(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 December 2016 (29.12.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/209847 A1

(51) International Patent Classification:
A61K 31/57 (2006.01) *C07J 7/00* (2006.01)

(21) International Application Number:
PCT/US2016/038582

(22) International Filing Date:
21 June 2016 (21.06.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/182,583 21 June 2015 (21.06.2015) US

(71) Applicant: **PREVACUS, INC.** [US/US]; 2580 Care Drive #3, Tallahassee, FL 32308 (US).

(72) Inventors: **LU, Xia**; Crystal Pharmatech, Co., Ltd., B4-101 Biobay, 219 Xinghu Street, Suzhou, 215123 (CN). **ZHANG, Yanfeng**; Crystal Pharmatech, Co., Ltd., B4-101 Biobay, 219 Xinghu Street, Suzhou, 215123 (CN). **LEVY, Daniel, E.**; 37 Lakewood Circle, San Mateo, CA 94402 (US). **LEWANDOWSKI, Michael**; 10191 Lake Grove Drive, Odessa, FL 33556 (US).

(74) Agents: **MANSO, Peter, J.** et al.; Mintz Levin Cohn Ferris Glovsky and Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2016/209847 A1

(54) **Title**: C-20 STEROID COMPOUNDS, COMPOSITIONS, AND USES THEREOF TO TREAT TRAUMATIC BRAIN INJURY (TBI), INCLUDING CONCUSSIONS

FIG. 1

Thinking/ Remembering	Difficulty thinking clearly	Feeling slowed down	Difficulty concentrating	Difficulty remembering new information
Physical	Headache	Nausea or vomiting (early on)	Sensitivity to noise or light	Feeling tired, having no energy
	Fuzzy or blurry vision	Dizziness	Balance problems	
Emotional/Mood	Irritability	Sadness	More emotional	Nervousness or anxiety
Sleep	Sleeping more than usual	Sleep less than usual	Trouble falling asleep	

(57) **Abstract**: The present invention relates to C-20 steroid compounds, compositions and methods of use thereof to treat, minimize and/or prevent traumatic brain injury (TBI), including severe TBI, moderate TBI and mild TBI, including concussions.

C-20 STEROID COMPOUNDS, COMPOSITIONS, AND USES THEREOF TO TREAT TRAUMATIC BRAIN INJURY (TBI), INCLUDING CONCUSSIONS

Cross-Reference To Related Application

This application relates and claims priority to and the benefit of United States Provisional Application No. 62/182,583, filed on June 21, 2015, which is entitled "C-20 Steroid Compounds, Compositions and Uses Thereof to Treat Traumatic Brain Injury (TBI), including Concussions" and is hereby incorporated by reference in its entirety as if fully set forth herein.

This application also relates to United State Provisional Patent Application No. 62/051,898, filed on 17-Sep-2014, United State Provisional Patent Application No. 62/052,457, filed on 18-Sep-2014, and United States patent Application, Serial No. 14/857,331, filed on 17-Sep-2015, each of which is entitled "C-20 Steroid Compounds, Compositions and Uses Thereof to Treat Traumatic Brain Injury (TBI), including Concussions" and each of which is hereby incorporated by reference in its entirety.

Field of the Invention

The present invention relates to novel C-20 steroid compounds, compositions and uses thereof for treating, minimizing and/or preventing traumatic brain injury (TBI), including severe TBI, moderate TBI, and mild TBI, including concussions. The present invention further relates to polymorphs of *ent*-19-norprogesterone.

Background

Today it is believed that more than 1.5 million people experience a traumatic brain injury (TBI) each year in the United States. Of those affected with TBI, it is thought that at least about 75 percent sustain mild traumatic brain injury or MTBI, as opposed to moderate or severe TBI. Even though these injuries are defined as mild, MTBI may cause long-term or permanent impairments and disabilities. Many people with MTBI have difficulty returning to routine, daily activities and may be unable to return to work for many weeks or months. In addition to the human toll of these injuries, MTBI costs the U.S. approximately $17 billion each year. See

Report to Congress on Mild Traumatic Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem, September 2003, available at http://www.cdc.govincipc/pub-res/mtbi/mtbireport.pdf. See also http://www.cdc.gov/traumaticbraininjury/get_the_facts.html; Faul M, Xu L, Wald MM, Coronado VG. Traumatic brain injury in the United States: emergency department visits, hospitalizations, and deaths. Atlanta (GA): Centers for Disease Control and Prevention, National Center for Injury Prevention and Control; 2010; Thurman D, Alverson C, Dunn K, Guerrero J, Sniezek J. Traumatic brain injury in the United States: a public health perspective. J Head Trauma Rehabil, 14(6):602-615 (1999); Injury Prevention & Control: Traumatic Brain Injury, Traumatic Brain Injury in the United States: Fact Sheet, available at Centers for Disease Control and Prevention at http://www.cdc.gov/traumaticbraininjury/get_the_facts.html and http://www.cdc.gov/TraumaticBrainlnjury/index.html; National Hospital Discharge Survey (NHDS), 2010; National Hospital Ambulatory Medical Care Survey (NHAMCS), 2010; National Vital Statistics System (NVSS), 2010; and Finkelstein E, Corso P, Miller T and associates. The Incidence and Economic Burden of Injuries in the United States. New York (NY): Oxford University Press; 2006; and Coronado, McGuire, Faul, Sugerman, Pearson. The Epidemiology and Prevention of TBI (in press) 2012.

TBI amongst U.S. military personnel is also a critically important health concern especially for veterans in the Operation Iraqi Freedom (01F) and Operation Enduring Freedom (OEF). According to a Defense and Veterans Brain Injury Center (DVBIC) analysis of surveillance data released by the Department of Defense (DoD), 33,149 U.S. military personnel were diagnosed with a TBI in 2011 alone. This number included service members (SMs) in the Army, Navy, Marine Corps, Air Force, and from the active duty and reserve components of the National Guard. See U.S. Dept. of Defense: http://www.health.mil/Research/TBI_Numbers.aspx. The U.S. Department of Veterans Affairs (VA) estimates that of the 771,874 veterans who sought care from a VA Medical Center from the start of OEF in October 1, 2001 to December 31, 2011, a total of 59,218 veterans were evaluated or treated for a condition possibly related to a TBI. See U.S. Dept. of Veterans Affairs, 2012 available at http://www.publichealth.va.govidocsiepidemiology/healthcare-iutilization-report-fy2012-qtr1.pdf.

TBI is a nondegenerative, noncongenital insult to the brain that can result from a bump, blow or jolt to the head or a penetrating head injury that disrupts the normal function of the brain possibly leading to permanent or temporary impairment of cognitive, physical, and psychosocial functions, with an associated diminished or altered state of consciousness. Not all blows or jolts to the head can cause a TBI. The severity of a TBI can range from "mild" to "severe". A "mild TBI" is characterized as a brief change in mental status or consciousness, whereas a "severe TBI" is characterized as an extended period of unconsciousness or memory loss after the injury. See http://www.cdc.govitraumaticbraininjury/get_the facts.html. See also Centers for Disease Control and Prevention (CDC), National Center for Injury Prevention and Control. Report to Congress on mild traumatic brain injury in the United States: steps to prevent a serious public health problem. Atlanta (GA): Centers for Disease Control and Prevention, 2003.

The Glasgow Coma Scale (GCS) defines the severity of a TBI within 48 hours of injury. Thus, as used herein, moderate to severe brain injuries are defined as follows:

- Moderate brain injury is defined as a brain injury resulting in a loss of consciousness from 20 minutes to 6 hours and a Glasgow Coma Scale of 9 to 12. See http://www.traumaticbraininjury.com/symptoms-of-tbi/severe-tbi-symptoms/;
- Severe brain injury is defined as a brain injury resulting in a loss of consciousness of greater than 6 hours and a Glasgow Coma Scale of 3 to 8. See http://www.traumaticbraininjury.com/symptoms-of-tbi/severe-tbi-symptoms/;
- Mild traumatic brain injury (mTBI) is defined as the result of the forceful motion of the head or impact causing a brief change in mental status (confusion, disorientation or loss of memory) or loss of consciousness for less than 30 minutes. While MRI and CAT scans are often normal, a person with a mild TBI may remain conscious or may experience a loss of consciousness for a few seconds or minutes. Other symptoms of mild TBI include headache, confusion, difficulty thinking, lightheadedness, dizziness, blurred vision or tired eyes, ringing in the ears, bad taste in the mouth, fatigue or lethargy, frustration, a change in sleep patterns, behavioral or mood swings, memory problems, concentration, attention, or thinking. See http://www.traumaticbraininjury.com/symptoms-of-tbi/mild-tbi-symptoms/.

A person with a moderate or severe TBI may present these same symptoms, but may also present a headache that gets worse or does not go away, repeated vomiting or nausea, convulsions

or seizures, an inability to awaken from sleep, dilation of one or both pupils of the eyes, slurred speech, weakness or numbness in the extremities, loss of coordination, and increased confusion, restlessness, or agitation. See http://www.ninds.nih.gov/disorders/tbi/tbi.htm

Today, most TBIs that occur each year are mild, commonly called concussions. See http://www.cdc.gov/traumaticbraininjury/get_the_facts.html. See also Centers for Disease Control and Prevention (CDC), National Center for Injury Prevention and Control. Report to Congress on mild traumatic brain injury in the United States: steps to prevent a serious public health problem. Atlanta (GA): Centers for Disease Control and Prevention; 2003. See also Injury Prevention & Control: Traumatic Brain Injury, Traumatic Brain Injury in the United States: Fact Sheet, available at Centers for Disease Control and Prevention, available at http://www.cdc.gov/traumaticbraininjury/get_the_facts.html. See also http://www.cdc.goviTraumaticBrainInjury/index.html. Thus, it is currently believed that concussion is the most common type of traumatic brain injury.

A concussion is a type of traumatic brain injury (TBI) caused by a bump, blow or jolt to the head with a temporary loss of brain function. Concussions can also occur from a fall or a blow to the body that causes the head and brain to rattle or move quickly back and forth. See http://www.cdc.goviconcussion/pdf/Fact_Sheet_ConcussTBI-a.pdf. See also Facts about Concussion and Brain Injury at http://www.cdc.goviconcussion/pdf/Fact_Sheet_ConcussTBI-a.pdf. Concussions are defined as a traumatically induced transient disturbance of brain function and involves a complex pathophysiological process and are a subset of MTBI, which are generally self-limited and at the less-severe end of the brain injury spectrum. See Harmon KG et al.: American Medical Society for Sports Medicine position statement: concussion in sport. Br J Sports Med. 47(3):184 (Feb., 2013).

It has been estimated that as many as 3.8 million concussions occur in the U.S.A. per year during competitive sports and recreational activities; however, as many as 50% of the concussions may go unreported. See Harmon KG et al.: American Medical Society for Sports Medicine position statement: concussion in sport. Br J Sports Med. 47(3):184 (Feb., 2013). In addition, concussion is big business in football in the U.S.A. In view of the fact that there are about 1,700 players in the NFL, about 66,000 student athletes playing college football, about another 1.1 million high school football players and approximately 250,000 youths who participate in Pop Warner football, there is a demand to find solutions to reducing risks associated with concussions,

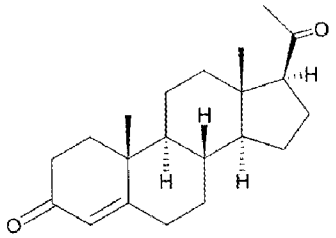
"...whose terrifying consequences regularly tear across the sports pages. And a wave of companies offering diagnostic tools and concussion treatments are just as eager to sell them a peace of mind." See Peter Keating: Concussion test may not be panacea - ImPACT sells tests and training to thousands, but some question program's validity, ESPN The Magazine, August 12, 2012 available at http://espn.go.com/espn/otl/story/ /id/8297794/neuropsychological-testing-concussions-not-panacea.

According to the Centers for Disease Control and Prevention, most people with a concussion recover quickly and fully. However, for some people, symptoms can last for days, weeks, or longer. In general, recovery may be slower among older adults, young children and teens. Those who have had a concussion in the past are also at risk of having another one and may find that it takes longer to recover if they have another concussion. Symptoms of concussion usually fall into four categories.

See **FIG. 1**. See also http://www.cdc.gov/concussion/pdf/Fact_Sheet_ConcussTBl-a.pdf.

The terms mild brain injury, mild traumatic brain injury (MTBI), mild head injury (MHO, minor head trauma, and concussion may be used interchangeably. See National Center for Injury Prevention and Control. Report to congress on mild traumatic brain injury in the United States: Steps to prevent a serious public health problem. Atlanta, GA: Centers for Disease Control and Prevention (2003), Petchprapai N, Winkelman C: Mild traumatic brain injury: determinants and subsequent quality of life. A review of the literature. Journal of Neuroscience Nursing, 39 (5):260-72 (2007). See also Guidelines for Mild Traumatic Brain Injury and Persistent Symptoms available at http://onf.org/system/attachments/60/original/Guidelines_for_Mild Traumatic_Brain_Injury_and_Persistent_Symptoms. Although the term "concussion" is still used in sports literature as interchangeable with "MHI" or "MTBI", the general clinical medical literature now uses "MTBI" instead. See Barth JT, Varney NR, Ruchinskas RA, Francis JP: Mild head injury: The new frontier in sports medicine. In Varney NR, Roberts RJ. The Evaluation and Treatment of Mild Traumatic Brain Injury. Hillsdale, New Jersey: Lawrence Erlbaum Associates. pp. 85-6. (1999); and http://en.wikipedia.org/wiki/Concussion. Nonetheless, even though the terms are used interchangeably, a "concussion" is a subset of "MTBI". See Harmon KG et al.: American Medical Society for Sports Medicine position statement: concussion in sport. Br J Sports Med. 47(3):184 (Feb., 2013).

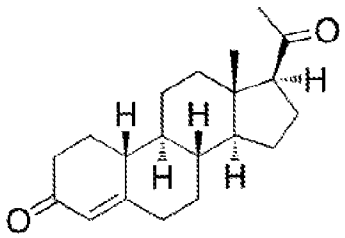
Progesterone is a C-21 steroid hormone. The chemical structure for progesterone is as follows:



progesterone

Progesterone is a progestogen, and it is one of the major naturally occurring human progestogens. Progesterone is involved in the female menstrual cycle, pregnancy and embryogenesis of humans and other species. Progesterone is naturally produced by the ovaries of mammals, but can also be produced by some plants and yeast.

19-Norprogesterone is a C-20 steroid hormone. The chemical structure for 19-Norprogesterone is as follows:



19-norprogesterone

19-Norprogesterone is believed to be a potent progesten with mineralcorticoid properties and high affinity for the progesterone receptor. See Paris J, Botella J, Fournau P, Bonnet P, Thevenot R: Extinction of mineralocorticoid effects in 19-norprociesterone derivatives: structure-activity relationships; *J. Pharmacol. Exp. Ther.* 243 (1): 288-91 (1987); and Botella, J. *et al*: Structure-activity and structure-affinity relationships of 19-nor-progesterone derivatives in rat uterus. *J Endocrinological Investigation.* 13(11):905-910 (1990).

19-Norprogesterone is a member of the family of 19-nor-corticosteroids that is produced in extra-adrenal tissue in biologically relevant quantities. Levels of this class of steroids are known to be increased and possibly pathogenic in certain states of human hypertension. See Melby JC, Dale SL, Holbrook M, Griffing GT: 19-Nor-corticosteroids in experimental and human hypertension. *Clin Exp Hypertens A*; 4 (9¬10):1851-67 (1982).

The use of progesterone and its analogues have many medical applications, both to address acute situations and to address the long-term decline of natural progesterone levels. Other uses of progesterone include, for example, the prevention of preterm birth, to control anovulatory bleeding, to increase skin elasticity and bone strength, and to treat multiple sclerosis.

Today, there is a belief that progesterone may be useful for the treatment of traumatic brain injury (TBI), which may result in substantial and sustained improvements in cytologic, morphologic, and functional outcomes. See Schumacher M, Weill-Engerer S, Liere P, *et al.*: Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. Prog Neurobiol; 71:3-29 (2003). For example, it has been reported that the administration of progesterone following brain injury may limit brain damage, reduce loss of neuronal tissue and improve functional recovery. See Goss CW, Hoffman SW, Stein DG. Behavioral effects and anatomic correlates after brain injury: a progesterone dose-response study. Pharmacol Biochem Behay. 76: 231-42 (2003). It has also been reported that progesterone may reduce poor outcomes following traumatic brain injury by inhibiting inflammatory factors (TNF-a and IL-13) and subsequently reducing brain edema. See Pan, D., et al.: *Biomed Environ Sci.* 20:432¬438 (2007); and Jiang, C., et al.: *Inflamm Res.* 58:619-624 (2009). Still further, it has been reported that progesterone-treated rats may demonstrate improvements on a Neurological Severity Score (test for motor and cognitive functioning) following traumatic brain injury. See Roof, R. L., *et al.*: *Restor Neurol Neurosci.* 4:425-427 (1992).

In addition, it has been reported that progesterone may effectively attenuate edema in both rodent sexes following injury (Djebaili, M., et al.: *J Neurotrauma.* 22, 106-118 (2005). Administering progesterone or its derivative allopregnanolone (ALLO) also results in a decrease of the presence of the factors of cell death (caspase-3) and gliosis (GFAP), Cutler, S. M., *et al.*: *J Neurotrauma.* 24:1475-1486 (2007), following injury, VanLandingham, J. W., *et al.*: *Neurosci Lett.* 425:94-98 (2007); Wright, D. W., et al.: *Ann Emerg Med.* 49:391-402, 402 e391-392 (2007). See also, Progesterone for the Treatment of Traumatic Brain Injury (ProTECT III), ClinicalTrials.gov Identifier:NCT00822900 and http://acutecareresearch.org/studies/current/progesterone-treatment-tbi-protect-iii; Efficacy and Safety Study of Intravenous Progesterone in Patients With Severe Traumatic Brain Injury (SyNAPSe), ClinicalTrials.gov Identifier:NCT01143064; Progesterone Treatment of Blunt Traumatic Brain Injury, ClinicalTrials.gov Identifier:NCT00048646; Blood Tests to Study Injury

Severity and Outcome in Traumatic Brain Injury Patients (BioProTECT), ClinicalTrials.gov Identifier:NCT01730443. See further, ProTECT™III at http://www.protectiii.com/; and http://em.emory.edu/protect/; and http://clinicaltrials.gov/show/NCT00822900. See also Progesterone for Traumatic Brain Injury Tested in Phase III Clinical Trial at http://www.sciencedaily.com/releases/2010/02/100219204407.htm. Still further, see BHR Pharma Investigational Traumatic Brain Injury Treatment Receives European Medicines Agency Orphan Medicinal Product Designation at http://synapse-trial.com/downloads/PREMAOrphan.pdf.

More recently, it has been reported that "...progesterone given to both male and female laboratory rats and mice can cross the blood—brain barrier...and reduce edema levels after TBI...; reduce lipid peroxidation and isoprostanes, which, in turn, contribute to postinjury ischemic conditions...; generate metabolites that reduce proapoptotic and increase antiapoptotic enzymes...and the expression of proinflammatory genes and their protein products...; influence the expression of aquaporins implicated in the resolution of edema...; in different models of cerebral ischemia, significantly reduce the area of necrotic cell death and improve behavioral outcomes...; protect neurons distal to the injury that would normally die...; enhance ligodendrocyte-induced remyelination in young and old rats with demyelinating disorders...; and produce significant sparing of cognitive, sensory, and spatial learning performance after bilateral medial frontal cortex injury...Progesterone has been shown to have beneficial effects in 22 different injury models; a number of extensive reviews discuss these data...To date, most research on progesterone and its metabolites has focused on the treatment of TBI...This line of research originated when researchers...found that, after bilateral contusion injury to the medial frontal cortex in young adult male and female rats, 5 days of treatment with progesterone significantly improved spatial learning and sensory performance, compared with controls given injections of the vehicle alone. The first successful clinical trial for the treatment of TBI in more than 30 years of research was recently completed. This National Institute of Neurological Disorders and Stroke (NINDS)—sponsored phase 2a single-center clinical trial for progesterone in the treatment of moderate-to-severe adult TBI...found that the mortality rate among patients given progesterone IV for 3 days after the injury was less than half that among control subjects given the standard-of-practice care but no hormone (13.6% vs 30.4%). Thirty-day functional outcomes for moderately injured patients in the progesterone group were significantly better than those for the placebo group [and]...that a National Institutes of Health—appointed data safety monitoring board found

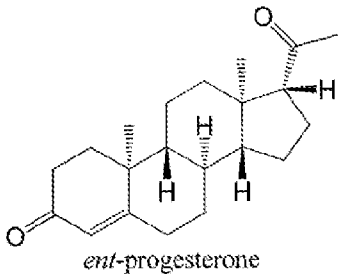
no serious adverse events attributable to progesterone treatment in this trial. A second independent randomized double-blind study from China examined 159 patients with severe TBI given a course of intramuscular injections of progesterone for 5 days. The investigators reported very similar beneficial outcomes on morbidity and mortality at both 30 days and 6 months after injury, again without any serious adverse events caused by the treatment..." See D.G. Stein and I. Sayeed: Is Progesterone Worth Consideration as a Treatment for Brain Injury? *AJR* (194):20-22 (January 2010).

In about June 2010, BHR Pharma initiated the SyNAPSe® study (Study of the Neuroprotective Activity of Progesterone in Severe Traumatic Brain Injuries) to study the effectiveness of an intravenous progesterone infusion formula. See http://www.synapse-trial.comi; http://www.besinscriticalcare.conn/progesterone-research/; and http://em.emory.edu/protect/. Nonetheless, it is reported that "BHR-100 must be administered within eight hours of the TBI and infused continuously over five days...The SyNAPSe® study's Independent Data and Safety Monitoring Board (DSMB) has released six analyses of the trial's safety data over the course of the study, concluding each time that SyNAPSe® should continue to its intended completion...The DSMB's formal interim analysis of primary six-month efficacy data from 400 SyNAPSe patients, conducted in January 2013, concluded that there was no reason to stop the study for futility...[and] The SyNAPSe® study is endorsed by the American Brain Injury Consortium (ABIC) and the European Brain Injury Consortium (EBIC)." See http://www.besinscriticalcare.conn/progesterone-research/. See also, BHR Pharma SyNAPSe® Trial DSMB Data Analyses Determine No Safety Issues; Study Should Continue to Conclusion at http://www.prnewswire.conninews-releases/bhr-pharnna-synapse-trial-dsnnb-data-analyses-determine-no-safety-issues-study-should-continue-to-conclusion-187277871.htnnl.

19-norprogesterone and its analogs may have medical applications. For example, this class of compounds is believed to facilitate axon remylination. See Hussain R, EI-Etr M, Gaci 0, Rakotomamonjy J, Macklin WB, Kumar N, Sitruk-Ware R, Schumacher M, Ghoumari AM: "Progesterone and Nestorone facilitate axon remyelination: a role for progesterone receptors", *Endocrinology*, 152 (10): 3820-31 (2011). Additionally, this class of compounds has been studied as potential oral contraceptives. See, e.g., Mueck AO, Sitruk-Ware R.: "Nomegestrol acetate, a novel progestogen for oral contraception", Steroids, 76 (6): 531-9 (2011). Additional useful activities may include inhibition of apoptosis. See Dressing GE, Pang Y, Donq J, Thomas

P.: "Progestin signaling through mPRa in Atlantic croaker granulosa/theca cell cocultures and its involvement in progestin inhibition of apoptosis", *Endocrinology*_l 51, (12): 5916-26 (2010).

Progesterone exists in a non-naturally occurring enantiomeric form known as ent-progesterone:



ent-progesterone

ent-Progesterone is believed to have equal efficacy to natural progesterone in reducing cell death, brain swelling, and inflammation while the enantiomer has three times the antioxidant activity of racemate under certain conditions. Similarly, *ent*-Progesterone is believed to have fewer sexual side effects such as suppression of spermatogenesis; inhibition of the conversion of testosterone to dihydrotestosterone; reduction in the size of the testes, epididymis, and leydig cells; and no hyper-coagulative risk as may be seen with natural progesterone. In addition, utilities for *ent*-progesterone have been described in U.S. Patent Application No. 13/645,881, which was filed on October 5, 2012 and is entitled "Nasal Delivery Mechanism for Prophylatic and Post-Acute Use for Progesterone and/or Its Enantiomer for Use in Treatment of Mild Traumatic Brain Injuries, U.S. Patent Application No. 13/645,854, which was filed on October 12, 2012 and is entitled "Prophylactic and Post-Acute Use of Progesterone and Its Enantiomer to Better Outcomes Associated with Concussion," and U.S. Patent Application No. 13/645,925, which was filed on October 12, 2012 and is entitled "Prophylactic and Post- 15 Acute Use of Progesterone in Conjunction with Its Enantiomer for Use in Treatment of Traumatic Brain Injuries, the entire contents and disclosures each of which are incorporated herein by reference in their entireties. See also VanLandingham et al.: The enantiomer of progesterone acts as a molecular neuroprotectant after traumatic brain injury, *Neuropharmacology*, 51:1078-1085 (2006).

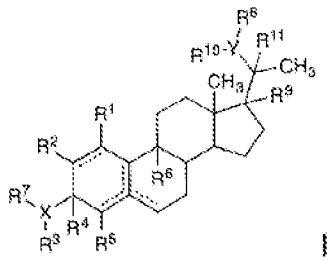
Due to side effects associated with long term progesterone treatments, it is believed that progesterone is not suitable for long-term chronic administration for indications outside of hormone replacement therapy and contraception. Even though there is a current belief that

intravenous progesterone may be useful for the treatment of moderate to severe traumatic brain injury (TBI), MTBI in the U.S. population, including among those who served in the military, is a public health problem, the magnitude and impact of which are underestimated by current civilian and military surveillance systems. There is no doubt that much research is needed to determine the full magnitude of MTBI, including concussions, to identify preventable and modifiable risk factors, develop and test strategies to reduce MTBIs in civilian and military life, and improve health and social outcomes and quality of life for those who sustain these injuries. Thus, there is a need for novel MTBI treatments that are effective, that can be conveniently administered on demand, that are tissue-specific and/or that do not induce side effects, such as those commonly associated with progesterone or the reproductive system.

