RC2 detected for the combination where the thyrotropin releasing hormone receptor truncated at amino acid 335 (TRHR335) is IG1, Rluc is RC1, beta-arrestin 1 (barr1) is IG2, EGFP is RC2 and OxR2 or TRHR is IG3.

This example demonstrates that a detectable signal can be generated when IG1 does not interact with IG2, in this case, a truncated TRHR that does not interact with barr1 (Heding *et al.* (2000) *Endocrinology* 141, 299-306). The lack of signal
5 observed in Figure 9 upon treatment of TRHR335/Rluc + EGFP/barr1 + OxR2 with TRH confirms that the signal observed upon OxA treatment of this agent combination is not due to IG1-IG2 association.

Therefore, this example provides further and distinct evidence for the molecular
10 association of the thyrotropin releasing hormone receptor with the orexin receptor, as the inability of IG1 to interact with IG2 is indicative of RC1 and RC2 proximity as a result of IG2-IG3-IG1 association and not IG1-IG2 association. This provides further evidence against signals originating from non-specific IG1-IG2 association in the absence of an IG1-specific ligand.

15 Furthermore, this example demonstrates that the signal results from IG2-IG3-IG1 association as opposed to IG3 activation causing transactivation of IG1, which then associates with IG2, thereby bringing RC1 and RC2 into close proximity without IG2 and IG3 associating.

20 **EXAMPLE 7 MEASUREMENT OF A DETECTABLE SIGNAL IN A CHARACTERISTIC DOSE-DEPENDENT MANNER INDICATIVE OF THE MOLECULAR ASSOCIATION OF TRHR WITH OXR2**

25 Referring now to Figures 10, 11 and 12, BRET signals were measured from cells transiently co-expressing: TRHR/Rluc and barr2/Venus with pcDNA3 (treated with increasing doses of TRH; Figure 10); OxR2/Rluc and barr2/Venus with pcDNA3 (treated with increasing doses of OxA; Figure 11); and TRHR/Rluc and barr2/Venus with OxR2 (treated with increasing doses of OxA; Figure 12).

30 This example shows: a TRH dose-response curve for TRHR as IG1, Rluc as RC1, barr2 as IG2, Venus as RC2 and in the absence of IG3 (Figure 10); an OxA dose-response curve for OxR2 as IG1, Rluc as RC1, barr2 as IG2, Venus as RC2 and

in the absence of IG3 (Figure 11); and OxA dose-response curves for the TRHR as IG1, Rluc as RC1, barr2 as IG2, Venus as RC2 and OxR2 as IG3 (Figure 12).

This example demonstrates that signals can be detected in a dose-dependent
5 manner. Furthermore, the EC_{50} values for signals resulting from the modulator (OxA) acting on IG3 (OxR2) and consequent proximity of IG1-RC1 (TRHR/Rluc) and IG2-RC2 (barr2/Venus; Figure 12) are comparable to those from OxA activation of IG1 (OxR2) resulting in proximity of IG1-RC1 (OxR2/Rluc) and IG2-RC2 (barr2/Venus; Figure 11), and distinct from those from TRH activation of IG1
10 (TRHR) resulting in proximity of IG1-RC1 (TRHR/Rluc) and IG2-RC2 (barr2/Venus; Figure 10).

Therefore, this example further demonstrates that the signal results from IG2-IG3-IG1 association as opposed to IG1-IG2 association.

15

The dose-response Hill slopes for OxA activation of IG1 (OxR2) resulting in proximity of IG1-RC1 (OxR2/Rluc) and IG2-RC2 (barr2/Venus; Figure 11); and TRH activation of IG1 (TRHR) resulting in proximity of IG1-RC1 (TRHR/Rluc) and IG2-RC2 (barr2/Venus; Figure 10) are both approximately 1. In contrast, the dose-
20 response Hill slopes for modulator (OxA) acting on IG3 (OxR2) resulting in proximity of IG1-RC1 (TRHR/Rluc) and IG2-RC2 (barr2/Venus; Figure 12) are substantially greater than 1.

Therefore, this example demonstrates the potential for identifying and monitoring
25 specific molecular associations using the Hill slope as an indicator.

This example further demonstrates that different forms of Rluc substrate (reporter component initiator), in this case coelenterazine h and EnduRen, can be used to generate data with similar EC_{50} values (Figure 12).

30

EXAMPLE 8 MEASUREMENT OF ADDITIVE DETECTABLE SIGNALS IN A DOSE-DEPENDENT MANNER INDICATIVE OF THE MOLECULAR ASSOCIATION OF TRHR WITH OXR2

5 Referring now to Figures 13 and 14, BRET signals were measured from cells transiently co-expressing TRHR/Rluc and EGFP/barr1 in the absence of OXR2 with increasing doses of TRH, as well as cells transiently co-expressing TRHR/Rluc and EGFP/barr1 with OXR2 with increasing doses of OxA with and without 10^{-6} M TRH, or increasing doses of TRH with 10^{-6} M OxA.

10

This example shows a curve mathematically generated by addition of the ligand-induced signal generated with 10^{-6} M TRH (from the TRH: TRHR/Rluc + EGFP/barr1 curve) to each of the points generated for the OxA: TRHR/Rluc + EGFP/barr1 + OXR2 curve (TRHR/Rluc + EGFP/barr1 + OXR2: TRH (10^{-6} M) +

15 OxA: Data calculated) overlain on a curve generated from data observed for the TRHR/Rluc + EGFP/barr1 + OXR2: TRH (10^{-6} M) + OxA combination (Figure 13).

Furthermore, this example shows a curve mathematically generated by addition of the ligand-induced signal generated with 10^{-6} M OxA (from the OxA: TRHR/Rluc + EGFP/barr1 + OXR2 curve) to each of the points generated for the TRH: TRHR/Rluc + EGFP/barr1 curve (TRHR/Rluc + EGFP/barr1 + OXR2: TRH + OxA (10^{-6} M): Data calculated) overlain on a curve generated from data observed for the TRHR/Rluc + EGFP/barr1 + OXR2: TRH + OxA (10^{-6} M) combination (Figure 14).

25

Therefore, this example clearly demonstrates the additive effect of combined treatment with IG1 ligand (TRH) and IG3 ligand (OxA; modulator) in a dose dependent manner.

30 Therefore, this example provides further evidence for the molecular association of the thyrotropin releasing hormone receptor with the orexin receptor, as this additive effect is indicative of RC1 and RC2 proximity as a result of IG1-IG2 association in addition to IG2-IG3-IG1 association. This provides further evidence

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against signals originating from non-specific IG1-IG2 association in the absence of an IG1-specific ligand. Without wishing to be bound by theory, this additive effect may also be partly due to IG1 ligand acting as a modulator to modulate the association of IG2 and IG3 via allosteric effects on IG3. Furthermore, this additive
5 effect may also be partly due to an active IG conformation (one that is bound to agonist) being more favourable for signal generation, perhaps enabling increased proximity of RC1 and RC2, or more favourable relative orientation of RC1 and RC2.

10 **EXAMPLE 9 MEASUREMENT OF ADDITIVE DETECTABLE SIGNALS IN A DOSE-DEPENDENT MANNER INDICATIVE OF THE MOLECULAR ASSOCIATION OF TRHR335 WITH OXR2**

Referring now to Figure 15, BRET signals were measured from cells transiently
15 co-expressing TRHR335/Rluc, barr2/Venus and OXR2 with increasing doses of TRH and OxA alone or in combination.

This example demonstrates, using dose response curves, that TRH addition does not result in a BRET signal due to RC1 (Rluc) and RC2 (Venus) proximity as a
20 result of interacting with IG1 (TRHR335) when IG1 (TRHR335) is not able to interact with IG2 (barr2). However, a BRET signal due to RC1 (Rluc) and RC2 (Venus) proximity as a result of interacting with IG3 (OXR2) is observed, indicating an association of IG1 (TRHR335) and IG3 (OXR2). This confirms the data in example 6.

25

This example further shows that, despite the lack of BRET signal resulting from TRH addition, an increased signal above that observed with OxA addition alone is observed upon addition of both TRH and OxA.

30 This demonstrates that activation of IG1 (TRHR335) does influence signal generation, despite not being able to contribute to IG1-IG2 (TRHR335-barr2) association. Without wishing to be bound by theory, this may imply that IG1 is influencing IG3 by an allosteric mechanism. This may also imply that an active IG

conformation (one that is bound to agonist) is more favourable for signal generation, perhaps enabling increased proximity of RC1 and RC2, or more favourable relative orientation of RC1 and RC2.

- 5 Therefore, this example further demonstrates that co-treatment of IG1 and IG3 can result in additional signal generation and/or information compared to treatment of IG3 alone and that such co-treatment is encompassed by the present invention.

10 **EXAMPLE 10 MEASUREMENT OF A DETECTABLE SIGNAL INDICATIVE OF THE MOLECULAR ASSOCIATION OF TRHR WITH OXR2 AT VARIOUS EXPRESSION LEVELS**

- Referring now to Figure 16, eBRET signals were measured from cells transiently
15 co-expressing TRHR/Rluc, EGFP/barr1 and OxR2 following addition of 10^{-6} M OxA.

- This example shows cumulative eBRET reads over time for each combination of receptors (IG1 and IG3; data captured over 83mins). The same amount of
20 EGFP/barr1 (IG2-RC2) is transfected for each experiment. TRHR/Rluc (IG1-RC1) is transfected at a constant amount (0.1 μ g DNA/well) while OxR2 (IG3) is transfected at varying amounts of DNA (0, 0.01, 0.05, 0.1, 0.5, 0.7 μ g DNA /well). The signal is only detected when OxR2 (IG3) is expressed (no signal was recorded at 0 μ g OxR2).

25

This example demonstrates that signal can be detected when DNA concentrations of OxR2 are as low as 0.01 μ g DNA/well.

- Furthermore, this example demonstrates that increasing the amounts of OxR2
30 DNA in each transfection results in increases in the detectable signal. The largest detectable signal is observed at a 1:1 ratio of DNA concentration (0.1:0.1 μ g DNA/well). Further increases in the OxR2 DNA concentration (0.5 or 0.7 μ g DNA/well) with levels higher than the amount of TRHR/Rluc DNA results in a

lower signal being detected.

This example implies that increasing the number of IG3 molecules (OxR2) leads to a point being reached beyond which the number of IG1 molecules (TRHR) becomes limiting for the formation of hetero-dimers/-oligomers. Consequently, there would be increasing propensity for IG3 molecules (OxR2) not associated with IG1 molecules (TRHR) to associate with IG2-RC2 (EGFP/barr1) upon interacting with the modulator (OxA) without a signal being generated. Therefore, signal generation would be inhibited due to the competition for IG2-RC2 (EGFP/barr1) association.

Therefore, this example provides further and distinct evidence for the molecular association of the thyrotropin releasing hormone receptor with the orexin receptor, as such decreases in signal with increases in IG3 concentration beyond that of IG1 concentration would not be expected to occur if the signal was not dependent upon specific molecular association of IG1 and IG3.

EXAMPLE 11 MEASUREMENT OF A DETECTABLE SIGNAL INDICATIVE OF THE MOLECULAR ASSOCIATION OF TRHR WITH OXR2 IN 384-WELL PLATES

Referring now to Figure 17, BRET signals were measured from cells transiently co-expressing TRHR/Rluc, barr2/Venus and OxR2 with increasing doses of OxA in 96-well and 384-well microplates.

BRET measurements were carried out using the same concentration of cells expressing the same concentration of agents, the same concentration of Rluc substrate (reporter component initiator) and the same concentration of ligand (modulator). The total volume added to each well of the 384-well plate was approximately half that added to each well of the 96-well plate.

This example demonstrates measurement of a detectable signal indicative of the molecular association of TRHR with OxR2 in a dose-dependent manner in 384-

well plates in addition to 96-well plates.

Therefore, this example demonstrates that the method described in the invention is able to be scaled down, thereby making it amenable to high-throughput
5 screening applications.

**EXAMPLE 12 MEASUREMENT OF A DETECTABLE SIGNAL INDICATIVE OF
THE MOLECULAR ASSOCIATION OF THE THYROTROPIN RELEASING
HORMONE RECEPTOR AS IG3 WITH THE OREXIN RECEPTOR AS IG1**

10

Referring now to Figure 18, eBRET signals were measured from cells transiently co-expressing OxR2/Rluc8 and barr2/Venus either with HA-TRHR or pcDNA3. Ligand treatments were either OxA or TRH.

15 Prior to ligand or vehicle treatment (added at 0 minutes), a baseline eBRET signal was recorded for each of the receptor combinations. Within the first 5 minutes, OxA treatment of cells co-expressing OxR2/Rluc8 and barr2/Venus with HA-TRHR, resulted in the eBRET signal rapidly reaching a peak of 0.1 and this signal remained high for the entire recording period (over an hour). A signal was also
20 observed following TRH treatment of cells co-expressing OxR2/Rluc8, barr2/Venus and HA-TRHR. This signal however gradually increased over time to reach 0.05. No ligand-induced eBRET signal was observed following TRH treatment of cells co-expressing OxR2/Rluc8 and barr2/Venus with pcDNA3.

