

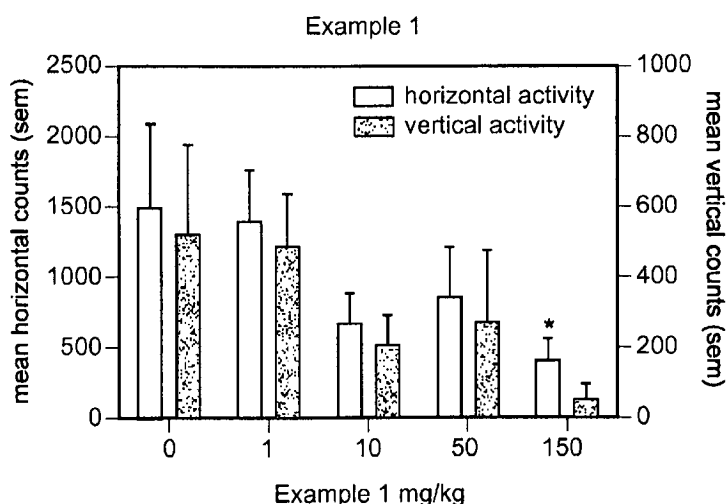


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(54) Title: USE OF GHRELIN RECEPTOR INVERSE AGONISTS OR ANTAGONISTS FOR TREATING SLEEP DISORDERS

FIG. 1



(57) Abstract: The present invention relates to methods of treating sleep disorders in patients comprising administration of a ghrelin receptor inverse agonist or antagonist. The invention also includes methods of treating sleep disorders comprising the administration of a pharmaceutical composition comprising a ghrelin receptor inverse agonist or antagonist and at least one pharmaceutically acceptable carrier, diluent, or excipient.

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USE OF GHRELIN RECEPTOR INVERSE AGONISTS OR ANTAGONISTS FOR TREATING SLEEP DISORDERS

FIELD OF THE INVENTION

5 The present invention relates to compounds that are ghrelin receptor inverse agonists/antagonists useful for treating sleep disorders. The invention also relates to pharmaceutical compositions containing such compounds.

BACKGROUND

10 Insomnia, the most common sleep disorder, affects approximately 50-70 million American adults. It is characterized by difficulty falling asleep, waking frequently during the night, waking too early and not being able to return to sleep, or waking up and not feeling refreshed.

 Early treatments for insomnia commonly employed central nervous system
15 (CNS) depressants such as barbiturates. These compounds typically have long half lives and have a well-known spectrum of side effects, including lethargy, confusion, depression and next day hangover effects. In addition, chronic use has been associated with a high potential for addiction involving both physical and psychological dependence. Treatments moved away from barbiturates and other CNS depressants
20 toward the benzodiazepine class of sedative-hypnotic agents. This class of compounds produces a calming effect that results in a sleep-like state in patients and animals, with a greater safety margin than prior hypnotics. However, many benzodiazepines possess side effects that limit their usefulness in certain patient populations. These problems include synergy with other CNS depressants (especially alcohol), the development of
25 tolerance upon repeat dosing, rebound insomnia following discontinuation of dosing, hangover effects the next day and impairment of psychomotor performance and memory. More recent treatments for insomnia have used non-benzodiazepine compounds. Ambien (zolpidem), Sonata (zaleplon) are examples of approved drug products. A need exists in the art for safe and therapeutically effective
30 non-benzodiazepine agents for treating sleep disorders.

 Night Eating Syndrome (NES) is generally associated with insufficient food intake in the first half of the day, evening hyperphagia and then insomnia and also sleep awakenings. See, e.g., Vander Wal, Jillon S., Clinical Psychology Review (2012) 32(1), 49-59. Despite increased awareness of NES, it is still not recognized, and more
35 importantly, approaches to treatment continue to be investigated without success.

The present invention relates to the use of ghrelin receptor inverse agonists or antagonists for treating sleep disorders.

SUMMARY OF THE INVENTION

5 The present invention relates to methods of treating sleep disorders in patients comprising administering to the patient, in need of such treatment, a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist.

10 In another embodiment, the present invention relates to methods of treating sleep disorders in patients comprising administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist and at least one pharmaceutically acceptable carrier, diluent, or excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

15 Figure 1 is a graphical illustration of the effects of Example 1 at reducing locomotor activity in male Wistar Han rats at 35-65 minutes post dosing via oral gavage.

Figure 2 is a graphical illustration of the somnolence effects of Example 1 in humans.

20 Figure 3 is an example of an animal's individual activity pattern after once a day (QD) administration of Example 1 or vehicle.

Figure 4 is an example of an animal's individual food intake pattern after QD administration of Example 1 or vehicle.

25

Figure. 5 provides the change in sleep duration within 3 hours following evening administration of Example 1.

30 Figure 6 provides the change in food intake within 3 hours following evening administration of Example 1.

DETAILED DESCRIPTION OF THE INVENTION

35 In another embodiment, the present invention relates to methods of treating primary insomnia in patients comprising administering to the patient, in need of such

treatment, a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist.

In another embodiment, the present invention relates to methods of treating excessive daytime sleepiness in patients comprising administering to the patient, in need of such treatment, a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist. The methods of present invention include treating excessive daytime sleepiness in patients diagnosed with Prader-Willi syndrome.

In another embodiment, the present invention relates to methods of treating NES in patients comprising administering to the patient, in need of such treatment, a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist.

In another embodiment, the present invention relates to methods of treating primary insomnia in patients comprising administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist and at least one pharmaceutically acceptable carrier, diluent, or excipient.

In another embodiment, the present invention relates to methods of treating excessive daytime sleepiness in patients comprising administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist and at least one pharmaceutically acceptable carrier, diluent, or excipient. The methods of present invention include treating excessive daytime sleepiness in patients diagnosed with Prader-Willi syndrome.

In another embodiment, the present invention relates to methods of treating NES in patients comprising administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of a ghrelin receptor inverse agonist or antagonist and at least one pharmaceutically acceptable carrier, diluent, or excipient.

In another embodiment, the present invention relates to a method of treating sleep disorders in patients comprising administering to the patient, in need of such treatment, a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method of treating primary insomnia in patients comprising administering to the patient, in need of such treatment, a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-

yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method of treating excessive daytime sleepiness in patients comprising administering to the patient, in need of such treatment, a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof. The methods of present invention are useful for treating excessive daytime sleepiness in patients diagnosed with Prader-Willi syndrome.

In another embodiment, the present invention relates to methods of treating NES in patients comprising administering to the patient, in need of such treatment, a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to methods of treating sleep disorders in patients comprising administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient.

In another embodiment, the present invention relates to methods of treating primary insomnia in patients comprising administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient.

In another embodiment, the present invention relates to methods of treating excessive daytime sleepiness in patients comprising administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient. The methods of present

invention are useful for treating excessive daytime sleepiness in patients diagnosed with Prader-Willi syndrome.

In another embodiment, the present invention relates to methods of treating NES in patients comprising administering to the patient, in need of such treatment, a
5 pharmaceutical composition comprising a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient.

In another embodiment, the present invention also provides:

10 the use of a ghrelin receptor inverse agonist or antagonist as described herein, for the manufacture of a medicament for treating a sleep disorder, particularly primary insomnia, excessive daytime sleepiness or NES;

a ghrelin receptor inverse agonist or antagonist as described herein for use as a medicament;

15 a ghrelin receptor inverse agonist or antagonist as described herein for use in the treatment of a sleep disorder, particularly primary insomnia, excessive daytime sleepiness or NES;

a pharmaceutical composition comprising a ghrelin receptor inverse agonist or antagonist as described herein and a pharmaceutically acceptable excipient; and

20 a pharmaceutical composition for treatment of a sleep disorder, particularly primary insomnia, excessive daytime sleepiness or NES, comprising a ghrelin receptor inverse agonist or antagonist as described herein;

particularly where the ghrelin receptor inverse agonist or antagonist may be (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone or a pharmaceutically acceptable
25 salt thereof.

(R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone or a pharmaceutically acceptable salt thereof may simply be generally referenced herein as Example 1.

30 In another embodiment, the present invention also provides any method discussed herein where the ghrelin receptor inverse agonist or antagonist, particularly (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone or a pharmaceutically acceptable salt thereof, is administered in combination with another pharmacologically
35 active agent.

In another embodiment, the ghrelin receptor inverse agonist or antagonist, particularly Example 1, may be employed in combination with other pharmacologically active agents, also referred to as compounds, which are known in the art, either administered separately or in the same pharmaceutical compositions, include, but are not limited to: insulin sensitizers including (i) PPAR. γ . antagonists such as glitazones (e.g. ciglitazone; darglitazone; englitazone; isaglitazone (MCC-555); pioglitazone; rosiglitazone; troglitazone; tularik; BRL49653; CLX-0921; 5-BTZD), GW-0207, LG-100641, and LY-300512, and the like); (iii) biguanides such as metformin and phenformin; (b) insulin or insulin mimetics, such as biota, LP-100, novarapid, insulin detemir, insulin lispro, insulin glargine, insulin zinc suspension (lente and ultralente); Lys-Pro insulin, GLP-1 (73-7) (insulintropin); and GLP-1 (7-36)-NH.sub.2); (c) sulfonylureas, such as acetohexamide; chlorpropamide; diabinese; glibenclamide; glipizide; glyburide; glimepiride; gliclazide; glipentide; gliquidone; glisolamide; tolazamide; and tolbutamide; (d) .alpha.-glucosidase inhibitors, such as acarbose, adiposine; camiglibose; emiglitate; miglitol; voglibose; pradimicin-Q; salbostatin; CKD-711; MDL-25,637; MDL-73,945; and MOR 14, and the like; (e) cholesterol lowering agents such as (i) HMG-CoA reductase inhibitors (atorvastatin, itavastatin, fluvastatin, lovastatin, pravastatin, rivastatin, rosuvastatin, simvastatin, and other statins), (ii) bile acid absorbers/sequestrants, such as cholestyramine, colestipol, dialkylaminoalkyl derivatives of a cross-linked dextran; Colestid.RTM.; LoCholest.RTM., and the like, (ii) nicotinyl alcohol, nicotinic acid or a salt thereof, (iii) proliferator-activator receptor .alpha. agonists such as fenofibric acid derivatives (gemfibrozil, clofibrate, fenofibrate and benzafibrate), (iv) inhibitors of cholesterol absorption such as stanol esters, beta-sitosterol, sterol glycosides such as tiqueside; and azetidinones such as ezetimibe, and the like, and (acyl CoA:cholesterol acyltransferase (ACAT)) inhibitors such as avasimibe, and melinamide, (v) anti-oxidants, such as probucol, (vi) vitamin E, and (vii) thyromimetics; (f) PPAR. α . agonists such as beclofibrate, benzafibrate, ciprofibrate, clofibrate, etofibrate, fenofibrate, and gemfibrozil; and other fibric acid derivatives, such as Atromid.RTM., Lopid.RTM. and Tricor.RTM., and the like, and PPAR. α . agonists as described in WO 97/36579 by Glaxo; (g) PPAR. δ . agonists; (h) PPAR .alpha./.delta. agonists, such as muraglitazar, and the compounds disclosed in U.S. Pat. No. 6,414,002; and (i) anti-obesity agents, such as (1) growth hormone secretagogues, growth hormone secretagogue receptor agonists/antagonists, such as NN703, hexarelin, MK-0677, SM-130686, CP-424,391, L-692,429, and L-163,255; (2) protein tyrosine phosphatase-1B (PTP-1B) inhibitors; (3) cannabinoid receptor ligands, such as

cannabinoid CB.sub.1 receptor antagonists or inverse agonists, such as rimonabant (Sanofi Synthelabo), AMT-251, and SR-14778 and SR 141716A (Sanofi Synthelabo), SLV-319 (Solvay), BAY 65-2520 (Bayer); (4) anti-obesity serotonergic agents, such as fenfluramine, dexfenfluramine, phentermine, and sibutramine; (5) .beta.3-

5 adrenoreceptor agonists, such as AD9677/TAK677 (Dainippon/Takeda), CL-316,243, SB 418790, BRL-37344, L-796568, BMS-196085, BRL-35135A, CGP12177A, BTA-243, Trecadrine, Zeneca D7114, SR 59119A; (6) pancreatic lipase inhibitors, such as orlistat (Xenical.RTM.), Triton WR1339, RHC80267, lipstatin, tetrahydrolipstatin, teasaponin, diethylumbelliferyl phosphate; (7) neuropeptide Y1 antagonists, such as BIBP3226, J-

10 115814, BIBO 3304, LY-357897, CP-671906, GI-264879A; (8) neuropeptide Y5 antagonists, such as GW-569180A, GW-594884A, GW-587081X, GW-548118X, FR226928, FR 240662, FR252384, 1229U91, GI-264879A, CGP71683A, LY-377897, PD-160170, SR-120562A, SR-120819A and JCF-104; (9) melanin-concentrating hormone (MCH) receptor antagonists; (10) melanin-concentrating hormone 1 receptor

15 (MCH1R) antagonists, such as T-226296 (Takeda); (11) melanin-concentrating hormone 2 receptor (MCH2R) agonist/antagonists; (12) orexin receptor antagonists, such as SB-334867-A, and those disclosed in patent publications herein; (13) serotonin reuptake inhibitors such as fluoxetine, paroxetine, and sertraline; (14) melanocortin agonists, such as Melanotan II; (15) other Mc4r (melanocortin 4 receptor) agonists,

20 such as CHIR86036 (Chiron), ME-10142, and ME-10145 (Melacure), CHIR86036 (Chiron); PT-141, and PT-14 (Palatin); (16) 5HT-2 agonists; (17) 5HT2C (serotonin receptor 2C) agonists, such as BVT933, DPCA37215, WAY161503, R-1065; (18) galanin antagonists; (19) CCK agonists; (20) CCK-A (cholecystokinin-A) agonists, such as AR-R 15849, GI 181771, JMV-180, A-71378, A-71623 and SR14613; (22)

25 corticotropin-releasing hormone agonists; (23) histamine receptor-3 (H3) modulators; (24) histamine receptor-3 (H3) antagonists/inverse agonists, such as hioperamide, 3-(1H-imidazol-4-yl)propyl N-(4-pentenyl)carbamate, clobenpropit, iodophenpropit, imoproxifan, GT2394 (Gliatech), and O-[3-(1H-imidazol-4-yl)propanol]-carbamates; (25)

.beta.-hydroxy steroid dehydrogenase-1 inhibitors (.beta.-HSD-1); 26) PDE

30 (phosphodiesterase) inhibitors, such as theophylline, pentoxifylline, zaprinast, sildenafil, aminone, milrinone, cilostamide, rolipram, and cilomilast; (27) phosphodiesterase-3B (PDE3B) inhibitors; (28) NE (norepinephrine) transport inhibitors, such as GW 320659, despiramine, talsupram, and nomifensine; (29) a second or third ghrelin receptor inverse agonist or antagonist; (30) leptin, including recombinant patient leptin (PEG-OB,

35 Hoffman La Roche) and recombinant methionyl patient leptin (Amgen); (31) leptin

derivatives; (32) BRS3 (bombesin receptor subtype 3) agonists such as [D-Phe6,beta-Ala11,Phe13,Nle14]Bn(6-14) and [D-Phe6,Phe13]Bn(6-13)propylamide, and those compounds disclosed in Pept. Sci. 2002 August; 8(8): 461-75); (33) CNTF (Ciliary neurotrophic factors), such as GI-181771 (GlaxoSmithKline), SR146131 (Sanofi

5 Synthelabo), butabindide, PD170,292, and PD 149164 (Pfizer); (34) CNTF derivatives, such as axokine (Regeneron); (35) monoamine reuptake inhibitors, such as sibutramine; (36) UCP-1 (uncoupling protein-1), 2, or 3 activators, such as phytanic acid, 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propeny-

10 l]benzoic acid (TTNPB), retinoic acid; (37) thyroid hormone .beta. agonists, such as KB-2611 (KaroBioBMS); (38) FAS (fatty acid synthase) inhibitors, such as Cerulenin and C75; (39) DGAT1 (diacylglycerol acyltransferase 1) inhibitors; (40) DGAT2 (diacylglycerol acyltransferase 2) inhibitors; (41) ACC2 (acetyl-CoA carboxylase-2) inhibitors; (42) glucocorticoid antagonists; (43) acyl-estrogens, such as oleoyl-estrone, disclosed in del Mar-Grasa, M. et al., Obesity Research, 9:202-9 (2001); (44) dipeptidyl

15 peptidase IV (DP-IV) inhibitors, such as isoleucine thiazolidide, valine pyrrolidide, NVP-DPP728, LAF237, MK-431, P93/01, TSL 225, TMC-2A/2B/2C, FE 999011, P9310/K364, VIP 0177, SDZ 274-444; (46) dicarboxylate transporter inhibitors; (47) glucose transporter inhibitors; (48) phosphate transporter inhibitors; (49) Metformin (Glucophage.RTM.); and (50) Topiramate (Topimax.RTM.); and (50) peptide YY, PYY

20 3-36, peptide YY analogs, derivatives, and fragments such as BIM-43073D, BIM-43004C (Olitvak, D. A. et al., Dig. Dis. Sci. 44(3):643-48 (1999)); (51) Neuropeptide Y2 (NPY2) receptor agonists such NPY3-36, N acetyl [Leu(28,31)] NPY 24-36, TASP-V, and cyclo-(28/32)-Ac-[Lys28-Glu32]-(25-36)-pNPY; (52) Neuropeptide Y4 (NPY4) agonists such as pancreatic peptide (PP), and other Y4 agonists such as 1229U91; (54)

25 cyclooxygenase-2 inhibitors such as etoricoxib, celecoxib, valdecoxib, parecoxib, lumiracoxib, BMS347070, tiracoxib or JTE522, ABT963, CS502 and GW406381, and pharmaceutically acceptable salts thereof; (55) Neuropeptide Y1 (NPY1) antagonists such as BIBP3226, J-115814, BIBO 3304, LY-357897, CP-671906, GI-264879A; (56) Opioid antagonists such as nalmefene (Revex.RTM.), 3-methoxynaltrexone, naloxone,

30 naltrexone; (57) 11.beta. HSD-1 (11-beta hydroxy steroid dehydrogenase type 1) inhibitor such as BVT 3498, BVT 2733; (58) a minorex; (59) amphechloral; (60) amphetamine; (61) benzphetamine; (62) chlorphentermine; (63) clobenzorex; (64) cloforex; (65) clominorex; (66) clortermine; (67) cyclexedrine; (68) dextroamphetamine; (69) diphemethoxidine, (70) N-ethylamphetamine; (71) fenbutrazate; (72) fenisorex; (73)

35 fenproporex; (74) fludorex; (75) fluminorex; (76) furfurylmethylamphetamine; (77)

levamfetamine; (78) levophacetoperane; (79) mefenorex; (80) metamfepramone; (81) methamphetamine; (82) norpseudoephedrine; (83) pentorex; (84) phendimetrazine; (85) phenmetrazine; (86) picilorex; (87) phytopharm 57; and (88) zonisamide.

5 The term "pharmaceutically acceptable salt" as used herein means those salts which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of patients and lower animals without undue toxicity, irritation, allergic response and the like and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well-known in the art. For example, S. M. Berge
10 et al. describe pharmaceutically acceptable salts in detail in Berge et al., J. Pharmaceutical Sciences, 1977, 66: 1-19. The salts can be prepared in situ during the final isolation and purification of Example 1 of the present invention or separately by reacting the free base of Example 1 with a suitable organic or inorganic acid. Representative acid addition salts include, but are not limited to acetate, adipate,
15 alginate, citrate, aspartate, benzoate, benzenesulfonate, bicarbonate, bisulfate, butyrate, camphorate, camphorsulfonate, citrate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isethionate), lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-
20 phenylpropionate, phosphate, picrate, pivalate, propionate, succinate, sulphate, tartrate, thiocyanate, and p-toluenesulfonate.

 The term "ghrelin receptor" as used herein means a G protein-coupled receptor, known as the growth hormone secretagogue receptor (GHSR1a or GHS-1aR).

 The term "sleep disorders" as used herein includes approximately 70 syndromes
25 characterized by disturbance in the patient's amount of sleep, quality or timing of sleep, or in behaviors or physiological conditions associated with sleep. Representative examples of sleep disorder syndromes include, but are not limited to, insomnia, primary insomnia, sleep apnea, narcolepsy, restless leg syndrome, circadian rhythm sleep disorder, REM sleep behavior disorder, somnambulism (sleepwalking), sleep bruxism
30 (teeth grinding), hypersomnia, exploding head syndrome, sleep paralysis, and excessive daytime sleepiness (EDS).

 EDS is one of the typical features of patients with Prader-Willi syndrome (PWS); caregivers describe PWS children as sleepy and with highly frequent daytime napping. EDS is demonstrated with reduced sleep onset latencies both at nighttime and during
35 the daytime on the multiple sleep latency test. Parental reports and questionnaires

show the presence of EDS in 90-100% of adults with PWS. PWS patients also have higher self-reported levels of EDS, compared with other intellectual disability groups and non-PWS controls.

“NES has been characterized in terms of eating patterns, sleep disturbances, clinical course, and familial aggregation. In comparison to healthy controls, persons with NES consume a significantly greater proportion of their calories after the evening meal, wake more often during the night, and are more likely to eat upon awakening than controls.” Vander Wal, *supra*, p 50, reference to an internal Table and citations omitted.