Summary of the Invention

In brief, it is believed that the present invention overcomes many of the disadvantages and shortcomings associated with the current state of mild traumatic brain injury (MTBI) treatment through the discovery of certain novel C-20 steroid compounds, namely, *ent*-19-norprogesterone (PRV-002), compositions and methods of use that are believed to be effective in the treatment of MTBI, including concussions a subset thereof, that can be administered either in accordance with a prescribed treatment regimen or conveniently on demand. Quite remarkably, the C-20 steroid compounds and/or compositions thereof of the present invention are believed to be tissue-specific and/or do not induce side effects, such as those associated with progesterone or the reproductive system. Uniquely, the C-20 steroid compounds and/or compositions thereof of the present invention can be conveniently administered by any route of administration, especially topically, e.g., pernasally, buccally and/or sublingually, on demand to deliver an effective amount to effectively and/or prophylactically treat and or prevent MTBI. Even more remarkably, the C-20 steroid compounds and compositions thereof as contemplated by the present invention are believed to be tissue-specific in the brain for treating MTBI and/or do not induce side effects commonly associated with progesterone or the reproductive system.

Generally speaking, the C-20 steroid compounds of the present invention have a common chemical structure as shown by Formula I below:



I

Wherein,

X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^1, R^2, R^5, and R^6 are independently H, C_1-C_6 alkyl, halogen, OR^{12}, $NR^{13}R^{14}$, SR^{15}, SOR^{16} or SO_2R^{17};

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or

R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

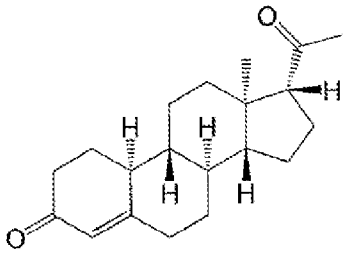
R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16} and R^{17} are independently H, C(O)-C_1-C_6 alkyl or C_1-C_6 alkyl; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

More specifically speaking, the C-20 steroid compounds of the present invention as depicted in Formula I possess the stereochemical configurations of natural steroids. In addition,

the C-20 steroid compounds of the present invention, as shown in Formula I, may be racemic. Still further, the C-20 steroid compounds of the present invention, as illustrated by Formula I, may have stereochemical configurations that are opposite to that of natural steroids.

One preferred C-20 steroid compound that is contemplated by the present invention is *ent*-19-norprogesterone (PRV-002). *Ent*-19-norprogesterone has a molecular formula of C_{20} H_{28} $O_2$and a molar mass of 300.435 g/mol. The chemical names for *ent*-19-norprogesterone include ent-19-norpregn-4-ene-3,20-dione. The chemical structure of ent-norprogesterone is as follows:



ent-19-norprogesterone

In accordance with the present invention, it has been discovered that *ent*-19-norprogesterone exist in at least two polymorphic forms, polymorph A and polymorph B, each having distinctively different physical properties, and methods of making same. The *ent*-19-norprogesterone polymorphs are useful in the treatment of TBI, including severe TBI, moderate TBI and mild TBI, including concussions. Thus, the present invention contemplates ent-19-norprogesterone in crystalline and amorphous forms, and preferably in crystalline form.

In preferred embodiments of the present invention, pure, single polymorphs as well as mixtures comprising two or more different polymorphs are contemplated. A pure, single polymorph may be substantially free from other polymorphs. "Substantially free" means that other polymorph(s) are present in an amount less than about 15 weight percent, more preferably less than about 10 weight percent, even more preferably less than about 5 weight percent, still more preferably less than about 2 weight percent, and most preferably less than about 1 weight percent. Someone with ordinary skill in the art would understand the phrase "in an amount less than about 15 weight percent" to mean that the polymorph of interest is present in an amount of more than about 85 weight percent. Likewise, the phrase "less than about 10 weight percent" would mean that the polymorph of interest is “substantially pure” in an amount of more than about 90 weight percent, and so on and so forth. Thus, “substantially pure” means that the polymorph of interest is present in an amount of at least about 85 weight percent, more preferably

at least about 90 weight percent, even more preferably at least about 95 weight percent, still more preferably at least about 98 weight percent, even more preferably at least about 98.8 weight percent, and most preferably at least about 99 weight percent.

As used herein, the term "substantially similar" means an analytical spectrum, such as XRD pattern, Raman spectroscopy, microscopic images, particle distribution, and etc., which resembles the reference spectrum to an appreciable degree in both the peak locations and their intensity.

In one embodiment, the Type A polymorph is substantially free of other ent-19-norprogesterone polymorphs. As used herein, substantially free means comprising less 15 weight percent, more preferably less than about 10 weight percent, even more preferably less than about 5 weight percent, still more preferably less than about 2 weight percent, and most preferably less than about 1 weight percent ent-19-norprogesterone polymorphs.

In another embodiment, the Type A polymorph has a purity of greater than or equal to at least about 90 weight percent, even more preferably at least about 95 weight percent, still more preferably at least about 98 weight percent, even more preferably about 98.8 weight percent, and most preferably at least about 99 weight percent.

In one embodiment, the Type A ent-19-norprogersterone polymorph exhibits about 15 XRPD peaks, or which about 14 XRPD peaks have an intensity (counts) of at least about 2500 at XRPD peak positions of about 11.2 ±1.0 degrees, 12.8 ±1.0 degrees, 13.9 ±1.0 degrees, 16.4 ±1.0 degrees, 17.3 ±1.0 degrees, 18.2 ±1.0 degrees, 21.1 ±1.0 degrees, 23.9 ±1.0 degrees, 25.1 ±1.0 degrees, 26.2 ±1.0 degrees, 26.6 ±1.0 degrees, 29.3 ±1.0 degrees, 33.8 ±1.0 degrees and 34.8 ±1.0 degrees 2-theta (deg), and of which 9 XRPD peaks have an intensity (counts) of at least about 5000 at XRPD peak positions of about 11.2 ±0.5 degrees, 12.8 ±0.5 degrees, 16.4 ±0.5 degrees, 17.3 ±0.5 degrees, 18.2 ±0.5 degrees, 21.1 ±0.5 degrees, 26.2 ±0.5 degrees, 29.3 ±0.5 degrees and 34.8 ±0.5 degrees 2-theta (deg), as depicted in **FIG. 12** and **FIG. 14**, or about 9 XRPD peaks have an intensity (counts) of at least about 5000 at XRPD peak positions of about 11.2 ±0.2 degrees, 12.8 ±0.2 degrees, 16.4 ±0.2 degrees, 17.3 ±0.2 degrees, 18.2 ±0.2 degrees, 21.1 ±0.2 degrees, 26.2 ±0.2 degrees, 29.3 ±0.2 degrees and 34.8 ±0.2 degrees 2-theta (deg), as depicted in **FIG. 12** and **FIG. 14**, respectively.

In yet another embodiment, ent-19-norprogersterone polymorph Type A exhibits an X-ray powder diffraction pattern which is substantially similar to the X-ray powder diffraction pattern

depicted in **FIG. 12**. In still another embodiment, ent-19-norprogersterone polymorph Type A exhibits an X-ray powder diffraction pattern which is substantially similar to the X-ray powder diffraction pattern depicted in **FIG. 14**. In still another embodiment, ent-19-norprogersterone polymorph Type A exhibits an X-ray powder diffraction pattern which is substantially similar to X-ray powder diffraction pattern depicted in **FIG. 21**. In still another embodiment, ent-19-norprogersterone polymorph Type A exhibits an X-ray powder diffraction pattern which is substantially similar to the X-ray powder diffraction pattern depicted in **FIG. 23**.

In other embodiments, ent-19-norprogersterone polymorph Type A exhibits TGA/DSC curves which is substantially similar to the TGA/DSC curves depicted in **FIG. 15**, a microscopic image which is substantially similar to the microscopic image depicted in **FIG. 16**, a particle size distribution which is substantially similar to the particle size distribution depicted in **FIG. 17A** and /or **FIG. 17B**, and a microscopic image which is substantially similar to the microscopic image depicted in **FIG. 18A**, **FIG. 18B**, **FIG. 18C** and/or **FIG. 18D.**

In another embodiment, ent-19-norprogersterone polymorph Type A exhibits at least one or more substantially similar XRPD peak positions, preferably any 1 to 4 substantially similar XRPD peak positions, more preferably any 1 to 9 substantially similar XRPD peak positions, and most preferably any 1 to anyone of the 15 substantially similar XRPD peak positions, as depicted on **FIG. 12** and/or **FIG. 14**, wherein each such substantially similar XRPD peak position is at about ±0.5 degrees, more preferably ±0.4 degrees, even more preferably ±0.3 degrees and most preferably ±0.2 degrees of its XRPD peak position as depicted in **FIG 12** or **FIG 14**, at 6.2, 9.2, 12.9, 14.0. 15.3, 16.6, 17.5, and 18.4 ±0.2 degrees 2-theta.

In another aspect, the present invention provides a crystalline form of ent-19-norprogesterone selected from the group consisting of a crystalline form having an X-ray powder diffraction pattern substantially the same as

(1) an X-ray powder diffraction pattern having at least four 2θ° peaks selected from the group consisting of about 11.2 ±1.0 degrees, 12.8 ±1.0 degrees, 13.9 ±1.0 degrees, 16.4 ±1.0 degrees, 17.3 ±1.0 degrees, 18.2 ±1.0 degrees, 21.1 ±1.0 degrees, 23.9 ±1.0 degrees, 25.1 ±1.0 degrees, 26.2 ±1.0 degrees, 26.6 ±1.0 degrees, 18.8 ±1.0 degrees, 29.3 ±1.0 degrees, 33.8 ±1.0 degrees and 34.8 ±1.0 degrees 2-theta (deg);

(2) an X-ray powder diffraction pattern having at least four 2θ° peaks selected from the group consisting of about 11.2 ±0.5 degrees, 12.8 ±0.5 degrees, 13.9 ±0.5 degrees, 16.4 ±0.5

degrees, 17.3 ±0.5 degrees, 18.2 ±0.5 degrees, 18.8 ±0.5 degrees, 21.1 ±0.5 degrees, 23.9 ±0.5 degrees, 25.1 ±0.5 degrees, 26.2 ±0.5 degrees, 26.6 ±0.5 degrees, 29.3 ±0.5 degrees, 33.8 ±0.5 degrees and 34.8 ±0.5 degrees 2-theta (deg); or

(3) an X-ray powder diffraction pattern having at least four 2θ° peaks selected from the group consisting of about 11.2 ±0.2 degrees, 12.8 ±0.2 degrees, 13.9 ±0.2 degrees, 16.4 ±0.2 degrees, 17.3 ±0.2 degrees, 18.2 ±0.2 degrees, 18.8 ±0.2 degrees, 21.1 ±0.2 degrees, 23.9 ±0.2 degrees, 25.1 ±0.2 degrees, 26.2 ±0.2 degrees, 26.6 ±0.2 degrees, 18.8 ±0.2 degrees, 29.2 ±0.5 degrees, 33.8 ±0.2 degrees and 34.8 ±0.2 degrees 2-theta (deg);

(4) an X-ray powder diffraction pattern having at least four 2θ° peaks selected from the group consisting of about 11 ±1.0 degrees, about 13±1.0 degrees, about 14 ±1.0 degrees, about 16.5 ±1.0 degrees, about 17 ±1.0 degrees, about 18±1.0 degrees, 19 ±1.0 degrees, about 21±1.0 degrees, about 24 ±1.0 degrees, about 25 ±1.0 degrees, about 26 ±1.0 degrees, about 26.5 ±1.0 degrees, about 29 ±1.0 degrees, about 30 ±1.0 degrees, about 34 ±1.0 degrees, about 38 ±1.0, degrees and about 35±1.0 degrees 2-theta (deg).

In another embodiment, the Type B ent-19-norprogersterone polymorph exhibits about 6 XRPD peaks which have an intensity (counts) of at least about 250 at XRPD peak positions of about 13 ±1.0 degrees, 14.5 ±1.0 degrees, 15.2 ±1.0 degrees 16.8 ±1.0 degrees, 18.5 ±1.0 degrees and 21.8 ±1.0 degrees, 2-theta (deg), as depicted in **FIG. 19**, or about 6 XRPD peaks which have an intensity (counts) of at least about 250 at XRPD peak positions of about 13 ±0.5 degrees, 14.5 ±0.5 degrees, 15.2 ±0.5 degrees, 16.8 ±0.5 degrees, 18.5 ±0.5 degrees and 21.8 ±0.5 degrees, 2-theta (deg), as depicted in **FIG. 19**.

In yet another embodiment, ent-19-norprogersterone polymorph Type B exhibits an X-ray powder diffraction pattern which is substantially similar to the ent-19-norprogersterone polymorph Type B exhibits an X-ray powder diffraction pattern depicted in **FIG. 12**. In still another embodiment, ent-19-norprogersterone polymorph Type B exhibits an X-ray powder diffraction pattern which is substantially similar to the ent-19-norprogersterone polymorph Type B exhibits an X-ray powder diffraction pattern depicted **FIG. 19**. In still another embodiment, ent-19-norprogersterone polymorph Type B exhibits an X-ray powder diffraction pattern which is substantially similar to the ent-19-norprogersterone polymorph Type B exhibits an X-ray powder diffraction pattern depicted **FIG. 21**.

In other embodiments, ent-19-norprogersterone polymorph Type B exhibits TGA/DSC curves which are substantially similar to the TGA/DSC curves depicted in **FIG. 20**.

In another aspect, the present invention provides a crystalline form of ent-19-norprogesterone selected from the group consisting of a crystalline form having an X-ray powder diffraction pattern substantially the same as

(1) an X-ray powder diffraction pattern having at least four 2θ° peaks selected from the group consisting of about 13 ±1.0 degrees, 14.5 ±1.0 degrees, 15.2 ±1.0 degrees 16.8 ±1.0 degrees, 18.5 ±1.0 degrees and 21.8 ±1.0 degrees, 2-theta (deg) 2-theta (deg); or

(2) an X-ray powder diffraction pattern having at least four 2θ° peaks selected from the group consisting of about 13 ±0.5 degrees, 14.5 ±0.5 degrees, 15.2 ±0.5 degrees 16.8 ±0.5 degrees, 18.5 ±0.5 degrees and 21.8 ±0.5 degrees, 2-theta (deg); or

(3) an X-ray powder diffraction pattern having at least four 2θ° peaks selected from the group consisting of about 13 ±0.2 degrees, 14.5 ±0.2 degrees, 15.2 ±0.2 degrees 16.8 ±0.2 degrees, 18.5 ±0.2 degrees and 21.8 ±0.2 degrees, 2-theta (deg).

In another aspect of the present invention, amorphous ent-19-norprogesterone exhibits an amorphous pattern substantially similar to the amorphous pattern depicted in **FIG. 22**. In another aspect of the present invention, amorphous ent-19-norprogesterone exhibits an amorphous pattern substantially similar to the amorphous pattern depicted in **FIG. 22**.

In accordance with the present invention, the C-20 steroid compounds of Formula I are believed to be useful for treating, minimizing and/or preventing neuronal damage, such as neuronal damage, resulting from various injuries involving TBI, whether the TBI is mild, moderate or severe. An especially preferred treatment in accordance with the present invention is treatment of MTBI, including a concussion, with ent-19-progesterone.

In accordance with the present invention, a C-20 steroid compound of Formula I may be administered as a single therapeutic agent.

It is further contemplated by the present invention that the C-20 steroid compounds of Formula I can be administered through routes of administration that include, e.g., oral, sublingual, intravenous, intraperitoneal, subcutaneous, intramuscular, ocular, otic, intranasal, topical, transdermal and rectal routes of administration. The present invention further envisions that the C-20 compounds of Formula I can be formulated into a novel composition or admixture and administered in the form of, e.g., a tablet, capsule, gelcap, caplet, powder, granule, liquid,

solution, suspension, dispersion, pellet, bead, eyedrop, gel, cream, ointment, salve, balm, lotion or suppository. Still further, the present invention envisions that the C-20 steroid compounds of Formula I, including ent-19-norprogesterone, may be administered as a formulation that is swallowed, injected, infused, inhaled, applied transdermally or topically, such as applied to the skin, eye, ear, nose, mucosal membrane or any other membrane or inserted into the rectum. Nonetheless, it should be understood by those versed in the art that preferred routes of administration to treat TBI, especially MTBI, as contemplated by the present invention, is the pernasal, inhalation or injection routes of administration.

It should be further understood that the above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description further exemplifies illustrative embodiments. In several places throughout the specification, guidance is provided through examples, which examples can be used in various combinations. In each instance, the examples serve only as representative groups and should not be interpreted as exclusive examples.

Brief Description of the Figures

The foregoing and other objects, advantages and features of the present invention, and the manner in which the same are accomplished, will become more readily apparent upon consideration of the following detailed description of the invention taken in conjunction with the accompanying figures and examples, which illustrate embodiments, wherein:

FIG. 1 is drawn to a table showing concussion facts;

FIG. 2 is drawn to a drawing illustrating a Morris thigmotaxis water maze;

FIG. 3 is drawn to a chart that shows no significant differences in motor function, as measured by neuroscore, which were observed at 24h post-injury;

FIG. 4 is drawn to a chart that shows that when rats are treated with either PRV-002 4mg/kg or PRV-002 16mg/kg, they have significantly better motor function, compared to vehicle-treated rats, at 48h post-injury. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$.

FIG. 5A is drawn to a chart that shows that when treatment is with either PRV-002 4mg/kg or PRV-002 16mg/kg, significantly attenuated TBI-related cognitive deficits are observed

during trial 1 of the Morris water maze task at 48h post-injury. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 5B is drawn to a chart that shows that when treatment is with either PRV-002 4mg/kg or PRV-002 16mg/kg, significantly attenuated TBI-related cognitive deficits are observed during trial 2 of the Morris water maze task at 48h post-injury. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 6A is drawn to a chart that shows vehicle-treated rats spend significantly more time in thigmotaxia compared during sham, PRV-002 4mg/kg-treated, or PRV-002 16mg/kg-treated rats during trial 1 of the Morris water maze task, 48h post-injury. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 6B is drawn to a chart that shows vehicle-treated rats spend significantly more time in thigmotaxia compared during sham, PRV-002 4mg/kg-treated, or PRV-002 16mg/kg-treated rats during trial 2 of the Morris water maze task, 48h post-injury. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 7A is drawn to a photograph that shows the nasal mucosa of a rat free of Evans Blue Dye;

FIG. 7B is drawn to a photograph that shows no Evans Blue Dye observable in nasal mucosa of a rat using pipette for IN administration;

FIG. 7C is drawn to a photograph that shows excellent intranasal penetration observed in nasal mucosa of a rat using micro Atomizer.

FIG. 8A is drawn to a chart that shows that when injured rats are treated with PRV-002 4mg/kg, they have significantly better cognitive performance, as compared to all other groups, during trial 1 of the Morris water maze task (top). * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 8B is drawn to a chart that shows that when injured rats are treated with PRV-002 significant group differences in cognitive performance are not observed during trial 2 of the Morris water maze task (bottom);

FIG. 9A is drawn to a chart that shows that no significant group differences are observed in time spent in thigmotaxia during trial 1 of the Morris water maze task (top). Uninjured (sham) and PRV-002 4mg/kg-treated rats spends significantly less time in thigmotaxia as compared to

vehicle-treated injured rats during trial 2 of the Morris water maze task (bottom). * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 9B is drawn to a chart that shows rats treated with PRV-002 005mg/kg spent significantly more time in thigmotaxia, compared to sham rats, during trial 2. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 10 is drawn to a chart that shows that when rats are treated with PRV-002 0.1 mg/kg or PRV-002 4mg/kg, they have significantly improved motor function, as compared to vehicle-treated rats at 24h post-injury. All PRV-002 treatment groups had motor performance scores that are not significantly different from sham rats. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 11 is drawn to a chart that shows sham rats and that when rats treated with either PRV-002 0.05mg/kg, PRV-002 0.1 mg/kg, or PRV-002 4mg/kg, the treated rats have significantly better motor function, as compared to vehicle-treated rats at 48h post-injury. PRV-002 0.05mg/kg- and PRV-002 1 mg/kg-treated rats have significantly worse performance, compared to sham rats at 48h post-injury. * indicates a significant difference from vehicle-treated, injured rats, $p < 0.05$;

FIG. 12 shows an XRPD pattern overlay of Type A and Type B of PRV-002;

FIG. 13 shows the interconversion of amorphous PRV-002 Type A and Type B;

FIG. 14 shows an XRPD pattern of PRV-002 Type A (807302-25-A);

FIG. 15 shows TGA/DSC curves of PRV-002 Type A (807302-25-A);

FIG. 16 is a microscopic image of PRV-002 Type A (807302-25-A);

FIG. 17A shows particle size distribution of PRV-002 Type A (807302-25-A) without sonication, (b) sonicated using a power of 30W for 30s;

FIG. 17B shows particle size distribution of PRV-002 Type A (807302-25-A) sonicated using a power of 30W for 30s;

FIG.18A shows a microscopic image of a PRV-002 Type A batch collected from screening;

FIG.18B shows a microscopic image of a different PRV-002 Type A batch collected from screening;

FIG.18C shows a microscopic image of another different PRV-002 Type A batch collected from screening;

FIG.18D shows a microscopic image of yet another different PRV-002 Type A batch collected from screening;

FIG. 19 shows an XRPD pattern of PRV-002 Type B (807302-42-A);

FIG. 20 shows TGA/DSC curves of PRV-002 Type B (807302-42-A);

FIG. 21 shows an XRPD pattern overlay of PRV-002 Type B before and after storage at ambient conditions;

FIG. 22 shows an XRPD pattern of an amorphous precipitate sample of PRV-002 (807302-27-A8);

FIG. 23 shows an XRPD pattern overlay of amorphous before and after storage at ambient conditions with characteristic peaks of PRV-002 Type A;

FIG. 24 shows an XRPD pattern overlay of anti-solvent addition experiments (I/IV) from a PRV-002 Type A sample;

FIG. 25 shows an XRPD pattern overlay of anti-solvent addition (II/IV) from a PRV-002 Type A sample;

FIG. 26 shows aXRPD pattern overlay of anti-solvent addition (III/IV) from a PRV-002 Type A sample;

FIG. 27 shows an XRPD pattern overlay of anti-solvent addition (IV/IV) from a PRV-002 Type A sample;

FIG. 28 shows an XRPD pattern overlay of reverse anti-solvent addition (I/II) from a PRV-002 Type A sample;

FIG. 29 shows an XRPD pattern overlay of reverse anti-solvent addition (II/II) from a PRV-002 Type A sample;

FIG. 30 shows an XRPD pattern overlay of slow cooling (I/II) from a PRV-002 Type A sample;

FIG. 31 shows an XRPD pattern overlay of slow cooling (II/II) from a PRV-002 Type A sample;

FIG. 32 shows an XRPD pattern overlay of slurry at RT (I/III) from a PRV-002 Type A sample;

FIG. 33 shows an XRPD pattern overlay of slurry at RT (II/III) from a PRV-002 Type A sample;

FIG. 34 shows an XRPD pattern overlay of slurry at RT (III/III) from a PRV-002 Type A sample;

FIG. 35 shows an XRPD pattern overlay of slurry at 5 ºC (I/II) from a PRV-002 Type A sample;

FIG.36 shows an XRPD pattern overlay of slurry at 5 ºC (II/II) from a PRV-002 Type A sample) ;

FIG. 37 shows an XRPD pattern overlay of solid vapor diffusion (I/II) from a PRV-002 Type A sample;

FIG. 38 shows an XRPD pattern overlay of solid vapor diffusion (II/II) from a PRV-002 Type A sample;

FIG. 39 shows an XRPD pattern overlay of solution vapor diffusion from a PRV-002 Type A sample;

FIG. 40 shows an XRPD pattern overlay of crystallization induced by polymer mixture A from a PRV-002 Type A sample;

FIG. 41 shows an XRPD pattern overlay of crystallization induced by polymer mixture from a PRV-002 Type A sample;

FIG. 42 shows an XRPD pattern overlay of slow evaporation (I/II) from a PRV-002 Type A sample;

FIG. 43 shows an XRPD pattern overlay of slow evaporation (II/II) from a PRV-002 Type A sample;

FIG. 44 shows an XRPD pattern overlay of grinding from a PRV-002 Type A sample;

FIG. 45A shows effects of intranasal administration of about 3 mg/mL PRV-002 upon the mean diastolic arterial pressure (n=4) cardiovascular parameter;

FIG. 45B shows effects of intranasal administration of about 3 mg/mL PRV-002 upon the mean heart rate (n=4) cardiovascular parameter;

FIG. 45C shows effects of intranasal administration of about 3 mg/mL PRV-002 upon the mean mean arterial pressure (n=4) cardiovascular parameter;

FIG. 45D shows effects of intranasal administration of about 3 mg/mL PRV-002 upon the mean systolic arterial pressure (n=4) cardiovascular parameter;

FIG. 46A shows effects of intranasal administration of about 10 mg/mL PRV-002 upon the mean diastolic arterial pressure (n=4) cardiovascular parameter;

FIG. 46B shows effects of intranasal administration of about 10 mg/mL PRV-002 upon the mean heart rate (n=4) cardiovascular parameter;

FIG. 46C shows effects of intranasal administration of about 10 mg/mL PRV-002 upon the mean mean arterial pressure (n=4) cardiovascular parameter;

FIG. 46D shows effects of intranasal administration of about 10 mg/mL PRV-002 upon the mean systolic arterial pressure (n=4) cardiovascular parameter;

FIG. 47A shows effects of intranasal administration of about 23 mg/mL PRV-002 upon the mean diastolic arterial pressure (n=4) cardiovascular parameter;

FIG. 47B shows effects of intranasal administration of about 23 mg/mL PRV-002 upon the mean heart rate (n=4) cardiovascular parameter;

FIG. 47C shows effects of intranasal administration of about 23 mg/mL PRV-002 upon the mean mean arterial pressure (n=4) cardiovascular parameter;

FIG. 47D shows effects of intranasal administration of about 23 mg/mL PRV-002 upon the mean systolic arterial pressure (n=4) cardiovascular parameter; and

FIG. 48 depicts PRV-002 (ent-19-norprogesterone) mean concentrations in plasma of dogs dosed 3 times at 4 hour intervals in one day by IN administration in an amount of about 1mL/nostril at each dosing interval for a total PRV-002 (ent-19-norprogesterone) dose of about 46 mg/dog per dosing interval.

Detailed Description

By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description and examples are given concerning the novel C-20 steroid compounds, compositions, and methods of manufacture and uses thereof of the present invention.