25 This example demonstrates that a signal resulting from the proximity of RC1 and RC2 is detected specifically for the combination where OxR2 is IG1, Rluc8 is RC1, beta-arrestin 2 (barr2) is IG2, Venus is RC2 and HA-TRHR is IG3, and when the modulator, in this case TRH, modulates the association of IG2 and IG3 as a result of interacting specifically with IG3.

30

This example demonstrates that the molecular association of the thyrotropin releasing hormone receptor with the orexin receptor is detected with the thyrotropin releasing hormone receptor as IG3 and the orexin receptor as IG1. This demonstrates detection of the molecular association of these receptors using

an alternative arrangement of IG's compared to previous examples.

This example also demonstrates the use of a second type of luciferase, Rluc8, which in this case is used as RC1 with Venus as RC2.

5

This example further demonstrates that the alternative method of calculating the eBRET signal described in Pfleger *et al.*, 2006 (*Cell Signal* 18, 1664-1670) and Pfleger *et al.*, 2006 (*Nat Protoc* 1, 336-344) can be used in the measurement of a detectable signal indicative of the molecular association of the thyrotropin-releasing hormone receptor and the orexin receptor.

As in example 4, this example demonstrates that IG3 can be tagged, such as by the addition of a hemagglutinin (HA) epitope-tag, however, this tag does not constitute a reporter component and does not interfere with and/or contribute to the signal generated by the proximity of RC1 and RC2. Such tagging enables additional information to be ascertained, such as the relative expression level of IG3.

20 **EXAMPLE 13 MEASUREMENT OF A DETECTABLE SIGNAL INDICATIVE OF THE MOLECULAR ASSOCIATION OF THE THYROTROPIN RELEASING HORMONE RECEPTOR WITH THE OREXIN RECEPTOR WITH A Z-FACTOR IN EXCESS OF 0.6**

Referring now to Figures 19, 20 and 21, eBRET signals were measured from cells transiently co-expressing TRHR/Rluc8 and barr2/Venus with HA-OxR2 aliquoted into all wells of a 96-well plate. Phosphate-buffered saline (PBS) was added to the first two rows and the last two rows of the 96-well plate (48 wells in total) as a vehicle control. OxA was added to the middle four rows of the 96-well plate (48 wells in total). Data are presented as fluorescence/luminescence.

30

Prior to ligand or vehicle treatment (added at 0 minutes), baseline readings were recorded. OxA treatment of cells co-expressing TRHR/Rluc8 and barr2/Venus with HA-OxR2 resulted in an increase in the fluorescence/luminescence ratio (Figure 20) that was not observed following treatment with phosphate-buffered saline (PBS) vehicle control (Figure 19). Analysis of the

35

fluorescence/luminescence ratios comparing 48-wells treated with OxA (defined as 'signal' with respect to z-factor calculation) and 48-wells treated with PBS (defined as 'background' with respect to z-factor calculation) results in a z-factor of 0.67 using the calculation described by Zhang *et al.*, 1999 (*J Biomol Screen* 4, 67-73). Means are shown as solid lines and 3 standard deviations from the mean are shown as dotted lines.

This example demonstrates that a signal resulting from the proximity of RC1 and RC2 is detected specifically for the combination where TRHR is IG1, Rluc8 is RC1, beta-arrestin 2 (barr2) is IG2, Venus is RC2 and HA-OxR2 is IG3, and when the modulator, in this case OxA, modulates the association of IG2 and IG3 as a result of interacting specifically with IG3.

This example demonstrates that the molecular association of the thyrotropin releasing hormone receptor with the orexin receptor is detected in a manner that results in a z-factor in excess of 0.6 and is therefore amenable to high-throughput screening.

This example further demonstrates a third method of representing BRET data that can be used in representing a detectable signal indicative of the molecular association of the thyrotropin-releasing hormone receptor and the orexin receptor.

As in examples 4 and 12, this example demonstrates that IG3 can be tagged, such as by the addition of a hemagglutinin (HA) epitope-tag, however, this tag does not constitute a reporter component and does not interfere with and/or contribute to the signal generated by the proximity of RC1 and RC2. Such tagging enables additional information to be ascertained, such as the relative expression level of IG3.

The Claims Defining the Invention are as Follows:

1. A hetero-dimeric or hetero-oligomeric receptor, comprising at least one thyrotropin releasing hormone receptor subunit associated with at least one orexin receptor subunit.
5
2. A method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.
- 10 3. A method according to claim 2 characterised in that the thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist is co-administered with an orexin receptor agonist, inverse agonist or antagonist.
- 15 4. A method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by administering a therapeutically effective amount of an orexin receptor agonist, inverse agonist or antagonist.
- 20 5. A method according to claim 4 characterised in that the orexin receptor agonist, inverse agonist or antagonist is co-administered with a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.
- 25 6. A method for the manufacture of a medicament for the treatment of a patient suffering from an orexin-related ailment comprising use of a therapeutically effective amount of a thyrotropin releasing hormone receptor agonist, inverse agonist or antagonist.
- 30 7. A method according to claim 6 characterised in that the medicament contains an orexin receptor agonist, inverse agonist or antagonist.
- 35 8. A method for the manufacture of a medicament for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment comprising use of a therapeutically effective amount of an orexin receptor agonist, inverse agonist or antagonist.
9. A method according to claim 8 characterised in that the medicament contains a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

10. A method for the treatment of a patient suffering from an orexin-related ailment by administering a therapeutically effective amount of a thyrotropin-releasing hormone-selective binding agent, or fragment thereof.
- 5
11. A method according to claim 10 characterised in that the thyrotropin-releasing hormone-selective binding agent is an antibody, including a humanised antibody, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody and/or an anti-idiotypic antibody.
- 10
12. A method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by administering a therapeutically effective amount of an orexin-selective binding agent, or fragment thereof.
- 15
13. A method according to claim 12 characterised in that the orexin-selective binding agent is an antibody, including a humanised antibody, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody and/or an anti-idiotypic antibody.
- 20
14. A method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective activity, the method comprising the steps of:
- 25
- a) determining whether, and/or the extent to which, the test compound interacts with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor; and
- 30
- b) if the test compound interacts with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor, determining whether, or the extent to which the test compound interacts with the orexin receptor in the absence of the thyrotropin releasing hormone receptor;
- 35
- such that a test compound that exhibits greater affinity and/or potency and/or efficacy when interacting with the orexin receptor while the orexin receptor is associated with the thyrotropin releasing hormone receptor is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.
15. A method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective activity, the method

comprising the steps of:

- 5 a) determining whether, and/or the extent to which, the test compound interacts with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor; and
- 10 b) if the test compound interacts with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor, determining whether, or¹ the extent to which the test compound interacts with the thyrotropin releasing hormone receptor in the absence of the orexin receptor;
- 15 such that a test compound that exhibits greater affinity and/or potency and/or efficacy when interacting with the thyrotropin releasing hormone receptor while the thyrotropin releasing hormone receptor is associated with the orexin receptor is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.

16. A method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective antagonism or partial agonism, the method comprising the steps of:
- 20 a) determining whether, and/or the extent to which, the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:
- 25 i). a first agent, comprising the orexin receptor coupled to a first reporter component;
- ii). a second agent, comprising an interacting group coupled to a second reporter component;
- 30 iii). a third agent, comprising the thyrotropin releasing hormone receptor;
- iv). an agonist of the orexin receptor, the thyrotropin releasing hormone receptor and/or the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 35 wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the thyrotropin releasing hormone receptor;
- b) detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an antagonist or partial

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- agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 5 c) if the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, determining whether, or the extent to which the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and the orexin receptor in the absence of the thyrotropin releasing hormone receptor; such that a test compound that exhibits greater antagonistic or partial agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.
- 10
- 15 17. A method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective antagonism or partial agonism, the method comprising the steps of:
- a) determining whether, and/or the extent to which, the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:
- 20 i). a first agent, comprising the thyrotropin releasing hormone receptor coupled to a first reporter component;
- ii). a second agent, comprising an interacting group coupled to a second reporter component;
- 25 iii). a third agent, comprising the orexin receptor;
- iv). an agonist of the orexin receptor, the thyrotropin releasing hormone receptor and/or the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 30 wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the orexin receptor;
- b). detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;
- 35 c) if the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-

oligomer, determining whether, or the extent to which the test compound is an antagonist or partial agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and the orexin receptor in the absence of the thyrotropin releasing hormone receptor; such that a test compound that exhibits greater antagonistic or partial agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.

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18. A method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer selective inverse agonism, the method comprising the steps of:

15 a) determining whether, and/or the extent to which, the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:

- 20 i). a first agent, comprising the orexin receptor coupled to a first reporter component;
- ii). a second agent, comprising an interacting group coupled to a second reporter component;
- iii). a third agent, comprising a constitutively active thyrotropin releasing hormone receptor;

25 wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the thyrotropin releasing hormone receptor;

30 b) detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;

35 c) if the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, determining whether, or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and the orexin receptor in the absence of the

- 62 -

thyrotropin releasing hormone receptor; such that a test compound that exhibits greater inverse agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.

19. A method for screening a test compound for thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer inverse agonism, the method comprising the steps of:

a) determining whether, and/or the extent to which, the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, by contacting said test compound with a system comprising:

i). a first agent, comprising the thyrotropin-releasing hormone receptor coupled to a first reporter component;

ii). a second agent, comprising an interacting group coupled to a second reporter component;

iii). a third agent, comprising a constitutively active orexin receptor; wherein proximity of the first and second reporter components generates a signal; and wherein the modulator modulates the association of the interacting group with the orexin receptor;

b) detecting a decrease in the signal as a determination of whether and/or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer;

c) if the test compound is an inverse agonist of the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer, determining whether, or the extent to which the test compound is an inverse agonist of the thyrotropin releasing hormone receptor in the absence of the orexin receptor and the orexin receptor in the absence of the thyrotropin releasing hormone receptor; such that a test compound that exhibits greater inverse agonistic properties when interacting with the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer is selective for the thyrotropin releasing hormone receptor / orexin receptor hetero-dimer/-oligomer.

20. Selective agonists and/or antagonists and/or inverse agonists of the thyrotropin releasing hormone receptor/orexin receptor hetero-dimer/-oligomer.
- 5 21. A method for the treatment of a patient suffering from a thyrotropin-releasing hormone-related ailment by administering a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist.
- 10 22. A method according to claim 21 characterised in that the selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist is co-administered with a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.
- 15 23. A method according to claim 21 characterised in that the selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist is co-administered with an orexin receptor agonist, inverse agonist or antagonist.
- 20 24. A method for the treatment of a patient suffering from a orexin-related ailment by administering a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist.
- 25 25. A method according to claim 24 characterised in that the selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist is co-administered with a thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.
- 30 26. A method according to claim 24 characterised in that the selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist is co-administered with an orexin receptor agonist, inverse agonist or antagonist.
- 35 27. A method for the manufacture of a medicament for the treatment of a patient suffering from an thyrotropin-releasing hormone -related ailment comprising use of a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist.

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28. A method according to claim 27 characterised in that the medicament contains an orexin receptor agonist, inverse agonist or antagonist.

5 29. A method according to claim 27 characterised in that the medicament contains an thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

10 30. A method for the manufacture of a medicament for the treatment of a patient suffering from an orexin-related ailment comprising use of a therapeutically effective amount of a selective orexin receptor / thyrotropin-releasing hormone receptor hetero-dimer / -oligomer agonist, inverse agonist or antagonist.

15 31. A method according to claim 30 characterised in that the medicament contains an orexin receptor agonist, inverse agonist or antagonist.

32. A method according to claim 30 characterised in that the medicament contains an thyrotropin-releasing hormone receptor agonist, inverse agonist or antagonist.