The term “patient” as used herein means a human.

The present invention also provides pharmaceutical compositions which comprise a ghrelin receptor inverse agonist or antagonist, particularly Example 1, formulated together with one or more non-toxic pharmaceutically acceptable carriers. The pharmaceutical compositions may be specially formulated for oral administration in solid or liquid form, for parenteral injection, or for rectal administration.

The term “pharmaceutically acceptable carrier” as used herein means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. The present invention provides pharmaceutical compositions which comprise a ghrelin receptor inverse agonist or antagonist, particularly Example 1, formulated together with one or more non-toxic pharmaceutically acceptable carriers. The pharmaceutical compositions can be formulated for oral administration in solid or liquid form, for parenteral injection or for rectal administration.

The pharmaceutical compositions of this invention can be administered to patients orally, parenterally, intraperitoneally, topically (as by powders, ointments or drops), buccally or as an oral or nasal spray. The term "parenterally," as used herein, refers to modes of administration which include intravenous, intramuscular,
5 intraperitoneal, intrasternal, subcutaneous, intraarticular injection and infusion.

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers,
10 diluents, solvents or vehicles include water, ethanol, polyols (propylene glycol, polyethylene glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. 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 dispersions, and by the use of
15 surfactants.

These compositions may also contain adjuvants such as preservative agents, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be
20 desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is often desirable to
25 slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is
30 accomplished by dissolving or suspending the drug in an oil vehicle.

Suspensions, in addition to the ghrelin receptor inverse agonist or antagonist, particularly Example 1, may contain suspending agents, as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, and mixtures
35 thereof.

If desired, and for more effective distribution, the ghrelin receptor inverse agonist or antagonist, particularly Example 1, of the present invention can be incorporated into slow-release or targeted-delivery systems such as polymer matrices, liposomes, and microspheres. They may be sterilized, for example, by filtration through a bacteria-
5 retaining filter or by incorporation of sterilizing agents in the form of sterile solid compositions, which may be dissolved in sterile water or some other sterile injectable medium immediately before use.

The ghrelin receptor inverse agonist or antagonist, particularly Example 1, can also be in micro-encapsulated form, if appropriate, with one or more pharmaceutically
10 acceptable carriers as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the ghrelin receptor inverse agonist or antagonist, particularly Example 1, can be admixed with at least one inert
15 diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also
20 be of such composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Injectable depot forms are made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the
25 ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides) Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

30 The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Injectable preparations, for example, sterile injectable aqueous or oleaginous
35 suspensions may be formulated according to the known art using suitable dispersing or

wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic, parenterally acceptable diluent or solvent such as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the ghrelin receptor inverse agonist or antagonist, particularly Example 1, is mixed with at least one inert pharmaceutically acceptable carrier such as sodium citrate or calcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and salicylic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay; and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to

the ghrelin receptor inverse agonist or antagonist, particularly Example 1, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

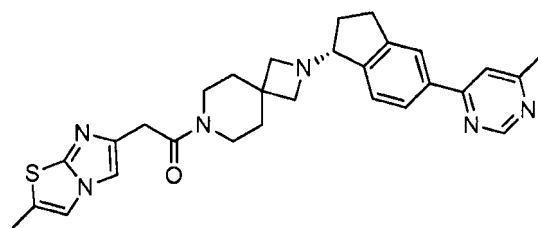
Actual dosage levels of the ghrelin receptor inverse agonist or antagonist, particularly Example 1, in the pharmaceutical compositions of this invention can be varied so as to obtain an amount of the ghrelin receptor inverse agonist or antagonist, particularly Example 1, which is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of the particular ghrelin receptor inverse agonist or antagonist, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated.

The total daily dose of the ghrelin receptor inverse agonist or antagonist, particularly Example 1, of this invention administered to a patient, from about 0.003 to about 10 mg/kg/day. For purposes of oral administration, more preferable doses can be in the range of from about 0.01 to about 5 mg/kg/day. Preferred doses range between 25 and 300 mgs per day. Most preferred is 75 to 200 mgs per day. If desired, the effective daily dose can be divided into multiple doses for purposes of administration, e.g. two to four separate doses per day.

All patents, patent applications, and literature references cited in the specification are herein incorporated by reference in their entirety.

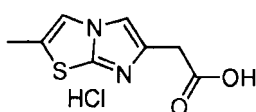
(R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone may be prepared using the synthetic methodology disclosed herein or in US 2011/0230461.

Example 1



(R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone

5



2-(2-methylimidazo[2,1-b]thiazol-6-yl)acetic acid hydrochloride

A solution of bromine (436 g, 2.73 mol) in acetic acid (750 mL) was added to a solution of ethyl 3-oxobutanoate (355 g, 2.73 mol) in acetic acid (1000 mL). The mixture was stirred at room temperature for 72 hours and was concentrated under reduced pressure at 45°C to remove the acetic acid. The residue was partitioned between methylene chloride (400 mL) and water (250 mL). The organic layer was washed with saturated sodium bicarbonate (2 x 300 mL), water (300 mL), brine (125 mL) and was dried over anhydrous magnesium sulfate. The solution was filtered and concentrated to give ethyl 4-bromo-3-oxobutanoate as a yellow oil (421 g).

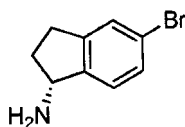
To a solution of 2-amino-5-methylthiazole (150 g, 1.31 mol) in acetone (1500 mL) was slowly added ethyl 4-bromo-3-oxobutanoate (345 g, 1.65 mol). The temperature of the reaction mixture was maintained between 22-40°C. The mixture turned into a thick paste and acetone (300 mL) was added to facilitate stirring. After stirring at room temperature overnight, the mixture was filtered and the filter cake was washed with acetone to provide a white solid. The solid was washed with hexanes and was dried in a vacuum oven at 40°C for 4 hours to give 4-(2-amino-5-methyl-thiazol)-3-oxobutyric acid ethyl ester hydrobromide (272 g).

To 4-(2-amino-5-methyl-thiazol)-3-oxobutyric acid ethyl ester hydrobromide (272 g, 0.84 mol) was added anhydrous ethanol (675 mL) and the thick mixture was heated at 90°C for 2 hours. During this time, the solids went into solution. The reaction mixture was concentrated to give a brown semi-solid which was triturated with ethanol to provide a white fluffy solid which was collected by filtration. The solids were washed

with Et₂O and dried under vacuum at 40°C for 4 hours to give ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate hydrobromide (226 g).

Ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate hydrobromide (226 g, 0.74 mol) was dissolved in water (350 mL) and the solution was adjusted to pH 7 by addition of potassium carbonate (51.0 g, 0.37 mol). The aqueous solution was extracted with methylene chloride (300 mL) and the organic phase was washed with brine (150 mL), dried over anhydrous magnesium sulfate, filtered and concentrated to give ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate as a brown oil (151.3 g).

Ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate (151.3 g, 0.67 mol) was dissolved in 10% aqueous HCl (435 mL) and the mixture was heated at reflux for 2 hours. The reaction mixture was cooled to room temperature and was concentrated *in vacuo* to give a yellow oil. Ethanol (100 mL) and diethylether (200 mL) were added and the resulting white precipitate was collected by filtration and dried in a vacuum oven overnight to give 144.3 g (93%) the title compound. MS (ES⁺) 197.1 (M+H)⁺. ¹H NMR (CD₃OD) δ 2.48 (s, 3H), 3.88 (s, 2H), 7.81 (s, 1H), 7.85 (s, 1H).



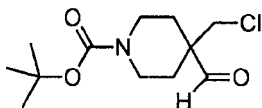
(R)-5-bromo-2,3-dihydro-1H-inden-1-amine

A 22 L 5-necked round-bottomed flask was charged with 5-bromo-1-indanone (1.0 kg, 4.72 mol), anhydrous THF (8 L) and (R)-methyl-CBS-oxazaborolidine (730 mL, 0.73 mol) and was cooled to -10°C under N₂. Borane-methylsulfide (10.0 M, 650 mL, 6.5 mol) was added dropwise over 1 hour while maintaining the temperature below -5°C. The mixture was stirred at -10°C to 0°C for 3 hours and was quenched with water (4 L) at such a rate to maintain the reaction temperature below 5°C. The mixture was extracted with EtOAc (3 x 3 L). The combined organic extracts were washed with brine (2 L), dried over MgSO₄, filtered and concentrated to give yellow solid. The crude product was passed through a short silica gel column (3 L silica gel packed with 1% Et₃N in hexanes) and eluted with EtOAc/hexanes(1/3). The filtrate was concentrated and the residue was slurried with 10% EtOAc in hexanes, filtered, and dried to give 585 g of an off-white solid as (S)-5-bromo-indan-1-ol. The mother liquors were re-concentrated, slurried with 10% EtOAc in hexanes and filtered to give another 200 g yellow solid as (S)-5-bromo-indan-1-ol. The combined lots (785 g, 78%) were carried on to the next step without further purification.

A solution of (S)-5-bromo-indan-1-ol (288 g, 1.35 mol) in toluene (2 L) was cooled in an ice bath under N₂ and treated with diphenylphosphoric azide (DPPA, 400 mL, 1.85 mol) in one portion followed by a solution of 1,8-diazabicyclo[5,4,0]undec-7-ene (300 mL, 2.01 mol) in toluene (600 mL). The reaction temperature was kept
5 between 3 and 10°C during the 3 hour addition and the mixture was warmed to 15°C over 3 hours (TLC indicated no starting material). The mixture was diluted with EtOAc (1 L) and washed with water (3 x 2 L). The organic layer was dried over MgSO₄, filtered and concentrated to give 516 g of a dark oil. The crude product was purified by silica gel column (packed with 1% Et₃N in hexanes, hexane eluant) to give (R)-1-azido-5-bromo-
10 indane (291 g, 90%) as an oil which was used directly in the next step.

A solution of (R)-1-azido-5-bromo-indane (154 g, 0.645 mol) was dissolved in methanol (2.4 L) and SnCl₂·2H₂O (265 g, 1.18 mol) was added. The mixture was stirred at room temperature overnight (TLC indicated no starting material) and was concentrated to dryness. The resulting residue was treated with 2N aqueous NaOH
15 (2.5 L) and EtOAc (1.5 L). The mixture was stirred for 1 hour and filtered through Celite® with the aid of EtOAc (3 x 250 ml). The organic solution was separated and the aqueous layer was extracted with EtOAc (3 x 2 L). The combined organic extracts were washed with 1 N HCl (2 x 2 L) followed by water (2 L). The pH of the combined aqueous layers was adjusted to 11 with cold saturated NaOH solution and the mixture was
20 extracted with EtOAc (3 x 2 L). The combined organic extracts were dried over MgSO₄, filtered and concentrated to give (87.5 g, 64.0%) (R)-5-bromo-2,3-dihydro-1H-inden-1-amine as a dark yellow oil which solidified upon refrigeration. MS (ES⁺) 213.9 (M+H)⁺. ¹H NMR (CDCl₃) δ 1.70-1.75 (m, 1 H), 2.40-2.45 (m, 1 H), 2.77-2.82 (m, 1 H), 2.93-2.97 (m, 1 H), 4.28-4.33 (m, 1 H), 7.18-7.23 (m, 1 H), 7.36-7.41 (m, 2 H).

25



tert-butyl 4-(chloromethyl)-4-formylpiperidine-1-carboxylate

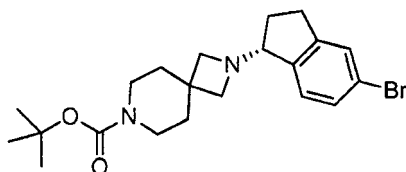
To a solution of diisopropylamine (22.6 mL, 159 mmol) in anhydrous THF (140 mL) in an oven-dried round-bottomed flask was added n-BuLi (65.4 mL, 163 mmol, 2.50
30 M in hexanes) dropwise at 0°C. The solution was stirred for 45 minutes and 1-tert-butyl 4-methyl piperidine-1,4-dicarboxylate (20 g, 80 mmol) in THF (60 mL) was added dropwise at 0°C and the mixture was stirred at 0°C for 1 hour. Chloriodomethane (17.9 mL, 239 mmol) was added dropwise and the mixture was stirred for 1 h. The mixture

was quenched with 250 mL of saturated aqueous NaHCO_3 followed by extraction with ethyl acetate (3 x 250 mL). The combined organic layers were washed (brine, 250 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil that was purified by silica chromatography using a Combiflash ISCO purification system

5 (Teledyne Isco Inc., Lincoln, NE) system to give 1-*tert*-butyl 4-methyl 4-(chloromethyl)piperidine-1,4-dicarboxylate (12 g, 52%). ^1H NMR (CDCl_3) δ 1.43 (s, 9H), 2.10-2.17 (m, 4H), 2.97 (br s, 2H), 3.56 (s, 2H), 3.74 (s, 3H), 3.83 (br s, 2H).

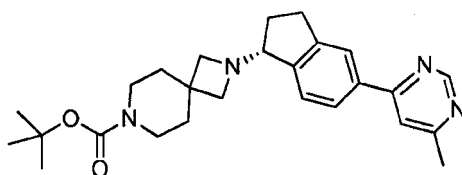
A solution of 1-*tert*-butyl 4-methyl 4-(chloromethyl)piperidine-1,4-dicarboxylate (11.7 g, 40.2 mmol) in anhydrous THF (100 mL) was cooled to 0°C . Lithium aluminum
10 hydride (1N in THF, 44.3 mL, 44.3 mmol), was added slowly (15 - 20 min) and the solution was stirred at 0°C for 25 minutes. The mixture was quenched by adding water (1.8 mL) dropwise with great caution. Aqueous 1N NaOH (1.8 mL) was added dropwise, and the mixture was stirred for 5 minutes. The cooling bath was removed, the solids were filtered off, and the cake was washed with Et_2O (2 x 100 mL). The filtrate
15 was washed with water (2 x 100 mL), brine (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give *tert*-butyl 4-(chloromethyl)-4-(hydroxymethyl)piperidine-1-carboxylate as a solid (9.96 g, 93.8%). ^1H NMR (CDCl_3) δ 1.43 (s, 9H), 1.48-1.55 (m, 4H), 3.36-3.41 (m, 4H), 3.57 (s, 2H), 3.59 (br s, 2H).

To a -78°C solution of oxalyl chloride (5.1 mL, 57 mmol) in dichloromethane (100
20 mL) in an oven-dried round-bottomed flask was added dimethylsulfoxide (8.2 mL, 114 mmol) in dichloromethane (17 mL). The mixture was stirred for 2 minutes and *tert*-butyl 4-(chloromethyl)-4-(hydroxymethyl)piperidine-1-carboxylate (13.7 g, 52 mmol) in dichloromethane (50 mL) was added over 10 minutes. The solution was stirred for 15 minutes at -78°C and triethylamine (36 mL, 260 mmol) was added. The mixture was
25 stirred at -78°C for 15 minutes and was warmed to room temperature. The mixture was stirred for 15 minutes at room temperature and was quenched with saturated aqueous NaHCO_3 (200 mL). The aqueous solution was washed with Et_2O (2 x 300 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to give the title compound as a yellow oil that solidified upon
30 standing under nitrogen atmosphere at room temperature (13.7 g, 99%). ^1H NMR (CDCl_3) δ 1.43 (s, 9H), 1.48-1.60 (m, 2H), 2.00-2.07 (m, 2H), 3.07 (t, 2H), 3.57 (s, 2H), 3.69-3.79 (m, 2H), 9.55 (s, 1H).



(R)-tert-butyl 2-(5-bromo-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonane-7-carboxylate

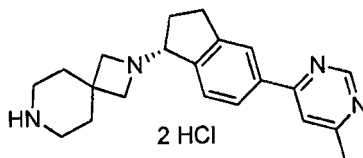
To a solution of (R)-5-bromo-2,3-dihydro-1H-inden-1-amine (1835 g, 8.66 mol) in anhydrous methanol (24 L) was added tert-butyl 4-(chloromethyl)-4-formylpiperidine-1-carboxylate (2310 g, 8.83 mol). The mixture was stirred at 50°C for 16 h, and cooled to rt. Sodium cyanoborohydride (1000 g, 15.9 mol) in THF (15 L) was added via a syringe pump over 2 hours. The mixture was stirred at 60°C for 24 hours under nitrogen with a vent into a bleach bath. The reaction was cooled to 20°C and transferred via a cannula into a vessel containing 24 L of 2.5M sodium hydroxide, and 30 L of DCM. The layers were separated and the aqueous layer was extracted with DCM (2 x 5 L). The aqueous layer was treated to destruct residual sodium cyanoborohydride. The combined organic layers were dried (MgSO₄) and concentrated under reduced pressure. The crude material was slurried in MTBE (7 L) by stirring at 40°C for 1 h and at rt for 1 h. The solid was filtered, and washed with MTBE (2 x 500 mL) and dried under vacuum oven at 50°C to give the title product as a white crystals (3657 g, 90%). MS (ES⁺) 422.3 (M+H)⁺. ¹H NMR (CDCl₃) δ 1.44 (s, 9H), 1.67 (dd, 4H), 1.84-1.93 (m, 1H), 2.07-2.16 (m, 1H), 2.72-2.81 (m, 1H), 2.95-3.15 (m, 5H), 3.31 (dd, 4H), 3.85 (br s, 1H), 7.12 (d, 1H), 7.28 (br s, 1H), 7.35 (br s, 1H). [α]_D²⁰ = +39.6 deg (c = 1.06 mg/mL, MeOH).



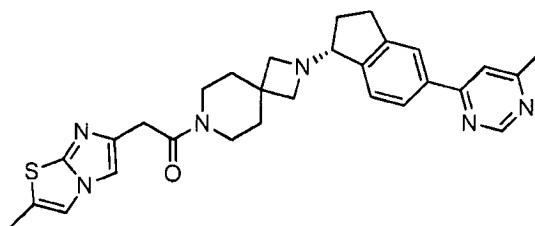
(R)-tert-butyl 2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonane-7-carboxylate

To a 50 mL flask charged with (R)-tert-butyl 2-(5-bromo-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonane-7-carboxylate (4.0 g, 9.49 mmol) was added bis(triphenylphosphine)palladium(II) chloride (0.17 g, 0.24 mmol), potassium acetate (3.73 g, 37.97 mmol), bis(pinacolato)diboron (2.65 g, 10.44 mmol) followed by degassing via vacuum then backfilling with nitrogen 5 times. De-oxygenated (nitrogen stream for 30 minutes prior to addition) toluene (40 mL) was added to the mixture and

the reaction was heated at 100°C for 1.5 hours. The reaction was monitored for completion by HPLC. Upon formation of the boronic ester intermediate, the reaction was cooled to 40°C and charged with a degassed solution of 4 M sodium hydroxide (11.87 mL, 47.46 mmol) followed by addition of 4-chloro-6-methylpyrimidine (1.53 g, 11.87 mmol). The resulting mixture was then heated to 90°C for 5 hours under nitrogen. The reaction was cooled to room temperature and charged with water (25 mL). After stirring for 20 minutes, the mixture was filtered to remove black solids. The organic layer was extracted to an aqueous solution containing 1.5 equiv of HCl (40 mL). The organic layer was removed and the resulting solution was treated with (4 g) ISOLUTE® Ultra Pure Si-Thiol silica gel for 1.5 hours and filtered. The aqueous solution was adjusted to pH 7.8 with 4N NaOH and extracted with toluene (40 mL). The toluene layer was concentrated to approximately 15 mL under vacuum at 45°C and heptane (75 mL) was added slowly and the mixture was stirred at 20°C for 1 hour. The product was filtered and dried under vacuum at 45°C for 8 hours to afford the title compound as a white solid (3.56 g, 86%). MS (ES⁺) 435.5 (M+H)⁺. ¹H NMR (CDCl₃) δ 1.46 (s, 9 H), 1.70 - 1.74 (m, 4 H), 1.90 - 2.01 (m, 1 H), 2.13 - 2.26 (m, 1 H), 2.59 (s, 3 H), 2.84 - 2.93 (m, 1 H), 3.04 - 3.21 (m, 5 H), 3.30 - 3.38 (m, 4 H), 3.95 - 4.02 (m, 1 H), 7.40 (d, 1 H), 7.56 (s, 1 H), 7.87 (d, 1 H), 7.95 (s, 1 H), 9.12 (s, 1 H).



2-[(R)-5-(6-methyl-pyrimidin-4-yl)-indan-1-yl]-2,7-diaza-spiro[3.5]nonane dihydrochloride (R)-tert-butyl 2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonane-7-carboxylate (72.6 g, 167 mmol) was suspended in methanol (363 mL) and 4M HCl in 1,4-dioxane (251 mL) was added. After stirring for 2 hours, the slurry was concentrated to dryness. The crude material was re-suspended in MeOH (500 mL) and concentrated (3x). The resulting solids were further dried under vacuum at 45°C to afford the title compound (74.1 g, 99.9%). MS (ES⁺) 335.2 (M+H)⁺. ¹H NMR (CD₃OD) δ 2.16-2.23 (m, 5H), 2.59 (br s, 1 H), 2.78-2.80 (m, 3H), 3.12 (br s, 1H), 3.19-3.24 (m, 4H), 3.37-3.49 (m, 1H), 4.14-4.23 (m, 3H), 4.49 (br s, 1H), 5.11 (br s, 1 H), 7.84 (d, 1H), 8.30-8.34 (m, 2H), 8.46 (s, 1H), 9.36 (s, 1H).