Definitions

As used in the description of the invention and the appended claims, the singular forms "a", "an" and "the" are used interchangeably and intended to include the plural forms as well and fall within each meaning, unless the context clearly indicates otherwise. Also, as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the listed items, as well as the lack of combinations when interpreted in the alternative ("or").

As used herein, the term "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. Thus, it is to be understood that when a value is recited in context of a condition, numerical value, result or a yield, the value may vary within a reasonable range of between about 0 and ±10%, such as ±10, ±5%, ±2.5%, ±1%, and ±0.2%. However, it should be understood that, when the term "about" is used in the context of blood, plasma or serum samples or concentrations, persons of ordinary skill in the art will understand the term "about" to mean and cover ±25%, and preferably to mean and cover plus 25% and minus 20%. When the term "about" is used before a 2θ° peak of an XRPD, persons of ordinary skill in the art will understand the term "about" to mean that the 2θ° value may vary in a range of between about ±0.5 and 0.00 degrees, such as ±1.0, ±0.9, ±0.8, ±0.7, ±0.6, ±0.5, ±0.4, ±0.3, ±0.2, ±0.1, ±0.05 and/or ±0.02 degrees. Thus, unless otherwise indicated, it is to be understood that all numbers expressing quantities, ratios, and numerical properties of ingredients, reaction conditions, and so forth used in the specification and claims are contemplated to be able to be modified in all instances by the term "about".

The terms "administration of or "administering" an active agent, i.e., the compounds of the present invention, should be understood to mean providing an active agent of the invention to the subject in need of TBI treatment in a form that can be introduced into that subject's body in a therapeutically useful form and therapeutically effective amount.

The term "alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to eight carbon atoms, and which is attached to the rest of the molecule by a single bond, such as illustratively, methyl, ethyl, n-propyl 1-methylethyl (isopropyl), n-butyl, n-pentyl, and 1,1-dimethylethyl (tert-butyl).

"Amorphous" refers to a composition comprising a compound that contains no or too little crystalline content of the compound to yield a discernable pattern by XRPD or other diffraction techniques. For example, glassy materials are a type of amorphous material. Amorphous materials do not have a true crystal lattice, and are glassy, technically resembling very viscous non-crystalline liquids. Glasses may better be described as quasi-solid amorphous material. As is known in the art, an amorphous material refers to a quasi-solid. A compound in an amorphous state may be produced by rapidly evaporating solvent from a solution of a compound, or by

grinding, pulverizing or otherwise physically pressurizing or abrading the compound while in a crystalline state.

As used herein, "at least one" means "one or more" of the listed elements.

"Blood sample" refers to whole blood taken from a subject, or any fractions of blood including plasma or serum.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. For example, a composition consisting essentially of the elements as defined herein would not exclude other elements that do not materially affect the basic and novel characteristic(s) of the claimed invention. "Consisting of" shall mean excluding more than trace amount of other ingredients and substantial method steps recited. Embodiments defined by each of these transition terms are within the scope of this invention.

"Crystalline" refers herein to a material that contains a specific compound or a salt of the compound, which may be hydrated and/or solvated, and has sufficient crystalline content to exhibit a discernable diffraction pattern by XRPD or other diffraction techniques. Crystallines can be characterized by their crystalline structure (X-ray diffraction pattern), their thermal properties (as determined by DSC and TGA), stability, solubility, etc. The X-ray diffraction pattern is presented as characteristic 2θ° peaks and one skilled in the art can readily identify a crystalline form of a compound or salt based on the characteristic 2θ° peaks of an X-ray diffraction pattern of the polymorph. When two X-ray diffraction patterns have at least 4, preferably at least 6, 8, or 10 2θ° peaks, or more preferably all peaks, that do not vary more than ±0.2, ±0.1, ±0.05 or ±0.02 degrees, it is deemed that the X-ray diffraction patterns are substantially the same. In some embodiments, characteristic peaks are those having a relative intensity of 25% or more. In some embodiments, characteristic peaks are those that have a relative intensity of 10% or more. In some embodiments, characteristic peaks are those that have a relative intensity of 5% or more.

A crystalline of a compound or a salt may be characterized by properties including one or more of the following as described in details herein, such as (i) its X-ray powder diffraction pattern (XRPD), (ii) its infrared spectrum (IR), (iii) its differential scanning calorimetry (DSC), (iv) its thermogravimetric analysis (TGA), (v) its vapor sorption curve, such as Gravimetric Vapour Sorption (GVS), and/or (vi) crystal structure, such as unit cell structure.

In some cases, a crystalline material that is obtained by direct crystallization of a compound dissolved in a solvent or solvent mixture or solution or interconversion of crystals obtained under different crystallization conditions, may have crystals that contain the solvent used in the crystallization. Such compositions may be referred to as a crystalline solvate. When the solvent is water, such compositions may be referred to as a crystalline hydrate. Also, the specific solvent system and physical embodiment in which the crystallization is performed, collectively termed as crystallization conditions, may result in the crystalline material having physical and chemical properties that are unique to the crystallization conditions. This may be due to the orientation of the chemical moieties of the compound with respect to each other within the crystal and/or the predominance of a specific polymorphic or pseudopolymorphic form of the compound in the crystalline material. General methods for precipitating and crystallizing a compound may be applied to prepare the various polymorphs or pseudopolymorphs described herein. These general methods are known to one skilled in the art of synthetic organic chemistry and pharmaceutical formulation, and are described, for example, by J. March, "Advanced Organic Chemistry: Reactions, Mechanisms and Structure," 4th Ed. (New York: Wiley-InterScience, 1992); Remington: The Science and Practice of Pharmacy, Editor-In-Chief Loyd V Allen, Jr , ed., 22nd edition, Pharmaceutical Press, London, UK, 2013 and Remington: The Science and Practice of Pharmacy, A. Gennaro, ed., 21st edition,-Lippincott, Williams & Wilkins, Philadelphia, Pa., 2006, each of which is incorporated herein by reference in their entireties.

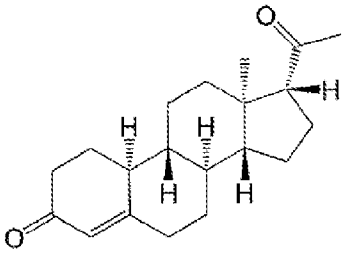
The term "cycloalkyl" denotes a non-aromatic mono or multicyclic ring system of 3 to 12 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and examples of multicyclic cycloalkyl groups include perhydronapththyl, adamantyl and norbornyl groups bridged cyclic group or spirobicyclic groups e.g., spiro(4,4)non-2-yl.

"Disease condition" refers to a disease state for which the compounds, compositions and methods of the present invention are being used against.

The term "effective amount", as used herein, means any amount or dosage strength of a C-20 steroid compound of the present invention, especially ent-19-norprogesterone, to treat, minimize and/or prevent traumatic brain injury, including severe, moderate and/or mild TBI, including concussions. Effective amount, as used herein, also means any amount or dosage amount considered by the U.S. Food and Drug Administration (FDA) or other governmental

agency or tribunal as being effective to treat, minimize and/or prevent traumatic brain injury, including severe, moderate and/or mild TBI, including concussions.

The term "*ent*-19-norprogesterone", "*ent*-19-norpregn-4-ene-3,20-dione" or "PRV-002" each refer to the compound of the structure:



ent-19-norprogesterone

This compound is described in U.S. Patent. Application No. 14/857,331 and in PCT/US2015/050633, both of which a5e incorporated herein by reference in their entireties.

The term "leaving group," or "LG", as used herein, refers to any group that leaves in the course of a chemical reaction involving the group and includes but is not limited to halogen, brosylate, mesylate, tosylate, triflate, p-nitrobenzoate, phosphonate groups, for example.

"Patient" refers to mammals and includes humans and non-human mammals.

"Polymorph" or "polymorphic form" refers to a crystalline form of a substance that is distinct from another crystalline form but that shares the same chemical formula. The different polymorphic forms of the same compound can have an impact on one or more physical properties, such as stability, solubility, melting point, bulk density, flow properties, bioavailability, etc.

"Pseudopolymorph" refers to a crystalline form of a hydrate or solvate of a compound. In contrast to polymorphs, pseudopolymorphs are chemically identical except differ in the amount of water or solvent bound in the crystal lattice. Depending on the solvent used during synthesis and/or crystallization some compounds form hydrates (with water) or solvates (with other solvents) in different stoichiometric ratio. Pseudopolymorphs may show different physical properties like habitus, stability, dissolution rate and bioavailability as known for polymorphs.

"Subject" refers to any animal, individual or Patient.

"Tautomer" refers to alternate forms of a compound that differ in the position of a proton, such as enol-keto and imine-enamine tautomers, or the tautomeric forms of heteroaryl groups

containing a ring atom attached to both a ring —NH— moiety and a ring =N— moiety such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles. For example, the proton of a salt of any compound of the present invention may be in different positions of the molecule. A salt of any compound of the present invention, including ent-19-norprogetserone, includes any and all structural variations due to the position of the salt proton unless otherwise indicated.

As used herein, the term "preventing" refers to the prophylactic treatment of a patient in need thereof. The prophylactic treatment can be accomplished by providing an appropriate dose of a therapeutic agent to a subject at risk of suffering from an ailment, thereby substantially averting onset of the ailment.

It will be understood by those skilled in the art that in human medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate inductive event or events may be unknown, latent, or the patient is not ascertained until well after the occurrence of the event or events. Therefore, as used herein the term "prophylaxis" is intended as an element of "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "protection," as used herein, is meant to include "prophylaxis."

"Treatment" or "treating" or "treat" means any treatment of a disease or disorder in a subject, including preventing, ameliorating and/or protecting against the disease or disorder, that is, (i) causing the clinical symptoms not to develop, (ii) inhibiting the disease or disorder, that is, arresting or suppressing the clinical symptoms or the development of clinical symptoms, and/or (iii) relieving the disease or disorder, that is, causing the regression or arrestation of the clinical symptoms. The particular treatment thus will depend on the disease and state of disease to be targeted and the current or future state of medicinal therapies and therapeutic approaches. A treatment may have associated toxicities.

"Therapeutically effective amount" refers to that amount of a compound of this invention, whether used alone or in a suitable pharmaceutical composition that is sufficient to effect treatment, as defined herein, when administered to a subject in need of such treatment, preferably without causing treatment limiting toxicity. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, such as, the weight, the heritage and the age of the subject, the condition of the subject, the severity of the disease condition, the particular compound chosen, the dosage form and the dosing regimen to be followed, timing of administration, the manner of administration and the like, all of which may be determined by one

of ordinary skill in the art. Thus, it should now be understood that a therapeutically effective amount can vary, depending on any of a number of factors, including, e.g., the compound and dosage amount and form selected, the route of administration, the treatment regimen, the condition of the subject, the severity of the disease being treated, as well as other factors understood by those in the art.

In one aspect, this invention provides salts, polymorphs, and pseudopolymorphs of the compounds of the present invention. The present invention contemplates any and all salt forms, polymorphs and pseudopolymorphs of the compounds, including ent-19-norprogesterone.Except where noted otherwise, capitalized and non-capitalized forms of all terms fall within each meaning. Singular word forms are intended to include plural word forms and are likewise used herein interchangeably where appropriate and fall within each meaning, unless expressly stated otherwise.All parts, percentages, ratios, etc. herein are by weight unless indicated otherwise.

It should also be understood that any and all articles, patents, patent publications, studies, abstracts, websites, etc. that are either referenced and/or cited herein are hereby incorporated herein by reference in their entireties.

It should be further understood that the terms "TBI", "MTBI" and "concussion" as used herein, have the meanings set forth herein above.

General Preparative Methods

The particular process to be utilized in the preparation of the C-20 steroid compounds used in this embodiment of the present invention depends upon the specific compound desired to be prepared. Such factors as the selection of the specific substituents play a role in the path to be followed in the preparation of the specific compounds of this invention. In some cases, those factors may be readily recognized by one of ordinary skill in the art.

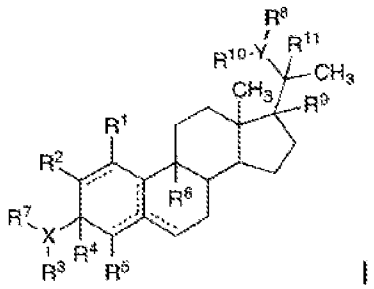
In accordance with the present invention, the following general preparative methods for synthesizing the C-20 steroid compounds of the present invention are described with more detailed in the reaction schemes/pathways and Examples presented below.

In accordance with certain synthetic transformations that may be employed in the synthesis of certain C-20 steroid compounds of the present invention and in the synthesis of certain intermediates involved in the synthesis of certain C-20 steroid compounds of the present invention, see for example, J. March. *Advanced Organic Chemistry*, 4th ed.; John Wiley: New

York (1992); R.C. Larock. *Comprehensive Organic Transformations*, 2nd ed.; Wiley-VCH: New York (1999); F.A. Carey; R.J. Sundberg. *Advanced Organic Chemistry*, 2nd ed.; Plenum Press: New York (1984); T.W. Greene; P.G.M. Wuts. *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley: New York (1999); L.S. Hegedus. *Transition Metals in the Synthesis of Complex Organic Molecules*, 2nd ed.; University Science Books: Mill Valley, CA (1994); L.A. Paquette, Ed. *The Encyclopedia of Reagents for Organic Synthesis*; John Wiley: New York (1994); A.R. Katritzky; O. Meth-Cohn; C.W. Rees, Eds. *Comprehensive Organic Functional Group Transformations*; Pergamon Press: Oxford, UK (1995); G. Wilkinson; F.G A. Stone; E.W. Abel, Eds. *Comprehensive Organometallic Chemistry*; Pergamon Press: Oxford, UK (1982); B.M. Trost; I. Fleming. *Comprehensive Organic Synthesis*; Pergamon Press: Oxford, UK (1991); A.R. Katritzky; C.W. Rees Eds. *Comprehensive Heterocylic Chemistry*; Pergamon Press: Oxford, UK (1984); A.R. Katritzky; C.W. Rees; E.F.V. Scriven, Eds. *Comprehensive Heterocylic Chemistry II*; Pergamon Press: Oxford, UK (1996); and C. Hansch; P.G. Sammes; J.B. Taylor, Eds. *Comprehensive Medicinal Chemistry*: Pergamon Press: Oxford, UK (1990), each of which is incorporated herein by reference in its entirety.

In addition, recurring reviews of synthetic methodology and related topics include Organic Reactions; John Wiley: New York; Organic Syntheses; John Wiley: New York; Reagents for Organic Synthesis: John Wiley: New York; The Total Synthesis of Natural Products; John Wiley: New York; The Organic Chemistry of Drug Synthesis; John Wiley: New York; Annual Reports in Organic Synthesis; Academic Press: San Diego CA; and Methoden der Organischen Chemie (Houben-Weyl); Thieme: Stuttgart, Germany. Furthermore, databases of synthetic transformations include Chemical Abstracts, each of which is incorporated herein by reference in its entirety and which may be searched using either CAS OnLine or SciFinder, Handbuch der Organischen Chemie (Beilstein), and which may be searched using SpotFire, and REACCS.

In one embodiment, the present invention provides for C-20 steroid compounds having a chemical structure of Formula I:



I

or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof,

wherein, X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^1, R^2, R^5, and R^6 are independently H, C_1-C_6 alkyl, halogen, OR^{12}, $NR^{13}R^{14}$, SR^{15}, SOR^{16} or SO_2R^{17};

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or

R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

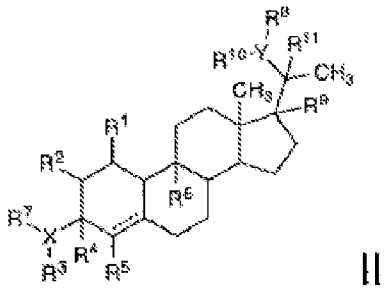
R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16} and R^{17} are independently H, C(O)-C_1-C_6 alkyl or C_1-C_6 alkyl; and

the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

In some embodiments, the C-20 steroid compounds of Formula I possess the stereochemical configuration of natural steroids. In other embodiments, the C-20 steroid compounds of Formula I are racemic. In still other embodiments, the C-20 steroid compounds of formula I possess a stereochemical configuration that is opposite to that of natural steroids.

In another embodiment, the present invention provides for C-20 steroid compounds having a chemical structure of Formula II:



II

wherein, X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^1, R^2, R^5, and R^6 are independently H, C_1-C_6 alkyl, halogen, OR^{12}, $NR^{13}R^{14}$, SR^{15}, SOR^{16} or SO_2R^{17};

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1 -2 nitrogen, oxygen or sulfur atoms, or R^8 is C_1-C_6 absent;

R^9 is H or alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

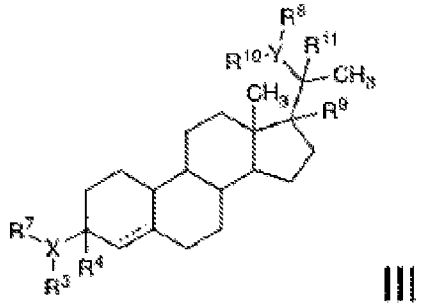
R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16} and R^{17} are independently H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

In some embodiments, the C-20 steroid compounds of Formula II possess the stereochemical configuration of natural steroids. In other embodiments, the C-20 steroid compounds of Formula II are racemic. In still other embodiments, the C-20 steroid compounds of formula II possess a stereochemical configuration that is opposite to that of natural steroids.

In yet another embodiment, the present invention provides for C-20 steroid compounds having a chemical structure of Formula III:



III

wherein;

X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or

R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

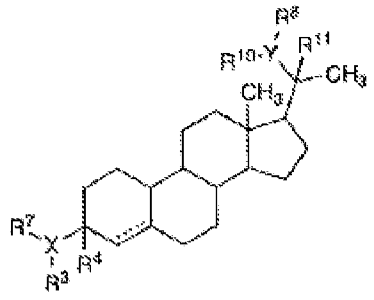
R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond,

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond; and

the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

In some embodiments, the C-20 steroid compounds of Formula III possesses the stereochemical configuration of natural steroids. In other embodiments, the C-20 steroid compounds of Formula III are racemic. In still other embodiments, the C-20 steroid compounds of formula III possess a stereochemical configuration that is opposite to that of natural steroids.

In yet still another embodiment, the present invention provides for C-20 steroid compounds having a chemical structure of Formula IV:



IV

wherein;

X is O, N or S;

Y is O, N or S; or, $YR^8R^{10}$is absent;

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or

R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl;

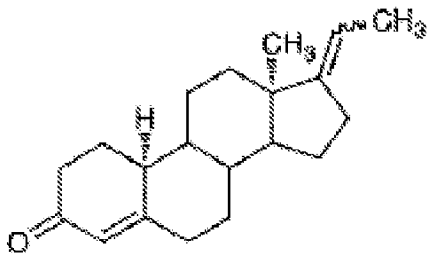
R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond; and

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond; and

the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

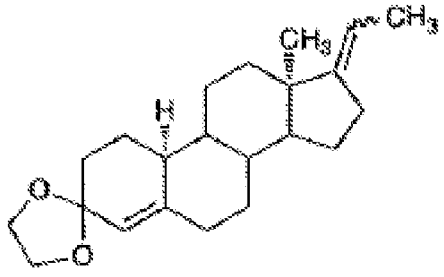
In some embodiments, the C-20 steroid compounds of Formula IV possesses the stereochemical configuration of natural steroids. In other embodiments, the C-20 steroid compounds of Formula IV are racemic. In still other embodiments, the C-20 steroid compounds of formula IV possess a stereochemical configuration that is opposite to that of natural steroids.

In one embodiment, the C-20 steroid compound of Formula I is Compound A:



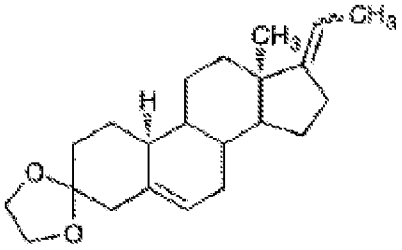
A.

In another embodiment, the C-20 steroid compound of Formula I is Compound B:



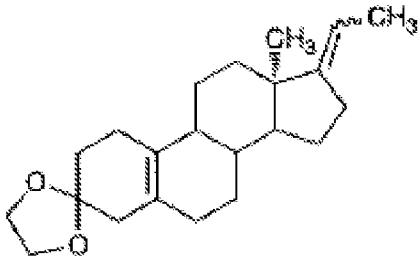
B.

In another embodiment, the C-20 steroid compound of Formula I is Compound C:



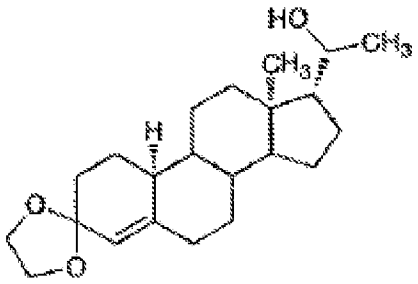
C.

In another embodiment, the C-20 steroid compound of Formula I is Compound D:



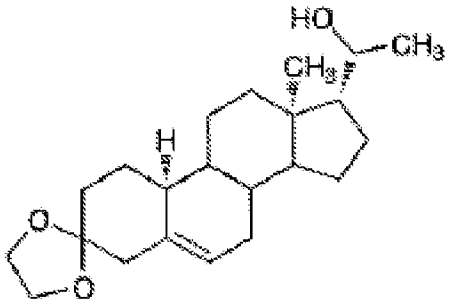
D.

In another embodiment, the C-20 steroid compound of Formula I is Compound E:



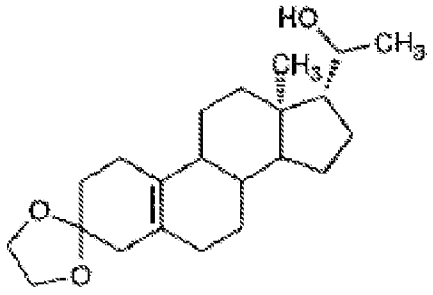
E.

In another embodiment, the C-20 steroid compound of Formula I is Compound F:



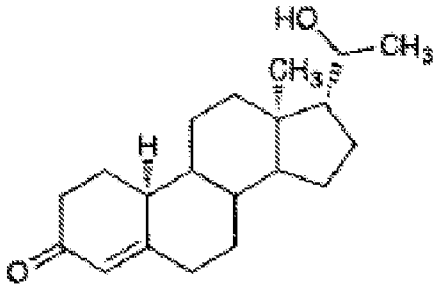
F.

In another embodiment, the C-20 steroid compound of Formula I is Compound



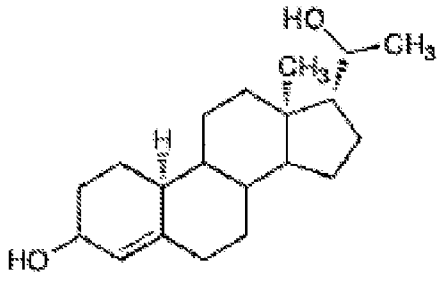
G.

In another embodiment, the C-20 steroid compound of Formula I is Compound H:



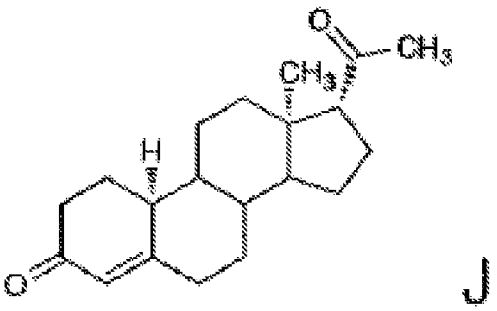
H.

In another embodiment, the C-20 steroid compound of Formula I is Compound I:



I.

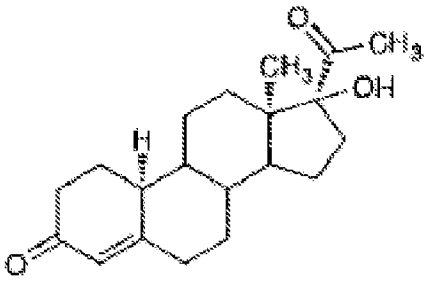
In another embodiment, the C-20 steroid compound of Formula I is Compound J:



J

(*ent*-19-Norprogesterone).

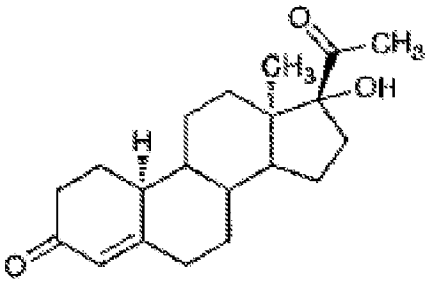
In another embodiment, the C-20 steroid compound of Formula I is Compound K:



K

(*ent*-17-((S)-Hydroxy)-19-norprogesterone).

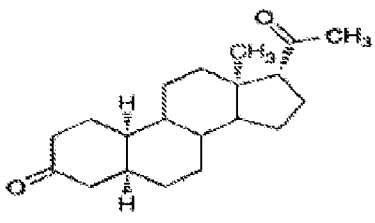
In another embodiment, the C-20 steroid compound of Formula I is Compound L:



L

(*ent*-17-((R)-Hydroxy)-19-norprogesterone).

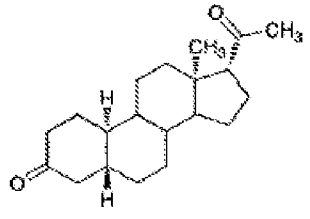
In another embodiment, the C-20 steroid compound of Formula I is Compound M:



M

(*ent*-5β-Dihydro-19-norprogesterone).

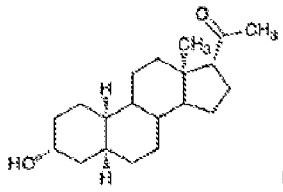
In another embodiment, the C-20 steroid compound of Formula I is Compound N:



N

(*ent*-5α-Dihydro-19-norprogesterone).

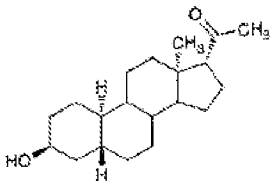
In another embodiment, the C-20 steroid compound of Formula I is Compound O:



O

(*ent*-19-Norepipregnanolone).