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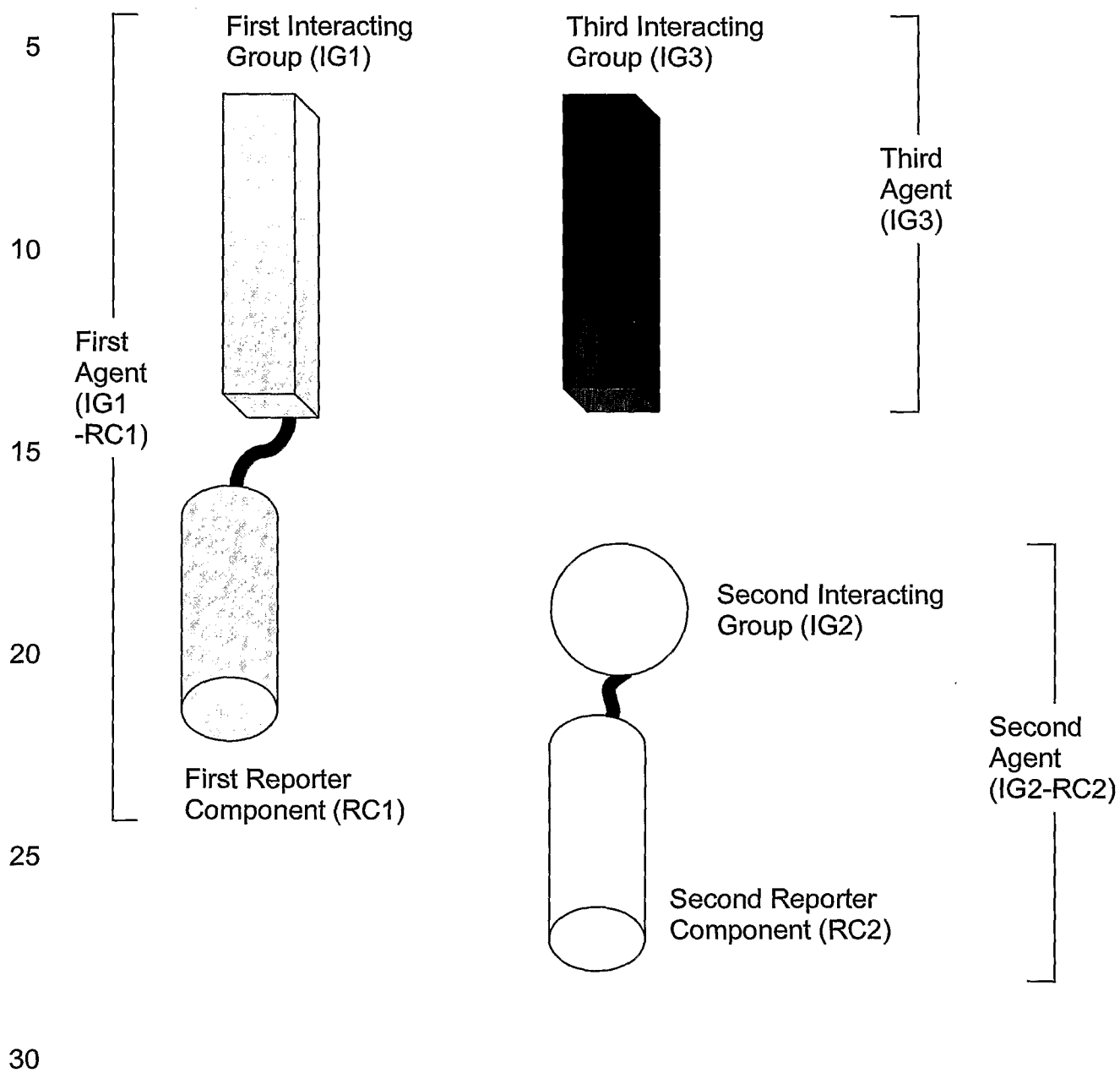


FIGURE 1

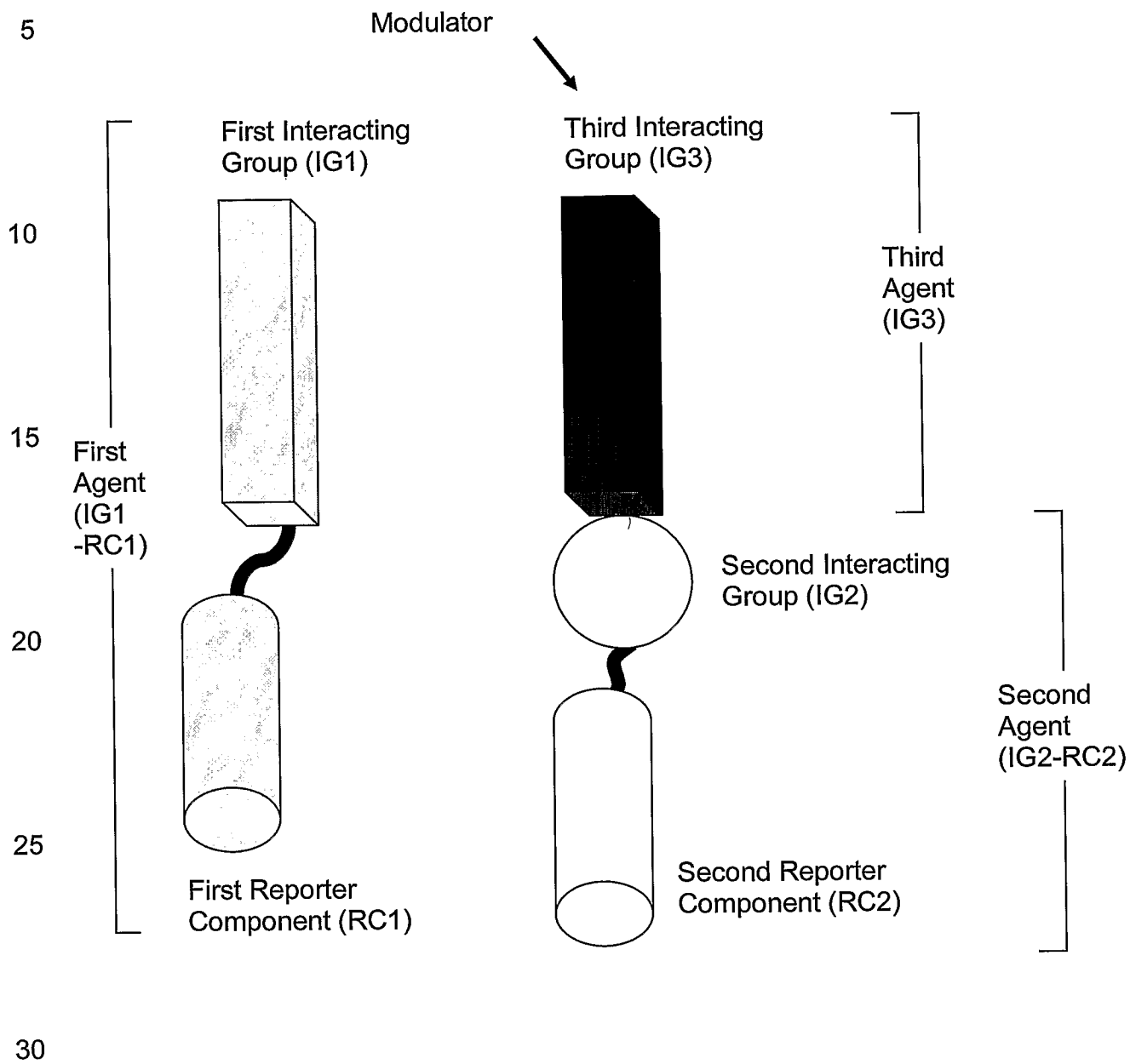


FIGURE 2

- 3/21 -

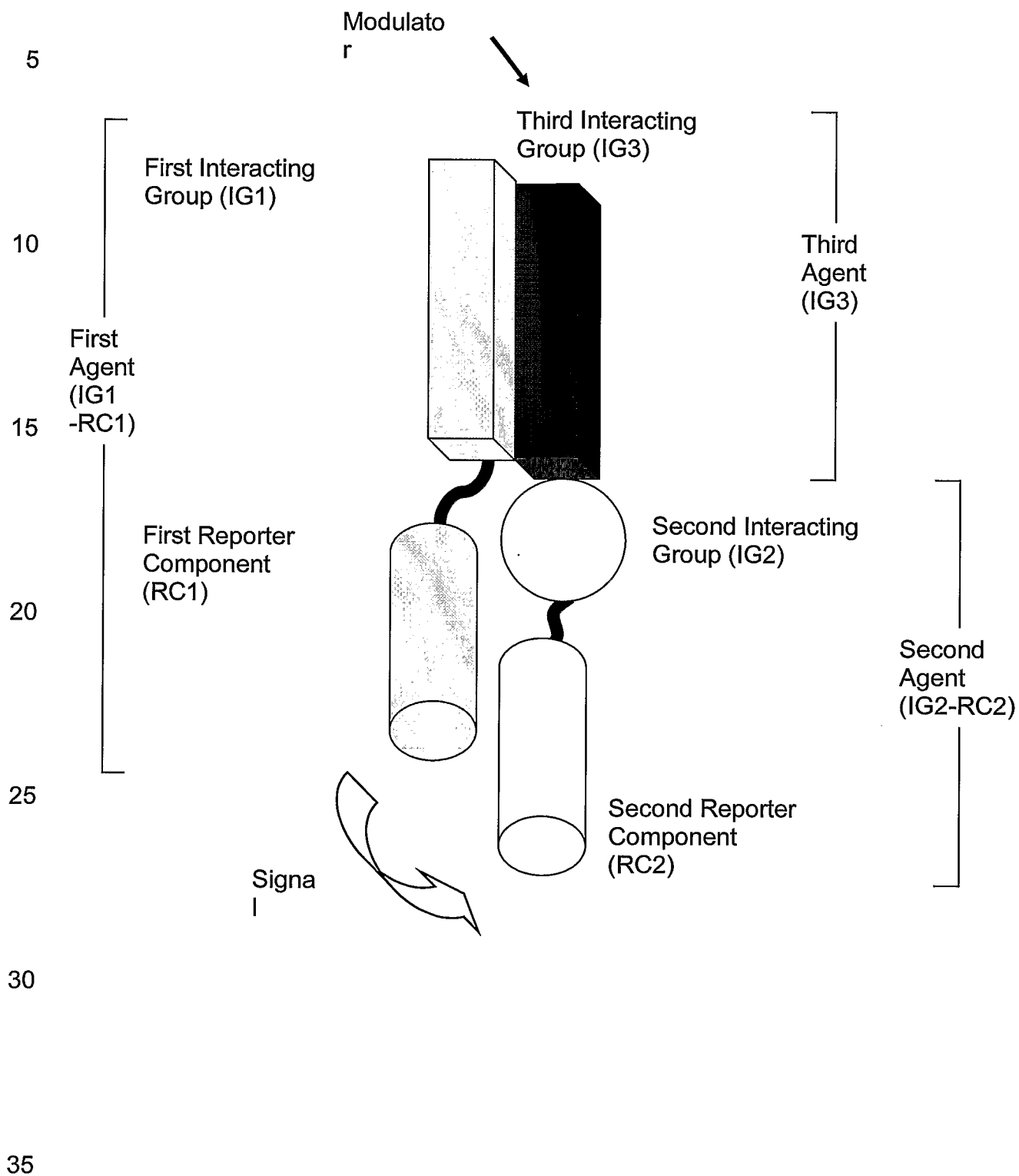


FIGURE 3

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TRHR/Rluc + barr2/Venus and other GPCRs