(R)-2-(2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone

To a suspension of 2-[(R)-5-(6-methyl-pyrimidin-4-yl)-indan-1-yl]-2,7-diaza-
 5 spiro[3.5]nonane dihydrochloride (540 mg, 1.22 mmol) in 10 mL of dichloromethane was added triethylamine (492 mg, 4.90 mmol). Once the mixture became a homogenous solution, it was added to a solution of 2-(2-methylimidazo[2,1-b]thiazol-6-yl)acetic acid hydrochloride (251 mg, 1.28 mmol) in 3 mL of dichloromethane. The mixture was stirred for 5 minutes and HBTU (462 mg, 1.22 mmol) in 2 mL of DMF was added. The reaction
 10 was stirred at room temperature for 1.5 hours. The reaction was quenched by the addition of 10 mL of NaHCO₃ and was diluted with 50 mL of dichloromethane. The organic layer was washed with saturated brine, dried over Na₂SO₄, filtered and the filtrate was concentrated. The residue was dissolved in 5 mL of CH₃CN and the solution was heated to 100°C for 1 hour with stirring. The mixture was cooled to room
 15 temperature and the resulting solids were vacuum filtered to afford the desired product as an off white powder (428 mg, 69%). MS (ES⁺) 513.5 (M+H)⁺. ¹H NMR (CD₃OD) δ 1.70-1.74 (m, 4 H), 1.88-1.96 (m, 1 H), 2.27 - 2.34 (m, 2 H), 2.40 (s, 3H), 2.58 (s, 3 H), 2.86-2.97 (m, 1 H), 3.11-3.15 (m, 1 H), 3.31-3.34 (m, 3 H), 3.52-3.55 (m, 4 H), 3.78 (s, 2 H), 4.05-4.09 (m, 1 H), 7.33 (s, 2 H), 7.55 (d, 1 H), 7.78 - 7.79 (m, 1 H), 7.94 - 8.03
 20 (m, 2 H), 8.93 (s, 1 H). [α]_D²⁰ = +45.3 deg (c = 2.5 mg/mL, MeOH).

IN VITRO ASSAYS

RADIOLIGAND BINDING ASSAYS

To measure the ability of a test compound to bind to the ghrelin receptor, and
 25 therefore have the potential to modulate ghrelin activity, radioligand displacement assays were performed. The SPA format was utilized for high throughput screening of test compounds and filter binding served for more comprehensive binding characterization. In both formats test compound affinity is expressed as K_i value, defined as the concentration of compound required to decrease [¹²⁵I] ghrelin binding by
 30 50% for a specific membrane batch at a given concentration of radioligand.

Patient Ghrelin SPA Binding Assay

Ghrelin SPA binding assays were performed in a final volume of 90 μ l containing 250 ng patient GHSR1a (HEK293 Tetracycline-Inducible cell line expressing the patient growth secretagogue receptor 1a; prepared as membranes) coupled to 0.5 mg SPA
5 beads (wheat germ agglutinin coated, GE Healthcare, RPNQ0060) and 50 pM [125 I] ghrelin (Perkin Elmer Life Sciences, NEX-388), plus varying concentrations of test compound or vehicle.

Briefly, assays were prepared at room temperature in 384-well plates (Matrix,
10 4322) containing 2 μ l of test compound in DMSO (or DMSO as vehicle). Assays were initiated by addition of 28 μ l assay buffer (50mM HEPES, 10mM MgCl₂, 0.2% BSA, EDTA-free protease inhibitors--1 tablet/50 ml buffer, pH 7.4), 30 μ l 8.3 μ g/ml hGHSR1a membrane and 30 μ l of 150 pM [125 I] ghrelin, both in assay buffer.

The mixture was incubated for 8 hours to allow binding to reach equilibrium and
15 the amount of receptor-ligand complex is determined by liquid scintillation counting using a 1450 Microbeta Trilux (Wallac).

Patient Ghrelin Filter Binding Assay

Ghrelin binding assays were performed in a final volume of 100 μ l containing 100
20 ng patient GHSR1a (HEK293 Tetracycline-Inducible cell line expressing the patient growth secretagogue receptor 1a; prepared as membranes) and 50 pM [125 I] ghrelin (Perkin Elmer Life Sciences, NEX-388), plus varying concentrations of test compound or vehicle.

Briefly, assays were prepared at room temperature in 96-well plates (Costar,
25 3357) containing 2 μ l of test compound in DMSO (or DMSO as vehicle). Assays were initiated by addition of 23 μ l assay buffer (50mM HEPES, 10mM MgCl₂, 0.2% BSA, EDTA-free protease inhibitor tablets --1 tablet/50 ml buffer, pH 7.4), 25 μ l 4 μ g/ml hGHSR1a membrane and 50 μ l of 100 pM [125 I] ghrelin, both in assay buffer.

The mixture was incubated for 90 minutes at room temperature followed by
30 transfer to a 0.3% PEI-treated, 96-well glass fiber filtration plate (Perkin Elmer, 6005174). The mixture was suctioned dry with vacuum and immediately washed 3 times with 200 μ l ice cold 50 mM Tris pH 7.5. Plates were allowed to dry overnight at room temperature and 30 μ l Supermix scintillant (Perkin Elmer, 1200-439) is added to

each well. The amount of receptor-ligand complex was determined by liquid scintillation counting using a 1450 Microbeta Trilux (Wallac).

Radioligand binding filtration format assays for dog (NM_001099945.1), monkey (XM_001084886.1), mouse (NM_177330), and rat (NM_032075) GHSR1a (all
5 expressed in unique HEK293 Tetracycline-Inducible cell lines) were performed in an identical manner as described for patient GHSR1a except that the final amount of membrane to be used is as follows: 2 μ g dog GHSR, 250 ng monkey GHSR, 200 ng mouse GHSR, or 125 ng rat GHSR.

10 Patient Ghrelin Functional Assay

To measure the ability of a test compound to modulate the activity of patient GHSR1a (agonize, antagonize, partially agonize, inversely agonize), a DELFIA GTP-binding assay (Perkin Elmer, AD0260 and AD0261) was performed. The assay
15 monitors the ligand-dependent exchange of GDP for GTP. GPCR activation results in an increase in fluorescence as receptor-bound GDP is replaced by Europium-labeled GTP. Antagonist binding prevents GDP-GTP exchange whereas binding of an inverse agonist pushes the receptor to the GDP bound (inactive) state, both resulting in decreased fluorescence.

Ghrelin functional assays were performed in a final volume of 39.5 μ l containing
20 720 ng patient GHSR1a (HEK293 Tetracycline-Inducible cell line expressing the patient growth secretagogue receptor 1a, prepared as membranes), 9 nM GTP-Europium and varying concentrations of test compound or vehicle. To test for receptor antagonism, membranes were incubated in the presence of agonist ghrelin (Anaspec, 24158) at the EC₈₀ concentration, plus test compound or vehicle.

25 Briefly, the test compound was prepared at room temperature in 384-well plates (Matrix, 4340). The test compound was first diluted in DMSO then added as 15 μ l to 10 μ l of basal buffer (50 mM HEPES pH 7.4, 3.7 mM MgCl₂, 250 μ M EGTA, 125 nM GDP) with and without 9 nM ghrelin peptide. Samples were then transferred as 6 μ l to 384-well filter plates (Pall, 5071) containing 30 μ l of 24 μ g/ml hGHSR1a membrane and 0.35
30 mg/ml saponin (Perkin Elmer, AD0261) in basal buffer.

The mixture was incubated 24 minutes at room temperature with gentle shaking, followed by the addition of 3.5 μ l of 100 nM GTP-Europium in 50 mM HEPES, pH 7.4. Samples were shielded from light and incubated for 90 minutes further at room temperature with gentle shaking. The reactions were suctioned dry with vacuum,

washed three times with 75 μ L ice cold 1x GTP Wash Solution (Perkin Elmer, AD0261), and immediately read on the Envision 2101 Multilabel Reader (Perkin Elmer) using excitation filter 320 nm and emission filter 615 nm.

5 Patient Dispersed Islet Cell Assay

Day 1: Patient islet cells in an intravenous (iv) bag are obtained. The islet cells are decanted by attaching a coupler to the iv bag and the liquid is decanted into 50 mL conical tubes. The bag is rinsed with 20 mL of media and pooled. The cells are spun 1 minute at 1000 revolutions per minute (rpm). The cells are then incubated overnight at 10 37 °C, 5% CO₂ (10 cm² suspension dishes, 10 mL media/plate).

Day 2: The islet cells are transferred to a 50 mL conical tube, Hank's Working Buffer without calcium is added and mixed, then the mixture is spun for 1 minute at 1000 rpm. The islets are then washed with Hank's Working Buffer without calcium, mixed and then spun at 1000 rpm for 1 minute. All but 15 mL of buffer is then removed 15 by pipette. 30 μ L of 500 mM EDTA [1 mM] is then added and then incubated 8 minutes at room temperature. To this is then added 75 μ L of 0.25 %Trypsin-EDTA and 15 μ L of 2mg/ml DNase I [2 μ g/ml]. The mixture is incubated for 10 minutes at 30 °C with shaking at 60 rpm. The clot is dispersed by triturating with a 1 mL pipette(50 times). 50 mL of Culture Media is added and passed each over 63 μ M nylon membrane. The 20 mixture is spun at 1000 rpm for 1 minute then the media is removed by pipette. Resuspend the pellet and washed cells again with approximately 25 mL Culture Media and spun at 1000 rpm for 1 minute. The supernatant is removed then the pellet is resuspended with approximately 5 mL Culture Media and the cells are counted. "V" bottom plates are seeded with 5000 cells/well (200 μ L/well). The plates are spun at 1000 25 rpm for 5 minutes and placed in cell culture incubation. 600,000 cells are removed for calcium imaging.

DAY 3: Dispersed Islet Assay

The culture media is replaced with 100 μ L of incubation buffer containing 3 mM glucose. The plates are spun for 5 minutes at 1000 rpm to re-pellet the islets. Incubate 30 the plates in a 37°C waterbath continuously gassed with 95%O₂/5%CO₂ for 45 minutes. Replace the pre-incubation buffer with 50 μ L of incubation buffer containing the various test compounds in the appropriate concentration of glucose (n = 4 for each sample). The plates are spun for 5 minutes at 1000 rpm to re-pellet the cells. The plates are returned to a waterbath continuously gassed with 95%O₂/5%CO₂ for 60 minutes.

Transferred 40 μ l to another plate and assay for insulin using an ELISA Patient Insulin Assay (ALPCO Patient Insulin ELISA; Cat. No. 80-INSHU-E10 available from ALPCO, Salem, New Hampshire, USA).

- The pharmacological data provided in Table 1 was obtained for Example 1. The
- 5 IC₅₀ (5.02 nM) and K_i data (4.37 nM) were obtained from the Patient Ghrelin SPA Binding Assay. The column denoted "n" is the number of times the test compound was assayed. The functionality of the test compound was determined to be inverse agonist using the Patient Ghrelin Functional Assay.

Table 1

In Vitro Assays	Example 1 Pre-incubation	K _d /K _i /EC ₅₀ /IC ₅₀ (nM)	K _i /EC ₅₀ /IC ₅₀ ng/mL	n
Human Binding				
Human GHS-1aR SPA	8 hours	4.37	2.24	10
Human GHS-1aR Filter	90 minutes	2.49	1.28	52
Human GHS-1aR Filter	24 hours	1.42	0.728	44
Human GHS-1aR Filter Motulsky	Equilibrium 4.07 hours	3.04	1.56	8
Preclinical Species Binding				
Rat GHS-1aR Filter	90 minutes	3.19	1.64	49
Mouse GHS-1aR Filter	90 minutes	7.70	3.95	15
Dog GHS-1aR Filter	90 minutes	3.56	1.83	9
Primate GHS-1aR Filter	90 minutes	1.83	0.938	8
Human Functional				
Eu-GTP Inverse Agonist	114 minutes	4.85	2.49	16
Eu-GTP Antagonist	114 minutes	6.57	3.37	16
Eu-GTP Competitive Antagonist	120 minutes	1.75	0.90	10

- 10 GHS-1aR = Growth hormone secretagogue Receptor; Eu-GTP = Europium-guanosine-5'-triphosphate; GH = Growth hormone.

- Example 1 is a potent (human K_d = 3 nM via Patient Ghrelin Filter Binding Assay; similar across species), selective (IC₅₀ > 1 μ M against a broad panel of receptors,
- 15 transporters, ion channels, and enzymes), and moderate on/off inverse agonist, competitive antagonist of the growth hormone secretagogue receptor (GHS-1aR).

IN VIVO ASSAYS

Male Wistar Han rats were administered a single dose (1, 10, 50, or 150 mg/kg) of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone (Example 1) via oral gavage to assess the effects of Example 1 on locomotor activity over a 21 hour period (Figure 1). Animals were habituated to the locomotor apparatus one hour prior to dosing. The lights were turned off at 4pm and turned on at 4am. Animals were dosed at 4pm. Activity was monitored post-dose for 20 hours. Plasma, brains and CSF were taken from a satellite group of animals at Tmax (30 minutes post dosing).

At 35-65 minutes post dosing, no biologically or statistically significant effect was seen at 1 mg/kg when compared to vehicle treated animals. At 10 mg/kg, Example 1 decreased rearing 56% and total locomotor activity 60% at 35-65 minutes post dosing. At 50 mg/kg, Example 1 decreased rearing 43% and total locomotor activity 48%. Example 1 at 150 mg/kg produced a reduction in total activity which was statistically significant when compared to vehicle control rats ($P < 0.5$) 35-65 minutes post dosing. Example 1 produced no statistically or biologically significant reductions in total activity 20 hours post dosing. Example 1 did not induce hyperactivity at any dose.

The plasma and brain levels of Example 1 were determined at 30 minutes post dosing, shown in Table 2.

Table 2

Dose	Plasma (nM free)	Brain (nM free)
Vehicle	0	0
1 mg/kg Ex. 1	18	1.6
10 mg/kg Ex. 1	184	16
50 mg/kg Ex. 1	856	80
150 mg/kg Ex. 1	1704	148

In three clinical studies (Figure 2), a dose-responsive relationship was observed with somnolence in humans where, at the highest doses of 100 mg BID for 14 days and 300 mg single doses, between 75 and 100% of subjects reported somnolence or a sleep-related effect. Patients were administered Example 1 as an extemporaneously prepared suspension in B3301001, B3301002, and the IR in B3301007 and as an extemporaneously prepared osmotic capsule (EPOC) in B3301007. Example 1 was safe and generally well tolerated in humans up to 100 mg BID for 14 days which equated to an estimated 80% systemic receptor occupancy for 20 hrs and 70% centrally

for approximately 3 hours. Dose related increase in heart rate (up to ~10 beats per minute) as well as attenuation of both ghrelin-induced growth hormone secretion and postprandial glucose were seen after acute dosing. All three of these effects appeared to fully tachyphylax by day 14 of BID dosing.

5 In a primate study, the effect of Example 1 on sleep duration and food intake three hours post evening dosing Example 1 was studied dosing either once a day (QD) or twice a day (BID). Eight healthy adult (5.8 to 9.7 years of age) male rhesus monkeys, (*Macaca mulatta*, of Indian origin) were used.

10 Animals were studied in parallel. According to a cross-over design, four of the animals received Example 1 (or Vehicle as a control) QD during the first phase of the study and BID during the second phase of the study. A reverse treatment schedule was used for the remaining four animals. When Example 1 was administered QD, the single dose was administered in the evening. Each treatment period lasted 28-30 consecutive days. Vehicle treatment lasted 10-14 days, immediately preceding the phase where
15 Example 1 was administered. Animals were also kept in their sleep/circadian chambers to become familiar with them. Treatment was administered as a liquid solution, dispensed automatically using a timer-controlled pump.

Throughout treatment periods, animals were maintained in light-controlled and sound attenuated sleep/circadian chambers, under constant conditions of dim light, in
20 order to determine the intrinsic rhythms of sleep/activity and self-administered food intake.

Data collection:

Sleep/activity cycle and sleep duration was documented using continuous on-line
25 actigraphic image analysis technique, as described in Masuda et al., *J Biol Rhythms*. 2010 Oct;25(5):361-71, Intrinsic activity rhythms in *Macaca mulatta*: their entrainment to light and melatonin. A software algorithm for actigraphic data analysis of sleep versus wakefulness in rhesus monkeys is explained in Zhdanova et al., *J Biol Rhythms*. 2011 Apr;26(2):149-59. Aging of intrinsic circadian rhythms and sleep in a diurnal nonhuman
30 primate, *Macaca mulatta*. Sleep onset and duration estimates were based on the comparisons between polysomnographic and actigraphic sleep in this species.

Each circadian chamber was equipped with an individual touch-screen monitor, connected to an automatic pellet dispenser (ENV-203-1000, Med Associates Inc., St. Albans, VT). This allowed for ad libitum around the clock self-administration of 1 g food

pellets (Nutritionally complete dustless precision pellets, Bio-Serv, Frenchtown, NJ) via pressing a moving on-screen target. The time each pellet dispensed was documented.

Data analysis was conducted using linear mixed model (SPSS), with the level of significance at $p < 0.05$. Data were presented as group means (standard error of the means (SEM)) for each treatment condition: QD or BID administration of vehicle or Example 1.

Figures 3 and 4 provide data on an individual animal's activity pattern (Fig. 3) or food intake pattern (Fig. 4) while receiving evening QD vehicle or Example 1. Actigraphic recordings from animals during a 12:12 hour light-dark cycle (LD) were taken, followed by constant dim light conditions thereafter (arrow along right side of graph). This individual had an intrinsic circadian period longer than 24 hours, illustrated by the entire activity pattern being delayed over time (gradual shift to the right). Vehicle or Example 1 was administered at the same clock time (17:00 hours). At the beginning of the vehicle treatment period (dashed line along right side of graph), this corresponded to a 2-hour prior-to-expected sleep onset (beginning of subjective night). By the time Example 1 treatment was initiated (solid line along right side of graph), this clock time corresponded to around a 3.5 hour prior-to-expected sleep onset.

Treatment with Example 1 significantly reduced sleep latency, facilitating sleep onset, and this effect continued throughout the treatment period. This effect also led to the overall shift of activity pattern to the left (phase advance in wake up time), with earlier sleep onset time resulting in similarly earlier time of awakening on subjective morning.

The Example 1 treatment significantly reduced food intake soon after administration, and this effect continued throughout the treatment period. This effect also led to the overall shift of food intake pattern to the left (phase advance in the time of morning meal). The food intake pattern in this animal followed the activity pattern. Compare Figure 3 and Figure 4.

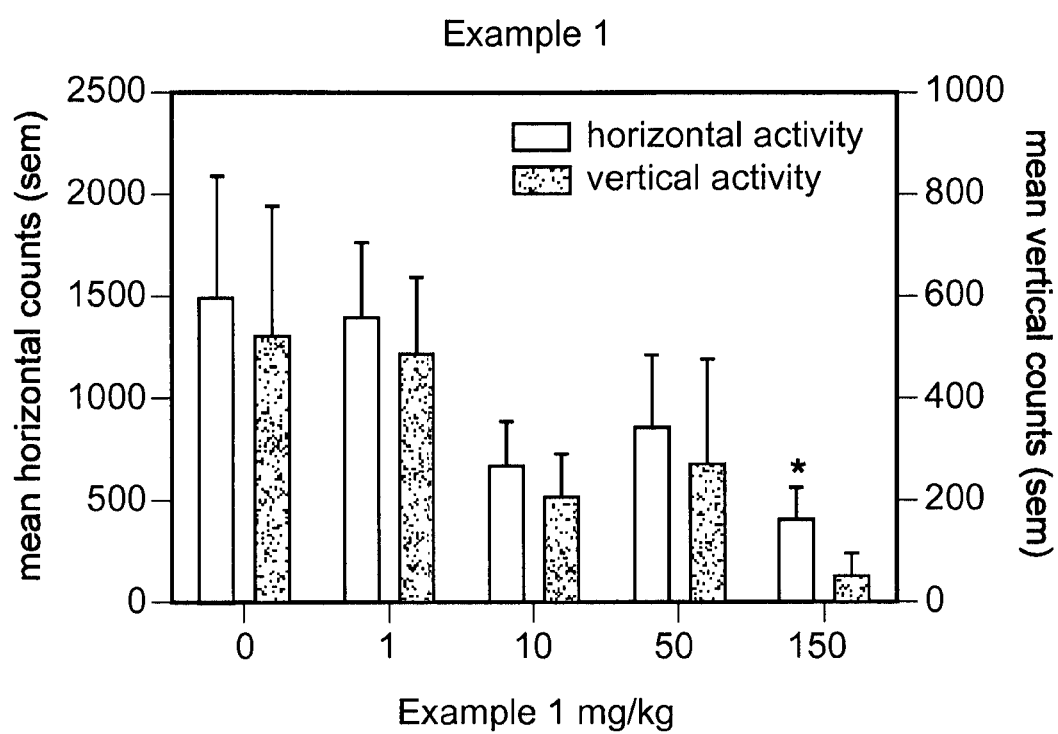
Figure 5 provides the change in sleep duration within 3 hours following evening treatment of Example 1 compared to vehicle (N=8 per group (QD vs BID)). For both groups, a significant increase in sleep duration was documented within 3 hours of treatment of Example 1 compared to administration of just vehicle. The data is expressed as a percent change of sleep duration for when animals receive vehicle compared to the other animals in the group receiving Example 1 (error bars providing the SEM; $p < 0.01$ vs. control).