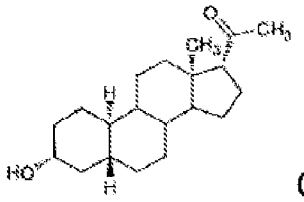
In another embodiment, the C-20 steroid compound of Formula I is Compound P:



P

(*ent*-19-Norallopregnanolone).

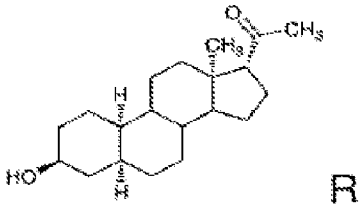
In another embodiment, the C-20 steroid compound of Formula I is Compound Q:



Q

(*ent*-19-Norisopregnanolone).

In another embodiment, the C-20 steroid compound of Formula I is Compound R:



R

(*ent*-19-orpregnanolone).

In another embodiment, the C-20 steroid compounds of Formula I represented by Compounds A-R exists as a single stereoisomer, wherein the stereochemistry at any center for which stereochemistry is not specified and can be either R or S.

In accordance with the present invention, the C-20 steroid compounds of Formulas I-IV are believed to be useful for treating, minimizing and/or preventing neuronal damage, such as neuronal damage, resulting from various injuries involving the brain, such as traumatic brain injury (TBI), whether the TBI is mild including concussions, moderate or severe traumatic brain injury.

Preferably, the C-20 steroid compounds of Formulas I-IV are believed to be useful to treat and/or prevent MTBI. In another embodiment, the C-20 steroid compounds of Formulas I-IV are believed to be useful to treat and/or prevent concussions.

In accordance with the present invention, the C-20 steroid compounds of formulas I-IV, especially ent-19 norprogesterone, may be administered in a dosage range of from about 0.05 mg/kg to 16 mg/kg, preferably from about 0.05 mg/kg to about 4 mg/kg and even more preferably from about 0.16 mg/kg to about 0.65 mg/kg or from about 1.13 mg/kg to about 45.2 mg/kg per 70 kg patient to treat, minimize and/or prevent TBI, including severe TBI, moderate TBI, mild TBI and concussions, preferably mild TBI, and even more preferable concussions. While the higher dosage ranges are preferred, it nevertheless should be understood that any effective amount, as used herein, to treat, minimize and/or prevent TBI, including severe TBI, moderate TBI, mild TBI and concussions, preferably mild TBI, and even more preferable concussions, is contemplated by the present invention. It is further contemplated that the C-20 steroid compounds of Formulas I-IV of the present invention can be administered through a number of routes of administration that include, e.g., oral, sublingual, intravenous, intraperitoneal, subcutaneous, intramuscular, intraabdominal, ocular, otic, intranasal, topical, transdermal, subcutaneous and rectal routes of administration.

The present invention further contemplates that in some embodiments, the C-20 steroid compounds can be formulated into, e.g., compositions or admixtures and administered in a dosage form selected from, e.g., a tablet, capsule, gelcap, caplet, powder, granule, liquid, solution, suspension, dispersion, pellet, bead, eyedrop, gel, cream, ointment, salve, balm, lotion or suppository. In other embodiments, the present invention contemplates that the C-20 steroid compounds may be administered as a formulation that is swallowed, injected, infused, inhaled, applied transdermally or topically, such as applied to the skin, eyes, ears, nose, lungs, mucosal membranes or any other membrane, or inserted into the rectum. Nonetheless, it should be understood by those versed in the art that preferred routes of administration to treat and/or prevent TBI, especially, mild TBI and concussions, as contemplated by the present invention, is the topical, e.g., pernasal or inhalation, or injection route of administration. In one embodiment, the present invention provides a C-20 steroid compounds of Formulas I-IV that is administered through a route selected from oral, sublingual, intravenous, intraperitoneal, ocular, intranasal, transdermal, subcutaneous, and rectal. In another embodiment, the C-20 steroid compounds of Formulas I-IV are administered orally. In another embodiment, the C-20 steroid compounds of Formulas I-IV are administered sublingually. In another embodiment, the C-20 steroid compounds of Formulas I-IV are administered by injection such as intravenously, intramuscularly, subcutaneously, or intraperitoneally. In another embodiment, the C-20 steroid compounds of Formulas I-IV are administered ocularly or otically. In another embodiment, the C-20 steroid compounds of Formulas I-IV are administered intranasally. In another embodiment, the C-20 steroid compound of Formulas I-IV are administered transdermally. In another embodiment, the C-20 steroid compounds of Formulas I-IV are administered subcutaneously. In another embodiment, the C-20 steroid compounds of Formulas I-IV is administered rectally. In another embodiment, the C-20 steroid compounds of Formulas I-IV are administered topically, including by inhalation.

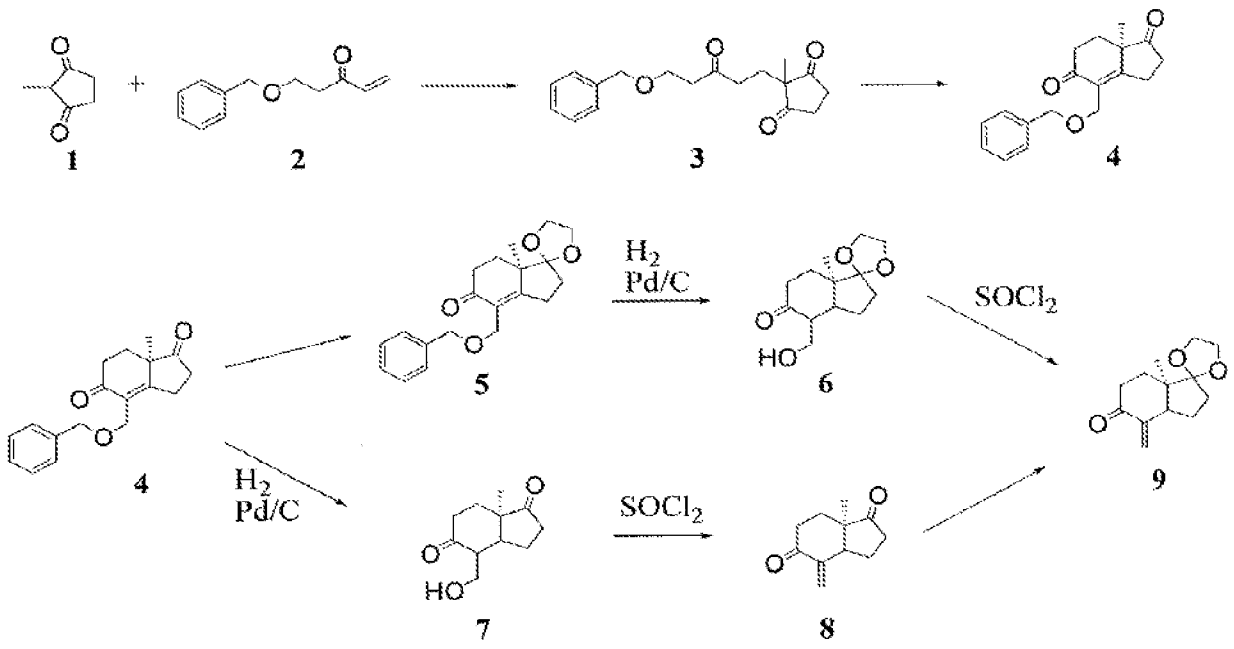
In one embodiment, the C-20 steroid compounds of Formulas I-IV are administered in a formulation selected from a tablet, capsule, gelcap, caplet, powder, solution, suspension, eyedrop, cream, ointment, lotion, gel or suppository. One of ordinary skill in the art will recognize that formulations that contain active agents of Formulas I-IV, may optionally contain co-therapeutic agents and inactive excipients. In addition one of ordinary skill in the art will recognize that liquid formulations contain a solvent and that said solvent may be either aqueous or organic.

In one embodiment, the C-20 steroid compounds of Formulas I-IV are administered as a formulation that is swallowed, injected, infused, inhaled, applied topically such as to the skin, eye, mucosal or other membranes and lungs, or inserted into the rectum. One of ordinary skill in the art will recognize that some formulations are intended for specific routes of administration while other formulations can be used in multiple routes of administration. For example, solution formulations may be injected, infused, deposited intraperitoneally, deposited subcutaneously, applied to the eye, sprayed or applied into the nose or inhaled as a nebulized liquid or suspension. Alternatively, tablets, capsules, gelcaps and caplets are intended to be swallowed.

Additionally, suppositories are intended for insertion into the rectum while creams, ointments and lotions are intended for topical applications.

The inventive methods of the present invention to make the C-20 steroid compounds of Formulas I-IV are illustrated in Schemes 1-15. In certain instances, reagents and solvents are listed. These reagents and solvents are exemplary and are not meant to be limited to the specific reagents or solvents shown.

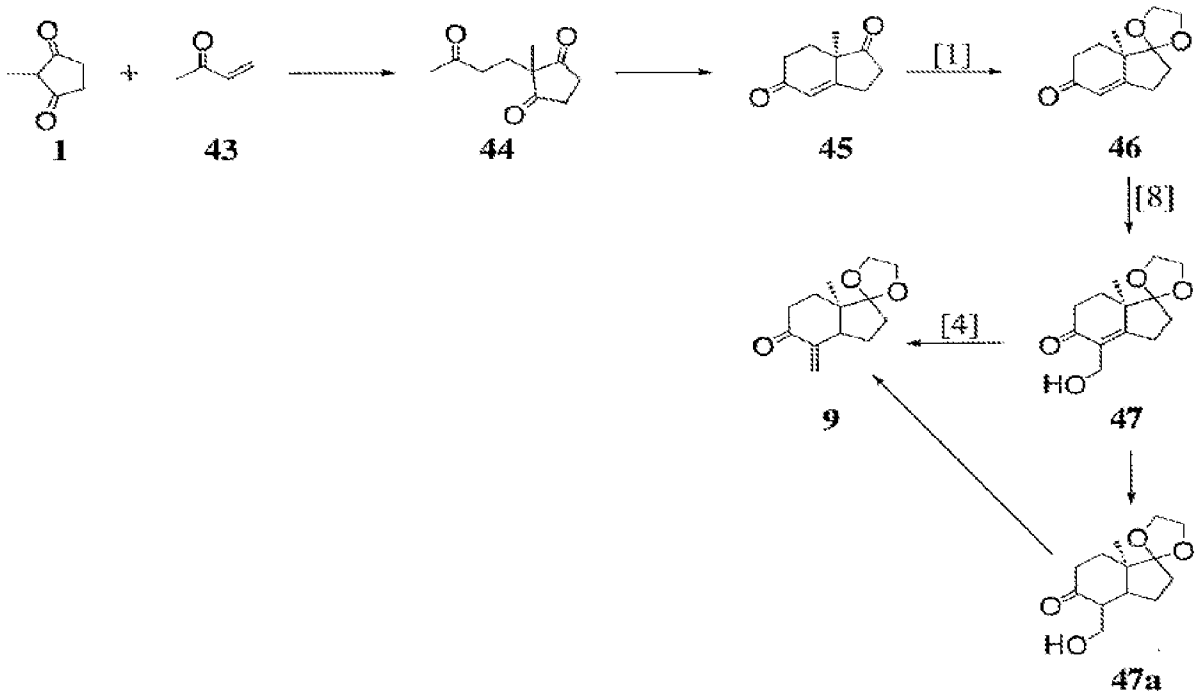
Scheme 1



Scheme 1 represents the formation of compound (9) via two alternative processes. In Scheme 1, (1) is reacted with (2) to produce (3). The preparation of compound (2) is described in Yamauchi, Noriaki; Natsubori, Yoshiaki; Murae, Tatsushi Bulletin of the Chemical Society of

Japan (2000), 73(11), 2513-2519). (3) is subjected to a stereoselective ring closing to form (4). Then (4) can be converted to (9) either: by selective protection of the carbonyl group to form (5) (as described in Bosch, M.P.; Camps, F.; Coll, J.; Guerrero, T.; Tatsuoka, T.; Meinwald, J. J. Org. Chem. 1986, 51, 773) followed by simultaneous hydrogenation of the ring double bond and cleavage of the benzyl ether to form (6) and elimination of the hydroxyl group therein with thionyl chloride; or by simultaneous hydrogenation of the ring double bond and cleavage of the benzyl ether to form (7) followed by elimination of the hydroxyl group therein with thionyl chloride to form (8) and protection of the carbonyl group (as described in Bosch, M.P.; Camps, F.; Coll, J.; Guerrero, T.; Tatsuoka, T.; Meinwald, J. J. Org. Chem. 1986, 51, 773).

Scheme 2



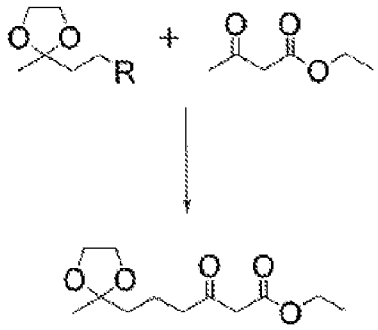
Scheme 2 represents an alternative to the formation of compound (9) of Scheme 1 from the combination of (1) and but-3-en-2-one (43). (1) and (43) are reacted to form (44) which is subjected to a stereoselective ring closing reaction to form (45). (45) is then selectively protected to form (46) (Bosch, M.P.; Camps, F.; Coll, J.; Guerrero, T.; Tatsuoka, T.; Meinwald, J. J. Org. Chem. 1986, 51, 773) which is subjected to a Baylis-Hillman reaction to form (47) (Satyanarayana reaction (Basavaiah, D.; Rao, A. J.; Satyanarayana, T. Chem. Rev. 2003, 103, 811). (47) is subjected to a Lewis acid facilitated reduction resulting in compound (9) of Scheme

1. Alternatively, (47) is hydrogenated giving (47a). Subsequent activation of the alcohol and elimination results in compound (9) of Scheme 1.

In certain embodiments, the conversion of (47a) to (9), and similar reactions, may utilize A1203 as a reagent.

One of ordinary skill in the art will recognize that activation of a beta-hydroxyketone and subsequent elimination reactions such as those described in Scheme 2 may be accomplished under a variety of conditions including, but not limited to KOH, methanesulfonyl chloride with diisopropylethylamine, para-toluenesulfonyl chloride with dimethylaminopyridine, DCC, pyridinium hydrochloride, alumina.

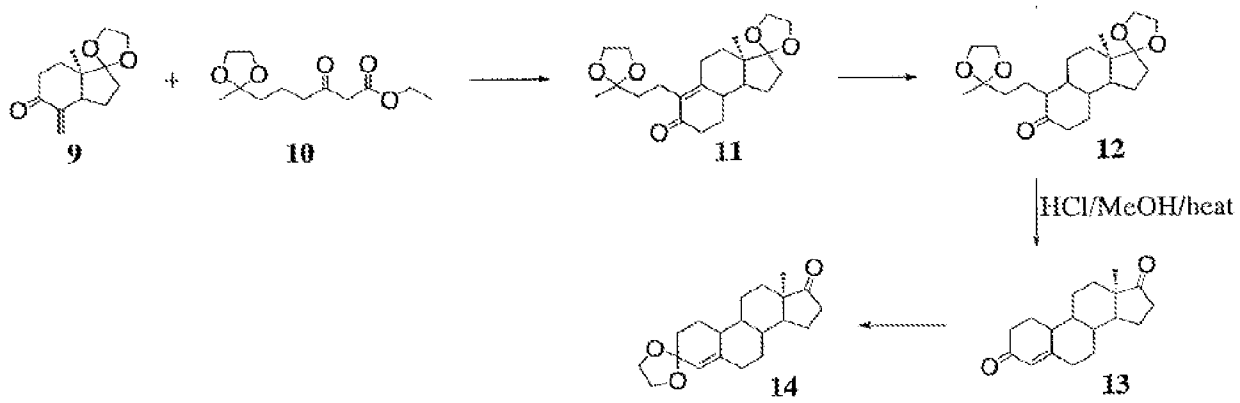
Scheme 3



10

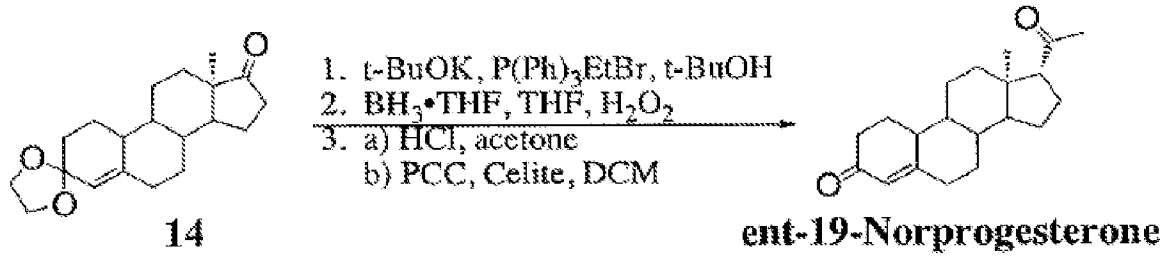
Scheme 3 represents a one step process to form compound (10) by reaction of substituted 2-ethyl-2-methyl-1,3-dioxolane a with ethyl 3-oxobutanoate. In certain embodiments, and without being limited thereto, leaving group R is -OTs, -OMs, -OTf, -CI, -Br, or -I. In still other embodiments, leaving group R is -OTs, -Br, or -I. In yet other embodiments, leaving group R is -Br.

Scheme 4



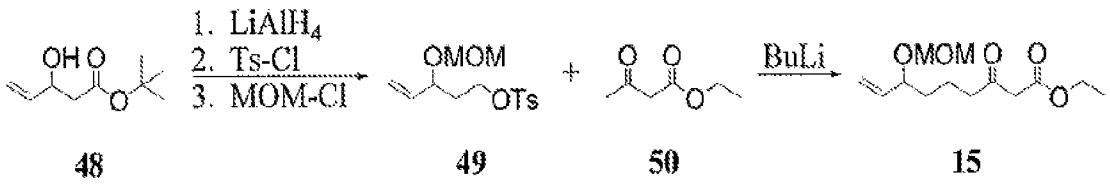
Scheme 4 represents the formation of compound (14) from the combination of (9) and (10). In Scheme 4, (9) and (10) are reacted to form (11) which is hydrogenated to form (12). (12) is then double deprotected and cyclized to form (13) which is selectively reprotected to form (14) (Tsunoda, T.; Suzuki, M.; Noyori, R. Tetrahedron Lett. 1980, 21, 1357).

Scheme 5



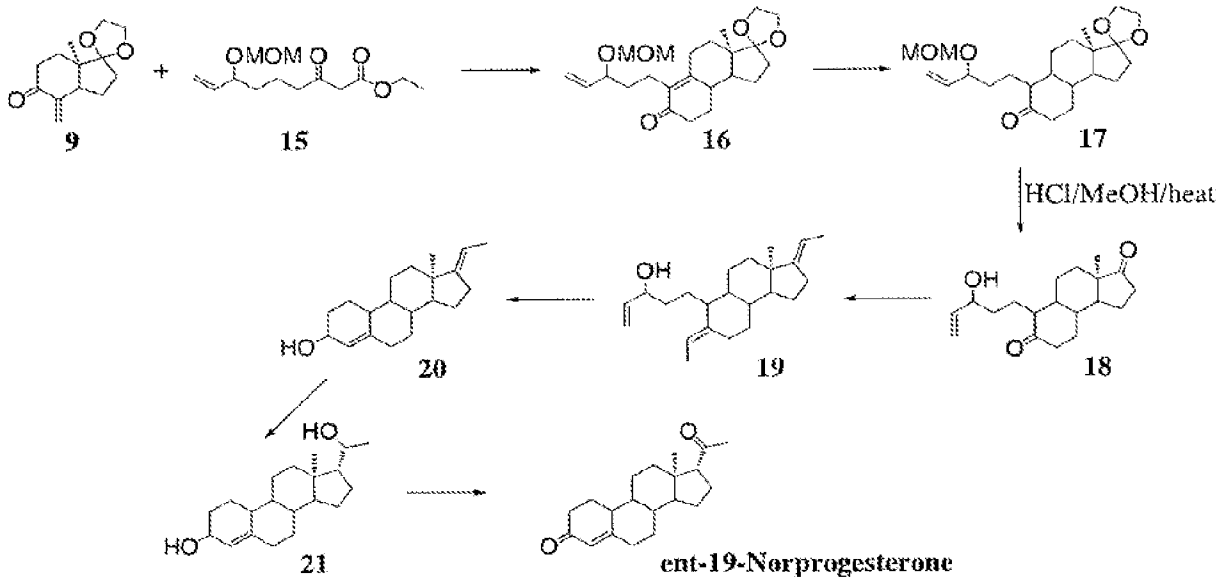
Scheme 5 represents the formation of ent-19-Norprogesterone from compound (14) of Scheme 4. In Scheme 5, (14) is reacted with potassium tert-butoxide and ethyl triphenylphosphonium bromide followed by hydroboration and oxidation to form ent-Progesterone. One of ordinary skill in the art will recognize that hydrolysis of the ketal protecting group can be done either before oxidation or after oxidation. One of ordinary skill in the art will further recognize that there are many reaction conditions and reagents suitable for the oxidation of an alcohol to a ketone and that alternatives to PCC include, but are not limited to, Swern, KMnO4, Dess-Martin, TEMPO and IBX.

Scheme 6



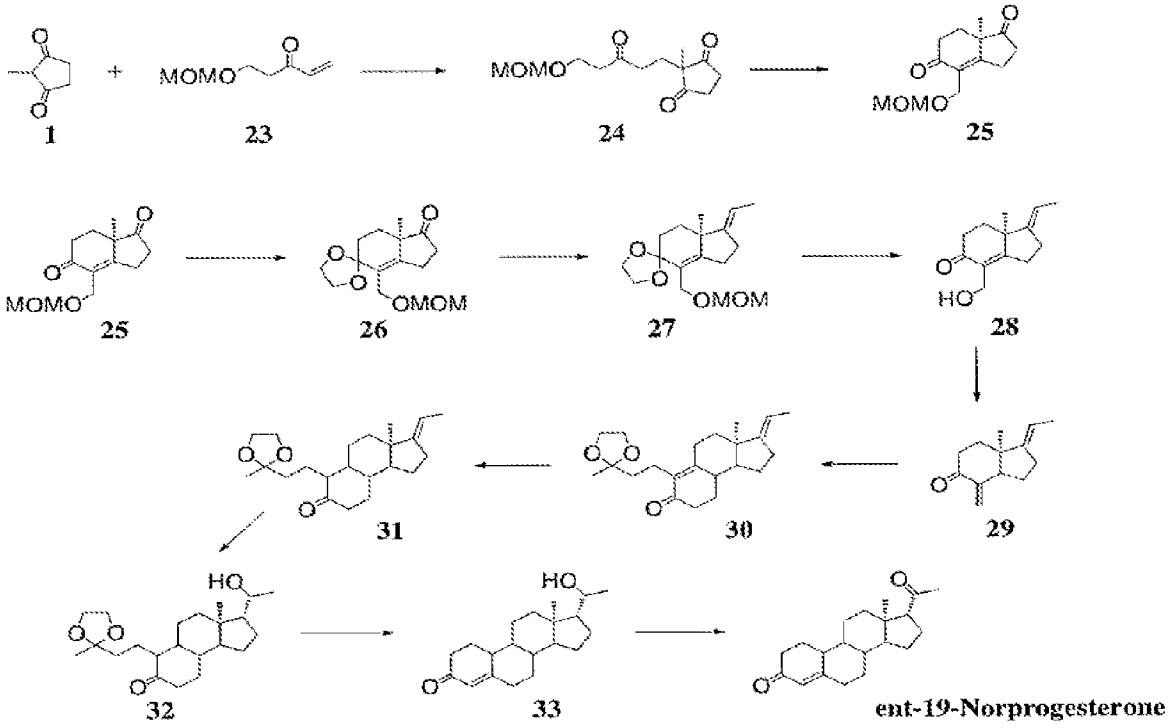
Scheme 6 represents the formation of compound (15) from the tert-butyl 3-hydroxypent-4-enoate (48) via reduction (Batt, Frederic and Fache, Fabienne, European Journal of Organic Chemistry, 2011(30), 6039-6055, S6039/1-S6039/46; 2011), formation of a tosylate and protection with a MOM (Methoxymethyl ether) protecting group to form (49). (49) is then reacted with ethyl 3-oxobutanoate (50) in the presence of a base to form (15).

Scheme 7



Scheme 7 represents the formation of ent-19-Norprogesterone from the combination of (9) from Scheme 1 and (15) from Scheme 6. In Scheme 7, (9) and (15) are reacted in a Robinson annulation to form (16) which is subjected to a Birch reduction or selective hydrogenation reaction to form (17). The MOM ether and ketal of (17) are simultaneously removed to form (18) which is then subjected to a double Wittig reaction to form (19). (19) then undergoes a ring closing metasthesis reaction to form (20) which is subjected to hydroboration reaction to form (21). Double oxidation of (21) results in formation of ent-19-Norprogesterone.