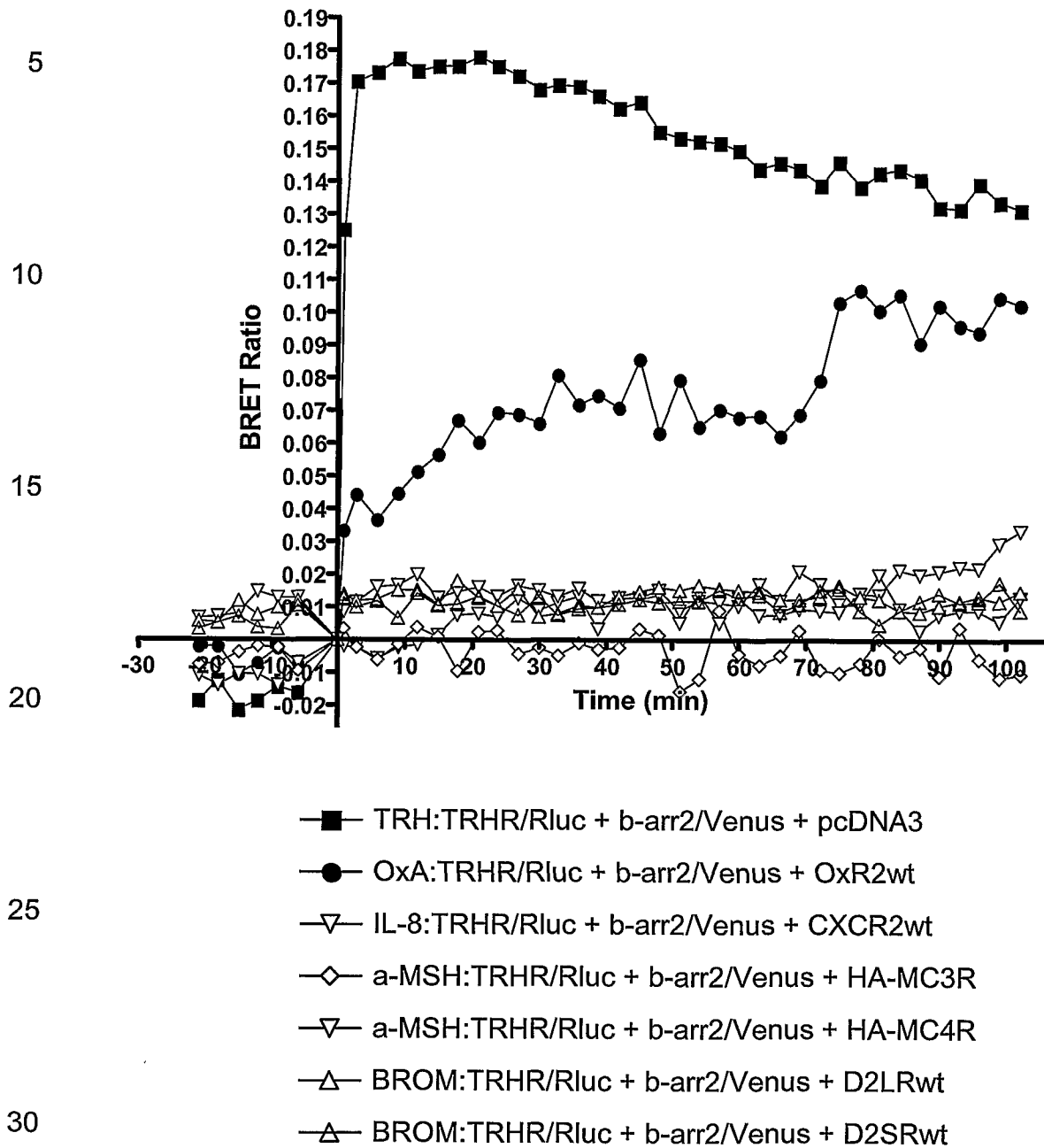
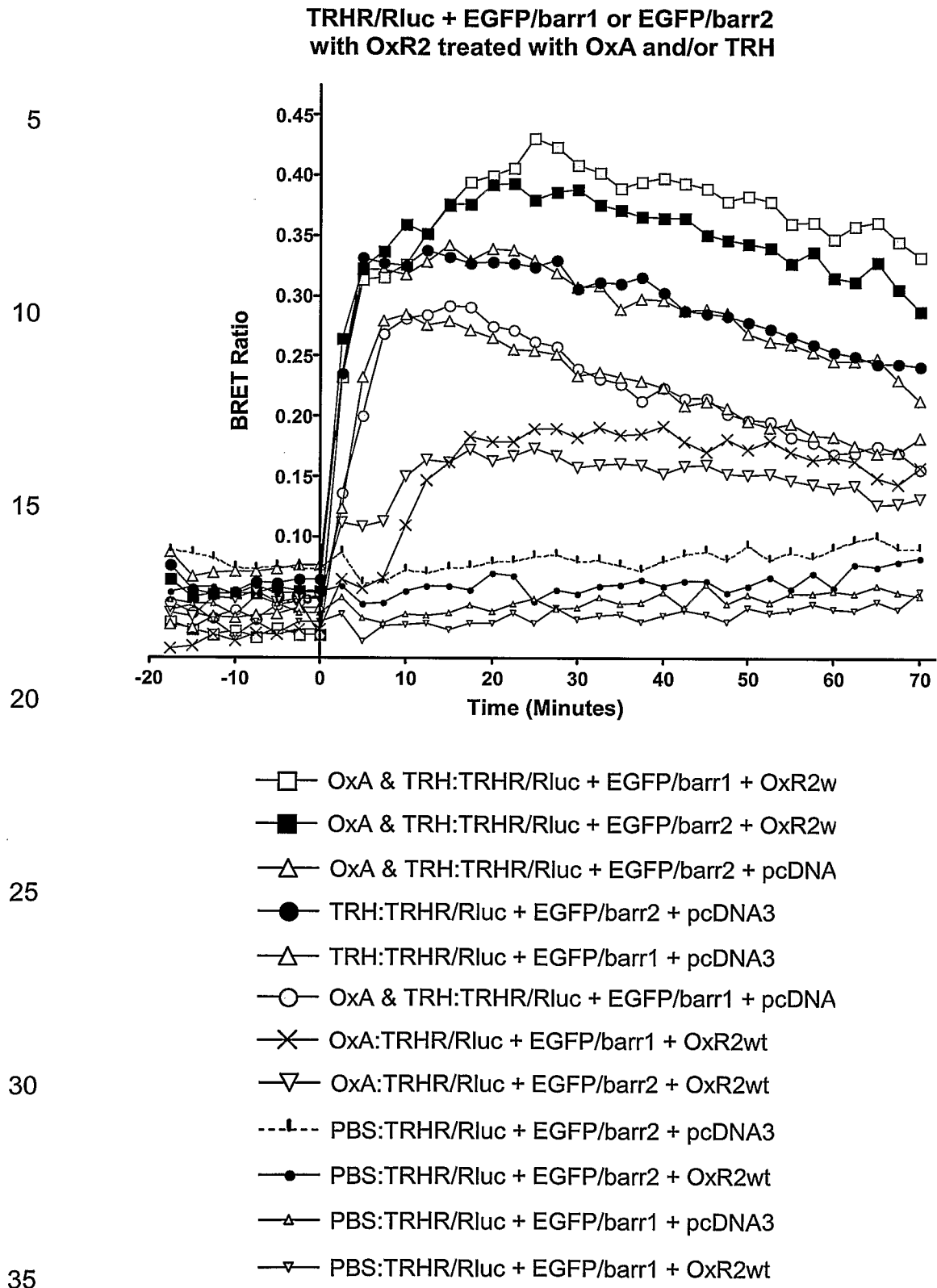
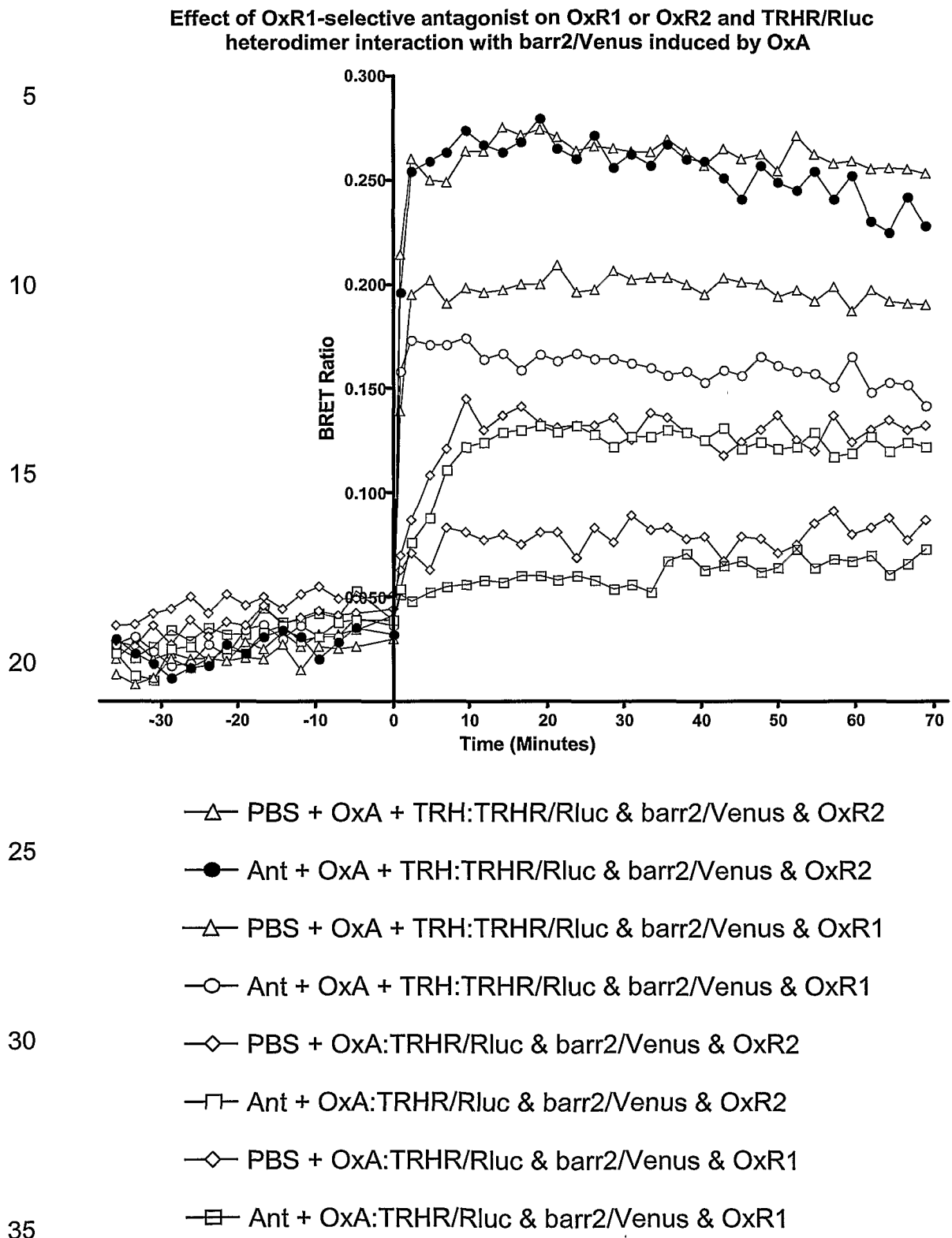


FIGURE 4

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**FIGURE 5**

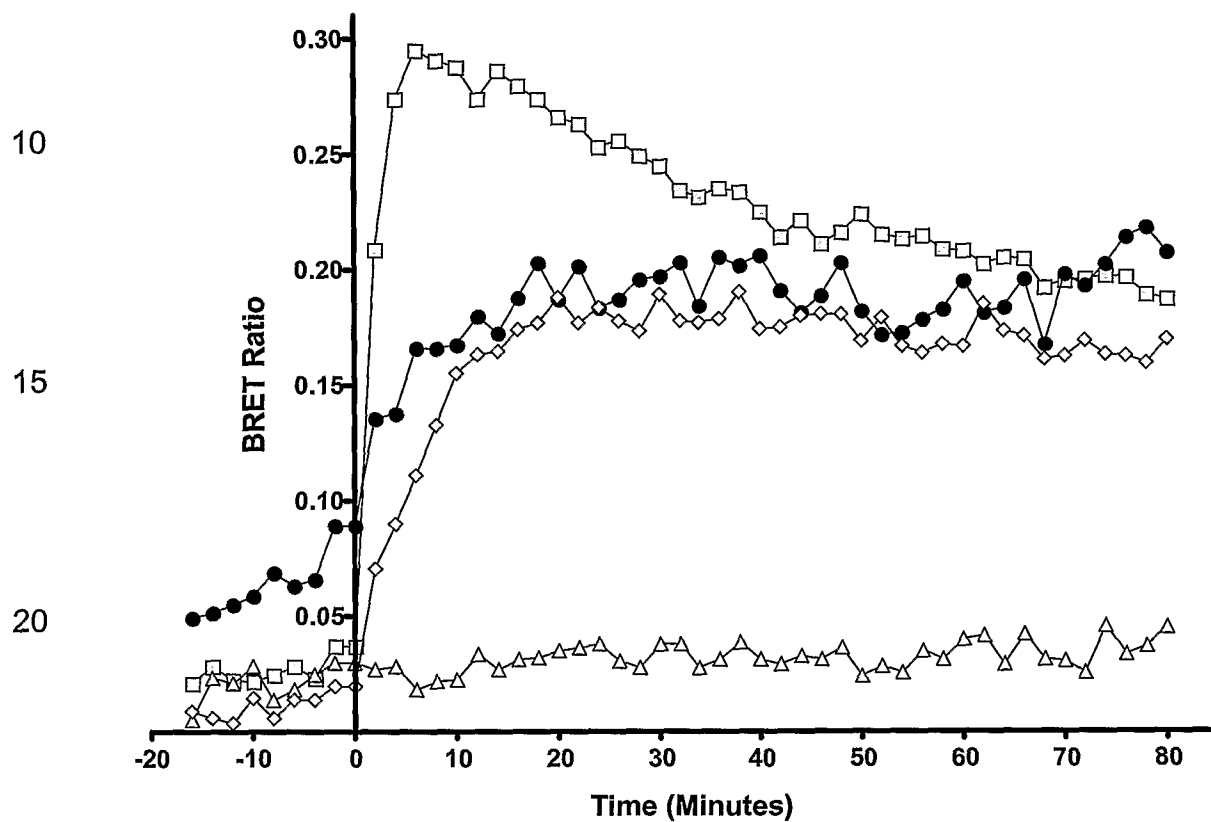
- 6/21 -

**FIGURE 6**

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HA-OXR2 and TRHR/Rluc + EGFP/barr1 or EGFP/barr2



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- TRH:TRHR/Rluc + EGFP/barr1 + pcDNA3
- OxA:TRHR/Rluc + EGFP/barr2 + HA-OxR2
- ◇— OxA:TRHR/Rluc + EGFP/barr1 + HA-OxR2
- △— OxA:TRHR/Rluc + EGFP/barr1 + pcDNA3