Figure 6 provides the change in evening food intake within 3 hours following evening administration of Example 1 or vehicle. Figure 6 provides the change for animals receiving Example 1 QD or BID (N=8 per group (QD vs BID)), for which a significant decrease in food intake was documented for both groups, when compared to administration of just vehicle, within 3 hours of administration of Example 1. The data is expressed as percent of food intake within 3 hours after administration of vehicle or Example 1 (error bars providing the SEM; $p < 0.01$ vs. vehicle).

We Claim:

1. A method of treating a sleep disorder in a patient comprising the step of administering to the patient, in need of such treatment, a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof.
2. The method according to claim 1 wherein the sleep disorder is primary insomnia.
3. The method according to claim 1 wherein the sleep disorder is excessive daytime sleepiness.
4. The method according to claim 3 wherein the patient has Prader-Willi syndrome.
5. The method according to claim 1 wherein the sleep disorder is Night Eating Syndrome.
6. A method of treating a sleep disorder in a patient comprising the step of administering to the patient, in need of such treatment, a pharmaceutical composition comprising a therapeutically effective amount of (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient.
7. The method according to claim 6 wherein the sleep disorder is primary insomnia.
8. The method according to claim 6 wherein the sleep disorder is excessive daytime sleepiness.
9. The method according to claim 8 wherein the patient has Prader-Willi syndrome.
10. The method according to claim 6 wherein the sleep disorder is Night Eating Syndrome.
11. The method of any of claims 1 to 10, wherein (R)-2-(2-methylimidazo[2,1-b]thiazol-6-yl)-1-(2-(5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1H-inden-1-yl)-2,7-diazaspiro[3.5]nonan-7-yl)ethanone, or a pharmaceutically acceptable salt thereof is administered in combination with another pharmacologically active agent.

1/5

FIG. 1

2/5

FIG. 2

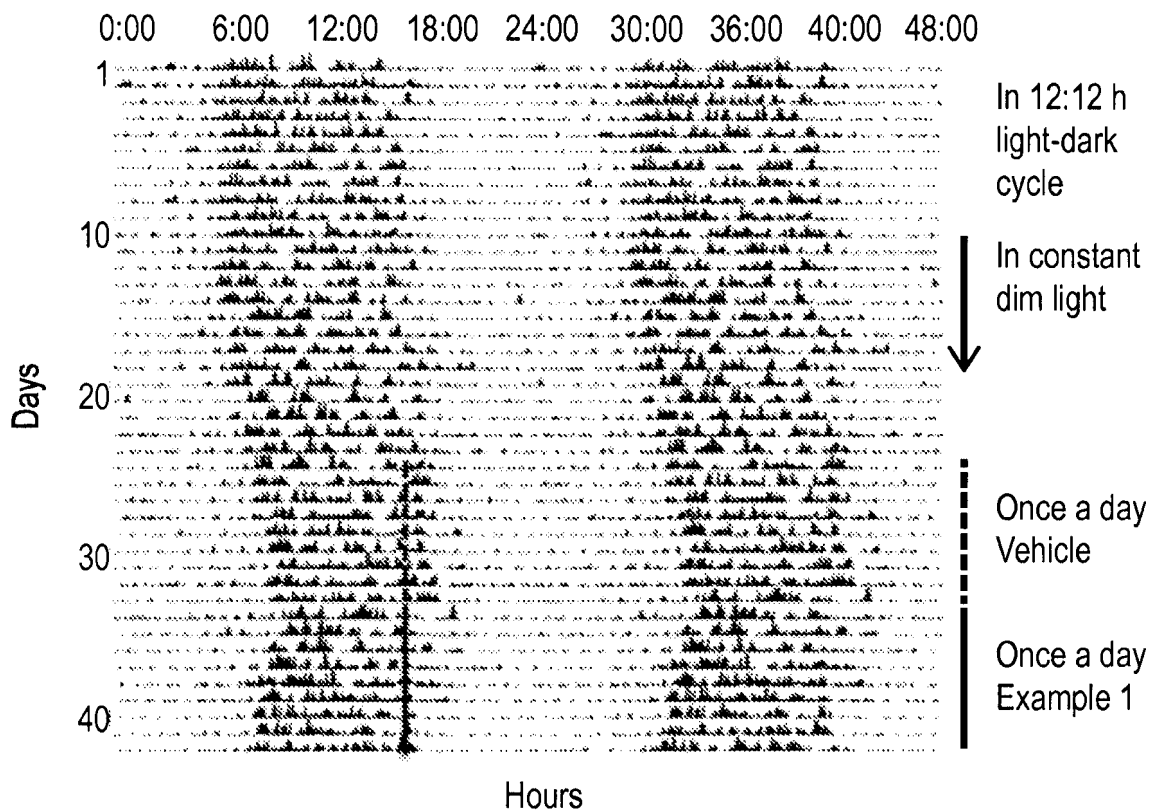
Study	Dose (mg)	Subjects (n/N)
B3301001	0	0/35
	2	0/6
	10	0/6
	50	3/6
	100	0/6
	100 fed	7/9
	150	5/8
	300	9/12
	40 divided, fed	0/6
	300 divided, fed	1/6

Study	Dose (mg)	Subjects (n/N)
B3301002	0	3/12
	2 BID	0/6
	10 BID	0/6
	40 BID	1/5
	100 BID	5/5

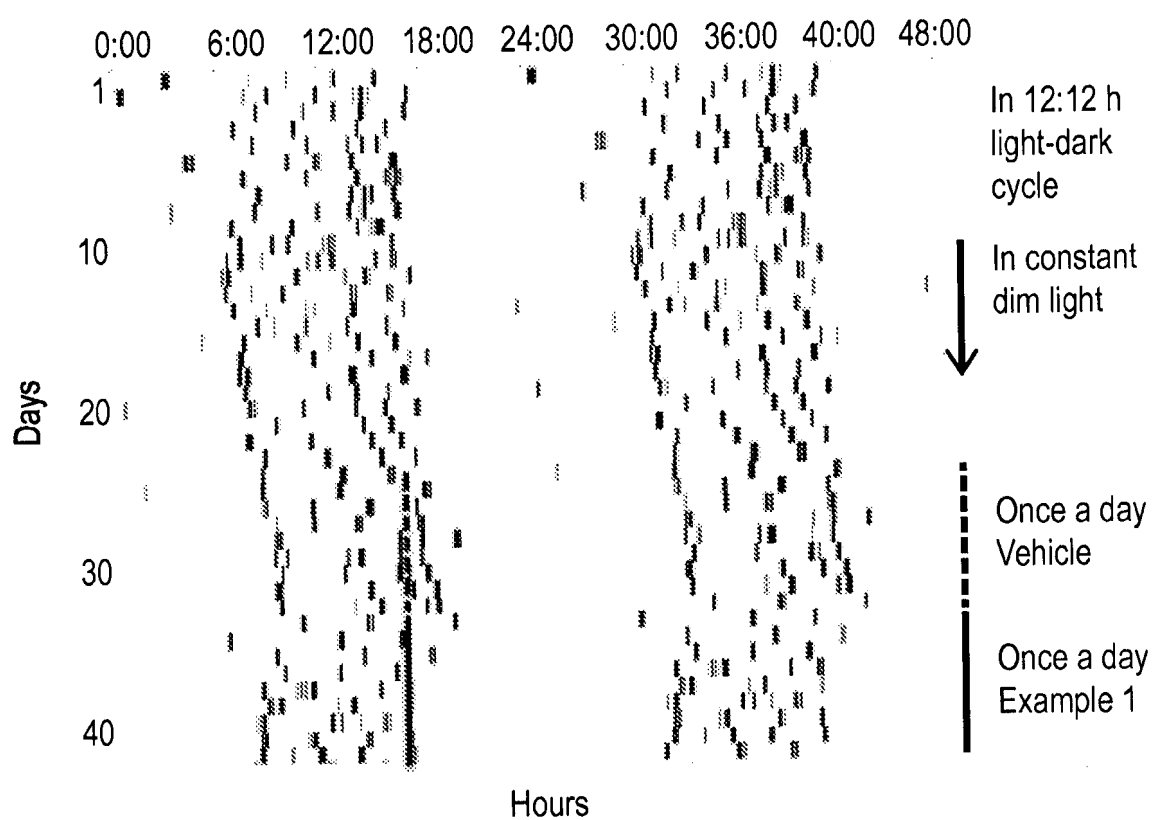
Study	Dose (mg)	Subjects (n/N)
B3301007	100 (IR)	4/16
	100 (EPOC 6 hr)	3/15
	100 (EPOC 6 hr), fed	0/8
	100 (EPOC 14 hr)	2/15
	100 (EPOC 14 hr), fed	0/7

3/5

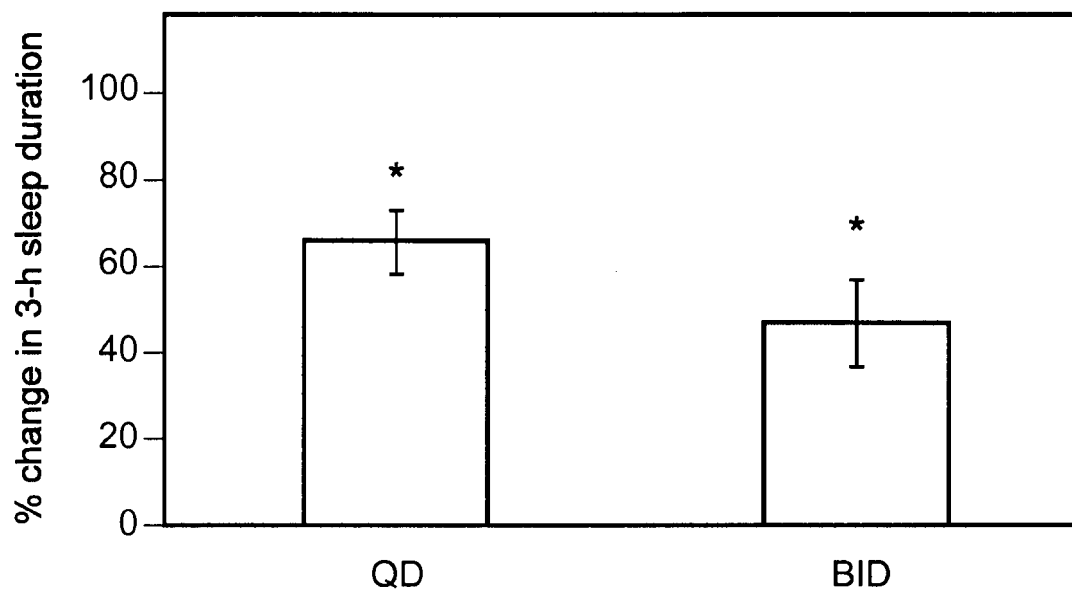
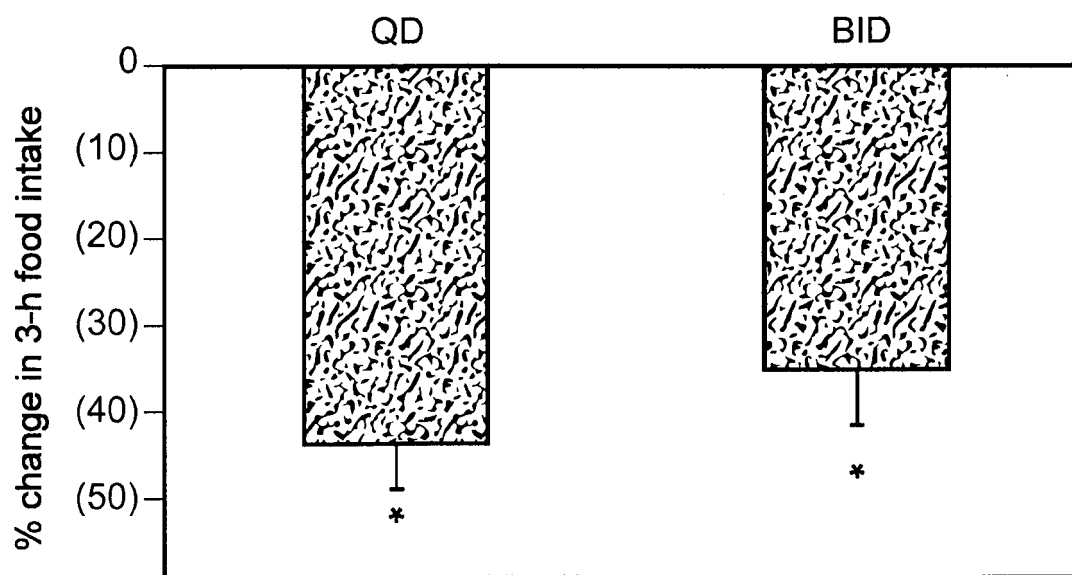
FIG. 3



4/5

FIG. 4

5/5

FIG. 5**FIG. 6**

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/054177

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K45/06 A61K31/397 A61K31/506
ADD.

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)
A61K A61P

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)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/230461 A1 (BHATTACHARYA SAMIT K [US] ET AL) 22 September 2011 (2011-09-22) cited in the application paragraphs [0002], [0086] - [0103] paragraph [0504]; compound 3F paragraph [0566]; table 1 -----	1-11
Y	WO 2008/008286 A2 (MERCK & CO INC [US]; GE MIN [US]; CLINE ERIC [US]; YANG LIHU [US]; MIL) 17 January 2008 (2008-01-17) page 22, lines 17-32 ----- -/-	1-11

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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G document member of the same patent family

Date of the actual completion of the international search

23 September 2013

Date of mailing of the international search report

27/09/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Hörtner, Michael

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2013/054177

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ALFONSO ABIZAID ET AL: "Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite", JOURNAL OF CLINICAL INVESTIGATION, vol. 116, no. 12, 1 December 2006 (2006-12-01), pages 3229-3239, XP055080458, ISSN: 0021-9738, DOI: 10.1172/JCI29867 page 3235, left-hand column, paragraph 3 - right-hand column, paragraph 1 -----	1,3-6,8,9,11
Y	US 2005/171131 A1 (KOSOGOF CHRISTI [US] ET AL) 4 August 2005 (2005-08-04) paragraphs [0002], [0009] -----	4,5,9-11
Y	ANTHONY P. GOLDSTONE: "Prader-Willi syndrome: advances in genetics, pathophysiology and treatment", TRENDS IN ENDOCRINOLOGY & METABOLISM, vol. 15, no. 1, 1 January 2004 (2004-01-01), pages 12-20, XP055080456, ISSN: 1043-2760, DOI: 10.1016/j.tem.2003.11.003 table 1 page 16, right-hand column, paragraph 1-2 -----	1,3,4,6,8,9,11
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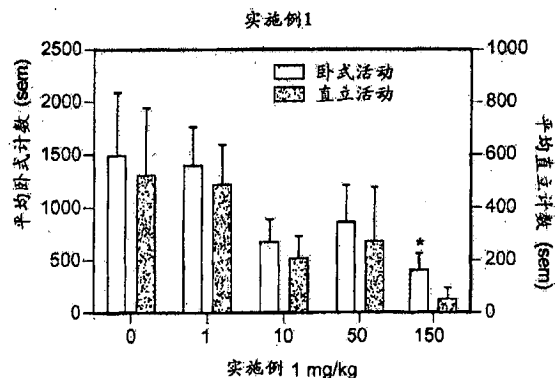
权利要求书1页 说明书19页 附图5页

(54) 发明名称

生长激素释放肽受体反向激动剂或拮抗剂用于
治疗睡眠障碍的用途

(57) 摘要

本发明涉及治疗患者的睡眠障碍的方法,包括给药生长激素释放肽受体反向激动剂或拮抗剂。本发明还包括治疗睡眠障碍的方法,其包括给药包含生长激素释放肽受体反向激动剂或拮抗剂和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。



1. 一种治疗患者的睡眠障碍的方法,包括向需要这样的治疗的患者给药治疗有效量的 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑 -6-基)-1-(2-(5-(6-甲基嘧啶 -4-基)-2,3-二氢 -1H-茚 -1-基)-2,7-二氮杂螺 [3.5] 壬烷 -7-基) 乙酮或其药学上可接受的盐的步骤。

2. 根据权利要求 1 的方法,其中所述睡眠障碍为原发性失眠。

3. 根据权利要求 1 的方法,其中所述睡眠障碍为日间过度嗜睡。

4. 根据权利要求 3 的方法,其中所述患者患有普拉德 - 威利综合征。

5. 根据权利要求 1 的方法,其中所述睡眠障碍为夜食综合征。

6. 一种治疗患者的睡眠障碍的方法,包括向需要这样的治疗的患者给药治疗有效量的药物组合物的步骤,所述药物组合物包含治疗有效量的 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑 -6-基)-1-(2-(5-(6-甲基嘧啶 -4-基)-2,3-二氢 -1H-茚 -1-基)-2,7-二氮杂螺 [3.5] 壬烷 -7-基) 乙酮或其药学上可接受的盐和至少一种药学上可接受的载体、稀释剂或赋形剂。

7. 根据权利要求 6 的方法,其中所述睡眠障碍为原发性失眠。

8. 根据权利要求 6 的方法,其中所述睡眠障碍为日间过度嗜睡。

9. 根据权利要求 8 的方法,其中所述患者患有普拉德 - 威利综合征。

10. 根据权利要求 6 的方法,其中所述睡眠障碍为夜食综合征。

11. 权利要求 1 至 10 中任一项的方法,其中 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑 -6-基)-1-(2-(5-(6-甲基嘧啶 -4-基)-2,3-二氢 -1H-茚 -1-基)-2,7-二氮杂螺 [3.5] 壬烷 -7-基) 乙酮或其药学上可接受的盐与另一种药理学活性剂共同给药。

生长激素释放肽受体反向激动剂或拮抗剂用于治疗睡眠障碍的用途

发明领域

[0001] 本发明涉及用于治疗睡眠障碍的化合物,其为生长激素释放肽 (ghrelin) 受体反向激动剂 / 拮抗剂。本发明还涉及含有这样的化合物的药物组合物。

背景技术

[0002] 失眠,最常见的睡眠障碍,影响约 5000-7000 万美国成年人。其特征在于难于入睡、频繁的夜间醒来、醒太早且不能恢复睡眠、或醒来且没有恢复精神的感觉。

[0003] 早期对于失眠的治疗普遍使用中枢神经系统 (CNS) 抑制剂比如巴比妥酸盐。这些化合物通常具有半衰期长,且具有熟知广谱的副作用,包括精神混乱、抑郁和第二天宿醉效应。另外,长期使用已经与包括生理和心理依赖的高成瘾潜能有关。治疗从巴比妥酸盐及其它 CNS 抑制剂转移到使用苯二氮卓类的镇静催眠剂。在患者和动物中这类化合物产生导致睡眠样状态的安静效应 (calming effect),比先前的安眠药具有更大的安全限度。然而,许多苯二氮卓具有副作用,其限制了其在某些患者人群中的有用性。这些问题包括与其它 CNS 抑制剂 (特别是酒精) 的增效作用、重复给药时耐受性发展、中止给药后的反弹性失眠、第二天的宿醉效应和精神活动能力和记忆的损伤。最新的失眠治疗已经使用非苯二氮卓类化合物。安必恩 (Ambien, 唑吡坦)、Sonata (扎来普隆) 是已批准药品的实例。本领域对于治疗睡眠障碍的安全且治疗有效的非苯二氮卓剂存在需要。

[0004] 夜食综合征 (Night Eating Syndrome, NES) 通常与上半天食物摄取不足、晚上摄食过多然后失眠以及睡眠觉醒。参见,例如 Vander Wal, Jillon S., Clinical Psychology Review (2012) 32 (1), 49-59。尽管对 NES 的认识增强了,但是仍然未将其弄清楚,且更重要地,一直研究治疗方法而没有成功。

[0005] 本发明涉及生长激素释放肽受体反向激动剂或拮抗剂用于治疗睡眠障碍的用途。

[0006] 发明简述

[0007] 本发明涉及治疗患者的睡眠障碍的方法,包括向需要这样的治疗的患者给药治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂。

[0008] 在另一个实施方案中,本发明涉及治疗患者的睡眠障碍的方法,包括向需要这样的治疗的患者给药包含治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。

附图说明

[0009] 图 1 为在雄性 Wistar Han 大鼠中,经由口服管饲法给药后 35-65 分钟,实施例 1 降低运动活性的影响的图解说明。

[0010] 图 2 为实施例 1 的在人类中瞌睡影响的图解说明。

[0011] 图 3 为在每天一次 (QD) 给药实施例 1 或载体之后动物的独立活动模式的实施例。

[0012] 图 4 为在每天一次 (QD) 给药实施例 1 或载体之后动物的独立食物摄取模式的实

施例。

[0013] 图 5 提供在晚上给药实施例 1 之后 3 小时内睡眠持续的变化。

[0014] 图 6 提供在晚上给药实施例 1 之后 3 小时内食物摄取的变化。

[0015] 发明详述

[0016] 在另一个实施方案中,本发明涉及治疗患者的原发性失眠的方法,包括向需要这样的治疗的患者给药治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂。