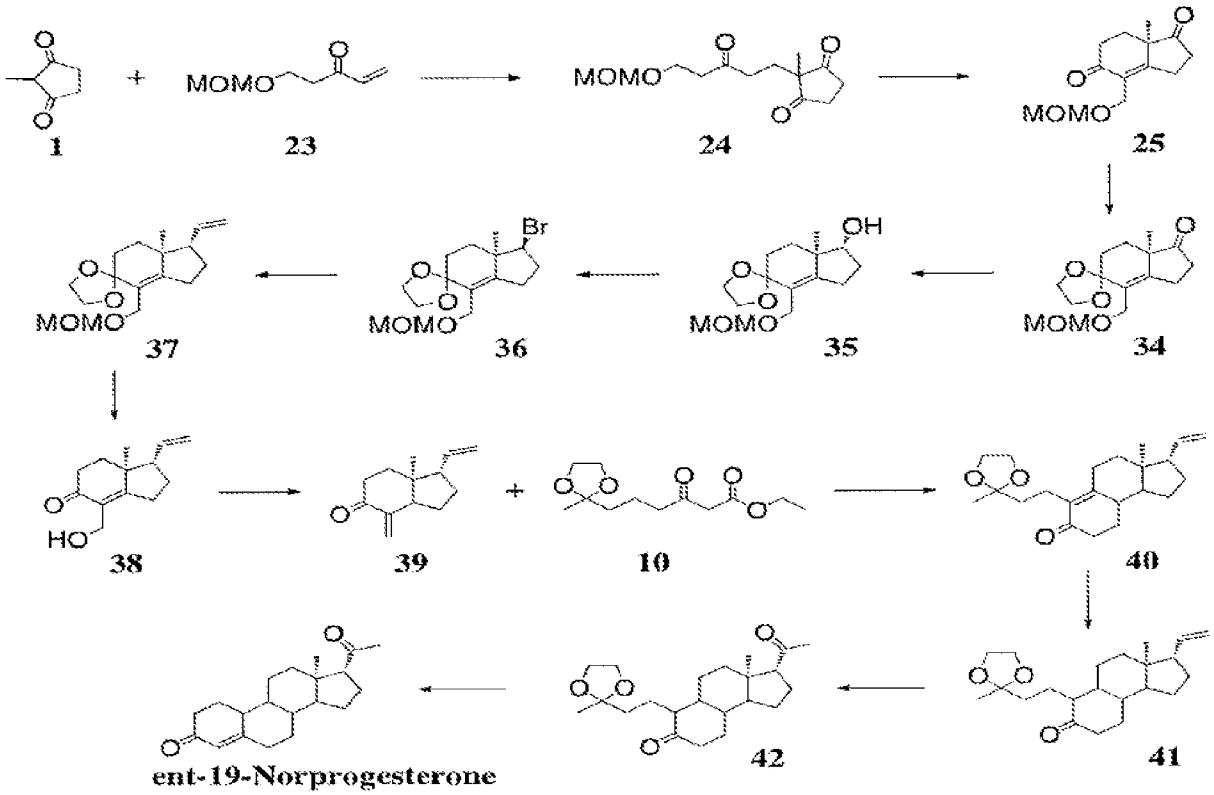
Scheme 8



Scheme 8 represents the formation of ent-19-Norprogesterone from the combination of (1) from Scheme 1 with a methoxymethylether protected compound (23). (1) and (23) are reacted to form (24) which is subjected to a stereoselective cyclization reaction to form (25). (25) is then selectively protected to form (26) (Tsunoda, T.; Suzuki, M.; Noyori, R. Tetrahedron Lett. 1980, 21, 1357) which is subjected to a Wittig reaction with ethyl triphenylphosphonium bromide to form (27). The MOM ether and the ketal of (27) are simultaneously hydrolyzed to form (28) which is then subjected to a Lewis acid facilitated reduction to form the exocyclic double bond in (29) (Das, Biswanath; Banerjee, Joydeep; Chowdhury, Nikhil; Majhi, Anjoy; Holla, Harish, Synlett (2006), (12), 1879-1882). (29) is subjected to a Robinson annulation with (10) from Scheme 3 to form (30) which is subjected to a Birch reduction or selective hydrogenation to form (31). (31) undergoes a hydroboration reaction to form (32). Hydrolysis of the ketal of (32) with tandem aldol cyclization forms (33). Oxidation of (33) results in ent-19-Norprogesterone.

In certain embodiments, the Lewis acid facilitated reduction is replaced by a hydrogenation and beta-elimination 2-step sequence.

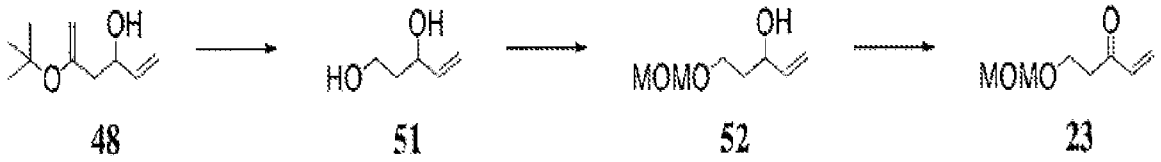
Scheme 9



Scheme 9 represents an alternative to formation of ent-19-Norprogesterone from Scheme 8. As illustrated, compound (25) is prepared as described in Scheme 8. Continuing, compound (25) is selectively protected to produce the acetal compound (34) (Tsunoda, T.; Suzuki, M.; Noyori, R. Tetrahedron Lett. 1980, 21, 1357) which is stereoselectively reduced to form the hydroxyl compound (35). (35) is brominated with inversion of stereochemistry to form (36) which is subjected to a nucleophilic displacement with a vinyl anion and inversion of stereochemistry to form (37). The MOM ether and ketal of (37) are simultaneously hydrolyzed to form (38) which is then subjected to Lewis acid facilitated reduction to form the exocyclic double bond in (39) (Das, Biswanath; Banerjee, Joydeep; Chowdhury, Nikhil; Majhi, Anjoy; Holla, Harish, Synlett (2006), (12), 1879-1882). (39) is reacted with compound (10) formed in Scheme 3 via a Robinson annulation to form (40) which is subjected to a Birch reduction or selective hydrogenation to form (41). (41) undergoes a Wacker oxidation to form (42). Tandem ketal hydrolysis and aldol cyclization of (42) results in ent-19-Norprogesterone.

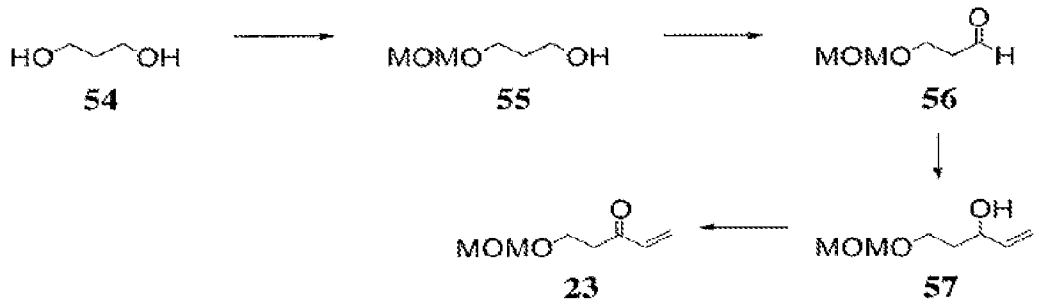
In certain embodiments, the Lewis acid facilitated reduction is replaced by a hydrogenation and beta-elimination 2-step sequence.

Scheme 10



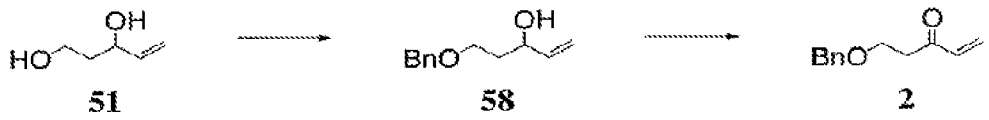
Scheme 10 represents the preparation of compound (23) illustrated in Scheme 9. This chemistry is adapted from a protocol for the preparation of a related compound (Batt, F.; Fache, F. Eur. J. Org. Chem. 2011, 6039). As illustrated, compound (48) is reduced to compound (50) (Scheme 6). The primary hydroxyl group of compound (51) (Batt, F.; Fache, F. Eur. J. Org. Chem. 2011, 6039) is then selectively converted to the corresponding methoxymethyl ether (52). Compound (52) is then oxidized to form compound (23).

Scheme 10a



Scheme 10a represents an alternative to the preparation of compound (23) illustrated in Scheme 10. This chemistry is adapted from a protocol for the preparation of a related compound (Batt, F.; Fache, F. Eur. J. Org. Chem. 2011, 6039). As illustrated, propylene glycol is converted to its mono-methoxymethyl ether compound (55). The free hydroxyl group is then oxidized to form the aldehyde of compound (56). The aldehyde is then converted to the allylic alcohol compound (57). Compound (57) is then oxidized to form compound (23).

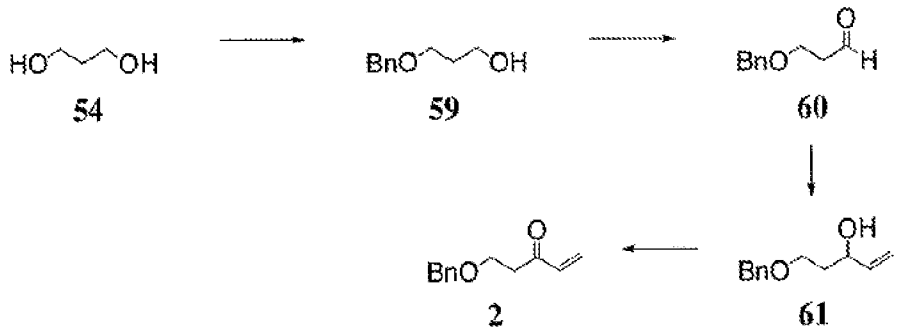
Scheme 11



Scheme 11 represents the preparation of compound (2) illustrated in Scheme 1. This chemistry is adapted from a protocol for the preparation of a related compound (Batt, F.; Fache, F.

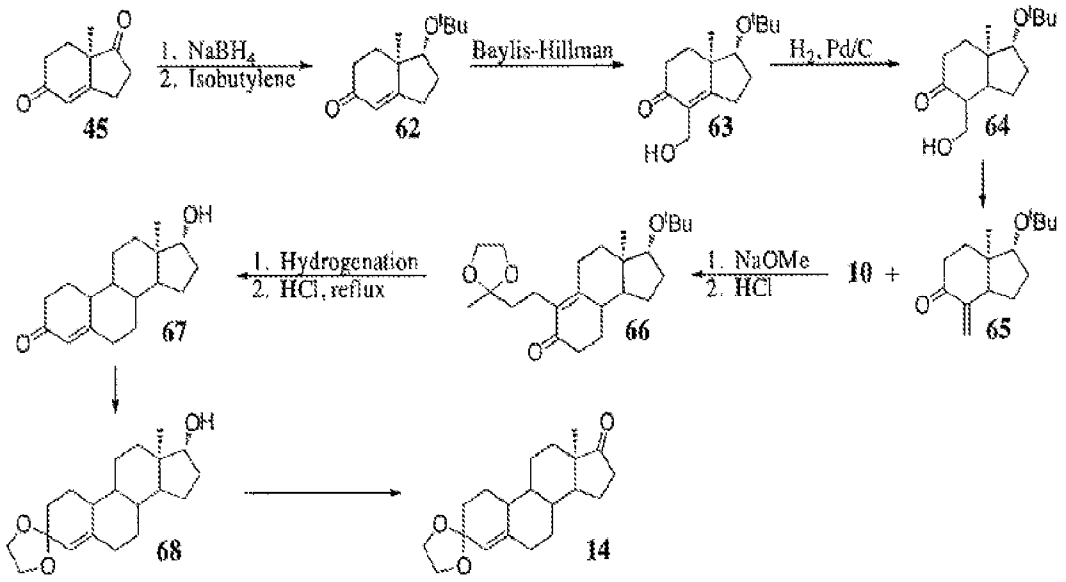
Eur. J. Org. Chem. 2011, 6039) and represents an alternative to the synthesis described in Yamauchi, Noriaki; Natsubori, Yoshiaki; Murae, Tatsushi Bulletin of the Chemical Society of Japan (2000), 73(11), 2513-2519). As illustrated, the primary hydroxyl group of compound (51) (Batt, F.; Fache, F. Eur. J. Org. Chem. 2011, 6039) is selectively converted to the corresponding benzyl ether (58). Compound (58) is then oxidized to form compound (2).

Scheme 11a



Scheme 11 a represents an alternative to the preparation of compound (2) illustrated in Scheme 11. This chemistry is adapted from a protocol for the preparation of a related compound (Batt, F.; Fache, F. Eur. J. Org. Chem. 2011, 6039) and represents an alternative to the synthesis described in Yamauchi, Noriaki; Natsubori, Yoshiaki; Murae, Tatsushi Bulletin of the Chemical Society of Japan (2000), 73(11), 2513-2519). As illustrated, propylene glycol is converted to its mono-benzyl ether compound (59). The free hydroxyl group is then oxidized to form the aldehyde of compound (60). The aldehyde is then converted to the allylic alcohol compound (61). Compound (61) is then oxidized to form compound (2).

Scheme 12



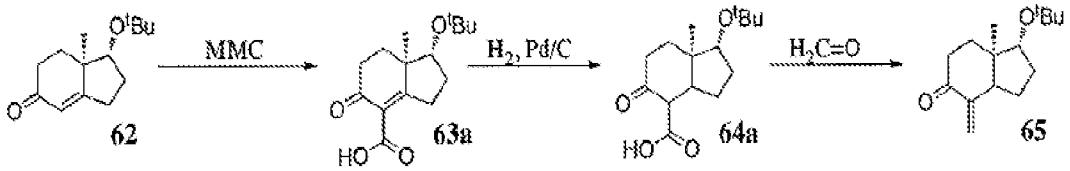
Scheme 12 provides an alternative synthesis of Compound (14) as described in Scheme 4. The synthesis includes the sequence converting compound (62) to compound (65) and the conversion of ent-19-nortestosterone (compound 67) to the dioxolane ketal compound (68).

Specifically, (45) is reduced and protected to form (62). (62) is subject to a Baylis-Hillman reaction to form (63) which is further reduced to form (64). (64) is subject to an elimination reaction to form the double bond in (65). (65) is reacted with Compound (10) from Scheme 3 to form (66) which is hydrogenated and cyclized to form ent-19-nortestosterone (67). ent-19-nortestosterone (67) is then ketal protected and reduced to form (14).

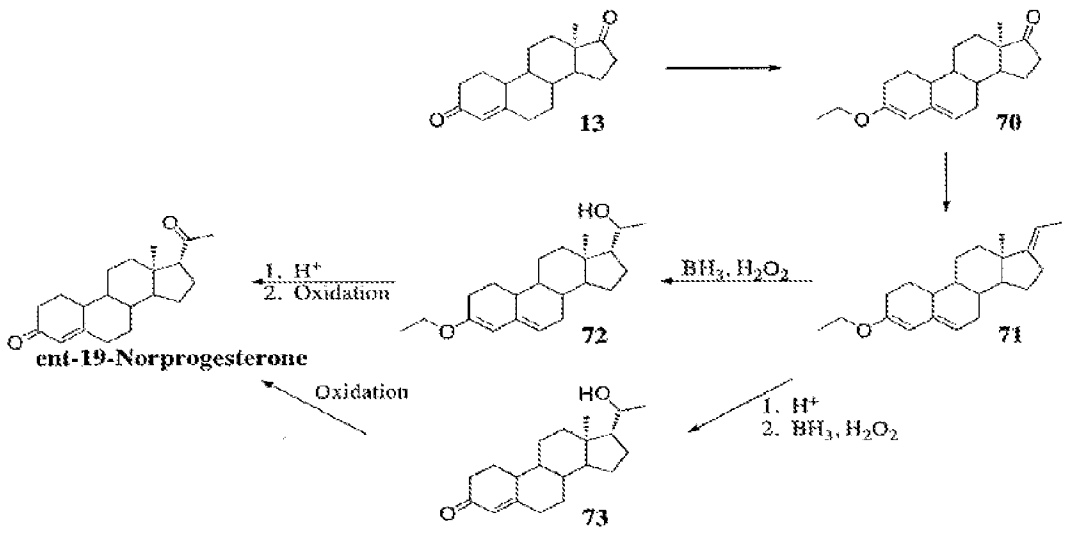
In certain embodiments, the conversion of compound (63) to compound (65) is accomplished in a single step comprising a Lewis acid facilitated reduction.

One of ordinary skill in the art will recognize that activation of a beta-hydroxyketone and subsequent elimination reactions such as those described in Scheme 12 may be accomplished under a variety of conditions including, but not limited to KOH, methanesulfonyl chloride with diisopropylethylamine, para-toluenesulfonyl chloride with dimethylaminopyridine, DCC, pyridinium hydrochloride, alumina.

Scheme 12a



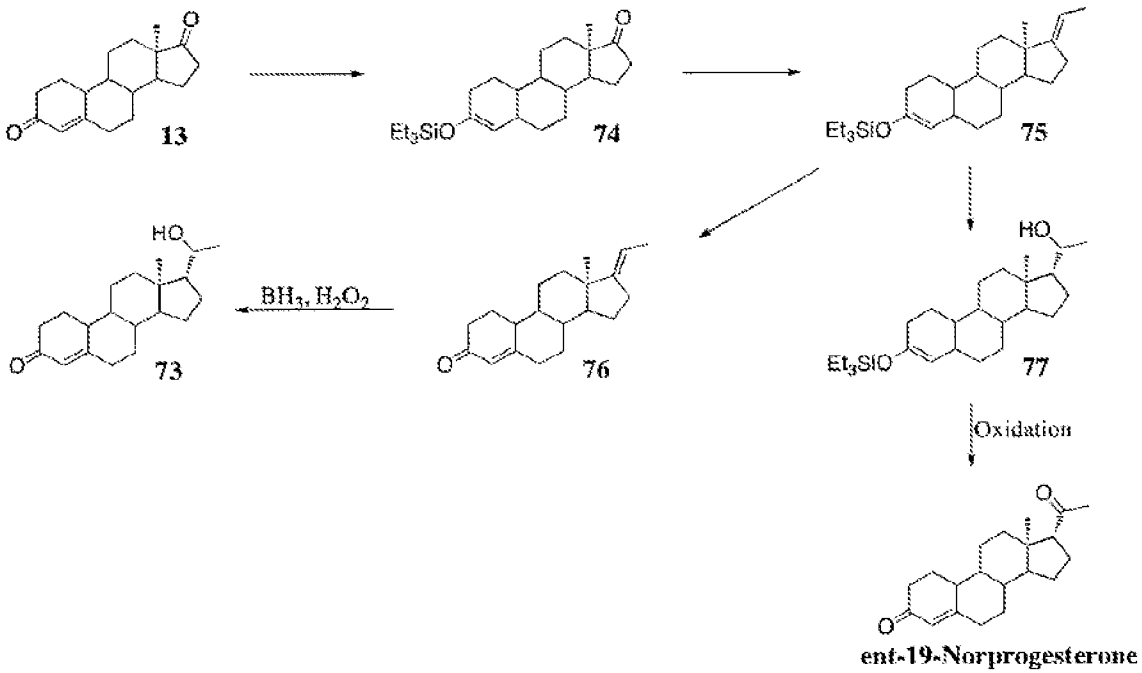
Scheme 12a provides an alternative conversion of compound (62) to compound (65). As illustrated, compound (62) is treated with methyl magnesium carbonate (MMC) forming the carboxylated product compound (63a). Catalytic hydrogenation reduces the olefin of compound (63a) forming compound (64a). Final decarboxylation in the presence of formaldehyde forms compound (65). In some embodiments, the conversion of compound (63a) to compound (64a) and the conversion of compound (64a) to compound (65) are distinct and separate synthetic steps. In other embodiments, the conversion of compound (63a) to compound (64a) and the conversion of compound (64a) to compound (65) are run in tandem. One of ordinary skill in the art will recognize that there are many catalysts useful for the reduction of a double bond to a single bond including, but not limited to, palladium on carbon, platinum on carbon, palladium hydroxide on carbon, palladium, platinum and Raney nickel.



Scheme 13

Scheme 13 represents an alternative continuation from compound (13) (Scheme 4) and depends upon the conversion of (13) to the ethyl enol ether compound (70) followed by the Wittig reaction generating compound (71). Reactions of this type are generally described by Antimo, et al., [Steroids 77 (2012) 250-254]. This sequence is completed by initial borane oxidation of (71) followed by hydrolysis of the enol ether and oxidation to form (72). Alternatively, (71) is initially hydrolyzed followed by borane oxidation giving compound (73).

Scheme 14



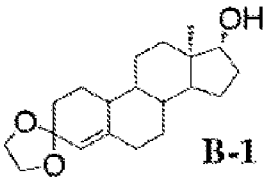
Scheme 14 represents an alternative to Scheme 13 and utilizes a reductive silylation to protect the enone of (13) to form (74). Protection of this type is generally described in Iwao, et al. [Tetrahedron Letters 49 (1972) 5085-5038] and Horiguchi, et al. [Journal of the American Chemical Society 111(16) (1989) 6259-6265]. Following borane oxidation of (75) to (77), oxidation of the alcohol and oxidative deprotection of the enone generates ent-19-Norprogesterone. Deprotection of this type is generally described by Yoshihiko, et al. [Journal of Organic Chemistry 43(5) (1978) 1011-1013].

Alternatively, the silyl enol ether (75) is initially oxidatively converted to (76) followed by borane oxidation to compound (73).

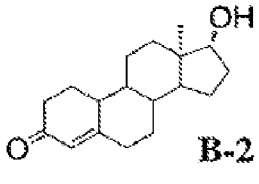
Active Intermediates

The particular process described in the methods of the invention can be utilized to prepare a number of useful intermediates. In certain embodiments, the intermediates have activity separate and apart from their usefulness in the preparation of ent-Progesterone. Specifically, in certain embodiments, the active intermediate compounds have activity in the treatment of traumatic brain injury. The present invention, in certain aspects, provides a method for the treatment of traumatic brain injury comprising administering a therapeutically effective amount of an active intermediate compound to a patient in need thereof.

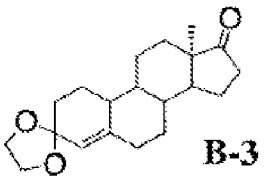
These active intermediate compounds include, but are not limited to,



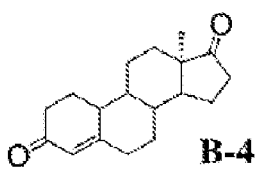
B-1



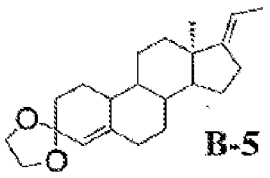
B-2



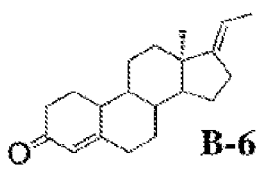
B-3



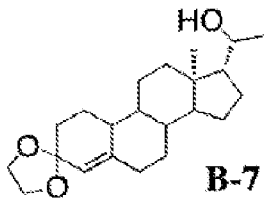
B-4



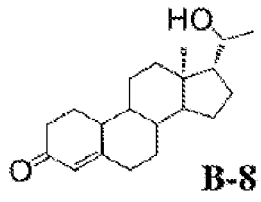
B-5



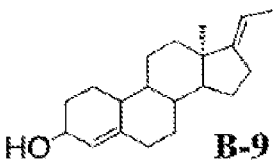
B-6



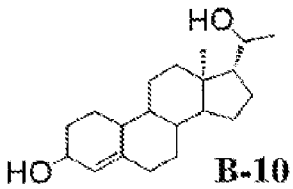
B-7



B-8

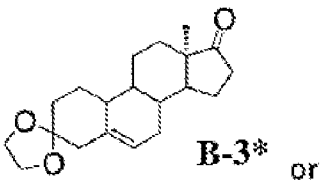


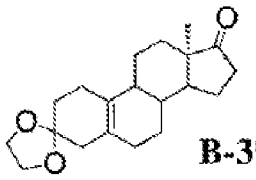
B-9



B-10

In each of the intermediates shown above, the double bond may migrate around the ring system, particularly into the second ring. For Example, intermediate B-3 may be represented as


B-3* or


B-3**.

Examples

Abbreviations and Acronyms

A comprehensive list of the abbreviations used by organic chemists of ordinary skill in the art appears in The ACS Style Guide (third edition) or the Guidelines for Authors for the Journal of Organic Chemistry. The abbreviations contained in said lists, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, each of which is incorporated herein by reference in its entirety.

More specifically, when the following abbreviations are used throughout this disclosure, they have the following meanings:

atm	atmosphere
br s	broad singlet
Buchi	rotary evaporator ®BUCHI Labortechnik AG
C	Celsius
$CDCl_3$	deuterated trichloromethane
Celite	diatomaceous earth filter agent ®Celite Corp.
d	doublet
dd	doublet of doublets
DIBAL-H	diisobutylaluminum hydride
DCM	dichloromethane
DMI	dimethyl-2-imidazolidinone
g	gram
h	hour, hours
$_1H$ NMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography

IN	inhalation
J	coupling constant (NMR spectroscopy)
L	liter
LAH	lithium aluminum hydride
LG	leaving group
M	mol L-1 (molar)
m	multiplet
MHz	megahertz
min	minute, minutes
mL	milliliter
pM	micromolar
mol	mole
MS	mass spectrum, mass spectrometry
m/z	mass-to-charge ratio
N	equivalents L-1 (normal)
NBS	N-bromo succinimide
NMO	N-Methylmorpholine-N-Oxide
NMR	Nuclear Magentic Resonance
pH	negative logarithm of hydrogen ion concentration
q	quartet
RBF	round bottom flask
r.t	room temperature
about RT	retention time (HPLC)
rt	room temperature
s	singlet
t	triplet
THE	tetrahydrofuran
TLC	thin layer chromatography
TsCI	tosyl chloride

The percentage yields reported in the following examples are based on the starting component that was used in the lowest molar amount. Air and moisture sensitive liquids and

solutions are transferred via syringe or cannula, and are introduced into reaction vessels through rubber septa. Commercial grade reagents and solvents are used without further purification. The term "concentrated under reduced pressure" refers to use of a Buchi rotary evaporator or equivalent equipment at approximately 15 mm of Hg. All temperatures are reported uncorrected in degrees Celsius (°C). Thin layer chromatography (TLC) is performed on pre-coated glass-backed silica gel 60 A F¬254 250 pm plates.

The structures of compounds of this invention are confirmed using one or more of the following procedures.

NMR

NMR spectra are acquired for each compound when indicated in the procedures below. NMR spectra obtained were consistent with the structures shown. Routine one-dimensional NMR spectroscopy was performed on a 300 MHz Brucker spectrometer. The samples were dissolved in deuterated solvents. Chemical shifts were recorded on the ppm scale and were referenced to the appropriate solvent signals, such as 2.49 ppm for DMSO-d6, 1.93 ppm for CD3CN, 3.30 ppm for CD3OD, 5.32 ppm for CD2Cl2 and 7.26 ppm for CDCI3 for 1H spectra.