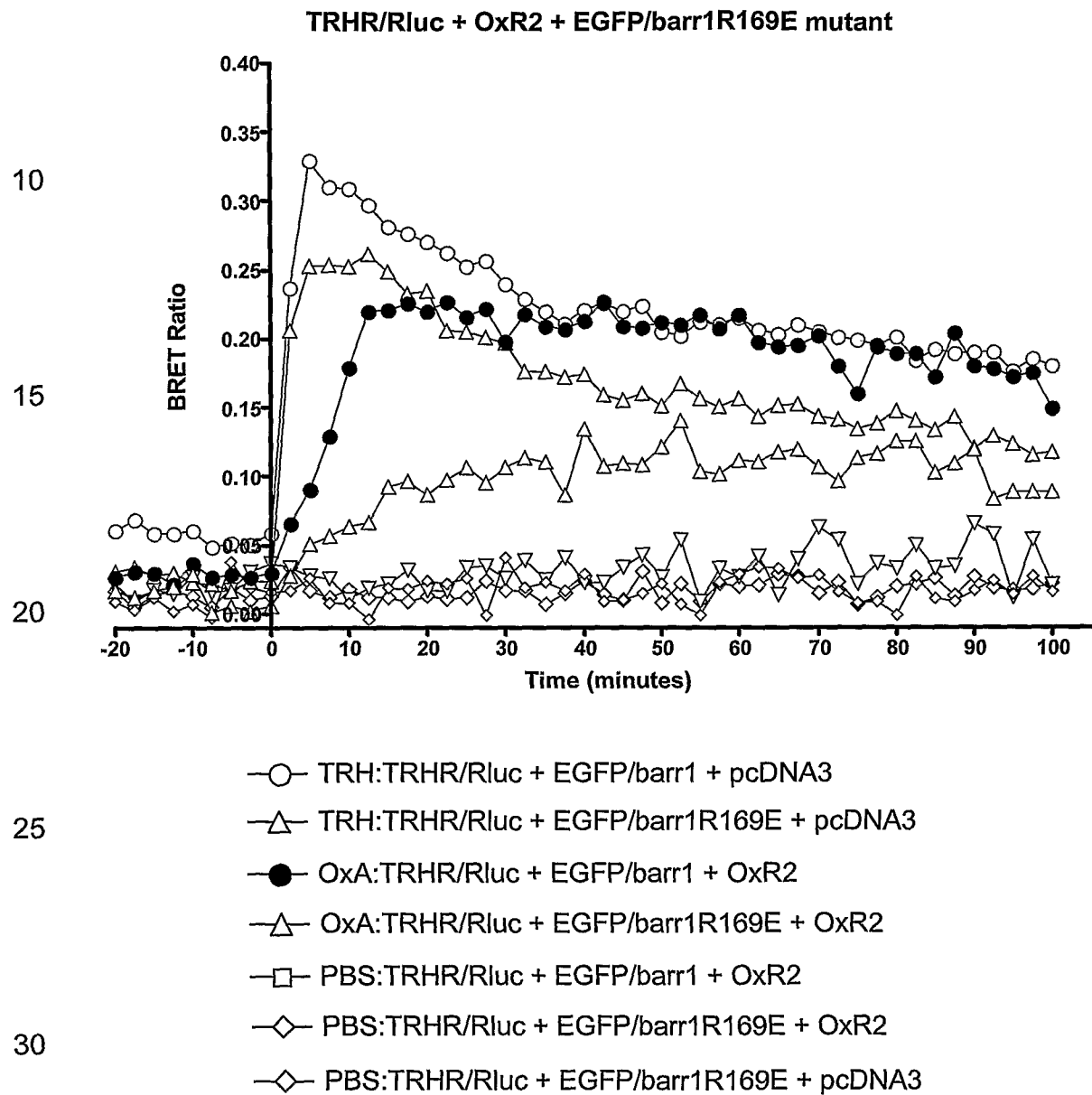
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FIGURE 7

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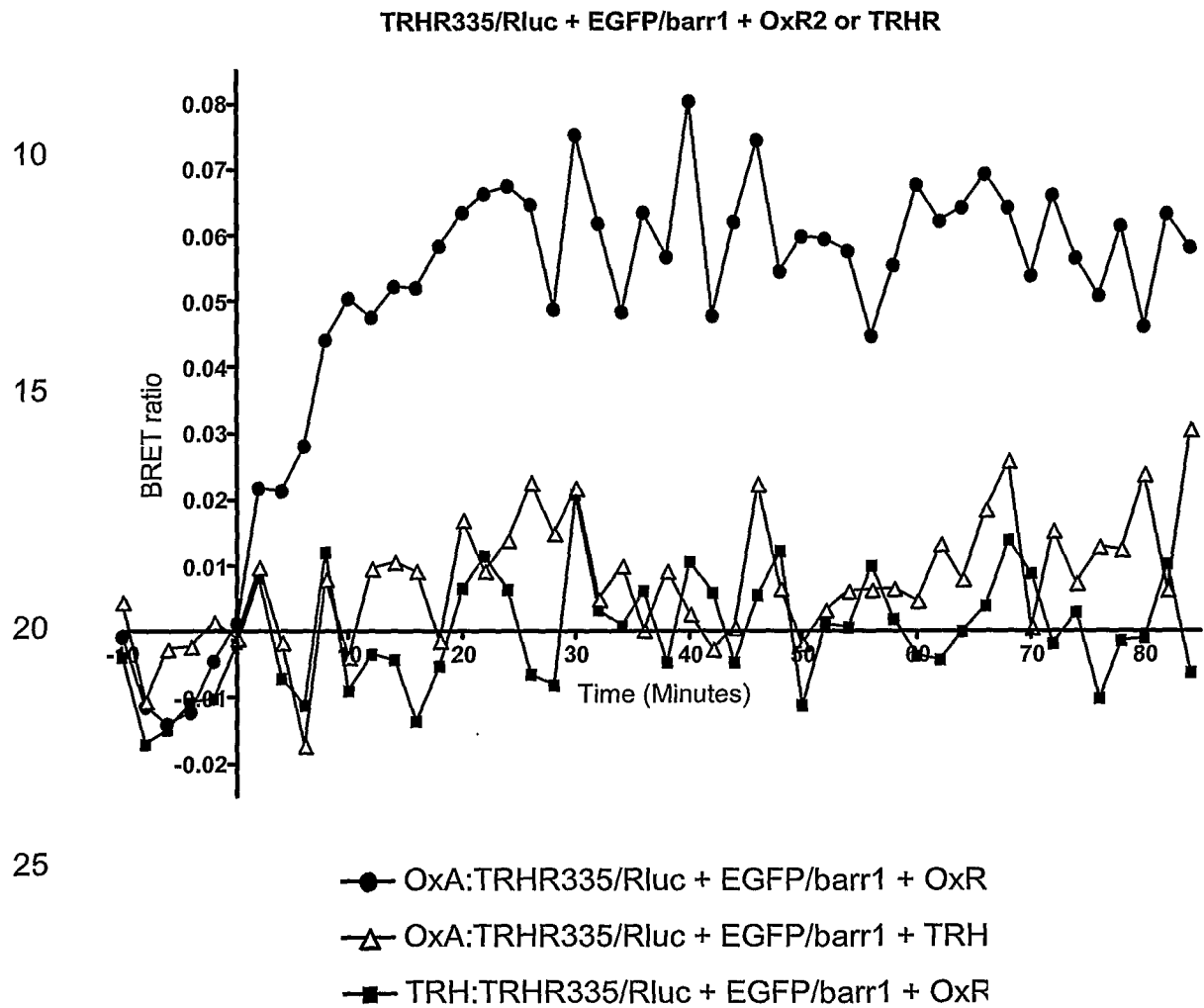
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FIGURE 8

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FIGURE 9

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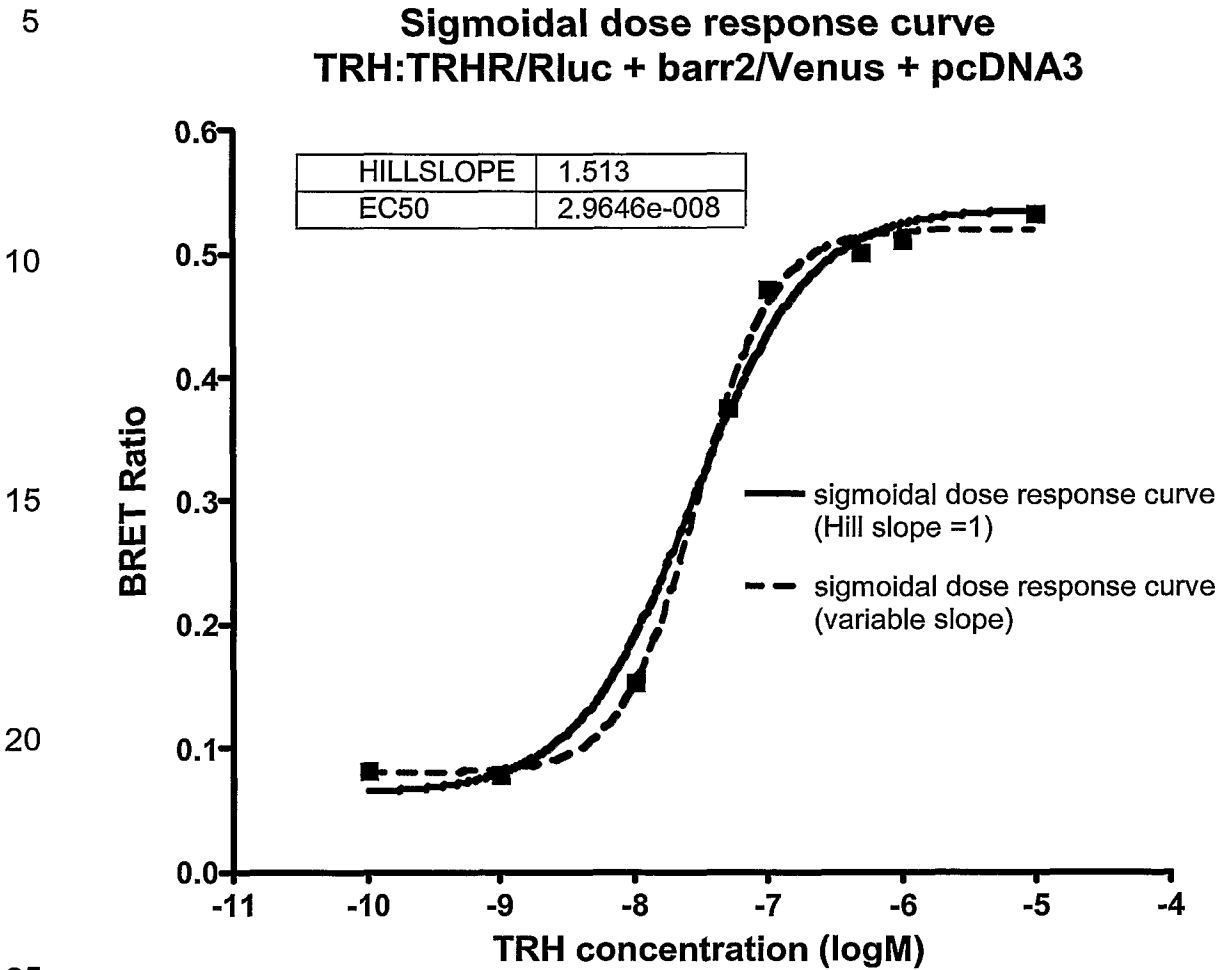


FIGURE 10

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Sigmoidal dose response curve
OxA:OxR2/Rluc + barr2/Venus + pcDNA3

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- — sigmoidal dose response curve (Hill Slope = 1)
- - sigmoidal dose response curve (variable slope)

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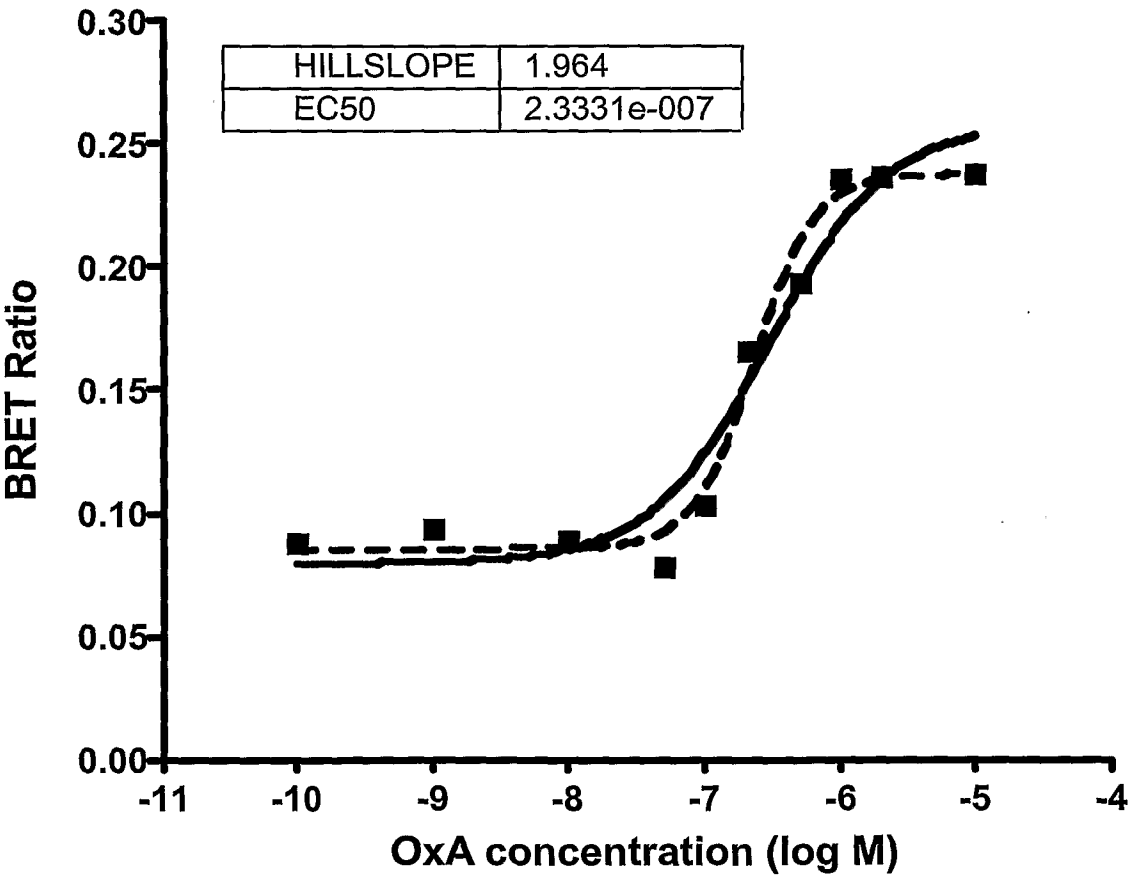