[0017] 在另一个实施方案中,本发明涉及治疗患者的日间过度嗜睡 (excessive daytime sleepiness) 的方法,包括向需要这样的治疗的患者给药治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂。本发明的方法包括治疗诊断患有普拉德-威利综合征 (Prader-Willi syndrome) 的患者的日间过度嗜睡。

[0018] 在另一个实施方案中,本发明涉及治疗患者的 NES 的方法,包括向需要这样的治疗的患者给药治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂。

[0019] 在另一个实施方案中,本发明涉及治疗患者的原发性失眠的方法,包括向需要这样的治疗的患者给药包含治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。

[0020] 在另一个实施方案中,本发明涉及治疗患者的日间过度嗜睡的方法,包括向需要这样的治疗的患者给药包含治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。本发明的方法包括治疗诊断患有普拉德-威利综合征的患者的日间过度嗜睡。

[0021] 在另一个实施方案中,本发明涉及治疗患者 NES 的方法,包括向需要这样的治疗的患者给药包含治疗有效量的生长激素释放肽受体反向激动剂或拮抗剂和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。

[0022] 在另一个实施方案中,本发明涉及一种治疗患者的睡眠障碍的方法,包括向需要这样的治疗的患者给药治疗有效量的 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺 [3.5] 壬烷-7-基) 乙酮或其药学上可接受的盐。

[0023] 在另一个实施方案中,本发明涉及一种治疗患者的原发性失眠的方法,包括向需要这样的治疗的患者给药治疗有效量的 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺 [3.5] 壬烷-7-基) 乙酮或其药学上可接受的盐。

[0024] 在另一个实施方案中,本发明涉及一种治疗患者的日间过度嗜睡的方法,包括向需要这样的治疗的患者给药治疗有效量的 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺 [3.5] 壬烷-7-基) 乙酮或其药学上可接受的盐。本发明的方法用于治疗诊断患有普拉德-威利综合征的患者的日间过度嗜睡。

[0025] 在另一个实施方案中,本发明涉及一种治疗患者的 NES 的方法,包括向需要这样的治疗的患者给药治疗有效量的 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺 [3.5] 壬烷-7-基) 乙酮或其药学上可接受的盐。

[0026] 在另一个实施方案中,本发明涉及一种治疗患者的睡眠障碍的方法,包括向需要这样的治疗的患者给药包含治疗有效量的(R)-2-(2-甲基咪唑并[2,1-b]噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茛-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮或其药学上可接受的盐和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。

[0027] 在另一个实施方案中,本发明涉及一种治疗患者的原发性失眠的方法,包括向需要这样的治疗的患者给药包含治疗有效量的(R)-2-(2-甲基咪唑并[2,1-b]噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茛-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮或其药学上可接受的盐和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。

[0028] 在另一个实施方案中,本发明涉及一种治疗患者的日间过度嗜睡的方法,包括向需要这样的治疗的患者给药包含治疗有效量的(R)-2-(2-甲基咪唑并[2,1-b]噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茛-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮或其药学上可接受的盐和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。本发明的方法用于治疗诊断患有普拉德-威利综合症的患者的日间过度嗜睡。

[0029] 在另一个实施方案中,本发明涉及一种治疗患者的NES的方法,包括向需要这样的治疗的患者给药包含治疗有效量的(R)-2-(2-甲基咪唑并[2,1-b]噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茛-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮或其药学上可接受的盐和至少一种药学上可接受的载体、稀释剂或赋形剂的药物组合物。

[0030] 在另一个实施方案中,本发明还提供:

[0031] 如本文描述的生长激素释放肽受体反向激动剂或拮抗剂在制备用于治疗睡眠障碍(特别是原发性失眠、日间过度嗜睡或NES)的药物中的用途;

[0032] 如本文描述的生长激素释放肽受体反向激动剂或拮抗剂,用作药物;

[0033] 如本文描述的生长激素释放肽受体反向激动剂或拮抗剂,用于治疗睡眠障碍,特别是原发性失眠、日间过度嗜睡或NES;

[0034] 药物组合物,包含如本文描述的生长激素释放肽受体反向激动剂或拮抗剂和药学上可接受的赋形剂;和

[0035] 用于治疗睡眠障碍(特别是原发性失眠、日间过度嗜睡或NES)的药物组合物,包含如本文描述的生长激素释放肽受体反向激动剂或拮抗剂;

[0036] 特别地,其中所述生长激素释放肽受体反向激动剂或拮抗剂可以是(R)-2-(2-甲基咪唑并[2,1-b]噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茛-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮或其药学上可接受的盐。

[0037] (R)-2-(2-甲基咪唑并[2,1-b]噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茛-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮或其药学上可接受的盐可以仅仅为本文如实施例1中一般提及的。

[0038] 在另一个实施方案中,本发明还提供本文讨论的任何方法,其中所述生长激素释放肽受体反向激动剂或拮抗剂,特别是(R)-2-(2-甲基咪唑并[2,1-b]噻

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唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮或其药学上可接受的盐与另一种药理学活性剂共同给药。

[0039] 在另一个实施方案中,所述生长激素释放肽受体反向激动剂或拮抗剂(特别是实施例1)可以与其它药理学活性剂组合应用,所述其它药理学活性剂也指本领域已知的化合物,分别给药或在同一药物组合物中给药,包括但不限于:胰岛素增敏剂,包括(i)PPAR γ 拮抗剂,比如格列酮类(例如环格列酮;达格列酮;恩格列酮;沙格列酮(isaglitazone)(MCC-555);吡格列酮;罗格列酮;曲格列酮;tularik;BRL49653;CLX-0921;5-BTZD)、GW-0207、LG-100641和LY-300512等);(iii)双胍类比如二甲双胍和苯乙双胍;(b)胰岛素或胰岛素模拟物,比如biota、LP-100、novarapid、地特胰岛素、赖脯胰岛素、甘精胰岛素、胰岛素锌混悬液(长效的和超长效的);Lys-Pro胰岛素、GLP-1(73-7)(insulintropin);和GLP-1(7-36)-NH₂);(c)磺酰脲类,比如醋酸己脲;氯磺丙脲;特泌脲;格列本脲;格列吡嗪;格列苯脲;格列美脲;格列齐特;格列太特;格列喹酮;格列索脲;妥拉磺脲;和甲苯磺丁脲;(d) α -葡萄糖苷酶抑制剂,比如阿卡波糖、脂解素;卡格列波糖;乙格列酯;米格列醇;伏格列波糖;普拉米星-Q;salbostatin;CKD-711;MDL-25,637;MDL-73,945;和MOR 14等;(e)胆固醇降低剂,比如(i)HMG-CoA还原酶抑制剂(阿托伐他汀、伊伐他汀、氟伐他汀、洛伐他汀、普伐他汀、rivastatin、罗苏伐他汀、辛伐他汀及其它他汀类),(ii)胆酸吸收剂/多价螯合剂,比如考来烯胺、考来替泊、交联右旋糖酐的二烷氨基烷基衍生物;Colestid.RTM.;LoCholest.RTM.等,(iii)烟醇,烟酸或其盐,(iii)增殖体-活化体受体 α 激动剂,比如非诺贝酸衍生物(吉非贝齐、氯贝丁酯、非诺贝特和苯扎贝特),(iv)胆固醇吸收抑制剂,比如甾烷醇酯、 β -谷甾醇、甾醇糖苷比如替奎安;和吡啶酮类化合物,比如伊泽替米贝等,和(酰基辅酶A:胆固醇酰基转移酶(ACAT))抑制剂,比如阿伐麦布和甲亚油酰胺,(v)抗氧化剂,比如普罗布考,(vi)维生素E,和(vii)拟甲状腺素药;(f)PPAR α 激动剂,比如苯氯贝特、苯扎贝特、环丙贝特、氯贝丁酯、依托贝特、非诺贝特和吉非贝齐;以及其它纤维酸衍生物,比如Atromid.RTM.、Lopid.RTM.和Tricor.RTM.等,和如Glaxo的W097/36579中所描述的PPAR α 激动剂;(g)PPAR δ 激动剂;(h)PPAR α/δ 激动剂;比如muraglitazar,以及US专利No.6,414,002中公开的化合物;和(i)抗肥胖剂,比如(1)生长激素促分泌素、生长激素促分泌素受体激动剂/拮抗剂,比如NN703、海沙瑞林、MK-0677、SM-130686、CP-424,391、L-692,429和L-163,255;(2)蛋白酪氨酸磷酸酶-1B(PTP-1B)抑制剂;(3)大麻素受体配体,比如如大麻素CB₁受体拮抗剂或反向激动剂,比如利莫那班(Sanofi Synthelabo)、AMT-251、和SR-14778以及SR 141716A(Sanofi Synthelabo)、SLV-319(Solvay)、BAY 65-2520(Bayer);(4)抗肥胖血清素能剂,比如芬氟拉明、右芬氟拉明、苯丁胺和西布曲明;(5) β 3-肾上腺素受体激动剂,比如AD9677/TAK677(Dainippon/Takeda)、CL-316,243、SB 418790、BRL-37344、L-796568、BMS-196085、BRL-35135A、CGP12177A、BTA-243、曲卡君(Trecadrine)、ZenecaD7114、SR 59119A;(6)胰脂肪酶抑制剂,比如奥利司他(Xenical.RTM)、Triton WR1339、RHC80267、利普司他汀、四氢利普司他汀、茶皂素、二乙基伞形酮磷酸酯(diethylumbelliferyl phosphate);(7)神经肽Y1拮抗剂,比如BIBP3226、J-115814、BIBO 3304、LY-357897、CP-671906、GI-264879A;(8)神经肽Y5拮抗剂,比如GW-569180A、GW-594884A、GW-587081X、GW-548118X、FR226928、FR 240662、FR252384、1229U91、GI-264879A、CGP71683A、LY-377897、PD-160170、SR-120562A、

SR-120819A 和 JCF-104 ; (9) 黑色素 - 浓缩激素 (MCH) 受体拮抗剂 ; (10) 黑色素 - 浓缩激素 1 受体 (MCH1R) 拮抗剂, 比如 T-226296 (Takeda) ; (11) 黑色素 - 浓缩激素 2 受体 (MCH2R) 激动剂 / 拮抗剂 ; (12) 食欲素受体拮抗剂, 比如 SB-334867-A, 和本文专利出版物中公开的那些 ; (13) 血清素再摄取抑制剂, 比如氟西汀、帕罗西汀和舍曲林 ; (14) 黑皮质素激动剂, 比如 Melanotan II ; (15) 其它 Mc4r (黑皮质素 4 受体) 激动剂, 比如 CHIR86036 (Chiron)、ME-10142 和 ME-10145 (Melacure)、CHIR86036 (Chiron) ; PT-141 和 PT-14 (Palatin) ; (16) 5HT-2 激动剂 ; (17) 5HT2C (血清素受体 2C) 激动剂, 比如 BVT933、DPCA37215、WAY161503、R-1065 ; (18) 甘丙肽拮抗剂 ; (19) CCK 激动剂 ; (20) CCK-A (胆囊收缩素 -A) 激动剂, 比如 AR-R 15849、GI 181771、JMV-180、A-71378、A-71623 和 SR14613 ; (22) 促肾上腺皮质激素释放激素激动剂 ; (23) 组胺受体 -3 (H3) 调节剂 ; (24) 组胺受体 -3 (H3) 拮抗剂 / 反向激动剂, 比如噻普酰胺 (hioperamide)、3-(1H-咪唑-4-基) 丙基 N-(4-戊烯基) 氨基甲酸酯、clobenpropit、iodophenpropit、imoproxifan、GT2394 (Gliatech) 和 O-[3-(1H-咪唑-4-基) 丙醇]-氨基甲酸酯类 ; (25). β -羟基类固醇脱氢酶-1 抑制剂 (β -HSD-1) ; (26) PDE (磷酸二酯酶) 抑制剂, 比如茶碱、己酮可可碱、扎普司特、西地那非、氨力农、米力农、西洛酰胺、咯利普兰和西洛司特 ; (27) 磷酸二酯酶 -3B (PDE3B) 抑制剂 ; (28) NE (去甲肾上腺素) 转运抑制剂, 比如 GW 320659、despiramine、他舒普仑和诺米芬辛 ; (29) 第二或第三生长激素释放肽受体反向激动剂或拮抗剂 ; (30) 瘦素, 包括重组患者瘦素 (PEG-OB, Hoffman La Roche) 和重组蛋氨酸人瘦素 (Amgen) ; (31) 瘦素衍生物 ; (32) BRS3 (铃蟾肽受体亚型 3) 激动剂, 比如 [D-Phe6, beta-Ala11, Phe13, Nle14]Bn(6-14) 和 [D-Phe6, Phe13]Bn(6-13) 丙酰胺, 和 Pept. Sci. 2002Aug ; 8(8) : 461-75 中公开的那些化合物 ; (33) CNTF (睫状神经生长因子), 比如 GI-181771 (GlaxoSmithKline)、SR146131 (Sanofi Synthelabo)、butabindide、PD170, 292 和 PD 149164 (Pfizer) ; (34) CNTF 衍生物, 比如阿索开 (Regeneron) ; (35) 单胺再摄取抑制剂, 比如西布曲明 ; (36) UCP-1 (未偶合蛋白质 -1)、2 或 3 活化剂, 比如植烷酸、4-[(E)-2-(5, 6, 7, 8-四氢-5, 5, 8, 8-四甲基-2-萘基)-1-丙烯基]苯甲酸 (TTNPB)、视黄酸 ; (37) 甲状腺激素 β 激动剂, 比如 KB-2611 (KaroBioBMS) ; (38) FAS (脂肪酸合成酶) 抑制剂, 比如浅蓝菌素和 C75 ; (39) DGAT1 (二酰基甘油酰基转移酶 1) 抑制剂 ; (40) DGAT2 (二酰基甘油酰基转移酶 2) 抑制剂 ; (41) ACC2 (乙酰基 - 辅酶 A 羧化酶 -2) 抑制剂 ; (42) 糖皮质激素拮抗剂 ; (43) 酰基 - 雌激素类, 比如油酰基 - 雌酮, 其公开在 del Mar-Grasa, M. 等人, Obesity Research, 9 : 202-9 (2001) 中 ; (44) 二肽基肽酶 IV (DP-IV) 抑制剂, 比如异亮氨酸噻唑烷 (thiazolidide)、缬氨酸吡咯烷 (pyrrolidide)、NVP-DPP728、LAF237、MK-431、P93/01、TSL 225、TMC-2A/2B/2C、FE 999011、P9310/K364、VIP 0177、SDZ 274-444 ; (46) 二羧酸酯转运蛋白抑制剂 ; (47) 葡萄糖转运蛋白抑制剂 ; (48) 磷酸酯转运蛋白抑制剂 ; (49) 二甲双胍 (Glucophage. RTM) ; 和 (50) 托吡酯 (Topimax. RTM) ; (50) 肽 YY、PYY 3-36、肽 YY 类似物、衍生物和片段比如 BIM-43073D、BIM-43004C (Olitvak, D. A. 等, Dig. Dis. Sci. 44(3) : 643-48 (1999)) ; (51) 神经肽 Y2 (NPY2) 受体激动剂, 比如 NPY3-36、N 乙酰基 [Leu(28, 31)]NPY 24-36、TASP-V 和环 -(28/32)-Ac-[Lys28-Glu32]-(25-36)-pNPY ; (52) 神经肽 Y4 (NPY4) 激动剂, 比如胰腺肽 (PP), 和其它 Y4 激动剂比如 1229U91 ; (54) 环氧化酶 -2 抑制剂, 比如艾托考昔、塞来考昔、伐地考昔、帕瑞考昔、鲁米考昔、BMS347070、tiracoxib 或 JTE522、ABT963、CS502 和 GW406381, 及其药学上可接受的盐 ; (55) 神经

肽 Y1 (NPY1) 拮抗剂, 比如 BIBP3226、J-115814、BIBO 3304、LY-357897、CP-671906、GI-264879A; (56) 阿片样物质拮抗剂, 比如纳美芬 (Revex, RTM.)、3- 甲氧基纳曲酮、纳洛酮、纳曲酮; (57) 11 β HSD-1 (1 型 11- β 羟基类固醇脱氢酶) 抑制剂, 比如 BVT3498、BVT 2733; (58) 阿米雷司; (59) amphetchloral (胺非氯醛); (60) 苯丙胺; (61) 苄非他明; (62) 氯苯丁胺; (63) 氯苄苯丙胺; (64) 氯苯丁胺酯; (65) 氯氨雷司; (66) 氯特胺; (67) 环己异丙甲胺 (cyclohexedrine); (68) 右旋苯丙胺; (69) 二苯甲哌啶乙醇 (diphenmethoxidine); (70) N- 乙基苯丙胺; (71) 苯丁吗酯; (72) 非尼雷司; (73) 芬普雷司; (74) 氟多雷司; (75) 氟氨雷司; (76) 糠甲基苯丙胺 (furfurylmethylamphetamine); (77) 左旋苯丙胺; (78) 左芬氟拉明 (levophacetoperane); (79) 美芬雷司; (80) 甲胺苯丙酮; (81) 脱氧麻黄碱; (82) 去甲伪麻黄碱; (83) 喷托雷司; (84) 苯甲曲秦; (85) 芬美曲秦; (86) 匹西雷司; (87) phytopharm57; 和 (88) 唑尼沙胺。

[0040] 如本文使用的术语“药学上可接受的盐”指那些盐, 其在合理医学判断范围内, 适用于接触患者和低等动物的组织而没有过度毒性、刺激、变态反应等, 且与合理的利益 / 风险比例相称。药学上可接受的盐是本领域熟知的。例如, S. M. Berge et al. 在 Berge 等, *J. Pharmaceutical Sciences*, 1977, 66:1-19 中详细描述的药理学上可接受的盐。所述盐可以在本发明的实施例 1 的最后分离和纯化期间原位制备, 或者通过实施例 1 的游离碱与适合的有机酸或无机酸反应分别制备。代表性的酸加成盐包括, 但不限于乙酸盐、己二酸盐、海藻酸盐、柠檬酸盐, 天冬氨酸盐、苯甲酸盐、苯磺酸盐、碳酸氢盐、硫酸氢盐、丁酸盐、樟脑酸盐、樟脑磺酸盐、柠檬酸盐、二葡萄糖酸盐、磷酸甘油、半硫酸盐、庚酸盐、己酸盐、富马酸盐、盐酸盐、氢溴酸盐、氢碘酸盐、2- 羟基乙磺酸盐 (羟乙基磺酸盐)、乳酸盐、马来酸盐、甲磺酸盐、烟酸盐、2- 萘磺酸盐、草酸盐、双羟萘酸盐、果胶盐 (pectinate)、过硫酸盐、3- 苯基丙酸盐、磷酸盐、苦味酸盐、新戊酸盐、丙酸盐、琥珀酸盐、硫酸盐、酒石酸盐、硫氰酸盐和对 - 甲苯磺酸盐。

[0041] 如本文使用的术语“生长激素释放肽受体”指 G 蛋白 - 偶联受体, 称为生长激素促分泌素受体 (GHSR1a 或 GHS-1aR)。

[0042] 如本文使用的术语“睡眠障碍”包括约 70 种以患者的睡眠量、睡眠质量或时间、或与睡眠相关的行为或生理条件方面的障碍为特征的综合征。睡眠障碍综合征的代表性实例包括, 但不限于失眠、原发性失眠、睡眠性呼吸暂停、发作性睡眠、不宁腿综合征、昼夜节律睡眠障碍、REM 睡眠行为障碍、梦游症 (梦行)、睡眠磨牙 (磨牙症)、睡眠过度、爆炸头综合征 (exploding head syndrome)、睡眠性麻痹和日间过度嗜睡 (EDS)。

[0043] EDS 是患有普拉德 - 威利综合征 (PWS) 的患者的典型特征之一; 护理者将 PWS 儿童描述为困倦且患有高度频繁的日间小睡 (daytime napping)。EDS 表现为在多次睡眠潜伏期测试时夜晚和白天期间入睡潜伏期缩短。父母的报道和调查问卷显示在患有 PWS 的 90-100% 成年人中存在 EDS。与其它智能障碍组和非 -PWS 对照相比, PWS 患者也具有较高的自报道 EDS 的水平。

[0044] “NES 已经在饮食方式、睡眠障碍、临床过程和家族聚集性方面进行了表征。与健康的对照相比, 患有 NES 的人在晚餐之后消耗显著更大比例的其卡路里、夜间更常醒来、且与对照相比更有可能在觉醒时进食。”Vander Wal, 上述, 第 50 页, 参考省略的内部表格和引文。

[0045] 如本文使用的术语“患者”指人类。

[0046] 本发明还提供药物组合物,其包括与一种或多种无毒药学上可接受的载体一起配制的生长激素释放肽受体反向激动剂或拮抗剂,特别是实施例1。药物组合物可以特别地配制用于以固体或液体形式口服给药,用于非肠道注射,或用于直肠给药。