Materials

Equipment used in the execution of the chemistry of this invention include but is not limited to the following:

- Low temperature vacuum pump — Zhengzhouchangcheng Experimental Equipment Co., Ltd (Model # DLSB-10/20)
- Rotary evaporator - Shanghaizhenjie Experimental Equipment Co., Ltd (Model # RE-52CS)
- Oil pump - Shanghai Vacuum pump factory (Model # 2XZ-4)
- Mechanical stirrer - Beijingshijiyuhua Experimental Equipment Co., Ltd (Model # DW-3-300)
- Vacuum drying oven - Beijinglianhekeyi Experimental Equipment Co., Ltd (Model # DZF-6020)
- LCMS — Agilent (Model # 1200-6100)
- GCMS — Agilent (Model # 7890A-5975C)
- GC — Agilent (Model # 7890A)
- Chiral HPLC — Shimadzu (Model # LC-20AT)

- NMR — Bruker (Model # AVANCEB1300)
- Liquid chromatograph — Agilent (Model # G1322A)
- High temperature oil bath — SMS (Model # 00508)
- Electronic balance — LBTEC (Model # XS205DU)

Chemicals and solvents that are used in the experimental workups are purchased from either Sigma Aldrich, Fisher Scientific or EMD unless otherwise stated and the solvents used are either ACS or HPLC grade with the two grades being used interchangeably. For TLC analysis, the silica 60 gel glass backed TLC plates are used.

EXAMPLE 1 - Preparation of compound 3 (Scheme 1)

2-Methyl-1,3-pentanedione (1 g, 1.2 eq.) is dissolved in anhydrous acetonitrile (40 mL) and 5-benzyloxy-pent-1-ene-2-one (1.5 g, 1.0 eq.) is added followed by triethylamine (50 mg, 0.05 eq.). The reaction is stirred at 25-30 deg C for 12 hours after which, it is concentrated to dryness. Purification of the residue on silica gel (Ethyl acetate/Hexane 1/5) gives compound 3 (1.8 g) as a colorless oil. 1H NMR (300 MHz, CDCI3): 6 1.10 (s, 3H), 1.90 (t, 2H), 2.50 (t, 2H), 2.65 (t, 2H), 2.70-2.90 (m, 4H), 3/0 (t, 2H), 4.50 (s, 2H), 7.25-7.4 (m, 5H). MS (M+ + 1) 303.1.

EXAMPLE 2 - Preparation of compound 46 (Scheme 2)

2-Ethyl-2-methyl-1,3-dioxolane (120mL) and compound 45 (20 g, 1.0 eq.) are combined under nitrogen. Ethylene glycol (1.2 mL, 0.14 eq.) is added followed by p-toluenesulfonic acid (390 mg, 0.02 eq.). The reaction is stirred at 25-30 deg C for 96 hours until the concentration of compound 45 is less than 20% as measured by HPLC. Ethyl acetate (100 mL) is added and the resulting mixture is washed with water (2 x 100 mL), is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/20) yielding compound 46 (8 g) as a colorless oil. 1H NMR (300 MHz, CDCI3): 6 1.20-1.35 (m, 7H), 1.60-1.70 (m, 1H), 1.90-2.00 (m, 1H), 2.10-2.80 (m, 6H), 3.85-4.05 (m, 4H), 5.85 (s, 1H). MS (M+ + 1) 209.1.

EXAMPLE 3 - Preparation of compound 47 (Scheme 2)

Compound 46 (8.0 g, 1.0 eq.) is added to a mixture of 1,4-dioxane (40 ml) and water (34 mL). Formaldehyde (3.1 g, 1.0 eq.) is then added followed by 1,4-diazabicyclo[2.2.2]octane

(DABCO, 8.5 g, 1.0 eq). The reaction is stirred at 25-30 deg C for 120 hours after which, ethyl acetate (100 mL) is added. The mixture is washed with water (2 x 100 mL), is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. Purification of the residue on silica gel (10% ethyl acetate in hexane) gives compound 47 (5 g) as a colorless oil. 1H NMR (300 MHz, CDCI3): 6 1.25 (m), 1.65 (m, 1H), 1.95 (m, 1H), 2.15-2.80 (m), 3.90-4.05 (m), 5.80 (s, 1H).

EXAMPLE 4 - Preparation of compound 47a (Scheme 2)

Compound 47 (2 g) is dissolved in anhydrous tetrahydrofuran (THF, 200 mL) under a nitrogen atmosphere. 10% Pd/C (200 mg) is added and the reaction is placed under a hydrogen atmosphere. The reaction is stirred at -10-0 deg C over 40 hours after which, the Pd/C is removed by filtration. The filtrate is concentrated to dryness and the residue is purified on silica gel (10% ethyl acetate/hexane) giving compound 47a (1.6 g) as a colorless oil. 1H NMR (300 MHz, DMSO-d6): 6 0.95-1.15 (m, 1H), 1.55-2.10 (m), 2.50 (t, 2H), 2.40-2.50 (m, 1H), 2.70-2.80 (q, 1H), 3.15-3.30 (m, 1H), 3.65-3.90 (m), 4.35 (dd, 1H). MS (M+ + 1) 241.1.

EXAMPLE 5 - Preparation of compound 9 (Scheme 2)

Compound 47a (300 mg, 1.0 eq.) is dissolved in dichloromethane (DCM, 3 mL) and triethylamine (TEA, 3.0 eq.) is added. The mixture is cooled to — 10 deg C under nitrogen and methanesulfonyl chloride (1.2 eq.) is added dropwise. Stirring is continued at 10-20 deg C for 4 hours after which, toluene (3 mL) is added followed by 1,8-diazabicycloundec-7-ene (DBU, 3.0 eq.). Stirring is continued at 25-30 deg C for an additional 40 hours after which, the reaction is washed with water (2 x 3 mL), is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/10) giving compound 9 (100 mg) as a colorless oil. 1H NMR (300 MHz, DMSO-d6): ö 1.00 (s, 3H), 1.40-1.60 (m, 2H), 1.70¬2.00 (m, 4H), 2.30-2.55 (m, 2H), 2.80 (m, 1H), 3.80-3.95 (m, 4H), 5.20 (s, 1H), 5.70 (s, 1H). MS (M+ + 1) 223.1.

EXAMPLE 6 - Preparation of compound 10 (Scheme 3)

Sodium hydride (426 mg, 1.2 eq.) is placed under nitrogen and cooled to 0 deg C. Tetrahydrofuran (THF, 10 mL) is added followed by hexamethylphosphoramide (HMPA, 326

mg, 0.25 eq.). Ethyl acetoacetate (1 mL, 1.0 eq.) is added and the mixture is stirred at 0 deg C for 10 minutes. n-Butyllithium (2.5M, 3.6 mL, 1.1 eq.) was added and the mixture is stirred at 0 deg C for an additional 10 minutes. 2-(2-methyl-1,3-dioxolan-2-yl)ethylbromide (1.6 g, 1.0 eq.) is added and the reaction is stirred at 0 deg C for 30 minutes. The reaction is quenched with aqueous oxalic acid (10%, 20 mL) and is washed with dichloromethane (DCM, 3 x 20 mL). The organic phase is additionally washed with saturated aqueous sodium bicarbonate (30 mL) and brine (30 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated. The residue is purified on silica gel (ethyl acetate/hexane 1/30) giving compound 10 (600 mg) as a yellow oil. 1H NMR (300 MHz, DMSO-d6): ö 1.25 (t, 3H), 1.30 (s, 3H), 1.60-1.80 (m, 4H), 2.60 (t, 2H), 3.45 (s, 2H), 3.90-4.00 (m, 4H), 4.15-4.25 (q, 2H).

EXAMPLE 7 - Preparation of compound 11 (Scheme 4)

Compound 9 (500 mg, 1.0 eq.) is dissolved in methanol (15 mL) and compound 10 (715 mg, 1.3 eq.) is added. Sodium methoxide (0.2eq)is added and the mixture is stirred at 30 deg C for 16 hours. Aqueous sodium hydroxide (5 M, 5.0 eq.) is added and the reaction is stirred for an additional 4 hours at 30 deg C. The methanol is then removed utilizing a rotary evaporator. Water (5 mL) is then added and the mixture is washed with toluene (2 x 3 mL). The aqueous phase is cooled to 0 deg C and is acidified to pH 6 with aqueous HCI (6 N). The mixture is washed with ethyl acetate and the organic extract is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/10) giving compound 11 (150 mg) as a colorless oil. MS (M+ + 1) 377.1.

EXAMPLE 8 - Preparation of ent-19-Norprogesterone (Scheme 5)

(a) Wittig Reaction

Ethyl triphenylphosphonium bromide (2.8 g, 3 equivalents) and potassium tert-butoxide (1.0 g, 3.0 equivalents) are combined in anhydrous tert-butanol (10 mL) under nitrogen. The mixture is heated to 75-80 deg C for 20 minutes after which, compound 14 (1.0 g, 1 equivalent) is added. The reaction is stirred at 75¬80 deg C for 3 hours after which, it is cooled to 20-25 deg C and is quenched with brine (20 mL). The resulting mixture is washed with ethyl acetate (3 x 20 mL). The combined organic extracts are dried over anhydrous sodium sulfate, are filtered and are

concentrated to dryness. The residue is purified on silica gel (10% ethyl acetate/hexane) giving the desired Wittig product in 90% yield. MS (M+ + 1) 329.3

(b) Borane Hydration

The Wittig product from part (a) (1.0 g, 1 equivalent) is placed under a nitrogen atmosphere and is dissolved in anhydrous tetrahydrofuran (THF, 100 mL). Borane-THF complex (1 M in THF, 3.0 mL, 1 equivalent) is added and the reaction is stirred at 20-25 deg C for 3 hours. The reaction is then concentrated to dryness and sodium hydroxide solution (10% in water, 50 mL) is added followed by hydrogen peroxide solution (30% in water, 0.5 mL). The resulting mixture is stirred at 20-25 deg C for an additional 1 hour after which, water (100 mL) is added. The mixture is then washed with dichloromethane (2 x 100 mL) and the combined organic extracts are washed with brine (50 mL). Concentration of the organic phase yields the crude alcohol which is used in the following step without purification.

(c) Ketal Hydrolysis

The crude product from step (b) (2.0 g, 1 equivalent) is dissolved in acetone (20 mL) and hydrochloric acid (30% in water, 20 mL) is added. The reaction is stirred at 20-25 deg C for 30 minutes after which, it is concentrated to dryness. The residue is dissolved in ethyl acetate (50 mL) and water (30 mL) is added. After stirring vigorously for 5 minutes, the phases are separated and the organic phase is washed with saturated aqueous sodium bicarbonate (2 x 25 mL) and brine (25 mL). The organic phase is then concentrated to dryness and the residue is purified on silica gel (10% ethyl acetate/hexane) giving the desired enone in 45% overall yield from the Wittig product. 1H NMR (300 MHz, DMSO-d6): 6 5.70 (s, 1H), 4.15 (d, 1H), 3.40-3.50 (m, 1H), 2.40-2.45 (m, 1H), 2.10-2.35 (m, 5H), 1.70-1.85 (m, 4H), 1.50-1.60 (m, 2H), 1.40-1.50 (m, 1H), 1.25-1.35 (m, 1H), 1.15-1.25 (m, 2H), 0.90-1.15 (m, 7H), 1.85-1.95 (m, 1H), 0.65 (s, 3H). MS (M+ + 1) 303.2.

(d) Oxidation to ent-19-Norprogesterone

Sodium acetate (1.20 g, 10 equivalents), pyridinium chlorochromate (PCC, 1.90 g, 4 equivalents), and the enone from step (c) (0.5 g, 1 equivalent) are combined with dichloromethane (50 mL) under nitrogen. The mixture is stirred at 20-25 deg C for 3 hours after which, it is filtered. The filter cake is washed with dichloromethane and the combined filtrates are concentrated to dryness. The residue is purified on silica gel (30% ethyl acetate/hexane) giving ent-19-norprogesterone in 90% yield. 1H NMR (300 MHz, DMSO-d6): 6 5.70 (s, 1H), 2.55-2.60 (t, 1H), 2.40-2.50 (m,

1H), 2.10-2.35 (m, 5H), 2.05 (s, 3H), 1.95-2.05 (m, 1H), 1.70-1.90 (m, 2H), 1.10-1.70 (m, 9H), 0.90-1.10 (m, 1H), 0.75-0.90 (m, 1 H), 0.60 (s, 3H). MS (M+ + 1) 301.1.

EXAMPLE 9 - Preparation of compound 48 (Scheme 6)

Compound 48 is prepared as described by Batt, et al. (Eur. J. Org. Chem., 2011, 6039-6055).

EXAMPLE 10 - Preparation of compound 49 (Scheme 6)

Compound 48 (100 g) is reduced to the corresponding alcohol using lithium aluminum hydride as described by Batt, et al. (Eur. J. Org. Chem., 2011, 6039-6055). The resulting diol (1 g, 1.0 eq.) is dissolved in dichloromethane (DCM, 10 mL) under nitrogen. Triethylamine (2.0 eq.) is added and the resulting mixture is cooled to 0 deg C. Para-toluenesulfonyl chloride (1.0 eq.) is added slowly and the reaction is stirred at 0 deg C for 30 minutes. The resulting mixture is washed with water (10 mL) after which, it is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/10) giving the desired primary tosylate (500 mg) as a yellow oil. The resulting primary tosylate (100 mg, 1.0 eq.) is dissolved in DCM (10 mL) under nitrogen. Diisopropylethyl amine (DIEA, 1.2 eq.) is added and the mixture is cooled to 0 deg C. Methoxymethyl chloride (1.0 eq) is added dropwise and the reaction is stirred from 0-25 deg C over 2 hours after which, it is washed with water (10 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (Ethyl acetate/hexane 1/20) giving the desired compound 49 (60 mg) as a yellow oil.

EXAMPLE 11 - Preparation of compound 24 (Scheme 9)

2-Methyl-1,3-cyclopentanedione (3.0 g, 1.2 eq.) is combined with compound 23 (3.1 g, 1.0 eq.) and acetonitrile (ACN, 30 mL). Triethylamine (TEA, 110 mg, 0.05 eq) is added and the reaction is stirred at 25 deg C for 4 hours. Dichloromethane (DCM, 100 mL) is then added and the mixture is washed with aqueous hydrochloric acid (2 x 30 mL) and saturated aqueous sodium bicarbonate (2 x 30 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/30)

giving compound 24 (2.6 g) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 1.10 (s, 3H), 1.90 (t, 2H), 2.50 (t, 2H), 2.65 (t, 2H), 2.70-2.90 (m, 4H), 3.35 (s, 3H), 3.75 (t, 2H), 4.60 (s, 2H).

EXAMPLE 12 - Preparation of compound 52 — 5-Methoxymethoxy-pent-1-ene-3-ol (Scheme 10)

Compound 48 (100 g) is reduced to the corresponding alcohol using lithium aluminum hydride as described by Batt, et al. (Eur. J. Org. Chem., 2011, 6039-6055). The resulting diol (13 g, 1 eq.) is added to a mixture of cyclohexane (26 mL), dichloromethane (DCM, 13 mL) and diisopropyl ethylamine (DIEA, 18 g, 1.1 eq.) under nitrogen. Methoxymethyl chloride (1 eq.) is added dropwise and the reaction is stirred at 20 deg C for 12 hours. DCM (100 mL) is then added and the mixture is washed with aqueous hydrochloric acid (2 M, 30 mL) and saturated aqueous sodium bicarbonate (2 x 30 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (10% ethyl acetate/hexane) giving the primary MOM ether (compound 52, 4 g) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 1.75-1.95 (m, 2H), 3.35 (s, 3H), 3.65-3.80 (m, 2H), 4.30-4.35 (m, 1H), 4.65 (s, 2H), 5.10-5.15 (m, 1H), 5.25-5.30 (m, 1H), 5.85-5.95 (m, 1H).

EXAMPLE 13 - Preparation of compound 23 - 5-Methoxymethoxy-pent-1-ene-3-one (Scheme 10)

Compound 52 (3.5 g, 1.0 eq.) is dissolved in dimethyl sulfoxide (DMSO, 20 mL) under nitrogen. 2-Iodoxybenzoic acid (IBX, 9.8 g, 1.5 eq.) is added and the reaction is stirred at 20 deg C for 12 hours. DCM (100 mL) is added and the resulting mixture is washed with saturated aqueous sodium sulfite (30 mL) and saturated aqueous sodium bicarbonate (30 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (Ethyl acetate/hexane 1/30) giving the desired compound 23 (3.1 g) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 2.90 (t, 2H), 3.35 (s, 3H), 3.90 (t, 2H), 4.65 (s, 2H), 5.90 (d, 1H), 6.20-6.45 (m, 2H).

EXAMPLE 14 - Preparation of compound 55 (Scheme 10a) - 3-Methoxymethyl propa n-1-01

Cyclohexane (180 mL), dichloromethane (90mL) and diisopropylethylamine (34 g, 1.1 eq.) are combined and propane-1,3-diol (20 g, 1.0 eq.) is added. Methoxymethyl chloride (20.9 g, 0.99 eq.) is added dropwise maintaining the internal reaction temperature at 20 deg C. The reaction is stirred at 20 deg C for 12 hours after which, dichloromethane (100 mL) is added. The mixture is washed with saturated aqueous sodium bicarbonate (2 x 30 mL), is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/5) giving compound 55 (5 g) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 1.80-1.90 (m, 2H), 3.40 (s, 3H), 3.70 (t, 2H), 3.80 (t, 2H), 4.65 (s, 2H).

EXAMPLE 15 - Preparation of compound 56 (Scheme 10a) - 3-Methoxymethyl pro pionaldehyde

Compound 55 (1g, 1.0 eq.) is dissolved in dimethylsulfoxide (10 mL) and 2-lodoxybenzoic acid (IBX, 3.5 g, 1.5 eq.) is added. The reaction is stirred at 20 deg C for 12 hours after which, it is washed with saturated aqueous sodium sulfite (20 mL) and is saturated aqueous sodium bicarbonate (20 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/20) giving compound 56 (0.3 g, 60% purity) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 1.80-1.90 (m, 2H), 3.40 (s, 3H), 3.70 (t, 2H), 3.80 (t, 2H), 4.65 (s, 2H).

EXAMPLE 16 - Preparation of compound 2 (Scheme 11)

Compound 2 is reported by Yamauchi, et al. (Bull. Chem. Soc. Jpn., 2001, 2513-2519). The Scheme 11 sequence for preparation of compound 2 is adapted from Batt, et al. (Eur. J. Org. Chem., 2011, 6039-6055).

EXAMPLE 17 - Preparation of compound 2 (Scheme 11a)

Propylene glycol (500 g) is combined with benzyl bromide (100 g, 1.0 eq.) under nitrogen. Sodium hydroxide (28 g, 1.2 eq.) is added and the mixture is stirred at 20 deg C for 4 hours. Ethyl acetate (800 mL) is then added and the mixture is washed with water (500 mL). The

organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness giving the desired crude 3-benzyloxypropanol (100 g) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 1.85-1.90 (m, 2H), 3.65 (t, 2H), 3.80 (t, 2H), 4.25 (t, 1H), 4.55 (s, 2H), 7.25-7.40 (m, 5H). Crude 3-benzyloxypropanol (100 g, 1.0 eq.) is combined with dimethyl sulfoxide (DMSO, 500 mL) and tetrahydrofuran (THF, 500 mL) under nitrogen. 2-Iodoxybenzoic acid (IBX, 253 g, 1.5 eq.) is added and the reaction is stirred at 20 deg C for 12 hours. Ethyl acetate (1500 mL) is then added and the mixture is washed with saturated aqueous sodium sulfite (500 mL) and saturated aqueous sodium bicarbonate (500 mL). The organic phase is washed with anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/20) giving the desired 3-benzyloxypropionaldehyde (30 g) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 2.70 (m, 2H), 3.80 (t, 2H), 4.55 (s, 2H), 7.25-7.40 (m, 5H), 9.80 (s, 1H). 3-benzyloxypropionaldehyde (30 g, 1.0 eq.) is dissolved in THF under nitrogen and is cooled to 0 deg C. Vinylmagnesium bromide(1M, 220 mL, 1.2 eq.) is added and the reaction is stirred at 0 deg C for 1 hour. Saturated aqueous ammonium chloride (100 mL) is then added and the mixture is extracted with dichloromethane (DCM, 3 x 100 mL). The organic extracts are dried over anhydrous sodium sulfate, are filtered and are concentrated to dryness giving crude 5-benzyloxy-pent-1-ene-3-ol. 1H NMR (300 MHz, CDCI3): 6 1.75-1.99 (m, 2H), 3.60-3.75 (m, 2H), 4.30-4.40 (m, 1H), 4.50 (s, 2H), 4.70 (s, 1H), 5.10-5.15 (m, 1H), 5.25-5.30 (m, 1H), 5.80-5.95 (m, 1H), 7.25-7.40 (m, 5H). This material is dissolved in DMSO (120 mL) and THF (120 mL) under nitrogen and IBX (65 g, 1.5 eq.) is added. The mixture is stirred at 20 deg C for 12 hours after which, ethyl acetate (500 mL) is added. The resulting mixture is washed with saturated aqueous sodium sulfite (200 mL) and saturated aqueous sodium bicarbonate (200 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel (ethyl acetate/hexane 1/20) giving the desired 5-benzyloxy-pent-1-ene-3-one (12.7 g) as a yellow oil. 1H NMR (300 MHz, CDCI3): 6 2.95 (t, 2H), 3.80 (t, 2H), 4.55 (s, 3H), 5.85 (d, 1H), 6.20-6.40 (m, 2H), 7.20-7.40 (m, 5H).

EXAMPLE 18 - Preparation of compound 62 (Scheme 12)

Compound 45 (300 g) is dissolved in dichloromethane (2.4 L) and ethanol (600 mL). The mixture is cooled to -15 deg C and sodium borohydride (20.85 g) is added portionwise while maintaining the reaction temperature at -15 deg C. The reaction is monitored by LCMS until the

content of compound 45 was <0.5%. The reaction is quenched with acetic acid (170 mL) and methanol (300 mL) is added. The resulting mixture is concentrated to 25% of its original volume and additional methanol (300 mL) is added. After concentrating to 25% of its original volume, a final portion of methanol (300 mL) is added and the mixture is concentrated to dryness. Dichloromethane (1.5 L) is added and the mixture is stirred for 20 minutes after which, it is filtered and the filter cake is washed with dichloromethane (600 mL). The combined filtrates are concentrated to dryness giving the desired crude alcohol (328 g). This crude material is dissolved in dichloromethane (3.28 L) and is cooled to -50 deg C. Borontrifluoride etherate (83.1 mL) and phosphoric acid (36.9 mL) are added and the mixture is stirred at -50 deg C for 30 minutes. Isobutylene (2.3 kg) is then added at -45 deg C. The mixture is stirred at -40 deg C for 1 hour after which, it is allowed to warm to room temperature. The reaction is monitored by LCMS during this period until the content of the alcohol is <10%. Aqueous ammonium hydroxide (13%, 2.3 L) is then added with vigorous stirring. The layers are separated and the aqueous phase is washed with dichloromethane (1.6 L). The combined organic phases are washed with saturated aqueous ammonium chloride (1.6 L) and brine (1.6 L). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is purified on silica gel giving the desired compound 62 (180 g, 44.3% yield) as a light yellow solid. 1H NMR (300 MHz, CDCI3): 6 5.75 (s, 1H), 3.60 (t, 1H), 2.65-2.75 (m, 1H), 2.45-2.55 (m, 1H), 2.30-2.40 (m, 2H), 1.95-2.05 (m, 2H), 1.65-1.85 (m, 2H), 1.20 (s, 9H), 1.10 (s, 3H).

EXAMPLE 19 - Preparation of compound 63 (Scheme 12)

Compound 62 (10 g, 1 equivalent) is combined with 1,4-dioxane (50 mL) and water (50 mL). Formaldehyde (37% in water, 3.7 g, 1 equivalent) is added followed by 1,4-diazabicyclo[2.2.2]octane (DABCO, 5.0 g, 1 equivalent). The reaction is stirred at 25-30 deg C for 40 hours and monitored by LCMS until the content of compound 63 was >60%. The reaction is then extracted with isopropanol/dichloromethane (1/3, 2 x 150 mL). The combined organic phases are dried over anhydrous sodium sulfate, are filtered and are concentrated to dryness. The residue is purified on silica gel (25% ethyl acetate/hexane) giving compound 63 (3.15 g, 27.6% yield) as a yellow oil. 1H NMR (300 MHz, DMSO-d6): 6 4.35 (t, 1H), 3.95-4.05 (m, 2H), 3.55-3.65 (m, 1H), 2.60-2.70 (dd, 1H), 2.50-2.60 (m, 1H), 2.45-2.50 (m, 1H), 2.20-2.25 (dd, 1H), 1.85-2.00 (m, 2H), 1.65-175 (m, 2H), 1.15 (s, 9H), 1.00 (s, 3H).

EXAMPLE 20 - Preparation of compound 63a (Scheme 12a)

Under a nitrogen atmosphere, compound 62 (50 grams) is combined with methyl magnesium carbonate (MMC, 2 M in dichloromethane, 400 mL). The mixture is heated to 115 deg C over 30 minutes with nitrogen bubbling through the reaction. The reaction is stirred for 1 hour at 115 deg C with monitoring by HPLC until the content of compound 63a was > 60%. The reaction is then cooled to 10 deg C and is added dropwise to a mixture of concentrated hydrochloric acid (220 mL) and ice (700 g) with rapid stirring. The layers are separated and the aqueous layer (pH = 3) is washed with methyl tert-butyl ether (MTBE, 500 mL then 250 mL). Water (250 mL) is added to the combined organic layers and the pH is adjusted to 10 on addition of 10% aqueous sodium carbonate solution. The layers are separated and the organic phase is washed with water (250 mL). The pH of the combined aqueous extracts is adjusted to 3 on addition of 10% aqueous hydrochloric acid solution. The resulting mixture is stirred at room temperature for 30 minutes until gas evolution ceases. The resulting solids are collected by filtration and are washed with water (50 mL). The solids are collected and are slurried in petroleum ether (150 mL) for 3 hours. The solids are collected by filtration and are washed with petroleum ether (50 mL). The resulting solids are dried in a vacuum oven at 30 deg C for 5 hours yielding compound 63a (30.2 g, 50.4% yield) as a light yellow solid. 1H NMR (300 MHz, CDCI3): 6 12.6-13.6 (br, 1H), 3.65-3.70 (dd, 1H), 3.10-3.40 (m, 2H), 2.60-2.85 (m, 2H), 2.00-2.15 (m, 2H), 1.75-1.95 (m, 2H), 1.20 (s, 12H).