FIGURE 11

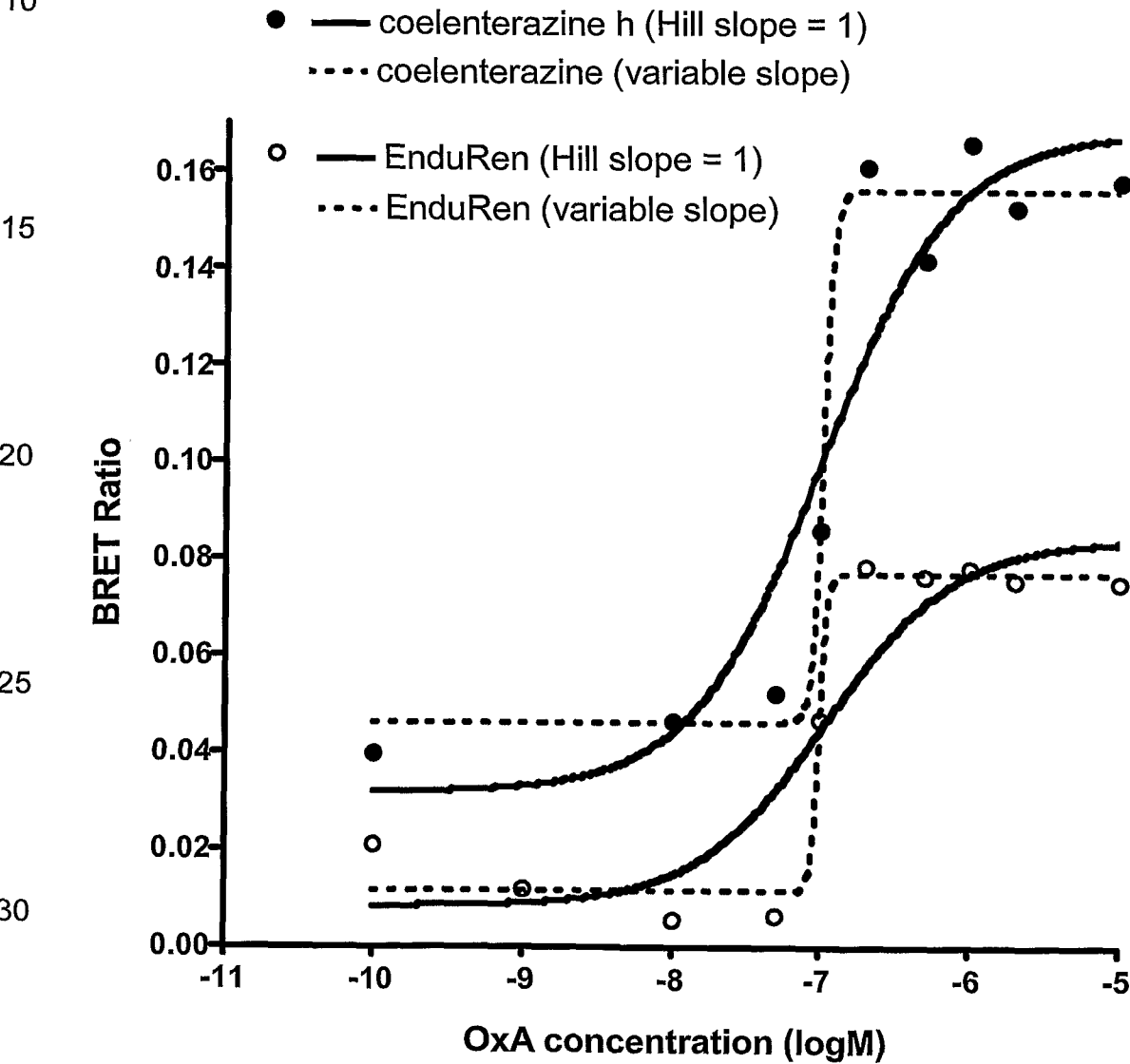
- 12/21 -

Sigmoidal dose response curve
OxA:TRHR/Rluc + barr2/Venus + OxA2

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	coelenterazine h	EnduRen
HILLSLOPE	13.07	17.24
EC50	1.0441e-007	9.9010e-008

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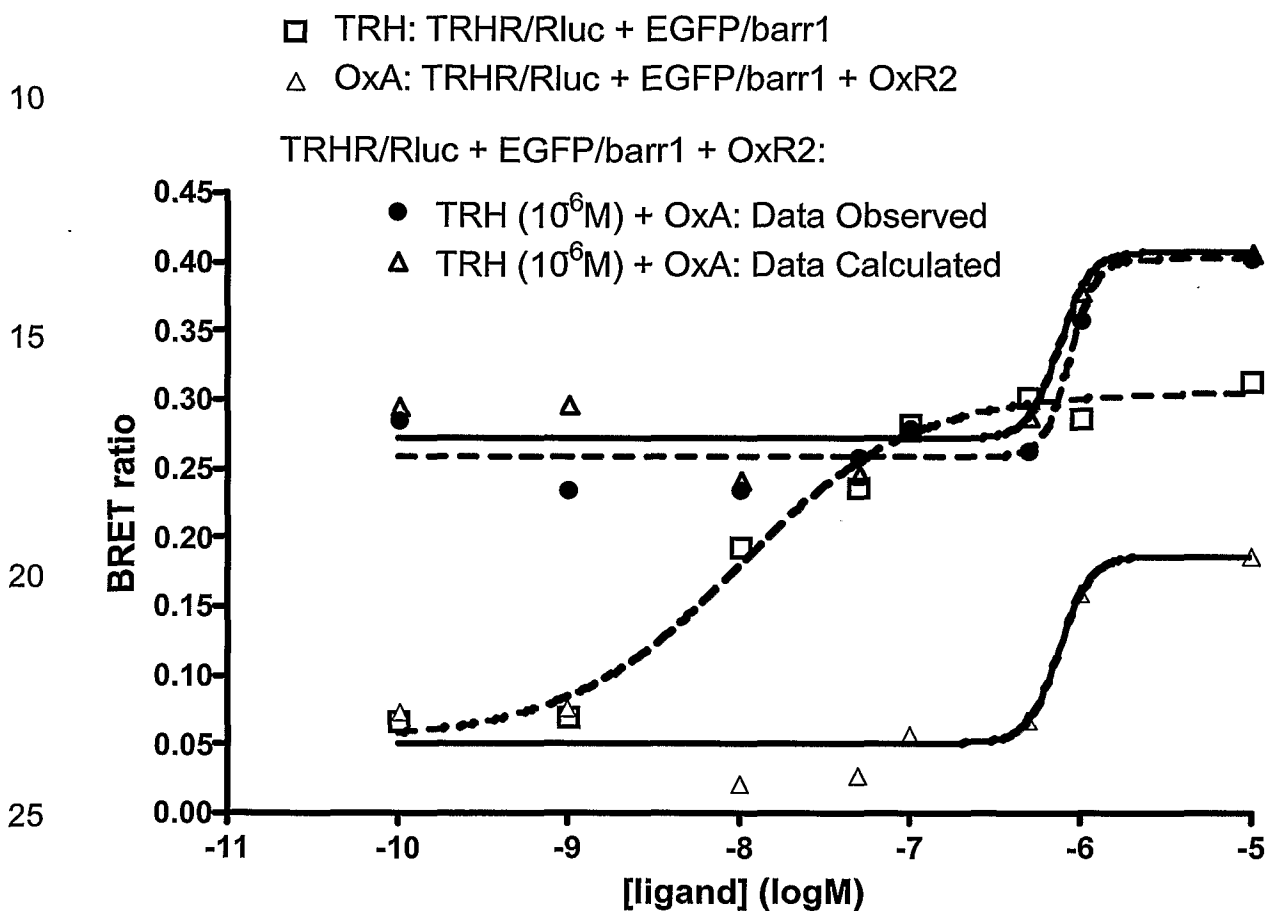


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FIGURE 12

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TRHR and OxR2 dose response curves

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FIGURE 13

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TRHR and OxR2 dose response curves (2)

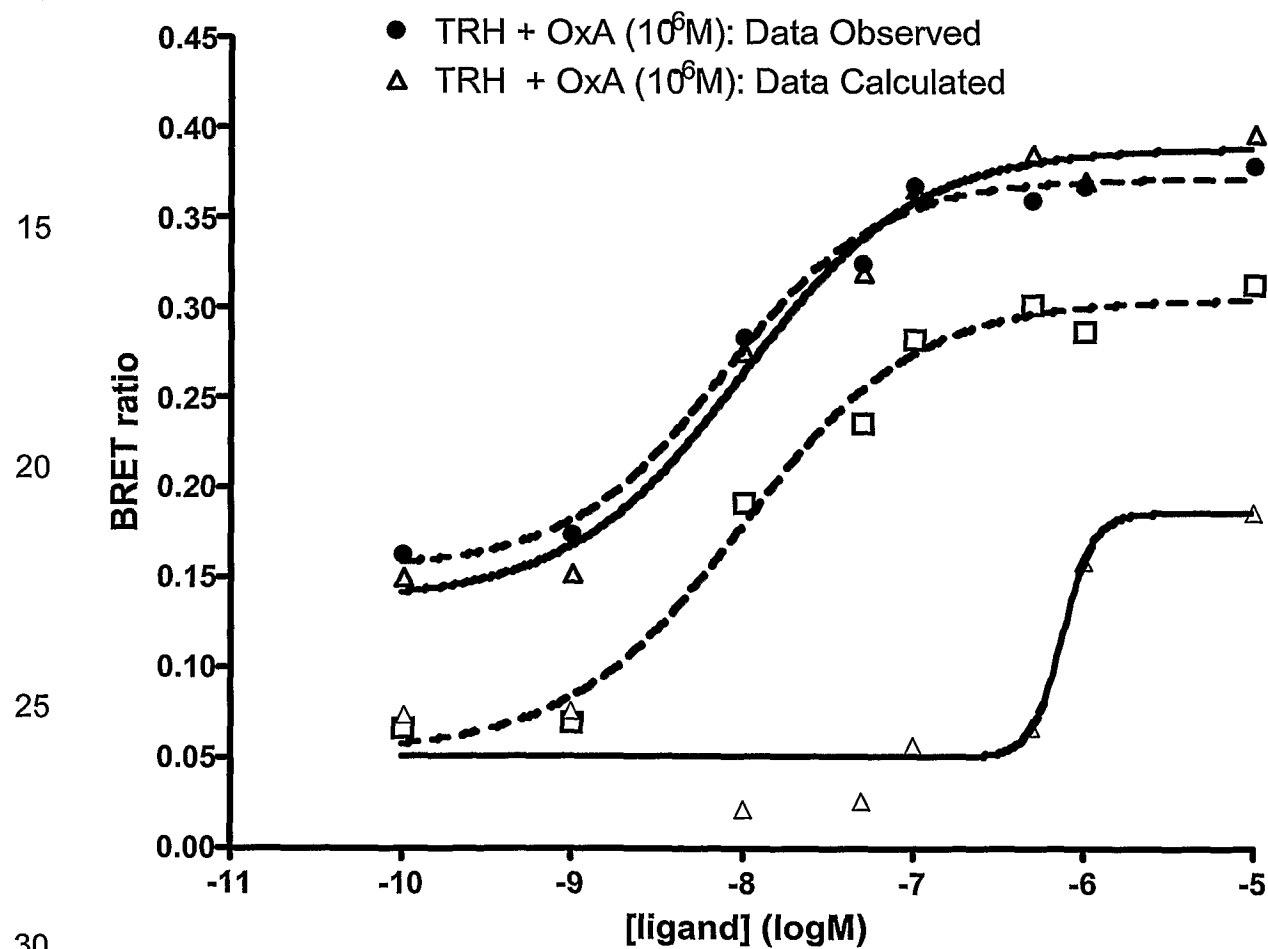
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□ TRH: TRHR/Rluc + EGFP/barr1

△ OxA: TRHR/Rluc + EGFP/barr1 + OxR2

TRHR/Rluc + EGFP/barr1 + OxR2:

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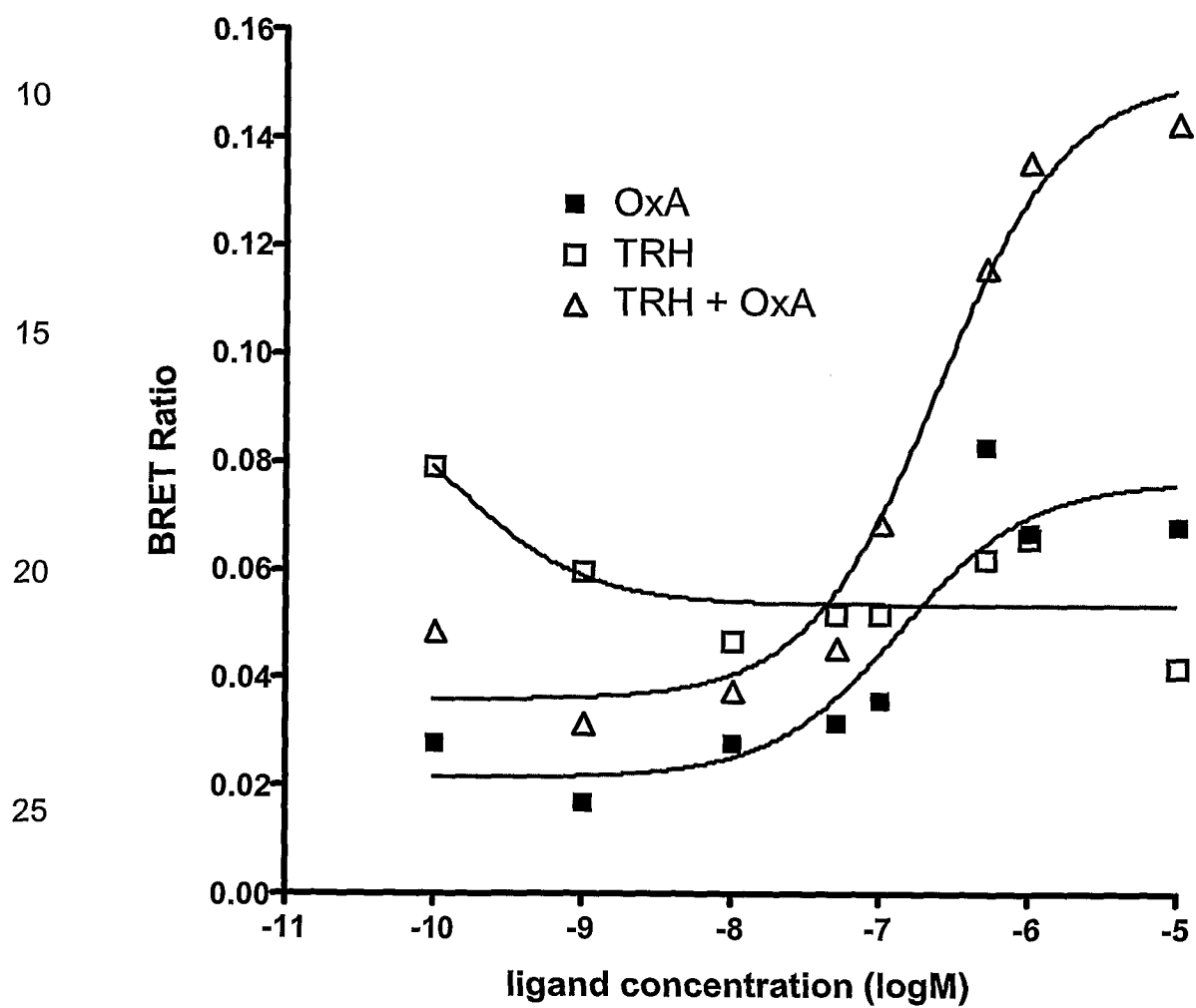
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FIGURE 14

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TRHR335/Rluc & barr2/Venus & OxR2



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FIGURE 15

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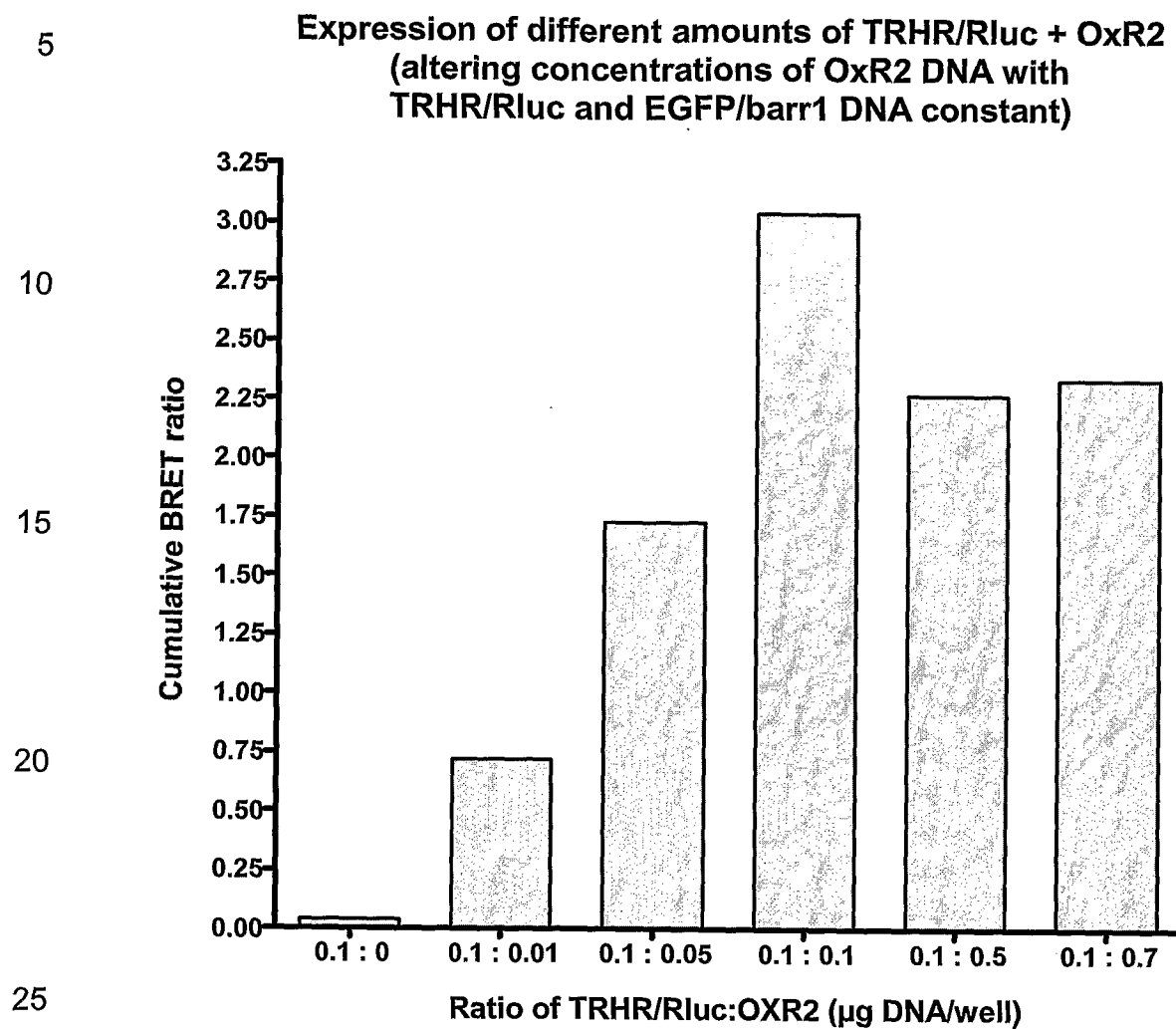
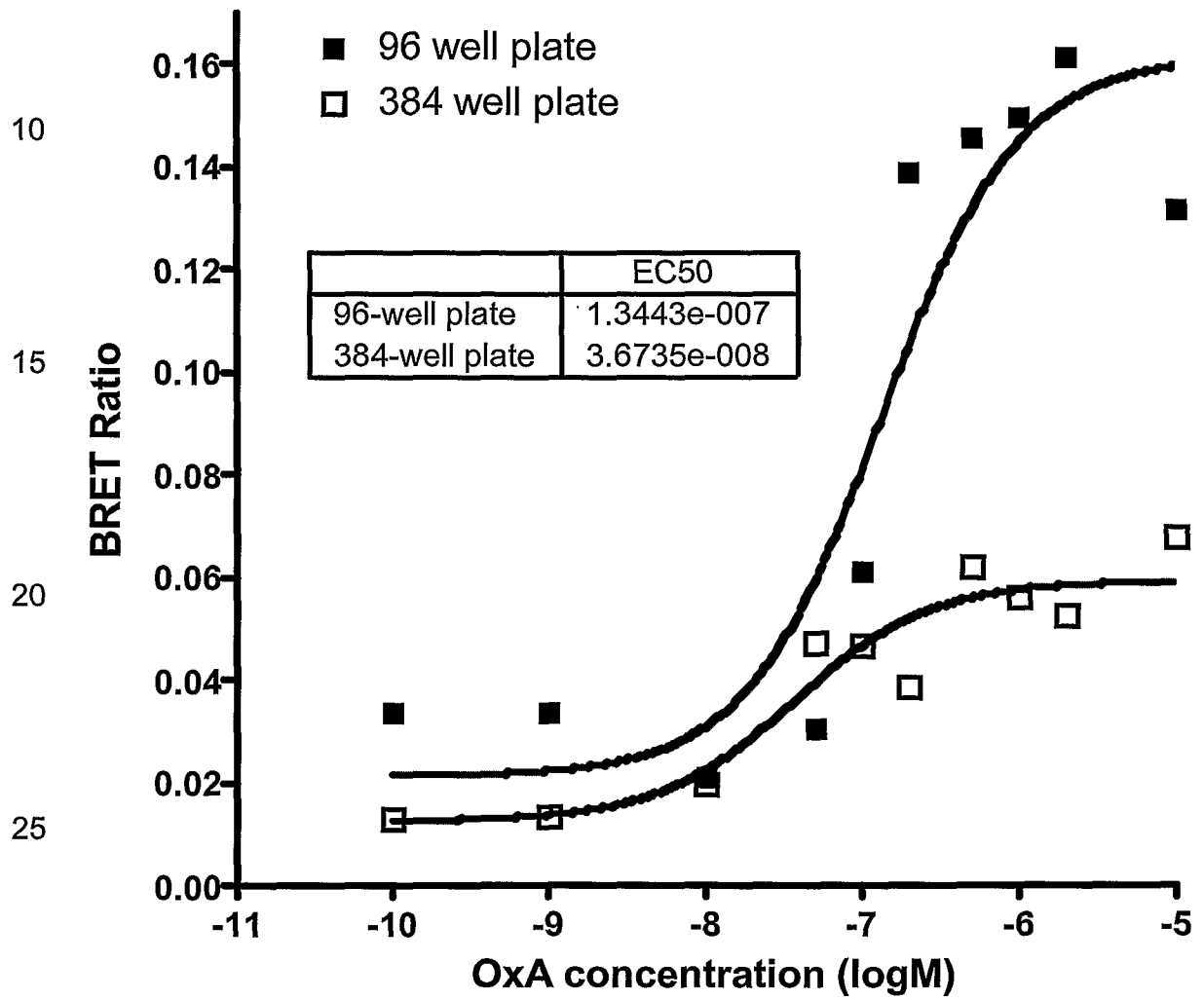


FIGURE 16

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96-well plate versus 384-well plate
OxA:TRHR/Rluc + barr2/Venus + OXR2



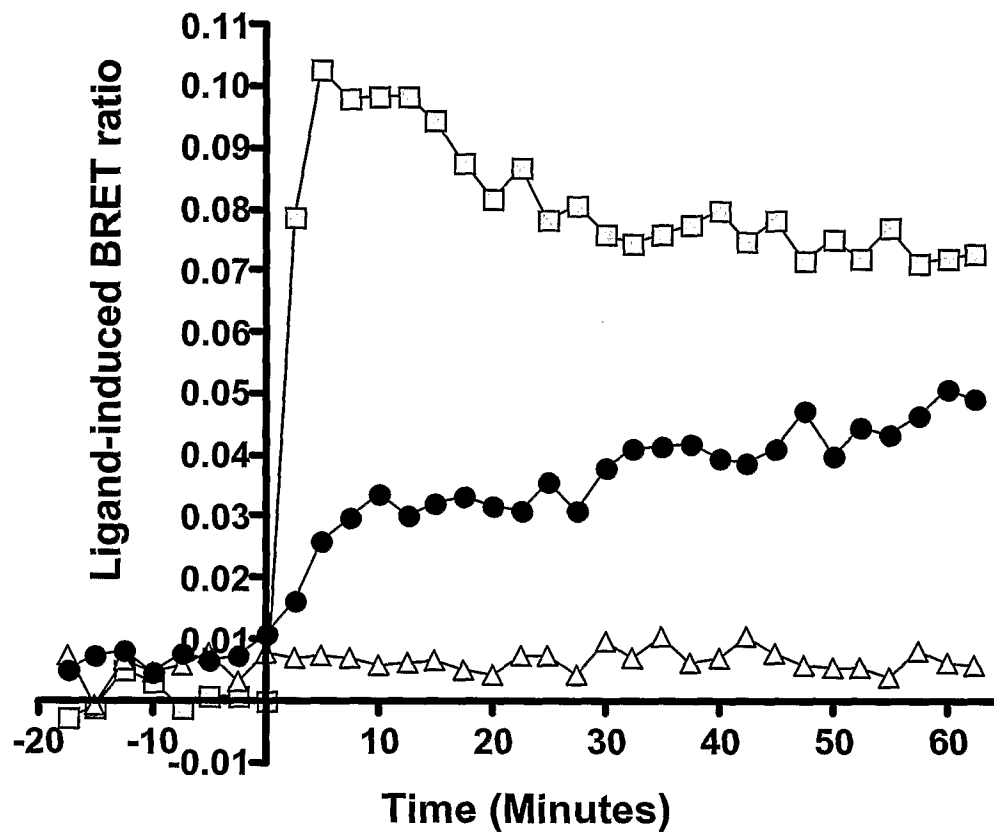
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FIGURE 17