[0047] 如本文使用的术语“药学上可接受的载体”指无毒、惰性固体、半固体或液体填充剂、稀释剂、包封材料或各种类型的制剂助剂。可以充当药学上可接受的载体的材料的某些实例为糖,比如乳糖、葡萄糖和蔗糖;淀粉,比如玉米淀粉和马铃薯淀粉;纤维素及其衍生物,比如羧甲基纤维素钠、乙基纤维素和醋酸纤维素;粉状西黄蓍胶;麦芽;明胶;滑石;赋形剂比如可可脂和栓剂蜡;油,比如花生油、棉籽油、红花油、芝麻油、橄榄油、玉米油和大豆油;二醇;如丙二醇;酯比如油酸乙酯和月桂酸乙酯;琼脂;缓冲剂,比如氢氧化镁和氢氧化铝;海藻酸;无热原水;等渗盐水;林格溶液、乙醇和磷酸盐缓冲液,以及其它无毒相容性润滑剂比如十二烷基硫酸钠和硬脂酸镁,以及着色剂、释放剂、包衣剂、甜味剂、调味剂和芳香剂,防腐剂和抗氧化剂也可以存在于该组合物中,根据配方设计师的判断而定。本发明提供药物组合物,其包括与一种或多种无毒药学上可接受的载体一起配制的生长激素释放肽受体反向激动剂或拮抗剂,特别是实施例1。所述药物组合物可以配制用于以固体或液体形式口服给药、用于非肠道注射剂或用于直肠给药。

[0048] 本发明的药物组合物可以按照如下方式给药予患者:口服、直肠、肠胃外、腹膜内、局部(如通过粉末、软膏剂或滴剂)、口腔或作为口腔或鼻腔喷雾剂。如本文使用的术语“肠胃外”指包括静脉内、肌内、腹膜内、胸骨内、皮下、关节内注射和输注的给药方式。

[0049] 用于非肠道注射的本发明的药物组合物包括药学上可接受的无菌水性或非水性溶液、分散液、悬浮液或乳剂、及重构成无菌可注射溶液或分散液的无菌粉末。合适的水性和非水载体、稀释剂、溶剂或介质的实例包括,但不限于水、乙醇、多元醇(丙二醇、聚乙二醇、甘油等)、其合适的混合物、植物油(比如橄榄油)和可注射的有机酯比如油酸乙酯。例如,可以通过使用包衣材料比如卵磷脂、在分散液的情况下通过保持期望的粒径和通过使用表面活性剂来保持合适的流动性。

[0050] 这些组合物也可包含助剂,比如防腐剂、润湿剂、乳化剂、分散剂、润滑剂和分散剂。可以通过各种抗菌剂和抗真菌剂比如尼泊金酯类、氯丁醇、苯酚、山梨酸等来确保预防微生物的作用。包括等渗剂例如糖、氯化钠等也可能是期望的。可以通过使用延迟吸收的试剂例如单硬脂酸铝和明胶来得到可注射药物的延长吸收形式。

[0051] 在某些情况下,为了延长药物的作用,通常期望减慢来自皮下注射或肌肉注射的药物的吸收。这可通过使用具有水溶性差的结晶材料或非晶形材料的液体悬浮液来实现。因而,药物的吸收率取决于其溶出速率,其反过来可能依赖于晶体大小和结晶形式。可选地,肠胃外给药药物形式的延迟吸收通过将药物溶解或悬浮在油性载体中实现。

[0052] 除了生长激素释放肽受体反向激动剂或拮抗剂(特别是实施例1)之外,悬浮液可以包含悬浮剂,例如乙氧基化异硬脂醇、聚氧乙烯山梨醇和脱水山梨醇酯、微晶纤维素、偏氢氧化铝(aluminum metahydroxide)、膨润土、琼脂、西黄蓍胶及其混合物。

[0053] 如果期望,且为了更均匀分布,本发明的生长激素释放肽受体反向激动剂或拮抗剂(特别是实施例1)可以并入缓释或靶向递送系统中,比如聚合基质、脂质体和微球。它们可以以无菌组合物形式通过例如过滤通过细菌保留过滤器,或加入杀菌剂来灭菌,所述

无菌固体组合物可以在即将使用之前溶于无菌水中或者某些其它无菌的可注射介质。

[0054] 生长激素释放肽受体反向激动剂或拮抗剂（特别是实施例 1），如果适当，与一种或多种如上所述药学上可接受的载体，也可以呈微囊封装的形式。可以用包衣和壳比如肠溶衣、释放控制包衣和药物制剂领域众所周知的其它包衣来制备片剂、锭剂、胶囊、丸剂和颗粒剂的固体剂型。在这样的固体剂中，生长激素释放肽受体反向激动剂或拮抗剂（特别是实施例 1）可以与至少一种惰性稀释剂（比如蔗糖、乳糖或淀粉）混合。按照常规实践，除了惰性稀释剂之外，这样剂型也可包括其他物质，例如压片润滑剂及其它压片助剂，例如硬脂酸镁和微晶纤维素。在胶囊、片剂和丸剂的情况下，所述剂型也可包括缓冲剂。它们可任选地包含遮光剂，并且也可以是它们仅仅或优先在肠道的某一部分中以延迟方式释放活性成分的组合物。可以使用的包埋组合物的实例包括聚合物和蜡。

[0055] 可注射的贮库形式是通过在可生物降解的聚合物比如聚交酯-聚乙醇酸交酯中形成药物的微囊化基质来制备。根据药物与聚合物的比例和使用的具体聚合物的性质，可以控制药物释放的速率。其它可生物降解的聚合物的实例包括聚（原酸酯）和聚（酸酐）。贮库可注射的制剂也通过将药物俘获在与身体组织相容的脂质体或微乳剂中制备。

[0056] 所述可注射的制剂可以例如以无菌固体组合物形式通过过滤穿过保留细菌的过滤器或通过加入杀菌剂灭菌，该无菌固体组合物可以仅仅在使用前溶解或分散在无菌水或其他无菌可注射介质中。

[0057] 根据已知技术，使用适合的分散剂或润湿剂和悬浮剂可以配制可注射的制剂，例如无菌可注射水性或油脂性悬浮液。所述无菌可注射的制剂也可以是在无毒肠胃外可接受的稀释剂或溶剂中的无菌可注射溶液、悬浮液或乳液，例如在 1, 3- 丁二醇中的溶液。其中可以使用的可接受的介质和溶剂为水、林格溶液 U. S. P. 和等渗氯化钠溶液。另外，无菌不挥发油通常用作溶剂或悬浮介质。为此目的，可使用任何温和的不挥发油，包括合成的甘油单酯或甘油二酯。另外，脂肪酸比如油酸用于可注射的制剂中。

[0058] 用于口服给药的固体剂型包括胶囊、片剂、丸剂、粉剂和颗粒剂。在这样的固体剂型中，生长激素释放肽受体反向激动剂或拮抗剂（特别是实施例 1）与至少一种惰性药学上可接受的载体比如柠檬酸钠或磷酸钙和 / 或 a) 填充剂或增量剂，比如淀粉、乳糖、蔗糖、葡萄糖、甘露醇和水杨酸；b) 粘合剂，比如羧甲基纤维素、海藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖和阿拉伯胶；c) 湿润剂，比如甘油；d) 崩解剂，比如琼脂、碳酸钙、马铃薯或木薯淀粉、海藻酸、某些硅酸盐、和碳酸钠；e) 溶液阻滞剂，比如石蜡；f) 吸收促进剂，比如季铵化合物；g) 润湿剂，比如鲸蜡醇和单硬脂酸甘油酯；h) 吸附剂，比如高岭土和膨润土；和 i) 润滑剂，比如滑石粉、硬脂酸钙、硬脂酸镁、固体聚乙二醇、十二烷基硫酸钠、及其混合物来混合。在胶囊、片剂和丸剂的情况下，所述剂型也可包括缓冲剂。

[0059] 类似类型的固体组合物也可用作填充的软和硬明胶胶囊的填充剂，其使用乳糖（lactose）或乳糖（milk sugar）以及高分子量聚乙二醇等。

[0060] 片剂、锭剂、胶囊、丸剂和颗粒剂的固体剂型可以用包衣和壳比如肠溶衣及药物制剂领域众所周知的其它包衣来制备。它们可任选地包含遮光剂，并且也可以是它们仅仅或优先在肠道的某一部分中以延迟方式释放活性成分的组合物。可以使用的包埋组合物的实例包括聚合物和蜡。

[0061] 用于口服给药的液体剂型包括药学上可接受的乳剂、微乳剂、溶液、悬浮液、糖浆

和酞剂。除了生长激素释放肽受体反向激动剂或拮抗剂（特别是实施例 1）之外，液体剂型可以包含本领域通常使用的惰性稀释剂比如例如水或其它溶剂、增溶剂和乳化剂比如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苄酯、丙二醇、1, 3- 丁二醇、二甲基甲酰胺、油（特别是棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油、和芝麻油）、甘油、四氢糠醇、聚乙二醇和脱水山梨醇的脂肪酸酯、及其混合物。

[0062] 除了惰性稀释剂之外，口服组合物也可以包括助剂，比如润湿剂、乳化剂和悬浮剂、甜味剂、着色剂、调味剂和芳香剂。

[0063] 为了获得一定量的生长激素释放肽受体反向激动剂或拮抗剂（特别是实施例 1），生长激素释放肽受体反向激动剂或拮抗剂（特别是实施例 1）在本发明药物组合物中的实际剂量水平可以变化，对于具体患者、组合物和给药方式，所述一定量可有效地获得期望的治疗反应。选择的剂量水平应当取决于具体生长激素释放肽受体反向激动剂或拮抗剂的活性、给药途径、待治疗病症的严重程度、病症和待治疗患者的既往医疗史。

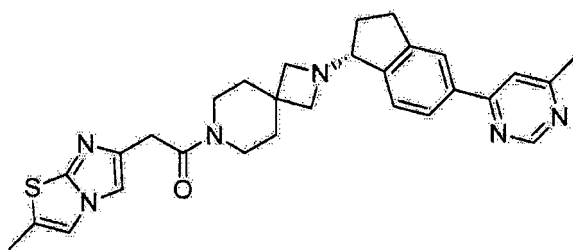
[0064] 向患者给药的本发明的生长激素释放肽受体反向激动剂或拮抗剂（特别是实施例 1）的总日剂量为约 0.003 至约 10mg/kg/ 天。为了口服给药的目的，更优选的剂量可以在约 0.01 至约 5mg/kg/ 天的范围内。优选的剂量介于 25 至 300mg/ 天之间。最优选的为 75 至 200mg/ 天。如果期望，用于给药目的，有效的日剂量可以分成多次剂量，例如每天两个至四个单独剂量。

[0065] 将本说明书中引用的所有专利、专利申请和文献参考的全部内容通过引用并入本文。

[0066] (R)-2-(2- 甲基咪唑并 [2,1-b] 噻唑 -6- 基)-1-(2-(5-(6- 甲基嘧啶 -4- 基)-2,3- 二氢 -1H- 茚 -1- 基)-2,7- 二氮杂螺 [3.5] 壬烷 -7- 基) 乙酮可以使用本文或 US 2011/0230461 中公开的合成方法制备。

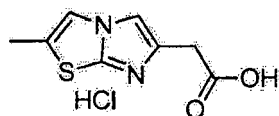
[0067] 实施例 1

[0068]



[0069] (R)-2-(2- 甲基咪唑并 [2,1-b] 噻唑 -6- 基)-1-(2-(5-(6- 甲基嘧啶 -4- 基)-2,3- 二氢 -1H- 茚 -1- 基)-2,7- 二氮杂螺 [3.5] 壬烷 -7- 基) 乙酮

[0070]



[0071] 2-(2- 甲基咪唑并 [2,1-b] 噻唑 -6- 基) 乙酸盐酸盐

[0072] 将溴 (436g, 2.73mol) 在乙酸 (750mL) 中的溶液加入到 3- 氧代丁酸乙酯 (355g, 2.73mol) 在乙酸 (1000mL) 中的溶液中。在室温下，搅拌该混合物 72 小时，并在减压

下在 45℃ 下浓缩以除去乙酸。将残余物分配到二氯甲烷 (400mL) 和水 (250mL) 之间。将有机层用饱和的碳酸氢钠 (2x 300mL)、水 (300mL)、盐水 (125mL) 洗涤, 并经无水硫酸镁干燥。将该溶液过滤并浓缩, 得到呈黄色油状物的 4-溴-3-氧代丁酸乙酯 (421g)。

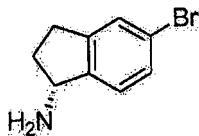
[0073] 向 2-氨基-5-甲基噻唑 (150g, 1.31mol) 在丙酮 (1500mL) 中的溶液中慢慢地加入 4-溴-3-氧代丁酸乙酯 (345g, 1.65mol)。使反应混合物的温度保持在 22-40℃。该混合物转变成稠浆状物, 并加入丙酮 (300mL) 以促进搅拌。在室温下搅拌过夜之后, 过滤该混合物, 并用丙酮洗涤滤饼, 得到白色固体。用己烷洗涤固体, 并在真空箱中于 40℃ 下干燥 4 小时, 得到 4-(2-氨基-5-甲基-噻唑)-3-氧代丁酸乙酯氢溴酸盐 (272g)。

[0074] 向 4-(2-氨基-5-甲基-噻唑)-3-氧代丁酸乙酯氢溴酸盐 (272g, 0.84mol) 中加入无水乙醇 (675mL), 并将稠混合物在 90℃ 下加热 2 小时。在该时间期间, 固体进入溶液中。浓缩反应混合物, 得到褐色半固体, 将其与乙醇一起研磨, 得到白色松散的固体, 通过过滤收集。将固体用 Et₂O 洗涤, 并在真空中于 40℃ 干燥 4 小时, 得到 (2-甲基咪唑并 [2, 1-b] [1, 3] 噻唑-6-基) 乙酸乙酯氢溴酸盐 (226g)。

[0075] 将 (2-甲基咪唑并 [2, 1-b] [1, 3] 噻唑-6-基) 乙酸乙酯氢溴酸盐 (226g, 0.74mol) 溶于水 (350mL) 中, 并通过加入碳酸钾 (51.0g, 0.37mol) 将溶液调节至 pH 7。用二氯甲烷 (300mL) 萃取该水溶液, 并用盐水 (150mL) 洗涤有机相, 经无水硫酸镁干燥, 过滤并浓缩, 得到呈褐色油状物的 (2-甲基咪唑并 [2, 1-b] [1, 3] 噻唑-6-基) 乙酸乙酯 (151.3g)。

[0076] 将 (2-甲基咪唑并 [2, 1-b] [1, 3] 噻唑-6-基) 乙酸乙酯 (151.3g, 0.67mol) 溶于 10% HCl 水溶液中 (435mL) 中, 并加热回流该混合物 2 小时。将反应混合物冷却至室温, 并在真空中干燥, 得到黄色油状物。加入乙醇 (100mL) 和乙醚 (200mL), 通过过滤收集得到的白色沉淀, 并在真空烘箱中干燥过夜, 得到 144.3g (93%) 的标题化合物。MS (ES⁺) 197.1 (M+H)⁺。¹H NMR (CD₃OD) δ 2.48 (s, 3H), 3.88 (s, 2H), 7.81 (s, 1H), 7.85 (s, 1H)。

[0077]



[0078] (R)-5-溴-2,3-二氢-1H-茚-1-胺

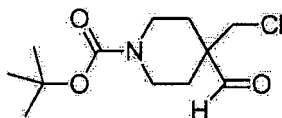
[0079] 给 22L 的 5-颈圆底烧瓶中装入 5-溴-1-茚满酮 (1.0kg, 4.72mol)、无水 THF (8L) 和 (R)-甲基-CBS-噻唑硼烷 (oxazaborolidine) (730mL, 0.73mol), 并在 N₂ 下冷却至 -10℃。经 1 小时, 滴加硼烷-甲基硫化物 (10.0M, 650mL, 6.5mol), 同时保持温度低于 -5℃。在 -10℃ 至 0℃ 下, 搅拌该混合物 3 小时, 并以保持反应温度低于 5℃ 的速率用水 (4L) 淬灭。用 EtOAc (3x 3L) 萃取该混合物。将合并的有机萃取物用盐水 (2L) 洗涤, 经 MgSO₄ 干燥, 过滤并浓缩, 得到黄色固体。使粗产物穿过短硅胶柱 (填充在己烷中的 1% Et₃N 的 3L 硅胶), 并用 EtOAc/己烷 (1/3) 洗脱。浓缩滤液, 并用己烷中的 10% EtOAc 使残余物浆液化, 过滤并干燥, 得到 585g 灰白色固体 (S)-5-溴-茚满-1-醇。将母液再浓缩, 用在己烷中的 10% EtOAc 浆液化并过滤, 得到另外 200g 黄色固体 (S)-5-溴-茚满-1-醇。使合并的批量物 (785g, 78%) 进行下一步, 而无需进一步纯化。

[0080] 在 N₂ 下, 在冰浴中冷却 (S)-5-溴-茚满-1-醇 (288g, 1.35mol) 在甲苯 (2L) 中的溶液, 并一次性用叠氮磷酸二苯酯 (DPPA, 400mL, 1.85mol) 处理, 接着用 1,8-二氮杂二环

[5, 4, 0] 十一碳-7-烯 (300mL, 2.01mol) 在甲苯 (600mL) 中的溶液处理。在 3 小时加入期间, 反应温度保持在 3 至 10℃, 经 3 小时使该混合物温热至 15℃ (TLC 指示没有起始原料)。用 EtOAc (1L) 稀释该混合物, 并用水 (3×2L) 洗涤。经 MgSO₄ 干燥该有机层, 过滤并浓缩, 得到 516g 的深色油状物。通过硅胶柱 (填充在己烷中的 1% Et₃N, 己烷洗脱液) 纯化粗产物, 得到呈油状物的 (R)-1-叠氨基-5-溴-茛满 (291g, 90%), 直接用于下一步中。

[0081] 将 (R)-1-叠氨基-5-溴-茛满 (154g, 0.645mol) 的溶液溶于甲醇 (2.4L) 中, 并加入 SnCl₂·2H₂O (265g, 1.18mol)。在室温下搅拌该混合物过夜 (TLC 指示没有起始原料), 并浓缩至干。用 2N NaOH 水溶液 (2.5L) 和 EtOAc (1.5L) 处理得到的残余物。搅拌该混合物 1 小时, 并借助于 EtOAc (3×250ml) 过滤穿过 **Celite®**。分离有机溶液, 并用 EtOAc (3×2L) 萃取水层。用 1N HCl (2×2L) 洗涤合并的有机萃取物, 接着用水 (2L) 洗涤。用冷的饱和 NaOH 溶液将合并的水层的 pH 调节至 11, 并用 EtOAc (3×2L) 萃取该混合物。将合并的有机萃取物经 MgSO₄ 干燥, 过滤并浓缩, 得到 (87.5g, 64.0%) 呈暗黄色油状物的 (R)-5-溴-2,3-二氢-1H-茛-1-胺, 当冷却时其固化。MS (ES⁺) 213.9 (M+H)⁺。¹H NMR (CDCl₃) δ 1.70-1.75 (m, 1H), 2.40-2.45 (m, 1H), 2.77-2.82 (m, 1H), 2.93-2.97 (m, 1H), 4.28-4.33 (m, 1H), 7.18-7.23 (m, 1H), 7.36-7.41 (m, 2H)。

[0082]



[0083] 4-(氯甲基)-4-甲酰基哌啶-1-羧酸叔丁酯

[0084] 在 0℃ 下, 向在烘干的圆底烧瓶中的二异丙胺 (22.6mL, 159mmol) 在无水 THF (140mL) 中的溶液中滴加 n-BuLi (65.4mL, 163mmol, 在己烷中 2.50M)。搅拌该溶液 45 分钟, 在 0℃ 下滴加在 THF (60mL) 的 4-甲基哌啶-1,4-二羧酸 1-叔丁酯 (20g, 80mmol), 并在 0℃ 下搅拌该混合物 1 小时。滴加氯碘甲烷 (17.9mL, 239mmol), 并搅拌该混合物 1 小时。用 250mL 的饱和 NaHCO₃ 水溶液淬灭该混合物, 接着用乙酸乙酯 (3×250mL) 萃取。将合并的有机层洗涤 (盐水, 250mL), 干燥 (Na₂SO₄) 并在减压下浓缩, 得到黄色油状物, 通过使用 Combiflash ISCO 纯化系统 (Teledyne Isco Inc., Lincoln, NE) 系统的二氧化硅色谱纯化, 得到 4-(氯甲基)哌啶-1,4-二羧酸 1-叔丁基 4-甲基酯 (12g, 52%)。 ¹H NMR (CDCl₃) δ 1.43 (s, 9H), 2.10-2.17 (m, 4H), 2.97 (br s, 2H), 3.56 (s, 2H), 3.74 (s, 3H), 3.83 (br s, 2H)。

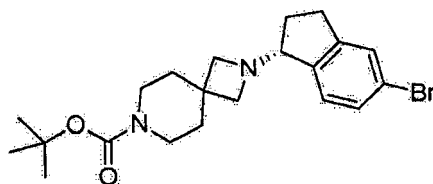
[0085] 将 4-(氯甲基)哌啶-1,4-二羧酸 1-叔丁基 4-甲基酯 (11.7g, 40.2mmol) 在无水 THF (100mL) 中的溶液冷却至 0℃。慢慢地 (15-20 分钟) 加入氢化铝锂 (在 THF 中 1N, 44.3mL, 44.3mmol), 并在 0℃ 下搅拌该溶液 25 分钟。通过极其小心地滴加水 (1.8mL) 淬灭该混合物。滴加 1N NaOH 水溶液 (1.8mL), 并搅拌该混合物 5 分钟。除去冷却浴, 过滤出固体, 并用 Et₂O (2×100mL) 洗涤滤饼。将滤液用水 (2×100mL)、盐水 (100mL) 洗涤, 干燥 (Na₂SO₄) 并在减压下浓缩, 得到呈固体的 4-(氯甲基)-4-(羟基甲基)哌啶-1-羧酸叔丁酯 (9.96g, 93.8%)。 ¹H NMR (CDCl₃) δ 1.43 (s, 9H), 1.48-1.55 (m, 4H), 3.36-3.41 (m, 4H), 3.57 (s, 2H), 3.59 (br s, 2H)。