EXAMPLE 21 - Preparation of compound 65 (Scheme 12a)

Compound 63a (10 g) is dissolved in anhydrous tetrahydrofuran (100 mL) under a nitrogen atmosphere. Anhydrous 10% palladium on carbon (1 g) is added and the mixture is cooled to 5-10 deg C. The cooled mixture is degassed three times by sequential evacuation and refilling with nitrogen. Following the third evacuation, the reaction vessel is filled with hydrogen. The mixture is stirred under a hydrogen atmosphere at 5-10 deg C for 1 hour and is monitored by LCMS until the reaction is complete. On completion of the hydrogenation, aqueous formaldehyde solution (37%, 20 mL) is added followed by piperidine (0.3 g, 0.10 eq). The mixture is stirred at 5-10 deg C for an additional 1 hour and monitored by LCMS until the reaction is complete. On completion of the reaction, brine (25 mL) and ice (25 g) are added and

stirring is continued for 15 minutes. The layers are separated and the organic phase is washed with saturated aqueous sodium bicarbonate (50 mL) and brine (50 mL). The organic phase is dried over anhydrous sodium sulfate, is filtered and is concentrated to dryness. The residue is slurried in methanol (5 mL) at 0 deg C for 10 minutes. On filtration, compound 65 (5 g, 56.3% yield) is isolated as a white solid. 1H NMR (300 MHz, CDCI3): 6 6.95 (s, 1H), 5.00 (s, 1H), 3.55-3.65 (dd, 1H), 2.45-2.60 (m, 2H), 2.35-2.45 (m, 1H), 2.05-2.15 (m, 1H), 1.95-2.05 (m, 1H), 1.55-1.80 (m, 4H), 1.15 (s, 9H), 0.80 (s, 3H).

EXAMPLE 22 - Preparation of compound 66 (Scheme 12)

Compound 10 (17.7 g, 1.2 equivalents) is dissolved in methanol (75 mL) and sodium methoxide solution (30% in methanol, 1.5 g, 0.2 equivalents) is added. The mixture is cooled to 5-10 deg C under nitrogen. Compound 65 (10 g, 1 equivalent) is dissolved in methanol (25 mL) and the resulting solution is added to the compound 10 solution dropwise over 2 hours while maintaining the reaction temperature between 0-5 deg C. The reaction is stirred at 20-25 deg C overnight after which, sodium hydroxide (5 M in water, 20 mL) is added. The reaction is stirred for an additional 2 hours after which, the methanol is removed under vacuum. Water (100 mL) and toluene (20 mL) are added and the mixture is stirred for 15 minutes. The layers are separated and the pH of the aqueous phase is adjusted to 3 with acetic acid. The aqueous mixture is then washed with ethyl acetate (100 mL and 50 mL). The combined organic extracts are concentrated to dryness and the residue is heated to 80 deg C under vacuum for 3 hours to complete the decarboxylation. The resulting residue is purified on silica gel (5% ethyl acetate/hexane) giving the desired compound 66 (10 g, 60% yield) as a light yellow solid. 1H NMR (300 MHz, DMSO-de): 6 3.8-3.9 (m, 4H), 3.45 (t, 1H), 2.65-2.75 (m, 1H), 2.20-2.50 (m, 6H), 1.85-1.95 (m, 2H), 1.75-1.80 (m, 1H), 1.10-1.65 (m, 8H), 1.25 (s, 3H), 1.10 (s, 9H), 0.80 (s, 3H).

EXAMPLE 23 - Preparation of compound 67 (ent-19-nortestosterone, Scheme 12)

Compound 66 (10 g, 1 equivalent) is dissolved in ethanol (100 mL) and triethylamine (10 mL) is added. Anydrous 10% palladium on carbon (1.0 g) is added. The mixture is degassed under vacuum and filled with a nitrogen atmosphere. This process of degassing and charging with nitrogen is repeated a total of 3 times. Following the third degassing, the reaction is charged with hydrogen. The reaction is heated to 30 deg C for 6 hours. Hydrochloric acid (6 M in water,

40 mL) is then added and the reaction is heated to reflux for an additional 2 hours. The reaction is cooled to 20-25 deg C and is filtered. The filtrate is collected and the ethanol is removed under vacuum. The resulting aqueous mixture is washed with dichloromethane (3 x 100 mL). The combined washes are concentrated to dryness and the residue is purified on silica gel (25% ethyl acetate/hexane) giving the desired compound 67 (4.70 g, 66.8% yield) as a light yellow solid. 1H NMR (300 MHz, CDCI3): 6 5.85 (s, 1H), 3.65-3.70 (t, 1H), 2.35-2.50 (m, 2H), 2.20-2.35 (m, 3H), 2.05-2.15 (m, 2H), 1.80-1.90 (m, 3H), 1.40-1.70 (m, 4H), 1.20-1.40 (m, 3H), 0.95-1.20 (m, 3H), 0.80-0.90 (m, 1 H), 0.80 (s, 3H). MS (M+ + 1) 275.1.

EXAMPLE 24 - Preparation of compound 68 (Scheme 12)

67 (100 g, 1 equivalent) is combined with ethylene glycol (312.2 g, 13.8 equivalents, p-toluenesulfonic acid (1.25 g, 0.02 equivalent) and toluene (3 L) in a 5 L flask that is equipped with a Dean-Stark trap assembly. The mixture is heated to reflux under a nitrogen atmosphere. Reflux is maintained for 3 hours and the reaction is monitored by TLC (50% ethyl acetate/petroleum ether) every hour during this period until all starting material is consuMed. The reaction is then cooled to 20-25 deg C and is poured into saturated aqueous sodium bicarbonate (1.5 L). The layers are separated and the aqueous phase is washed with dichloromethane (2 x 1 L). The combined organic layers are dried over anhydrous sodium sulfate, are filtered and are concentrated to dryness. The crude product is purified on silica gel (petroleum ether/ethyl acetate 100:1 to 20:1 with 0.5% triethylamine) giving compound 68 (96.5 g, 83.2% yield) as a mixture of isomers pertaining to the position of the olefin. 1H NMR (300 MHz, DMSO): 6 5.75 (s, 0.2H), 5.20-5.45 (m, 0.3H), 4.40-4.50 (m, 1H), 3.80-3.90 (m, 4H), 3.40-3.50 (m, 1H), 2.00-2.25 (m, 2H), 1.75-2.00 (m, 5H), 1.45-1.75 (m, 6H), 1.30-1.40 (m, 1H), 1.00-1.30 (m, 6H), 0.65 (s, 3H).

EXAMPLE 25 - Preparation of compound 14 (Scheme 12)

Compound 68 (96.5 g, 1 equivalent) is combined with acetonitrile (386 mL) under a nitrogen atmosphere. 2-lodoxybenzoic acid (IBX, 170 g, 2 equivalents) is added and the reaction is heated to 50-55 deg C for 3 hours. During this time, the reaction is monitored by TLC (50% ethyl acetate/petroleum ether) every hour until the starting material is consuMed. The reaction is then cooled to 20-25 deg C and the resulting solids are removed by filtration. The filter cake is washed with acetonitrile (2 x 193 mL) and the combined filtrates are concentrated giving crude

product. The crude product is purified on silica gel (petroleum ether/ethyl acetate 100:1 to 20:1 with 0.5% triethylamine) giving compound 14 (86.5 g, 87.1% yield) as a mixture of isomers pertaining to the position of the olefin. 1H NMR (300 MHz, DMSO): 6 5.75 (s, 0.2H), 5.25-5.40 (m, 0.3H), 3.80-3.90 (m, 4H), 2.35-2A5 (m, 1H), 1.80-2.30 (m, 8H), 1.45-1.80 (m, 6H), 1.10-1.45 (m, 5H), 0.80 (s, 3H).

EXAMPLE 26 - Intraperitoneal administration of PRV-002 attenuates motor and cognitive deficits in a rat model of traumatic brain injury

The goal of this study is to evaluate the motor and cognitive function of rats treated with PRV-002, an analogue of the enantiomer of progesterone, following traumatic brain injury. Male, Sprague-Dawley rats, approximately six weeks of age, received a mid-line cortical impact to induce traumatic brain injury. Rats receive intraperitoneal injections of either vehicle solution (45% cyclodextrin), PRV-002 4mg/kg, or PRV-002 16mg/kg at 15 min., 6h, and 24h post-injury. A sham group, which does not undergo impact or treatment is used as a control. Motor function is evaluated using a neurobehavioral battery, known as neuroscore, at 24h and 48h post-injury. Cognitive function is assessed using the Morris water maze (MWM) - memory score at 48h post-injury. Time spent swimming in close proximity to the wall of the Morris water maze (thigmotaxia) is used to evaluate spatial acquisition deficits and potential TBI-induced anxiety.

Significant motor and cognitive deficits are observed in vehicle-treated rats following injury. Injured rats are treated with either PRV-002 4mg/kg or PRV-002 16mg/kg shows significant improvement in neuroscore - motor performance, at 48h post-injury. Cognitive deficits, is measured by MWM-memory score and time spent in thigmotaxia, are also ameliorated in rats treated with either PRV-002 4mg/kg or PRV-002 16mg/kg. These findings provide support for potential clinical use of PRV-002 for the treatment of concussion and traumatic brain injury.

Methods

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA), six weeks of age and weighing between 225 — 275 g at the time of injury, are used. Rats are housed in standard

Plexiglas cages and are maintained on a 12-12 light cycle with lights on at 0700. Food and water are available ad libitum.

Injury

Prior to surgery, rats are anesthetized via inhalation with an initial induction of 5% isofluorane. The rat's scalp is shaved and cleaned with a 70% isopropanol solution and 10% betadine solution. During the surgery, anesthesia is maintained at 2.5% isofluorane with oxygen at a rate of 500 — 1000 mL/min. The rat's head is secured in a stereotaxic apparatus and a medial incision is made and the scalp is pulled back with bulldog clips over the frontal bone. A 6 mm circular piece of skull is removed with a Micromotor drill that utilized a removable 6 mm circular drill bit. The bone, above the medial frontal cortex (MFC), is removed using fine, curved tipped forceps, leaving the dura intact. An electrically-controlled injury device with a 3 mm metal impactor is used to produce the traumatic brain injury. A piston is placed on the dura. Electrical signals from the piston to a transducer signal correct placement. The piston is then used to produce a contusion at a depth of 3 mm. This procedure is used extensively by researchers conducting work on traumatic brain injury and represents one of the most consistent and reproducible forms of injury. Following injury the tissue is closed with 4¬0 monofilament sutures. Rats are placed in a heated recovery cage following surgery and are returned to their home cage following recovery.

Treatment

Rats are randomly placed in one of four treatment groups: 1) sham injury group (SHAM), 2) vehicle-treated injury group (VEHICLE), 3) PRV-002 4mg/kg-treated injury group (PRV-002 4mg/kg), or 4) PRV-002 16mg/kg-treated injury group. Rats receive intraperitoneal injections of either vehicle solution (45% cyclodextrin in sterile water) or PRV-002 solution (PRV-002 powder is dissolved into 45% cyclodextrin solution) at 15 minutes, 6 hours, and 24 hours post-injury.

Neuroscore

Testing of motor function, using a neurobehavioral battery known as neuroscore is conducted at 24 and 48 hours post-injury. The rats are exposed to a series of four neurobehavioral tests and are observed for abnormal twisting behavior. Rats receive scores from +4 uninjured to (-) nonfunctional for both left and right forelimbs in the forelimb extension task and forelimb paw placement, the left and right hind limbs in hind limb flexion, and left and right sides for the lateral pulsion test. If no twisting is observed the rat would score as normal +1, and if there is twisting

present the rat would score as abnormal (-). The total possible score is 33. The testing criteria is as follows:

Forelimb extension

Suspend the rat by its tail and determine the forelimb extension toward floor.

Score separately for both the left and right forelimb.

- +4 Normal: Rat extends both forelimbs fully and equally towards floor
- +3 Slightly impaired: There is a slight forelimb flexion
- +2 Moderately impaired: There is moderate forelimb flexion
- +1 Severely impaired: There is severe forelimb flexion
- - Nonfunctional: Forelimb remains tucked close to body.

Lateral Pulsion

During free walking, gently push the rat to the left and right side and determine the decrease in resistance to lateral pulsion. Score for both the left and right side of the rat.

- +4 Normal: Rat should resist equally when pushed to each side.
- +3 Slightly impaired: Rat maintains moderate resistance
- +2 Moderately impaired: Rat maintains slight resistance
- +1 Severely impaired: Rat does not resist when pushed
- - Non-functional: Rat does not resist when pushed and falls to its side

Forelimb Paw Placement

Suspend the rat by its tail and with a slight swinging motion observe the ability of the rat to grasp the object with the right and left paw. Score separately for both the left and right forelimb.

- +4 Normal: Rat can strongly grasp the object with both paws
- +3 Slightly impaired: Rat weakly grasps the object with paw misplacement
- +2 Moderately impaired: Rat is weak and unable to maintain grasp of the object
- +1 Severely impaired: Rat is unable to grasp the object
- Nonfunctional: Rat shows no attempt to grasp the object

Hind limb Flexion

Hold the rat by its tail and lift the hind limbs off of the ground. Determine the hind limb flexion for both the right and left limbs.

- +4 Normal: Rats have normal extension of hind limbs, no crossing or splaying
- +3 Slightly impaired: hind limbs have slight deviation from normal extension, slight clasping or splaying of hind limbs
- +2 Moderately impaired: Moderate crossing over or splaying of hind limbs
- +1 Severely impaired: Severe deviation from normal extension with severe crossing over or splaying of hind limbs
- - Nonfunctional: Hind limbs are crossed or splayed with no normal extension or function

Twisting

When the rat is suspended, observe if there is twisting

- +1 Normal: no twisting
- - Abnormal: twisting

Morris Water Maze - Memory Score. See **FIG. 2**.

Prior to injury, rats are trained to find a hidden escape platform submerged in location A in a circular pool of water. Forty-eight hours after injury, the platform is removed from the pool and the rats are given two, 60 seconds trials in the pool. Uninjured sham (normal) animals will remember the location of the platform and spend most of their time swimming through and around Zone A . Brain-injured animals whose memory is damaged by the TBI typically swim randomly around the pool, not remembering the location of the hidden platform. The amount of time spent swimming in concentric rings radiating from the escape platform area (zones A, B, and C, respectively) is measured and used to calculate the memory score. The Morris Water Maze memory score is calculated using the equation: (zone A x 20)+(zone B x 5)+(zone C) = memory score , where zones A, B, and C are annuli of increasing size that encompass and surround the area that formerly held the escape platform.

Morris Water Maze — Thigmotaxia

Thigmotaxis is a measure of the amount of time rats spend "wall hugging" or swimming around the edge of the tank. Time spent traveling in the thigmotaxia area is measured and is indicative of high anxiety and spatial acquisition deficits in injured animals. See **FIG. 2**.

Statistical Analysis

A one-way analysis of variance (ANOVA) is used to evaluate group differences in MWM memory score and MWM thigmotaxia. When warranted, post-hoc analysis of pair-wise comparisons is carried out using Fisher's Protected Least Significant Differences (PLSD) test. Neuroscore data is analyzed using the Kruskall-Wallis test to evaluate group differences. When warranted, pair-wise comparisons are carried out using the Mann-Whitney U Test.

Results

Neuroscore

Kruskal-Wallis tests are carried out to evaluate group differences on median neuroscore at 24h and 48h post-injury. These tests failed to reveal significant differences at 24h [x2 (3, n = 32) = 4.218, p = 0.239] (figure 1) but do reveal significant group differences at 48h post-injury [x2 (3, n=32) = 16.066, p = 0.001] (figure 2). Pair-wise comparisons are carried out using the Mann-Whitney U test at both 24h (see **Table 1**) and 48h (see **Table 2**) time points. Rats treated with either PRV-002 4mg/kg or PRV-002 16mg/kg have significantly better motor performance, compared to vehicle-treated rats, at 48h post-injury. See **FIG. 3** and **FIG. 4**.

Table 1. Neuroscore Pair-Wise Comparisons — 24h post-injury

	VEHICLE	PRV-002 4mg/kg	PRV-002 16mg/kg
SHAM	U = 1.0, p = 0.084	U = 19.0, p = 0.772	U = 34.0, p = 0.848
VEHICLE		U = 14.0, p = 0.177	U = 22.0, p = 0.087
PRV-002 4mg/kg			U = 33.0, p = 0.439

Table 2. Neuroscore Pair-Wise Comparisons — 48h post-injury

	VEHICLE	PRV-002 4mg/kg	PRV-002 16mg/kg
SHAM	U = 1.0, p = 0.004*	U = 19.5, p = 0.829	U = 28.0, p = 0.452
VEHICLE		U = 0.0, p = 0.002*	U = 0.0, p =0.001*
PRV-002 4mg/kg			U = 35.5, p = 0.580

* Indicates a significant difference, $p < 0.05$

Morris Water Maze — Memory Score

A one-way analysis of variance (ANOVA) is used to evaluate group differences in MWM memory score. Post-hoc analysis of pair-wise comparisons is carried out using Fisher's Protected Least Significant Differences (PLSD) test. Analysis reveals significant group differences in memory score during both trial 1 [F (3, 32) = 3.863, p 0.019] and trial 2 [F (3, 32) = 3.580, p = 0.026] of the MWM task. Post-hoc analysis shows that vehicle-treated injured rats have significantly worse cognitive function than sham, PRV-002 4mg/kg-, and PRV-002 16mg/kg-treated rats during both trials of the MWM task. See **FIG. 5A** and **FIG. 5B**.Morris Water Maze — Time Spent in Thigmotaxia. See **FIG. 2**.

A one-way analysis of variance (ANOVA) is used to evaluate group differences in time spent in thigmotaxia during the MWM task. Post-hoc analysis of pair-wise comparisons is carried out using Fisher's Protected Least Significant Differences (PLSD) test. Analysis reveals significant group differences in time spent in thigmotaxia during both trial 1 [F (3, 32) = 3.329, p = 0.033] and trial 2 [F (3, 32) = 4.7665, p = 0.008] of the MWM task. Post-hoc analysis shows that vehicle-treated injured rats spend significantly more time in thigmotaxia than sham, PRV-002 4mg/kg-, and PRV-002 16mg/kg-treated rats during both trials of the MWM task. See **FIG. 6A** and **FIG. 6B**.

Discussion

Neuroscore, MWM-memory score, and MWM-time spend in thigmotaxia all reveal significant motor and cognitive deficits in vehicle-treated rats following experimental traumatic brain injury. Though no significant group differences are seen in neuroscore at 24h post-injury, by 48h rats treated with PRV-002 4mg/kg or PRV-002 16mg/kg show significant attenuation of TBI-induced motor function deficits. Rats treated with PRV-002 4mg/kg or PRV-002 16mg/kg show amelioration of TBI-induced cognitive deficits, as measured by the MWM-memory score at 48h hours post-injury. Rats treated with either PRV-002 4mg/kg or PRV-002 16mg/kg spend less time in the thigmotaxia area during the water maze task, compared to vehicle-treated injured rats, indicating a reduction spatial acquisition deficits. The decreased time spent in thigmotaxia may also indicate that treatment with PRV-002 4mg/kg or PRV-002 16mg/kg may induce anxiolytic effects following TBI.

The results of this study reveal the efficacy of PRV-002 in counteracting TBI-induced motor and cognitive deficits in the cortical impact model of TBI in rats. These findings, coupled with previous work investigating the role of PRV-002 in attenuating neurodegeneration and death in cell culture models of TBI, provide support for the use of this compound for the treatment of concussion and TBI in humans. Studies investigating changes in protein expression in the brains of rats that are treated with either vehicle solution or PRV-002 following experimental brain injury will help to elucidate the mechanism by which this compound exerts in neuroprotective effect.

Example 27 - Intranasal administration of PRV-002 attenuates motor and cognitive deficits in a rat model of traumatic brain injury

The goal of this study is to evaluate the motor and cognitive function of rats treated via intranasal administration with PRV-002, an analogue of the enantiomer of progesterone, following traumatic brain injury. Prior to the initiation of the treatment study, an anatomical evaluation is performed using PRV002 labeled with Evans Blue dye to determine the optimal intranasal/intracerebral penetration of compound using intranasal administration via a miniature atomizer vs. a manual pipette. Post-mortem evaluation determined a clear advantage of the miniature atomizer over the pipette technique with respect to maximal nasal mucosal penetration.

Male, Sprague-Dawley rats, approximately six weeks of age, received a mid-line cortical impact to induce traumatic brain injury. Rats received an intranasal administration, via a miniature atomizer, of either vehicle solution (45% cyclodextrin), PRV-002 0.05 mg/kg (n=4), PRV-002 0.01mg/kg (n=11), PRV002 1mg/kg (n=4) or PRV002 4mg/kg (n=3) at 15 min., 6h, and 24h post-injury. A sham group, which did not undergo impact or receive treatment was used as a control. Motor function is evaluated using a neurobehavioral battery, known as neuroscore, at 24h and 48h post-injury. Cognitive function is assessed using the Morris water maze (MWM) - memory score at 48h post-injury. Time spent swimming in close proximity to the wall of the Morris water maze (thigmotaxia) is used to evaluate spatial acquisition deficits and potential TBI-induced anxiety.

Significant motor and cognitive deficits are observed in vehicle-treated, brain-injured rats following injury. Brain-injured rats treated IN with 4mg/kg PRV002 shows significant improvement in cognitive function (post-traumatic memory) tested at 48 h post-injury/treatment. Time spent in thigmotaxia is also significantly reduced in brain-injured animals receiving IN PRV002 (4mg/kg). Post-traumatic motor deficits at 24 h post-injury are significantly improved in animals treated with either PRV002 (0.1ring/kg) or PRV002 (4mg/kg). By 48 hr post-injury, brain-injured animals treated with PRV002 (0.05mg/kg), PRV002 (0.1 mg/kg) or PRV002 (4mg/kg) when compared with brain-injured, vehicle-treated animals. These findings provide support for potential clinical use of PRV-002 for the treatment of concussion and traumatic brain injury.

Methods

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA), six weeks of age and weighing between 225 — 275 g at the time of injury, are used. Rats are housed in standard Plexiglas cages and are maintained on a 12-12 light cycle with lights on at 0700. Food and water are available ad libitum.

Traumatic Brain Injury Model

Prior to surgery, rats are anesthetized via inhalation with an initial induction of 5% isofluorane. The rat's scalp is shaved and cleaned with a 70% isopropanol solution and 10% betadine solution. During the surgery, anesthesia is maintained at 2.5% isofluorane with oxygen

at a rate of 500 —1000 mL/min. The rat's head is secured in a stereotaxic apparatus and a medial incision is made and the scalp is pulled back with bulldog clips over the frontal bone. A 6 mm circular piece of skull is removed with a Micromotor drill that utilized a removable 6 mm circular drill bit. The bone, above the medial frontal cortex (MFC), is removed using fine, curved tipped forceps, leaving the dura intact. An electrically-controlled injury device with a 3 mm metal impactor is used to produce the traumatic brain injury. A piston is placed on the dura. Electrical signals from the piston to a transducer signal correct placement. The piston is then used to produce a contusion at a depth of 3 mm. This procedure is used extensively by researchers conducting work on traumatic brain injury and represents one of the most consistent and reproducible forms of injury. Following injury the tissue is closed with 4¬0 monofilament sutures. Rats are placed in a heated recovery cage following surgery and are returned to their home cage following recovery.

Treatment

Rats are randomly placed in one of four treatment groups: 1) sham injury group (SHAM-anesthesia and surgical incision without TBI), 2) brain-injured, vehicle-treated injury group (VEHICLE), or TBI followed by intranasal (IN) administration of PRV002 (0.05mg/kg, n=4), PRV002 (0.1mg/kg, n=11), PRV002 (1mg/kg, n=4), or PRV002 (4mg/kg, n=3). Experimental subjects receive an IN spray of either vehicle solution (45% cyclodextrin in sterile water) or PRV-002 solution (PRV-002 powder dissolved into 45% cyclodextrin solution) at 15 minutes, 6 hours, and 24 hours post-injury using a micro atomizer.

Neuroscore

Testing of motor function, using a neurobehavioral battery known as neuroscore is conducted at 24 and 48 hours post-injury. The rats are exposed to a series of four neurobehavioral tests and are observed for abnormal twisting behavior. Rats receive scores from +4 uninjured to (-) nonfunctional for both left and right forelimbs in the forelimb extension task and forelimb paw placement, the left and right hind limbs in hind limb flexion, and left and right sides for the lateral pulsion test. If no twisting is observed the rat would score as normal +1, and if there is twisting present the rat scores as abnormal (-). The total possible score is 33. The testing criteria is as follows:

<u>Forelimb extension</u>

Suspend the rat by its tail and determine the forelimb extension toward floor. Score separately for both the left and right forelimb.

- +4 Normal: Rat extends both forelimbs fully and equally towards floor
- +3 Slightly impaired: There is a slight forelimb flexion
- +2 Moderately impaired: There is moderate forelimb flexion
- +1 Severely impaired: There is severe forelimb flexion
- - Nonfunctional: Forelimb remains tucked close to body.

Lateral Pulsion

During free walking, gently push the rat to the left and right side and determine the decrease in resistance to lateral pulsion. Score for both the left and right side of the rat.

- +4 Normal: Rat should resist equally when pushed to each side.
- +3 Slightly impaired: Rat maintains moderate resistance
- +2 Moderately impaired: Rat maintains slight resistance
- +1 Severely impaired: Rat does not resist when pushed
- - Non-functional: Rat does not resist when pushed and falls to its side

Forelimb Paw Placement

Suspend the rat by its tail and with a slight swinging motion observe the ability of the rat to grasp the object with the right and left paw. Score separately for both the left and right forelimb.

- 0 +4 Normal: Rat can strongly grasp the object with both paws
- 0 +3 Slightly impaired: Rat weakly grasps the object with paw misplacement
- 0 +2 Moderately impaired: Rat is weak and unable to maintain' grasp of the object
- o+1 Severely impaired: Rat is unable to grasp the object
- o— Nonfunctional: Rat shows no attempt to grasp the object

Hind limb Flexion

Hold the rat by its tail and lift the hind limbs off of the ground. Determine the hind limb flexion for both the right and left limbs.

- 0 +4 Normal: Rats have normal extension of hind limbs, no crossing or splaying
- o+3 Slightly impaired: hind limbs have slight deviation from normal extension, slight clasping or splaying of hind limbs
- o+2 Moderately impaired: Moderate crossing over or splaying of hind limbs
- 0 +1 Severely impaired: Severe deviation from normal extension with severe crossing over or splaying of hind limbs
- - Nonfunctional: Hind limbs are crossed or splayed with no normal extension or function

Twisting

When the rat is suspended, observe if there is twisting

- +1 Normal: no twisting
- - Abnormal: twisting

Cognition: Morris Water Maze — Memory Score. See **FIG. 2**.