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HA-TRHR and OxR2/Rluc8 + barr2/Venus



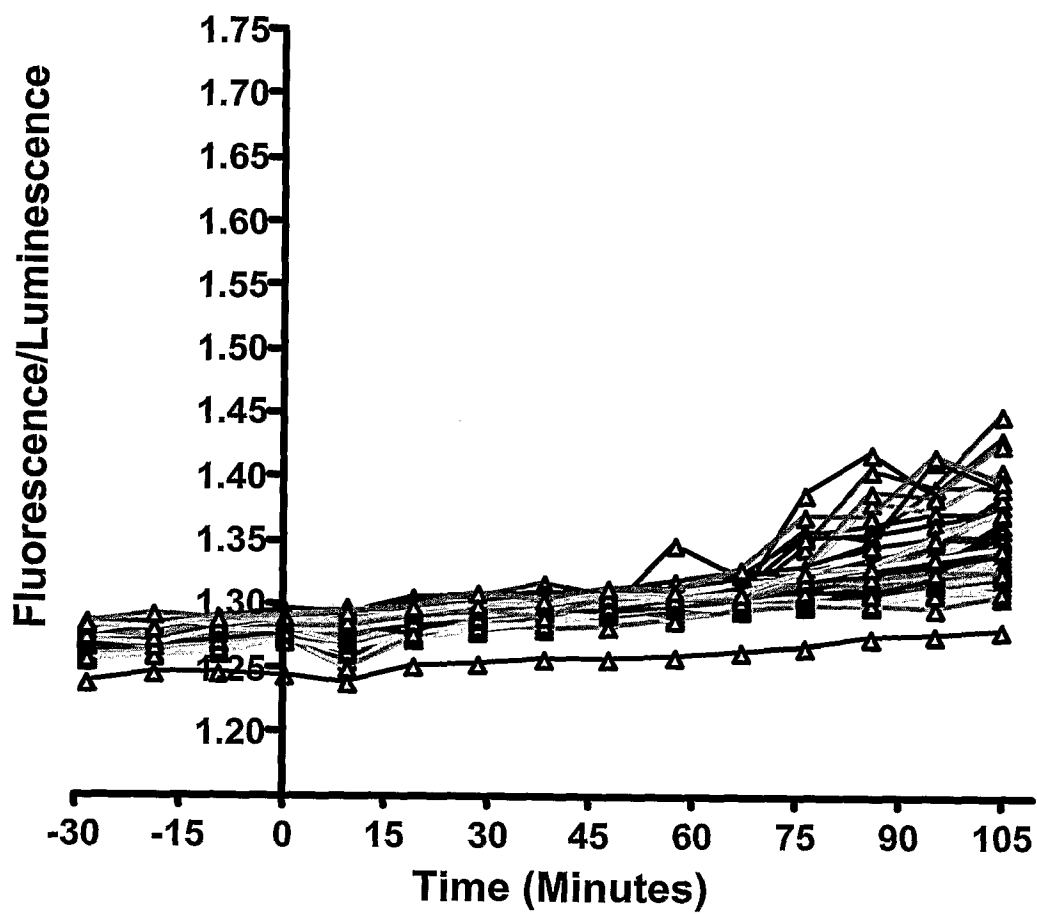
- OxA: OxR2/Rluc8 + barr2/Venus + HA-TRHR
- TRH: OxR2/Rluc8 + barr2/Venus + HA-TRHR
- △— TRH: OxR2/Rluc8 + barr2/Venus + pcDNA3

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15 **FIGURE 18**

- 19/21 -

PBS: TRHR/Rluc8 + barr2/Venus + HA-OxR2

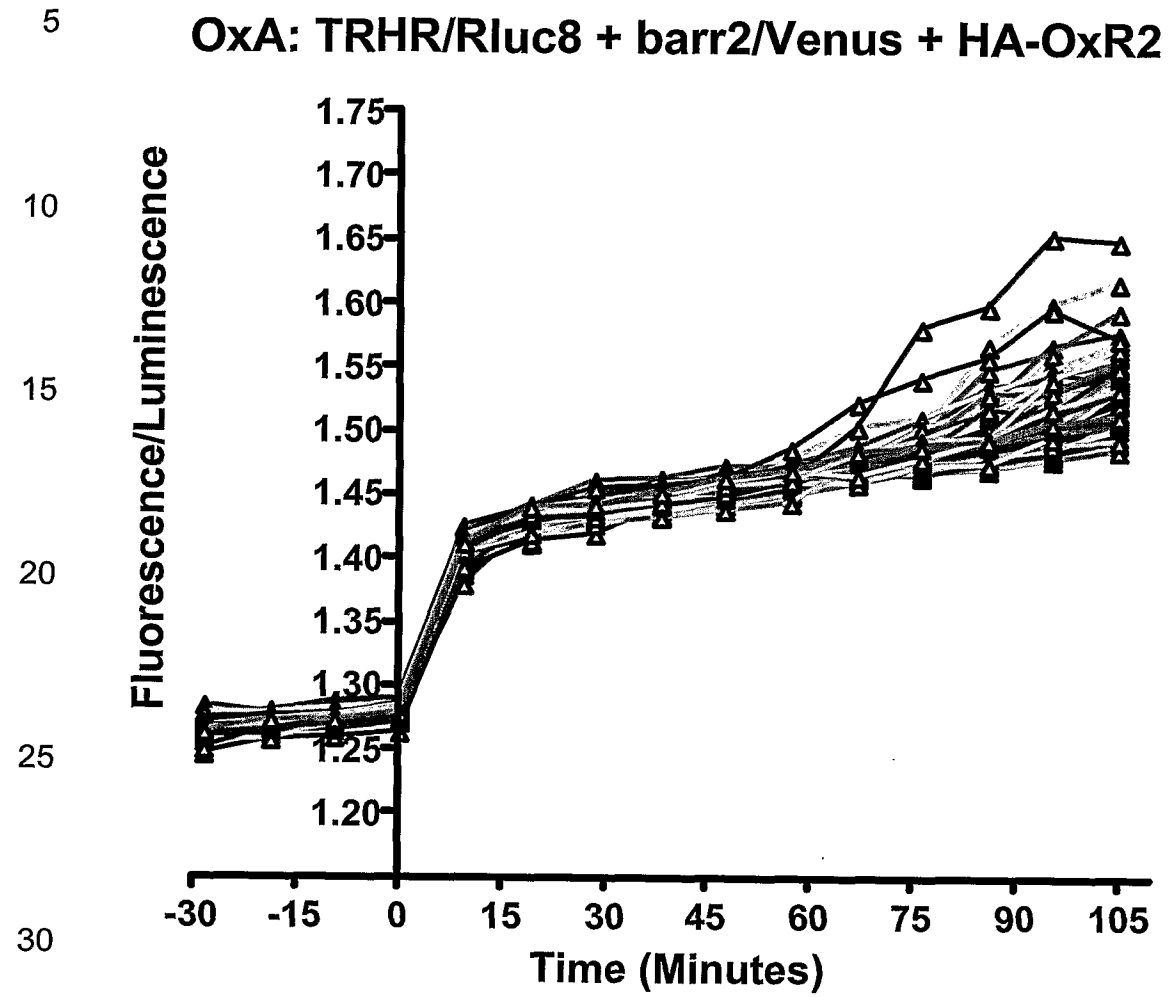
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20 **FIGURE 19**

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FIGURE 20

- 21/21 -

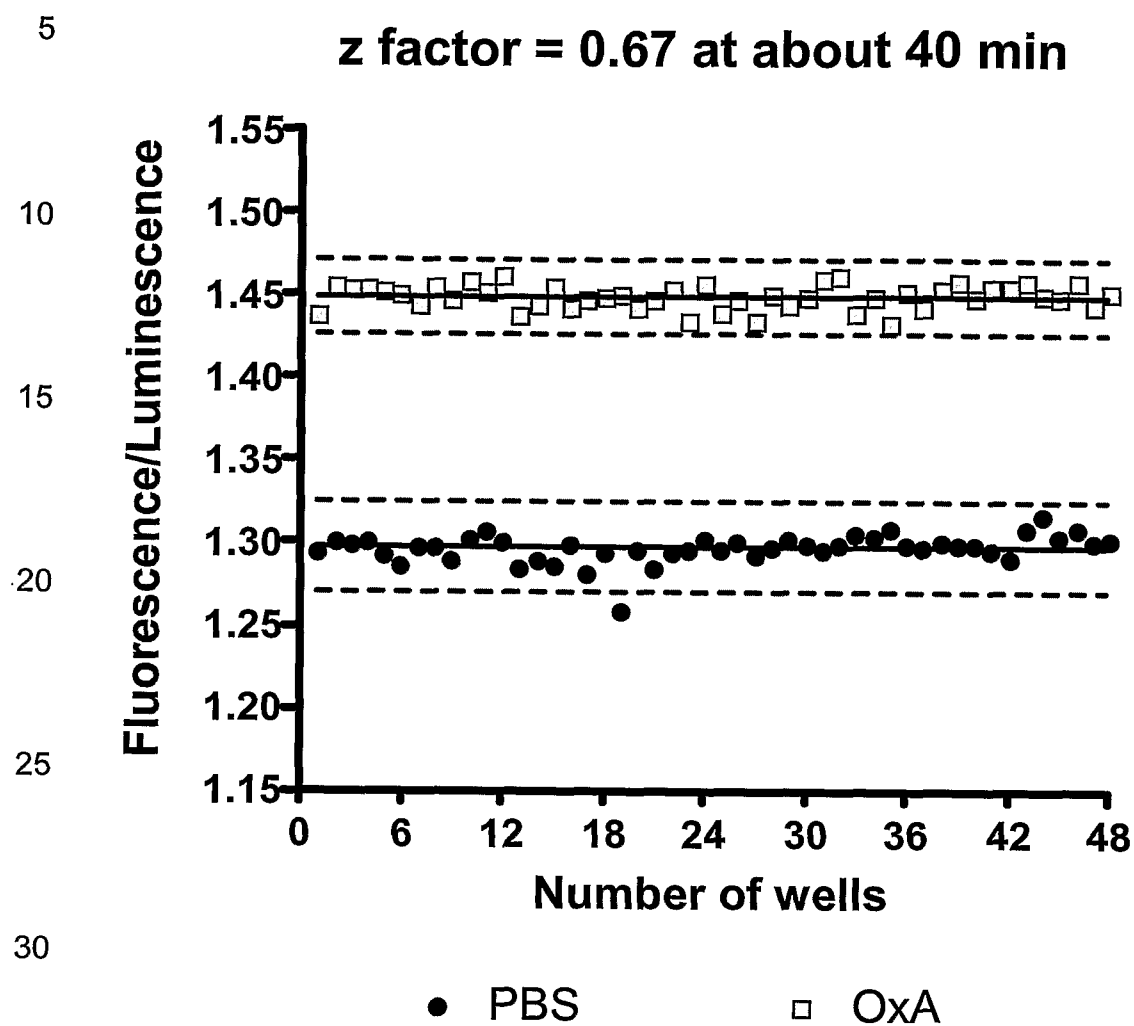


FIGURE 21

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/AU2007/001723
A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.	C07K 14/72 (2006.01)	C07K 16/28 (2006.01)	G01N 33/74 (2006.01)
	A61K 38/22 (2006.01)	G01N 33/566 (2006.01)	G01N 33/78 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See electronic databases below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: CA, WPIDS, MEDLINE, BIOSIS - keyword search (orexin, thyrotropin releasing hormone, receptor)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PFLEGER et al. "Receptors for hypothalamic releasing hormones TRH and GnRH: oligomerization and interactions with intracellular proteins" <i>Seminars in cell & Developmental Biology</i> 2004 , 15, 269-280 See p269	1-32
A	YAMADA et al. "A selective orexin-1 receptor antagonist, SB334867, blocks 2-DG-induced gastric acid secretion in rats" <i>Neuroscience Letters</i> 2005 , 376, 137-142 See abstract	1-32
A	RIEHL et al. "Chronic Oral Administration of CG-3703, a thyrotropin Releasing Hormone Analog, Increase Wake and Decreases Cataplexy in canine Narcolepsy" <i>Neuropsychopharmacology</i> 2000 , 23(1), 34-45 Cited in the application See p43, paragraph 3	1-32

☒ Further documents are listed in the continuation of Box C☐ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

 Date of the actual completion of the international search
27 November 2007

Date of mailing of the international search report

10 DEC 2007
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2007/001723

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HILAIRET et al. "Hypersensitization of the Orexin 1 Receptor by the CB1 Receptor" <i>J. Biol. Chem.</i> 2003, 278(26), 23731-23737 Cited in the application See abstract	1-32