[0086] 向在烘干的圆底烧瓶中的草酰氯 (5.1mL, 57mmol) 在二氯甲烷 (100mL) 中的 -78℃ 溶液中加入在二氯甲烷 (17mL) 中的二甲亚砜 (8.2mL, 114mmol)。搅拌该混合物 2 分钟,

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并经 10 分钟加入在二氯甲烷 (50mL) 中的 4-(氯甲基)-4-(羟基甲基)哌啶-1-羧酸叔丁酯 (13.7g, 52mmol)。在 -78℃ 下, 搅拌该溶液 15 分钟, 并加入三乙胺 (36mL, 260mmol)。在 -78℃ 下, 搅拌该混合物 15 分钟, 并温热至室温。在室温下, 搅拌该混合物 15 分钟, 并用饱和的 NaHCO₃ 水溶液 (200mL) 淬灭。用 Et₂O (2×300mL) 洗涤该水溶液。将合并的有机层用盐水洗涤, 干燥 (Na₂SO₄) 并在减压下浓缩, 得到呈黄色油状物的标题化合物, 当其在室温下在氮气气氛静置时固化 (13.7g, 99%)。¹H NMR(CDCl₃) δ 1.43(s, 9H), 1.48-1.60(m, 2H), 2.00-2.07(m, 2H), 3.07(t, 2H), 3.57(s, 2H), 3.69-3.79(m, 2H), 9.55(s, 1H)。

[0087]

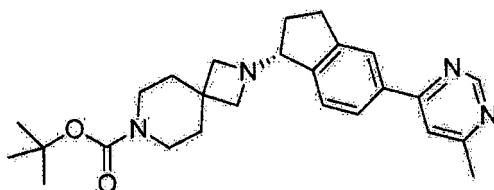


[0088] (R)-2-(5-溴-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺[3.5]壬烷-7-羧酸叔丁酯

[0089] 向 (R)-5-溴-2,3-二氢-1H-茚-1-胺 (1835g, 8.66mol) 在无水甲醇 (24L) 中的溶液中加入 4-(氯甲基)-4-甲酰基哌啶-1-羧酸叔丁酯 (2310g, 8.83mol)。在 50℃ 下搅拌该混合物 16 小时, 并冷却至室温。经 2 小时, 经由注射泵加入在 THF (15L) 中的氰基硼氢化钠 (1000g, 15.9mol)。在氮气下, 在 60℃ 下搅拌该混合物 24 小时, 经排液口排入漂白浴中。将反应冷却至 20℃, 经过套管转移到包含 24L 的 2.5M 氢氧化钠和 30L 的 DCM 的容器中。分离各层, 并用 DCM (2×5L) 萃取水层。处理水层破坏残余氰基硼氢化钠。将合并的有机层干燥 (MgSO₄), 并在减压下浓缩。通过在 40℃ 搅拌 1 小时, 并在室温下搅拌 1 小时, 使粗物质在 MTBE (7L) 中浆液化。过滤固体, 用 MTBE (2×500mL) 洗涤, 并在真空烘箱下于 50℃ 下干燥, 得到呈白色晶体的标题产物 (3657g, 90%)。MS (ES+) 422.3 (M+H)⁺。¹H NMR(CDCl₃) δ 1.44(s, 9H), 1.67(dd, 4H), 1.84-1.93(m, 1H), 2.07-2.16(m, 1H), 2.72-2.81(m, 1H), 2.95-3.15(m, 5H), 3.31(dd, 4H), 3.85(br s, 1H), 7.12(d, 1H), 7.28(br s, 1H), 7.35(br s, 1H)。

$[\alpha]_D^{20} = +39.6 \text{ deg}$ (c = 1.06mg/mL, MeOH)。

[0090]

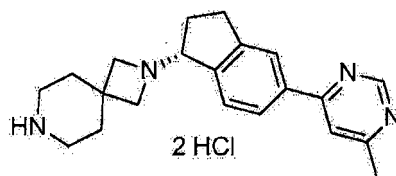


[0091] (R)-2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺[3.5]壬烷-7-羧酸叔丁酯

[0092] 向装入 (R)-2-(5-溴-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺[3.5]壬烷-7-羧酸叔丁酯 (4.0g, 9.49mmol) 的 50mL 烧瓶中加入双(三苯基膦)氯化钯 (II) (0.17g, 0.24mmol)、乙酸钾 (3.73g, 37.97mmol)、双(频哪醇)二硼 (2.65g, 10.44mmol), 接着经真空脱气, 然后再充入氮气 5 次。将去氧化的 (在加入前, 通入氮气流 30 分钟) 甲苯 (40mL) 加入到混合物中, 并在 100℃ 下加热该反应 1.5 小时。通过 HPLC 监测反应完成。在形成

硼酯中间体时,反应冷却至 40℃,装入 4M 氢氧化钠的脱气溶液 (11.87mL, 47.46mmol),接着加入 4-氯-6-甲基嘧啶 (1.53g, 11.87mmol)。然后,在氮气下,将得到的混合物加热至 90℃ 5 小时。使反应冷却室温,并装入水 (25mL)。在搅拌 20 分钟之后,过滤该混合物以除去黑色固体。将有机层萃取成包含 1.5 当量的 HCl (40mL) 的水溶液。除去有机层,用 (4g) **ISOLUTE®** Ultra Pure Si-Thiol 硅胶处理得到的溶液 1.5 小时,并过滤。将该水溶液用 4N NaOH 调节至 pH 7.8,并用甲苯 (40mL) 萃取。在 45℃ 下,在真空下将甲苯层浓缩为约 15mL,慢慢地加入庚烷 (75mL),并在 20℃ 下搅拌该混合物 1 小时。过滤产物,并在 45℃ 下,在真空下干燥 8 小时,得到呈白色固体的标题化合物 (3.56g, 86%)。MS (ES+) 435.5 (M+H)⁺。¹H NMR (CDCl₃) δ 1.46 (s, 9H), 1.70-1.74 (m, 4H), 1.90-2.01 (m, 1H), 2.13-2.26 (m, 1H), 2.59 (s, 3H), 2.84-2.93 (m, 1H), 3.04-3.21 (m, 5H), 3.30-3.38 (m, 4H), 3.95-4.02 (m, 1H), 7.40 (d, 1H), 7.56 (s, 1H), 7.87 (d, 1H), 7.95 (s, 1H), 9.12 (s, 1H)。

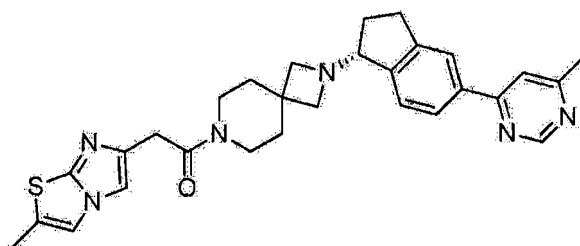
[0093]



[0094] 2-[(R)-5-(6-甲基-嘧啶-4-基)-茚满-1-基]-2,7-二氮杂-螺[3.5]壬烷二盐酸盐

[0095] 将 (R)-2-(5-(6-甲基嘧啶-4-基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺[3.5]壬烷-7-羧酸叔丁酯 (72.6g, 167mmol) 悬浮在甲醇 (363mL) 中,并加入在 1,4-二噁烷中的 4M HCl (251mL)。在搅拌 2 小时之后,将浆液浓缩至干。将粗物质再悬浮在 MeOH (500mL) 中并浓缩 (3x)。在 45℃ 下,在真空下进一步干燥得到的固体,得到标题化合物 (74.1g, 99.9%)。MS (ES+) 335.2 (M+H)⁺。¹H NMR (CD₃OD) δ 2.16-2.23 (m, 5H), 2.59 (br s, 1H), 2.78-2.80 (m, 3H), 3.12 (br s, 1H), 3.19-3.24 (m, 4H), 3.37-3.49 (m, 1H), 4.14-4.23 (m, 3H), 4.49 (br s, 1H), 5.11 (br s, 1H), 7.84 (d, 1H), 8.30-8.34 (m, 2H), 8.46 (s, 1H), 9.36 (s, 1H)。

[0096]



[0097] (R)-2-(2-甲基咪唑并[2,1-b]噻唑-6-基)-1-(2-(5-(6-甲基嘧啶-4-

[0098] 基)-2,3-二氢-1H-茚-1-基)-2,7-二氮杂螺[3.5]壬烷-7-基)乙酮

[0099] 向 2-[(R)-5-(6-甲基-嘧啶-4-基)-茚满-1-基]-2,7-二氮杂-螺[3.5]壬烷二盐酸盐 (540mg, 1.22mmol) 在 10mL 的二氯甲烷中的悬浮液中加入三乙胺 (492mg, 4.90mmol)。一旦该混合物变成均相溶液,将其加入到 2-(2-甲基咪唑并[2,1-b]噻唑-6-基)乙酸盐 (251mg, 1.28mmol) 在 3mL 的二氯甲烷中的溶液中。搅拌该混合物

5 分钟,并加入在 2mL 的 DMF 中的 HBTU(462mg,1.22mmol)。在室温下搅拌该反应 1.5 小时。通过加入 10mL 的 NaHCO_3 淬灭该反应,并用 50mL 的二氯甲烷稀释。用饱和的盐水洗涤有机层,经 Na_2SO_4 干燥,过滤并浓缩滤液。将残余物溶于 5mL 的 CH_3CN 中,并使该溶液加热至 100°C 1 小时,同时搅拌。将该混合物冷却至室温,并在真空中过滤得到的固体,得到呈灰白色粉末的期望产品(428mg,69%)。MS(ES+) 513.5(M+H)⁺。¹H NMR(CD_3OD) δ 1.70-1.74(m, 4H), 1.88-1.96(m, 1H), 2.27-2.34(m, 2H), 2.40(s, 3H), 2.58(s, 3H), 2.86-2.97(m, 1H), 3.11-3.15(m, 1H), 3.31-3.34(m, 3H), 3.52-3.55(m, 4H), 3.78(s, 2H), 4.05-4.09(m, 1H), 7.33(s, 2H), 7.55(d, 1H), 7.78-7.79(m, 1H), 7.94-8.03(m, 2H), 8.93(s, 1H)。 $[\alpha]_D^{20} = +45.3 \text{ deg}$ (c = 2.5mg/mL, MeOH)。

[0100] 体外测定

[0101] 放射性配体结合测定

[0102] 为了测量试验化合物结合生长激素释放肽受体的能力且因此具有调节生长激素释放肽活性的潜能,进行放射性配体置换测定。将 SPA 形式用于测试化合物的高通量筛选以及将滤膜结合用作更全面的结合表征。在两种形式中,将测试化合物的亲和力以 K_i 值表达,其定义为对于具体的膜批次而言,在给定的放射性配体浓度下,将 [^{125}I] 生长激素释放肽结合降低 50% 所需要的化合物浓度。

[0103] 患者生长激素释放肽 SPA 结合测定

[0104] 在 90 μl 的最终体积中进行生长激素释放肽 SPA 结合测定,所述最终体积中包含偶联至 0.5mg SPA 珠(小麦胚芽凝集素涂覆,GE Healthcare, RPNQ0060)的 250ng 患者 GHSR1a(表达患者生长促分泌素受体 1a 的 HEK293 四环素可诱导的细胞系;制备为膜)和 50pM [^{125}I] 生长激素释放肽(Perkin Elmer Life Sciences, NEX-388),加上浓度变化的测试化合物或介质。

[0105] 简而言之,在室温下于含有在 DMSO 中的 2 μl 的测试化合物(或 DMSO 作为介质)的 384 孔板(Matrix, 4322)中制备测定样品。通过加入 28 μl 测定缓冲液(50mM HEPES、10mM MgCl_2 、0.2% BSA、不含 EDTA 的蛋白酶抑制剂——1 片/50ml 缓冲液, pH 7.4),都在测定缓冲液中的 30 μl 的 8.3 $\mu\text{g/ml}$ hGHSR1a 膜和 30 μl 的 150pM [^{125}I] 生长激素释放肽,开始测试。

[0106] 将混合物培养 8 小时,以结合至达到平衡,并通过液体闪烁计数使用 1450Microbeta Trilux(Wallac)测定受体-配体复合物的量。

[0107] 患者生长激素释放肽滤膜结合测定

[0108] 在 100 μl 的最终体积中进行生长激素释放肽结合测定,所述最终体积中含有 100ng 患者 GHSR1a(表达患者生长促分泌素受体 1a 的 HEK293 四环素可诱导的细胞系;制备为膜)和 50pM [^{125}I] 生长激素释放肽(Perkin Elmer Life Sciences, NEX-388),加上浓度变化的测试化合物或介质。

[0109] 简而言之,在室温下于含有在 DMSO 中的 2 μl 的测试化合物(或 DMSO 作为介质)的 96 孔板(Costar, 3357)中制备测试样品。通过加入 23 μl 测定缓冲液(50mM HEPES、10mM MgCl_2 、0.2% BSA、不含 EDTA 的蛋白酶抑制剂——1 片/50ml 缓冲液, pH 7.4),都在测定缓冲液中的 25 μl 4 $\mu\text{g/ml}$ hGHSR1a 膜和 50 μl 的 100pM [^{125}I] 生长激素释放肽,开始测试。

[0110] 在室温下,将混合物孵育 90 分钟,接着转移至经 0.3% PEI- 处理的 96- 孔玻璃纤

维过滤板 (Perkin Elmer, 6005174)。将混合物用真空吸干并立即用 200 μ l 冰冷的 50mM Tris pH 7.5 洗涤 3 次。将板在室温下干燥过夜,并向每个孔加入 30 μ l Supermix 闪烁体 (Perkin Elmer, 1200-439)。通过液体闪烁计数,使用 1450Microbeta Trilux (Wallac) 测定受体-配体复合物的量。

[0111] 以对患者 GHSR1a 所述的相同方式,进行对狗 (NM_001099945.1)、猴 (XM_001084886.1)、小鼠 (NM_177330) 和大鼠 (NM_032075) GHSR1a (全部在独特的 HEK293 四环素可诱导的细胞系中表达) 的放射性配体结合过滤形式测定,除了要使用的膜的最终量为如下:2 μ g 狗 GHSR, 250ng 猴 GHSR, 200ng 小鼠 GHSR 或 125ng 大鼠 GHSR。

[0112] 患者生长激素释放肽功能测定

[0113] 为了测量测试化合物调节患者 GHSR1a 活性 (激动、拮抗、部分激动、反向激动) 的能力,进行 DELFIA GTP- 结合测定 (Perkin Elmer, AD0260 和 AD0261)。该测定监测将 GDP 用 GTP 交换的配体依赖性。在受体结合的 GDP 被铋标记的 GTP 替代时,GPCR 活化导致荧光增加。结合拮抗剂防止 GDP-GTP 交换,而结合反向激动剂将受体推动到 GDP 结合 (非活化) 状态,二者均导致降低的荧光。

[0114] 在 39.5 μ l 的最终体积中进行生长激素释放肽功能测定,所述最终体积含有 720ng 患者 GHSR1a (表达患者生长促分泌素受体 1a 的 HEK293 四环素可诱导的细胞系;制备为膜)、9nM GTP- 铋和浓度变化的测试化合物或介质。为了测试受体拮抗作用,将膜在激动剂生长激素释放肽 (Anaspec, 24158) 的存在下,于 EC₈₀ 浓度,加上测试化合物或介质培养。

[0115] 简而言之,在室温下在 384 孔板 (Matrix, 4340) 中制备测试化合物。首先,将测试化合物在 DMSO 中稀释,然后加入含或不含 9nM 生长激素释放肽的 15 μ l 至 10 μ l 的基础缓冲液 (50mM HEPES pH 7.4, 3.7mM MgCl₂, 250 μ M EGTA, 125nM GDP)。然后,将样品以 6 μ l 转移至包含在基础缓冲液中的 30 μ l 的 24 μ g/ml hGHSR1a 膜和 0.35mg/ml 皂苷 (Perkin Elmer, AD0261) 的 384 孔过滤板 (Pall, 5071)。

[0116] 在室温下,将混合物培养 24 分钟,同时轻轻振摇,接着加入 3.5 μ l 在 50mM HEPES, pH 7.4 中的 100nM GTP- 铋。使样品避光,并进一步在室温下培养 90 分钟,同时轻轻振摇。用真空吸干反应物,用 75 μ l 冰冷的 1x GTP Wash Solution (Perkin Elmer, AD0261) 洗涤三次,并立即在 Envision 2101Multilabel Reader (Perkin Elmer) 上使用激发滤光器 320nm 和发射滤光器 615nm 读取。

[0117] 患者分散胰岛细胞测定

[0118] 第 1 天:获得静脉内 (iv) 袋中的患者胰岛细胞。将该胰岛细胞通过连接连接器倾析至 iv 袋并将液体倾析到 50mL 锥形管中。将所述的袋用 20mL 介质漂洗并汇集。将细胞以 1000 转/分 (rpm) 旋转 1 分钟。然后,将细胞在 37°C, 5% CO₂ (10cm² 悬浮盘, 10mL 介质/板) 中培养过夜。

[0119] 第 2 天:将胰岛细胞转移至 50mL 锥形管,加入不含钙的 Hank's Working Buffer 并混合,然后将混合物以 1000rpm 旋转 1 分钟。之后,用不含钙的 Hank's Working Buffer 洗涤胰岛,混合,然后以 1000rpm 旋转 1 分钟。然后,通过移液管移除差不多 15mL 缓冲液。然后,加入 30 μ L 的 500mM EDTA [1mM], 并且之后在室温下培养 8 分钟。接着,向其中加入 75 μ L 的 0.25% 胰蛋白酶-EDTA 和 15 μ l 的 2mg/ml DNase I [2 μ g/ml]。在 30°C 下,将混

合物培养 10 分钟,同时以 60rpm 振摇。通过用 1mL 移液管研磨 (50 次) 将凝块分散。加入 50mL 的 Culture Media,并将每个通过 $63\mu\text{M}$ 尼龙膜。将混合物以 1000rpm 旋转 1 分钟,然后通过移液管移除介质。将小球重新悬浮,并用约 25mL Culture Media 再次洗涤细胞,以 1000rpm 旋转 1 分钟。除去上清液,然后,将约 5mL Culture Media 将小球再悬浮并对细胞计数。以 5000 细胞 / 孔 ($200\mu\text{L}$ / 孔) 接种到“V”形底部的板。将板以 1000rpm 旋转 5 分钟,并置于细胞培养孵育中。为了钙造影,除去 600,000 个细胞。

[0120] 第 3 天:分散胰岛测定

[0121] 将培养基用 $100\mu\text{L}$ 含有 3mM 葡萄糖的培养缓冲液替换。将板以 1000rpm 旋转 5 分钟,以将胰岛重新造粒。在用 95% O_2 /5% CO_2 连续脱气 45 分钟的 37℃ 水浴中培养所述板。用以合适的葡萄糖浓度 (对于每种样品, $n = 4$) 含有不同测试化合物的 $50\mu\text{L}$ 培养缓冲液替换预培养缓冲液。将板以 1000rpm 旋转 5 分钟,以将胰岛重新造粒。将板返回用 95% O_2 /5% CO_2 连续脱气 60 分钟的水浴。将 $40\mu\text{L}$ 转移至另一块板,并对于胰岛素使用 ELISA Patient Insulin Assay (ALPCO Patient Insulin ELISA; Cat. No. 80-INSHU-E10, 可从 ALPCO, Salem, New Hampshire, USA 获得) 测定。

[0122] 对于实施例 1,获得表 1 中提供的药理学数据。 IC_{50} (5.02nM) 和 K_i 数据 (4.37nM) 是从患者生长激素释放肽 SPA 结合测定获得。标记为“n”的列是测定化合物的次数。使用患者生长激素释放肽功能测定,将测试化合物的功能确定为反向激动剂。

[0123] 表 1

[0124]

体外测定	实施例 1 预培养	$\text{K}_d/\text{K}_i/\text{EC}_{50}/\text{IC}_{50}$ (nM)	$\text{K}_i/\text{EC}_{50}/\text{IC}_{50}$ ng/mL	n
人结合				
人 GHS-1aR SPA	8 小时	4.37	2.24	10
人 GHS-1aR Filter	90 分钟	2.49	1.28	52
人 GHS-1aR Filter	24 小时	1.42	0.728	44
人 GHS-1aR Filter Motulsky	平衡 4.07 小时	3.04	1.56	8
临床前物种结合				
大鼠 GHS-1aR Filter	90 分钟	3.19	1.64	49
小鼠 GHS-1aR Filter	90 分钟	7.70	3.95	15
狗 GHS-1aR Filter	90 分钟	3.56	1.83	9

[0125]

灵长类动物 GHS-1aR Filter	90 分钟	1.83	0.938	8
人功能				
Eu-GTP 反向激动剂	114 分钟	4.85	2.49	16
Eu-GTP 拮抗剂	114 分钟	6.57	3.37	16
Eu-GTP 竞争性拮抗剂	120 分钟	1.75	0.90	10

[0126] GHS-1aR = 生长激素促分泌素受体 ;Eu-GTP = 铕 - 鸟苷 -5'-三磷酸酯 ;GH = 生长激素。

[0127] 实施例 1 是生长激素促分泌素受体 (GHS-1aR) 的有效的 (经由患者生长激素释放肽滤膜结合测定, 人 $K_d = 3\text{nM}$; 类似的跨物种)、选择性的 (对于广泛组的受体、转运蛋白、离子通道和酶, $IC_{50} > 1\mu\text{M}$) 和适度的 on/off 反向激动剂、竞争性拮抗剂。

[0128] 体内测定

[0129] 经由口服管饲法向雄性 Wistar Han 大鼠给药单剂量 (1、10、50 或 150mg/kg) 的 (R)-2-(2-甲基咪唑并 [2,1-b] 噻唑 -6-基)-1-(2-(5-(6-甲基嘧啶 -4-基)-2,3-二氢 -1H- 茚 -1-基)-2,7-二氮杂螺 [3.5] 壬烷 -7-基) 乙酮 (实施例 1), 以评价经 21 小时期间实施例 1 对运动活性的影响 (图 1)。在给药前 1 小时, 使动物习惯该运动装置。在 4pm 关闭灯光, 并在 4am 打开灯光。在 4pm 给动物给药。在给药后 20 小时监测活动。在 Tmax (给药后 30 分钟) 从伴随组 (satellite group) 的动物采集血浆、脑和 CSF。

[0130] 在给药后 35-65 分钟, 当与介质处理的动物相比时, 在 1mg/kg 下没有看到任何生物学或统计学显著的作用。在 10mg/kg 下, 在给药后 35-65 分钟, 实施例 1 使跳跃降低了 56%, 且使总运动活性降低了 60%。在 50mg/kg 下, 实施例 1 使跳跃降低了 43%, 且使总运动活性降低了 48%。在 150mg/kg 下, 在给药后 35-65 分钟之后, 与介质对照大鼠相比, 实施例 1 降低了总活性, 其是统计学显著的 ($P < 0.5$)。在给药后 20 小时, 实施例 1 没有使总活性产生统计学或生物学显著地降低。在任何剂量下, 实施例 1 都没有诱导活动过度。

[0131] 在给药后 30 分钟, 测定实施例 1 的血浆和脑水平, 显示在表 2 中。

[0132] 表 2

[0133]

剂量	血浆 (nM 游离)	脑 (nM 游离)
介质	0	0
1mg/kg 实施例 1	18	1.6
10mg/kg 实施例 1	184	16
50mg/kg 实施例 1	856	80
150mg/kg 实施例 1	1704	148

[0134] 在三个临床研究 (图 2) 中, 在其中最高剂量 100mg BID 14 天和 300mg 单剂量的人

类中观察到瞌睡的剂量 - 反应性关系,75 至 100% 的受试者报道瞌睡或睡眠相关影响。向患者给药实施例 1,如在 B3301001、B3301002 中临时制备的悬浮液,和在 B3301007 中的 IR,及在 B3301007 中临时装备的渗透性胶囊 (EPOC)。实施例 1 在人类中是安全的,且通常是良好耐受的,至多 100mg BID 14 天,其等同于估计的 80% 全身受体占用达 20 小时和 70% 中心受体占用达约 3 小时。在急性给药之后,看到心率的剂量相关性增加 (至多 ~ 10 次搏动 / 分钟) 以及生长激素释放肽 - 诱导生长激素分泌和餐后葡萄糖二者的减少。通过 14 天的 BID 给药,所有这三种作用似乎都是完全快速耐受 (tachyphylax)。

[0135] 在灵长类动物研究中,每天一次 (QD) 或每天两次 (BID) 给药研究在晚上给药实施例 1 后三小时实施例 1 对睡眠期间和食物摄取的影响。使用八只健康的成年 (5.8 至 9.7 岁) 雄性猕猴 (印度来源的恒河猴)。

[0136] 对动物进行平行研究。根据交叉设计,在研究的第一阶段期间,四只动物 QD 接受实施例 1 (或作为对照的介质),和在研究的第二阶段期间,BID 接受实施例 1 (或作为对照的介质)。对于其余四只动物使用相反的治疗方案。当 QD 给药实施例 1 时,晚上给药单剂量。每个治疗期持续 28-30 个连续日。介质处理持续 10-14 天,在给药实施例 1 的阶段前立即处理。将动物也保持其睡眠 / 昼夜节律室中以使动物对其熟悉。呈液体溶液给药治疗,使用定时控制泵自动分配。

[0137] 在整个治疗期期间,动物保持在光 - 控制且静音 (sound attenuated) 的睡眠 / 昼夜节律室中,处于恒定的暗光条件下,以便测定睡眠 / 活动的内在节律和自我管理的 (self-administered) 食物摄取。

[0138] 数据收集:

[0139] 使用连续在线活动记录检查图像分析技术记录睡眠 / 活动周期和睡眠期间,如在 Masuda 等, J Biol Rhythms. 2010Oct ;25(5):361-71, Intrinsic activity rhythms in *Macaca mulatta*: their entrainment to light and melatonin 中描述的。猕猴的睡眠对比清醒的活动记录检查数据分析的软件算法为 Zhdanova 等, J Biol Rhythms. 2011Apr ; 26(2):149-59. Aging of intrinsic circadian rhythms and sleep in a diurnal nonhuman primate, *Macaca mulatta* 中解释的。基于该物种的多导睡眠描记和活动记录检查睡眠之间的比较,进行睡眠开始和睡眠期间的估计。

[0140] 每个昼夜节律室装有连接到自动小球分配器的单独的触屏监视器 (ENV-203-1000, Med Associates Inc., St. Albans, VT)。这允许经由按压屏幕上移动的目标昼夜不停随意地自我给药 1g 食物小球 (Nutritionally complete dustless precision pellets, Bio-Serv, Frenchtown, NJ)。记录分配每个小球的时间。

[0141] 使用线性混合模式 (SPSS) 进行数据分析,显著性水平 $p < 0.05$ 。数据呈现为每个处理条件下: QD 或 BID 给药介质或实施例 1 的各组平均值 (平均标准误差 (SEM))。

[0142] 图 3 和 4 提供关于单个动物的活动模式 (图 3) 或食物摄取模式 (图 4) 的数据,所述动物晚上 QD 接受介质或实施例 1。采集 12:12 小时光 - 暗循环 (LD) 期间来自动物的活动检查记录,此后是恒定的暗光条件 (沿着图右侧的箭头)。该个体具有比 24 小时更长的固有昼夜节律周期,通过随时间推移显示延迟的整体活动模式 (逐渐右移)。在同一时钟时间 (17:00 点钟) 给药介质或实施例 1。在介质处理期开始时 (沿着图右侧的虚线),这对应于预期睡眠开始之前的 2 小时 (主观的夜晚开始)。到了开始实施例 1 处理 (沿着图

右侧的实线)时,该时钟时间对应于预期睡眠开始之前约 3.5 小时。

[0143] 用实施例 1 处理显著地减少了睡眠潜伏期,促进了睡眠开始,该作用在整个治疗期间持续存在。该作用也导致活动模式向左整体移动(醒来时间的相位前移),睡眠开始时间越早,引起在主观的早晨醒来的时间类似地越早。

[0144] 实施例 1 处理在给药后不久显著地减少了食物摄取,该作用在整个治疗期间持续存在。该作用也导致食物摄取模式向左整体移动(早晨进食时间的相位前移)。该动物中的食物摄取模式遵循活动模式。比较图 3 和图 4。

[0145] 图 5 提供在与介质相比晚上用实施例 1 处理后的 3 小时内睡眠期间的变化(每组 $N = 8$ (QD 相对 BID))。对于两组,与仅给药介质相比,实施例 1 处理的 3 小时之内记录到睡眠期间的显著增加。该数据表示为与接受实施例 1 的组中的其他动物相比当动物接受介质时睡眠期间的百分比变化(误差线提供 SEM;与对照相比, $p < 0.01$)。

[0146] 图 6 提供在晚上给药实施例 1 或介质之后 3 小时内晚上食物摄取的变化。图 6 提供对于 QD 或 BID 接受实施例 1 的动物而言的变化(每组 $N = 8$ (QD 相对 BID)),当与仅给药介质相比时,在给药实施例 1 的 3 小时之内,记录到两组食物摄取的显著降低。数据表示为在给药介质或实施例 1 后 3 小时之内食物摄取的百分数(误差线提供 SEM;与介质相比, $p < 0.01$)。

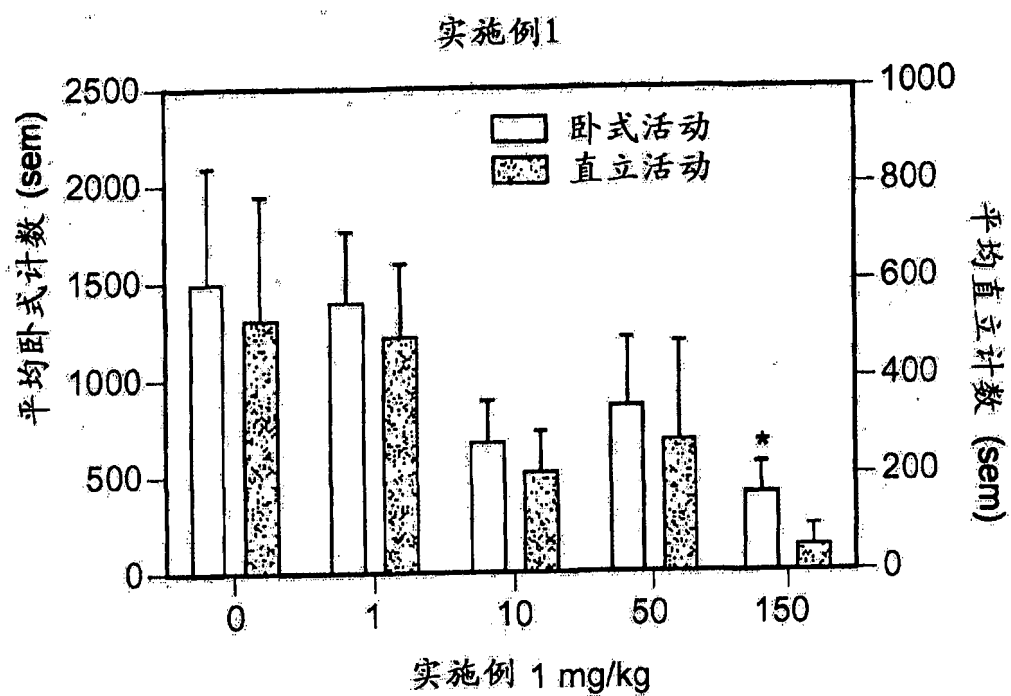


图 1

研究	剂量 (mg)	受试者 (n/N)
B3301001	0	0/35
	2	0/6
	10	0/6
	50	3/6
	100	0/6
	100	7/9
	150	5/8
	300	9/12
	40 分剂量, 进食	0/6
	300 分剂量, 进食	1/6

研究	剂量 (mg)	受试者 (n/N)
B3301002	0	3/12
	2 BID	0/6
	10 BID	0/6
	40 BID	1/5
	100 BID	5/5

研究	剂量 (mg)	受试者 (n/N)
B3301007	100 (IR)	4/16
	100 (EPOC 6 hr)	3/15
	100 (EPOC 6 hr), 进食	0/8
	100 (EPOC 14 hr)	2/15
	100 (EPOC 14 hr), 进食	0/7

图 2

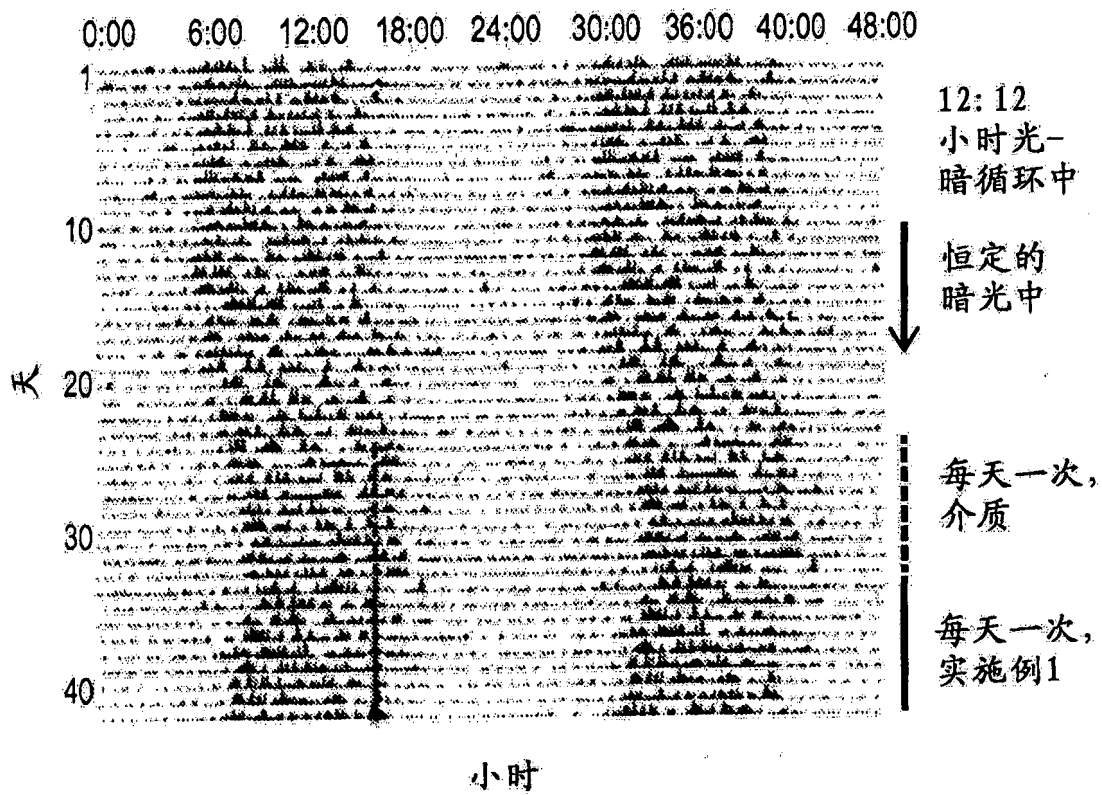


图 3

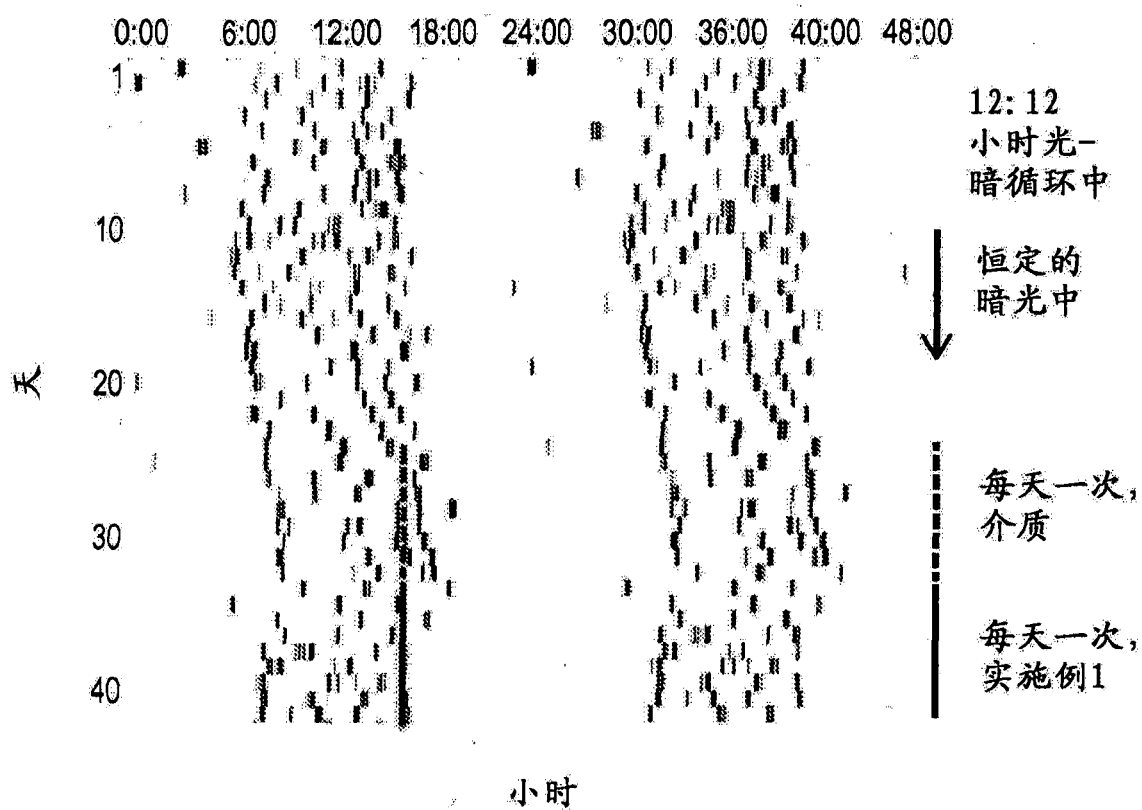


图 4

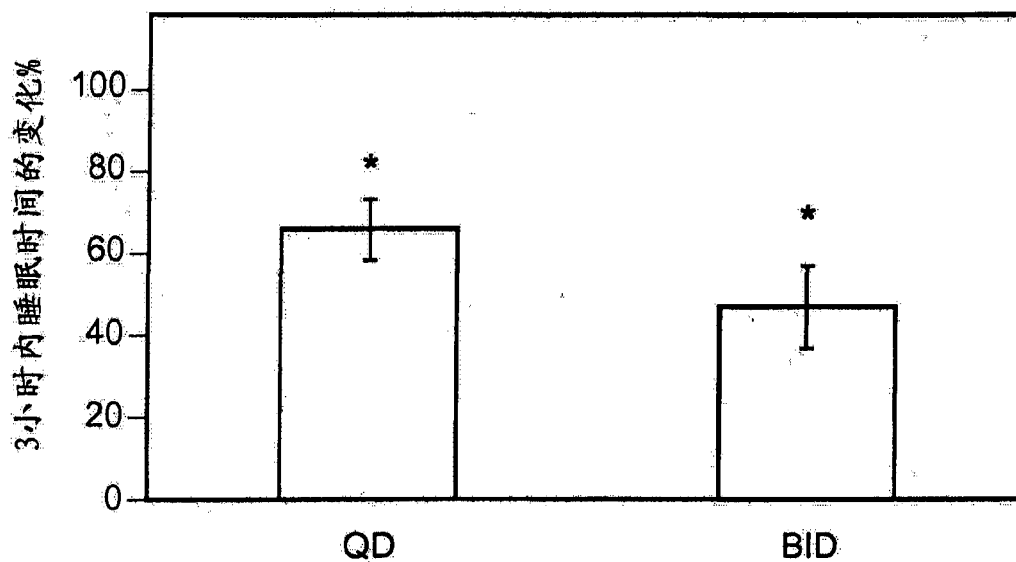


图 5

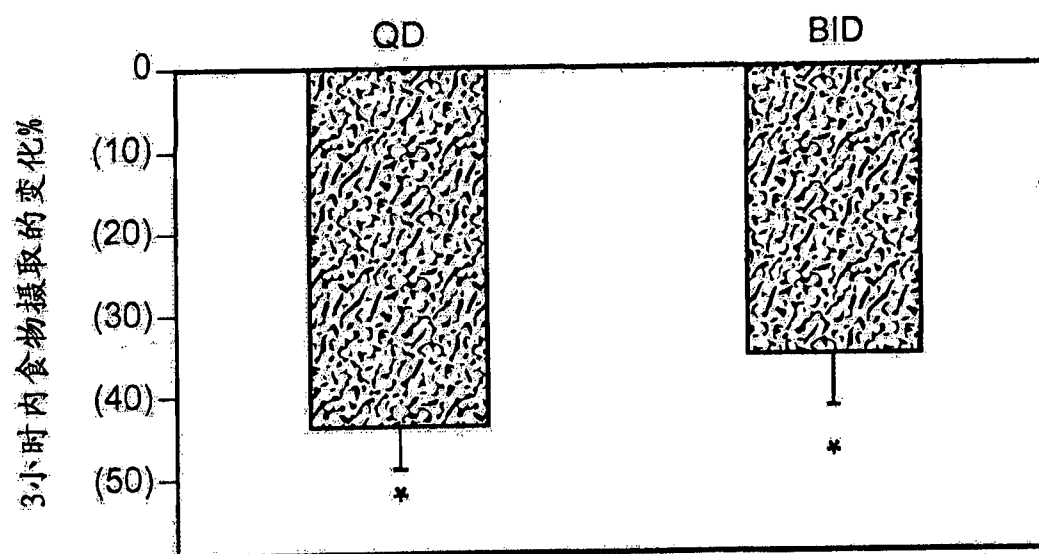


图 6