Prior to injury, rats are trained to find a hidden escape platform submerged in location A in a circular pool of water. Forty-eight hours after injury, the platform is removed from the pool and the rats are given two, 60 seconds trials in the pool. Uninjured sham (normal) animals will remember the location of the platform and spend most of their time swimming through and around Zone A . Brain-injured animals whose memory is damaged by the TBI typically swim randomly around the pool, not remembering the location of the hidden platform. The amount of time spent swimming in concentric rings radiating from the escape platform area (zones A, B, and C, respectively) is measured and used to calculate the memory score. The Morris Water Maze memory score is calculated using the equation:

(Zone A x 20) (Zone B x + Zane C = -memory score, where zones A, B, and C are annuli of increasing size that encompass and surround the area that formerly holds the escape platform.

Morris Water Maze — Thigmotaxia. See **FIG. 2**.

Thigmotaxis is a measure of the amount of time rats spend "wall hugging" or swimming around the edge of the tank. Time spent traveling in the thigmotaxia area is measured and is indicative of high anxiety and spatial acquisition deficits in injured animals. See **FIG. 2**.

Statistical Analysis

A one-way analysis of variance (ANOVA) is used to evaluate group differences in MWM memory score and MWM thigmotaxia. When warranted, post-hoc analysis of pair-wise comparisons is carried out using Fisher's Protected Least Significant Differences (PLSD) test. Neuroscore data is analyzed using the Kruskall-Wallis test to evaluate group differences. When warranted, pair-wise comparisons are carried out using the Mann-Whitney U Test.

Results

See **FIG. 7A**, **FIG. 7B** and **FIG. 7C**.

COGNITION

Morris Water Maze — Memory Score. See **FIG. 2**.

A one-way analysis of variance (ANOVA) is used to evaluate group differences in MWM memory score. Post-hoc analysis of pair-wise comparisons is carried out using Fisher's Protected Least Significant Differences (PLSD) test. Analysis reveals significant group differences in memory score during trial 1 [$F(5, 31) = 4.433$, $p = 0.005$] (figure 1, top) but not during trial 2 [$F(5, 31) = 0.928$, $p = 0.479$] (figure 1, bottom) of the MWM task. Post-hoc analysis shows all groups have significantly lower memory scores than PRV-002 4mg/kg-treated rats during trial 1. See **FIG. 8A** and **FIG. 8B**.

Morris Water Maze — Time Spent in Thigmotaxia. See **FIG. 2**.

A one-way analysis of variance (ANOVA) is used to evaluate group differences in time spent in thigmotaxia during the MWM task. Post-hoc analysis of pair-wise comparisons is carried out using Fisher's Protected Least Significant Differences (PLSD) test. Analysis reveals significant group differences in time spent in thigmotaxia during both trial 1 [$F(5, 31) = 1.857$, $p = 0.137$] (figure 2, top) and trial 2 [$F(5, 31) = 3.103$, $p = 0.025$] (figure 2, bottom) of the MWM task.

Post-hoc analysis shows that sham and PRV-002 4mg/kg-treated rats spent significantly less time in thigmotaxia, compared to vehicle-treated rats. See **FIG. 9A** and **FIG. 9B**.

MOTOR FUNCTION

Neuroscore

Kruskal-Wallis tests are carried out to evaluate group differences on median neuroscore at 24h and 48h post-injury. These tests reveal significant differences at 24h [X2 (3, n = 32) = 13.529, p = 0.019] (**FIG. 3**) and at 48h post-injury [x2 (3, n=32) = 18.153, p = 0.003] (**FIG. 4**). Pair-wise comparisons are carried out using the Mann-Whitney U test at both 24h (**Table 3**) and 48h (**Table 4**) time points. Rats treated with PRV-002 0.1mg/kg or PRV-002 4mg/kg have significantly improved motor function, compared to vehicle-treated rats at 24h post-injury. All PRV-002 treatment groups had motor performance scores that are not significantly different from sham rats at 24h post-injury (**Table 3**). Sham rats and rats treated with either PRV-002 0.05mg/kg, PRV-002 0.1 mg/kg, or PRV-002 4mg/kg have significantly better motor function, as compared to vehicle-treated rats at 48h post-injury. PRV-002 0.05mg/kg- and PRV-002 1 mg/kg-treated rats have significantly worse performance, compared to sham rats at 48h post-injury (**Table 4**). See also **FIG. 10** and **FIG. 11**.

Table 3. Neuroscore — 24h Post-Injury

	VEHICLE	PRV-002 0.05mg/kg	PRV-002 0.1mg/kg	PRV-002 1mg/kg	PRV-002 4mg/kg
SHAM	U = 1.0, p = 0.027*	U = 2.5, p = 0.106	U = 15.0, p = 0.356	U = 3.0, p = 0.139	U = 4.5, p = 0.629
VEHICLE		U = 2.5, p =0.064	U = 3.5 p = 0.006*	U = 2.0, p = 0.050	U = 0.0, p = 0.025*
PRV-002 0.05mg/kg			U = 18.0, p = 0.598	U = 8.0, p = 1.0	U = 1.0, p = 0.067
PRV-002 0.1mg/kg				U = 16.0, p = 0.430	U = 7.0, p = 0.134
PRV-002 1mg/kg					U = 1.0, p = 0.077

* indicates a significant difference, $p < 0.05$.

Table 4. Neuroscore — 48h Post-Injury

	VEHICLE	PRV-002 0.05mg/kg	PRV-002 0.1mg/kg	PRV-002 1mg/kg	PRV-002 4mg/kg
SHAM	U = 0.0, p = 0.014*	U = 1.0, p = 0.042*	U = 12.5, p = 0.209	U = 0.0, p = 0.019*	U = 4.0, p = 0.463
VEHICLE		U = 1.0, p =0.027*	U = 0.0 p = 0.002*	U = 3.5, p = 0.108	U = 0.0, p = 0.025*
PRV-002 0.05mg/kg			U = 11.0, p = 0.149	U = 7.0, p = 0.767	U = 1.5, p = 0.108
PRV-002 0.1mg/kg				U = 9.0, p = 0.086	U = 12.0, p = 0.479
PRV-002 1mg/kg					U = 0.0, p = 0.032*

* indicates a significant difference, $p < 0.05$

Discussion

Neuroscore, MWM-memory score, and MWM-time spent in thigmotaxia all reveal significant motor and cognitive deficits in brain-injured, vehicle-treated rats following experimental traumatic brain injury. Brain-injured rats treated IN with 4mg/kg PRV002 show significant improvement in cognitive function (post-traumatic memory) tested at 48 h post-injury/treatment. Time spent in thigmotaxia is also significantly reduced in brain-injured animals receiving IN PRV002 (4mg/kg). The decrease in time spent in thigmotaxia may also indicate that IN treatment with PRV-002 may induce anxiolytic effects following TBI.

Post-traumatic motor deficits at 24 h post-injury are significantly improved in animals treated with either PRV002 (0.1mg/kg) or PRV002 (4mg/kg). By 48 hr post-injury, brain-injured animals treated with PRV002 (0.05mg/kg), PRV002 (0.1mg/kg) or PRV002 (4mg/kg) show significantly improved motor function when compared with brain-injured, vehicle-treated animals. The results of this study reveal the efficacy of PRV-002 in counteracting TBI-induced motor and cognitive deficits in the cortical impact model of TBI in rats.

These observations, coupled with previous work showing improvement of cognitive and motor function following systemic (intraperitoneal) administration of PRV002 and studies investigating the role of PRV-002 in attenuating neurodegeneration and death in cell culture models of TBI, provide support for the use of this compound for the treatment of concussion and TBI in humans. Studies investigating changes in protein expression in the brains of rats treated with either vehicle solution or PRV-002 following experimental brain injury, coupled with MRI studies, will help to elucidate the mechanism(s) by which this compound exerts in neuroprotective effect in the injured brain following TBI.

Example 28 - ent-19-norprogesterone (PRV-002) solution

An Example of an ent-19-norprogesterone (PRV-002) solution that is used in accordance with Examples 26 and 27 is illustrated in **Table 5**.

Table 5. *ent*-19-Norprogesterone (PRV-002) Solution

Reagent	Amount added	Mix time, min	Resultant solution	Total volume after solubilization	Final Calculated concentration
Solution 1					
2- Hydroxypropyl - β -cyclodextrin (H107 –Sigma-Aldrich)	2.5 g			~ 7 ml	35.8%
Water	5 ml	10	Clear		
PRV-002 Formulation					
Solution 1	1 ml			~ 1 ml	35.8%
+ PRV-002 ent-19-norprogesterone	30 mg	60	Hazy		
+ Solution 1	0.1 ml	120	Hazy	~ 1.1 ml	
+ Solution 1	0.1 ml	O/N	Hazy to Clear	~ 1.2 ml	
+ Solution 1	0.1 ml	120	Mostly clear with slight haziness*	~ 1.3 ml	23 mg/ml

*- No precipitation is observed after O/N at RT.

Apparent solubility of PRV-002 in 35.8% of 2- Hydroxypropyl -6-cyclodextrin is about 23 mg/ml.

The PRV-002 solution is prepared by adding about 30 mg of PRV-001 compound to about 1 ml of 35.8% 2-Hydroxypropyl -p-cyclodextrin. Solution is hazy after mixing for about 60 min. Then about 0.1 ml of about 35.8% 2-Hydroxypropyl -P-cyclodextrin is added to about 1 ml of

PRV-002—Cyclodextrin mixture. Solution is hazy after mixing for about 120 min. Additional 0.1 ml of about 35.8% 2-Hydroxypropyl -p-cyclodextrin is added to about 1.1 ml of PRV-002—Cyclodextrin mixture and left on mixing overnight (0/N). Next day resultant solution is notably clearer but still hazy. About 0.1 ml more of about 35.8% 2-Hydroxypropyl-p-cyclodextrin is added to about 1.2 ml of PRV-002—Cyclodextrin mixture. Addition of another about 0.3 ml (0.1+0.1+0.1) aliquot of about 35.8% 2-Hydroxypropyl-p-cyclodextrin only slightly improves the clarity of PRV-002 solution. It is believed that PRV-002 is in solution at about 23mg/mi, and slight haziness is some sort of an artifact.

EXAMPLE 29 - Polymorph screening for selection of crystalline phase for PRV-002

Summary

The purpose of this project is to perform a polymorph screening for compound PRV-002. This screening is designed to evaluate polymorphism in the compound and select an appropriate crystalline phase for development with associated risks analyzed.

Using anhydrous Type A as the starting material, a polymorph screening is performed under 107 conditions through methods of anti-solvent addition, evaporation, slow cooling, slurry conversion, vapor diffusion, polymer-induced crystallization and grinding. A meta-stable anhydrate, Type B, and amorphous phase are identified, both of which will convert into Type A at ambient conditions.

Based on the screening results, Type A is an anhydrous form suitable for further development. Crystal morphology control is recommended as formation of large rods, observed from some screening conditions, may cause decreased flowability in down-stream processes.

Characterization of solid forms

Two crystal forms of PRV-002 Type A and B are isolated and characterized, with the XRPD comparison displayed in **FIG. 12**. Amorphous is also prepared, and XRPD characterization of Type B and amorphous samples after storage at ambient conditions do reveal that both Type B and amorphous convert back to Type A. A brief inter-conversion diagram is illustrated in **FIG. 13**.

Type A is recommended as the stable anhydrous form for further development. See **Figs. 12 and 13**.

PRV-002 Type A (*ent*-19-norprogesterone)

The starting material (Batch PH-PRV-1302-GLP-0B-A-1, with a Crystal Pharmatech ID of 807302- 25-A) as-received is crystalline, with the crystal form named as Type A.

X-ray powder diffraction (XRPD) pattern is displayed in **FIG. 14**. Thermogravimetric analysis (TGA) and differential scanning calorimeter (DSC) data are displayed in **FIG. 15**. A weight loss of about 0.4% is observed up to about 150 °C in TGA, and DSC results show a melting endotherm at about 143.3 °C (onset temperature), indicating Type A is an anhydrate.

Polarized light microscope (PLM) image in **FIG. 16** indicates the crystals are rod-like. The existence of agglomeration is concluded from particle size distribution (PSD) results in **Table 6** and **FIG. 17A** and **FIG. 17B**, as the percentage of large particles decrease after sonication. Particles of Type A can grow into big rods with enlarged length/width ratios, as shown in **FIG. 18A**, **FIG. 18B, FIG. C** and **FIG. B**, and this may impact the flowability of powders in down-stream processes without any crystal morphology control.

Table 6. Particle size distribution of PRV-002 Type A (807302-25-A)

Ultrasonic Condition	MV (μm)	SD (μm)	D10 (μm)	D50 (μm)	D90 (μm)
0s	152.8	93.0	50.0	137.9	274.3
30s/30W	99.46	43.3	41.0	105.3	146.7

Type B (*ent*-19-norprogesterone)

Type B can be generated via slow evaporation in IPAc or THF/n-heptane (about 4:1, v/v) solution at about RT. Type B sample (807302-42-A) is obtained via well-controlled evaporation of THF/n-heptane (about 4:1, v/v) solution at about RT. Solids are isolated and characterized before the solution is evaporated to dryness. XRPD pattern is shown in **FIG. 19** and TGA/DSC data are displayed in **FIG. 20**. A weight loss of about 1.4% up to about 150 °C is observed in TGA, and one exotherm at about 51.2 °C is observed before melting at about 142.3 °C (onset temperature) in DSC.

As shown in **FIG. 21**, the XRPD pattern collected after sample 807302-42-A is stored at ambient conditions for about 5 hours conformed to Type A. This indicates Type B is meta-stable to Type A and converts readily at ambient conditions. The exothermic event in the DSC of Type B is believed to be most likely caused by the phase transition from Type B to Type A.

Amorphous

Precipitates (807302-27-A8) appear after adding H_2O into DMSO solution, and XRPD pattern in **FIG. 22** reveals that amorphous sample is obtained. Characteristic peaks of Type A can be observed from **FIG. 23**, after sample 807302-27-A8 is kept at ambient conditions for about two weeks.

Conclusion

For compound PRV-002, 107 polymorph screening experiments are performed using anhydrous PRV-002 Type A (ent-19-norprogesterone) as the starting material. Type B (ent-19-norprogesterone) and amorphous are obtained, and both of them converted into Type A at ambient conditions. Based on the screening results and inter-conversion relationships, anhydrous Type A is recommended for further development.

EXAMPLE 30 - Polymorph screening experimental methods and parameters

Solubility

The solubility of starting Type A (807302-25-A) is estimated in 21 solvents at about room temperature (RT, 25 ± 3 °C). Approximately 2 mg solids are added into a 3-mL glass vial.

Solvents in **Table 7** are then added stepwise (100 μL per step) into the vials until the solids are dissolved or a total volume of about 2 mL is reached. Results summarized in **Table 7** are used to guide the solvent selection in polymorph screening.

Table 7. Solubility of Type A (807302-25-A) at about RT

Solvent	Solubility (mg/mL)	Solvent	Solubility (mg/mL)
MeOH	28.0<S<56.0	1,4-dioxane	S>38.0
EtOH	19.0 < S<38.0	ACN	S>34.0
IPA	12.0<S<16.0	$CHCl_3$	S>60.0
acetone	S>48.0	n-heptane	S<1.5
MIBK	S>66.0	toluene	S>48.0
EtOAc	S>48.0	DMAc	S>56.0
IPAc	29.0<S<58.0	DMSO	13.5<S<18.0
MTBE	10.7<S<16.0	NMP	S>68.0
THF	S>48.0	acetic acid	S>42.0
2-MeTHF	S>44.0	H_2O	S<1.0
DCM	S>58.0	--	--

Anti-solvent addition

A total of 20 anti-solvent addition experiments are carried out. About 15 mg of Type A sample (807302-25-A) is dissolved in about 0.2-2.2 mL solvent to obtain a clear solution, and the solution is magnetically stirred, then followed by addition of the relative anti-solvent to induce precipitation or the total amount of anti-solvent reaches about 15.0 mL. The precipitate is isolated for XRPD analysis. Clear solutions are transferred to agitation at about 5 ºC for about 4 days, and solids are then tested by XRPD. The final clear solutions are transferred to evaporation at about RT. XRPD patterns are displayed from **FIG. 24. FIG. 25, FIG. 26 and FIG. 27**. Results are summarized in **Table 8**, which show that only Type A is produced.

Table 8. Summary of anti-solvent addition experiments

Experiment ID	Solvent	Anti-Solvent	Solid Form
807302-27-A1	MeOH		Type A

807302-27-A2	EtOH	Type A
807302-27-A3	ACN	Type A
807302-27-A4	Acetone	Type A
807302-27-A5	NMP	Type A
807302-27-A6	THF	Type A
807302-27-A7	1,4-dioxane	Type A
807302-27-A8	DMSO	Amorphous
807302-27-A9	Acetic acid	Type A
807302-27-A10	DMAc	Type A
807302-27-A11	EtOH	Type A
807302-27-A12	IPA	Type A
807302-27-A13	MIBK	Type A
807302-27-A14	EtOAc	Type A
807302-27-A15	MTBE	Type A
807302-27-A16	THF	Type A
807302-27-A17	2-MeTHF	Type A
807302-27-A18	DCM	Type A
807302-27-A19	Toulene	Type A
807302-27-A20	Acetic acid	Type A

Reverse anti-solvent addition

About 15 mg of Type A sample (807302-25-A) is dissolved in appropriate solvent to obtain a saturated solution. The saturated solution is added into a 20-mL glass vial with about 6 mL of relative anti-solvent and mixture is stirred at about RT to induce precipitation. The precipitate is agitated for about 30 seconds and isolated for XRPD analysis. The final clear solutions are transferred to evaporation at about RT. XRPD patterns are displayed in **FIG. 28** and **FIG. 29**. Results are summarized in **Table 9**, which show that no new crystal form is produced.

Table 9. Summary of reverse anti-solvent addition experiments

EXPERIMENT ID	SOLVENT	ANTI-SOLVENT	SOLID FORM
807302-28-A1	MeOH		Type A
807302-28-A2	EtOH		Type A
807302-28-A3	ACN		Type A
807302-28-A4	acetone		Type A
807302-28-A5	NMP	H_2O	Type A
807302-28-A6	1,4-dioxane		Type A
807302-28-A7	DMSO		Type A
807302-28-A8	acetic acid		Type A
807302-28-A9	DMAc		Type A
807302-28-A10	THF	n-heptane	Type A

Slow cooling

Slow cooling experiments are conducted in nine solvent systems. About 20 mg of Type A sample (807302-25-A) is suspended in about 1.0 mL of solvent in a 3-mL glass vial at about RT. The suspension is then heated to about 50 ºC, equilibrated for about 2 hrs and is filtered to a new vial using a Nylon membrane (pore size of about 0.45 μm). Filtrates are slowly cooled down to about 5 ºC at a rate of about 0.1 ºC/min. The obtained solids are kept isothermal at about 5 ºC before they are isolated for XRPD analysis. XRPD patterns are displayed in **FIG. 30** and **FIG. 31**. Results are summarized in **Table 10**, which indicate that only Type A is produced.

Table 10. Summary of slow cooling experiments

Experiment ID	Solvent (v/v)	Solid Form
807302-29-A1	MTBE	Type A*
807302-29-A2	IPA	Type A*

807302-29-A3	EtOH/H_2O (4:1)	Type A
807302-29-A4	ACN/H_2O (4:1)	Type A*
807302-29-A5	acetic acid/H_2O (4:1)	Type A*
807302-29-A6	DMAc/H_2O (4:1)	Type A*
807302-29-A7	NMP/H_2O (4:1)	N/A
807302-29-A8	1,4-dioxane/H_2O (4:1)	Type A
807302-29-A9	THF/n-heptane (4:1)	Type A*

N/A: no solid was obtained.

*: solid is obtained via evaporation at about RT

Slurry at room temperature

Slurry conversion experiments are conducted at about RT in different solvent systems. About 15 mg of Type A sample (807302-25-A) is suspended in about 0.5 mL of solvent in a 1.5-mLglass vial. After the suspension is stirred for about 4 days at about RT, the remaining solids are isolated for XRPD analysis. XRPD patterns are displayed in **FIG. 32**, **FIG. 33** and **FIG. 34**. Results are summarized in **Table 11**, which show that only Type A is obtained.

Table 11. Summary of slurry conversion experiments at RT

Experiment ID	Solvent (v/v)	Solid Form
807302-30-A1	n-heptane	Type A
807302-30-A2	IPA	Type A*
807302-30-A3	IPA/H_2O (982/18, a_w=0.199)	Type A*
807302-30-A4	IPA/H_2O (956/44, a_w=0.401)	Type A*
807302-30-A5	IPA/H_2O (919/81, a_w=0.597)	Type A*
807302-30-A6	IPA/H_2O (847/153, a_w=0.804)	Type A*
807302-30-A7	H_2O	Type A
807302-30-A8	MTBE	Type A
807302-30-A9	DMSO	Type A
807302-30-A10	ACN/H_2O (1:3)	Type A
807302-30-A11	acetone/H_2O (1:3)	Type A
807302-30-A12	THF/n-heptane (1:3)	Type A

807302-30-A13	2-MeTHF/n-heptane (1:3)	Type A

a_w: approximate water activity.

Slurry at about 5 °C

Slurry conversion experiments are also conducted at 5 °C in different solvent systems. About 15 mg of Type A sample (807302-25-A) is suspended in about 0.5 mL of solvent in a 1.5-mL glass vial. After the suspension is stirred for about 4 days at about 5 °C, the remaining solids are isolated for XRPD analysis. XRPD patterns are displayed in **FIG. 35** and **FIG. 36**. Results are summarized in **Table 12**, which show no form change.

Table 12. Summary of slurry conversion experiments at 5 °C

Experiment ID	Solvent (v/v)	Solid Form
807302-31-A1	n-heptane	Type A
807302-31-A2	H_2O	Type A
807302-31-A3	IPA	Type A
807302-31-A4	MTBE	Type A
807302-31-A5	EtOH/H_2O (1:3)	Type A
807302-31-A6	THF/H_2O (1:3)	Type A
807302-31-A7	DMF/H_2O (1:3)	Type A
807302-31-A8	acetic acid/H_2O (1:3)	Type A
807302-31-A9	EtOAc/n-heptane (1:3)	Type A

Solid vapor diffusion

Solid vapor diffusion experiments are conducted using 14 different kinds of solvent. Approximate 15 mg of Type A sample (807302-25-A) is weighed into a 3-mL vial, which is placed into a 20-mL vial with about 2 mL of volatile solvent. The 20-mL vial is sealed with a cap and kept at about RT for about 2 days allowing solvent vapor to interact with sample. XRPD patterns are displayed in **FIG. 37** and **FIG. 38**. The solids are tested by XRPD and the results are summarized in **Table 13**, which indicate no form change.

Table 13. Summary of solid vapor diffusion experiments

Experiment ID	Solvent	Solid Form

807302-32-A1	H_2O	Type A
807302-32-A2	DCM	N/A
807302-32-A3	EtOH	Type A
807302-32-A4	MeOH	Type A
807302-32-A5	ACN	Type A
807302-32-A6	THF	N/A
807302-32-A7	$CHCl_3$	N/A
807302-32-A8	acetone	Type A
807302-32-A9	DMF	Type A
807302-32-A10	EtOAc	Type A
807302-32-A11	1,4-dioxane	Type A
807302-32-A12	IPA	Type A
807302-32-A13	acetic acid	N/A
807302-32-A14	DMSO	Type A

N/A: no solid was obtained.

Solution to vapor diffusion

Ten solution vapor diffusion experiments are conducted. Approximate 15 mg of Type A sample (807302-25-A) is dissolved in about 1.0 mL of appropriate solvent to obtain a clear solution in a 3-mL vial. This solution is then placed into a 20-mL vial with about 3 mL of volatile solvents. The 20-mL vial is sealed with a cap and kept at about RT allowing sufficient time for organic vapor to interact with the solution. The precipitates are isolated for XRPD analysis. XRPD patterns are displayed in **FIG. 39**. The results are summarized in **Table 14**, which show that only Type A is obtained.

Table 14. Summary of solution vapor diffusion experiments

EXPERIMENT ID	SOLVENT	ANTI-SOLVENT	SOLID FORM
807302-33-A1	MeOH		Type A
807302-33-A2	EtOH		Type A
807302-33-A3	IPA		Type A
807302-33-A4	ACN	H_20	Type A*

807302-33-A5	Acetone		Type A
807302-33-A6	THF		N/A
807302-33-A7	1,4-dioxane		Type A*
807302-33-A8	Acetic acid		N/A
807302-33-A9	2-MeTHF	n-heptane	Type A*
807302-33-A10	THF	n-heptane	Type A*

N/A: no solid is obtained
*: solid is obtained via evaporation at about RT

Polymer-induced crystallization

Polymer-induced crystallization experiments are performed with two sets of polymer mixtures in five different solvents. Approximate 15 mg of Type A sample (807302-25-A) is dissolved in about 1.0 mL of appropriate solvent to obtain a clear solution in a 3-mL vial. About 2 mg of polymer mixture is added into 3-mL glass vial. All the samples are sealed using parafilm and then are transferred to evaporation at about RT to induce precipitation. The solids are isolated for XRPD analysis. XRPD patterns are displayed in **FIG. 40** and **FIG. 41**. Results are summarized in **Table 15**, which show that a mixture of Type A and B is generated.

Table 15. Summary of polymer-induced crystallization experiments

Experiment ID	Solvent (v/v)	Polymer Mixture	Solid Form
807302-34-A1	IPAc	A	Mixture of Type A and B
807302-34-A2	MIBK		Type A
807302-34-A3	$CHCl_3$		Type A
807302-34-A4	THF/n-heptane (4:1)		Type A
807302-34-A5	MeOH/H_2O (4:1)		Type A
807302-34-B1	IPAc	B	Type A
807302-34-B2	MIBK		Type A
807302-34-B3	$CHCl_3$		Type A

807302-34-B4	THF/n-heptane (4:1)	Type A
807302-34-B5	MeOH/H_2O (4:1)	Type A

Polymer mixtureA: polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyvinylchloride (PVC), polyvinyl acetate (PVAC), hypromellose (HPMC), methyl cellulose (MC) (mass ratio of 1:1:1:1:1:1) Polymer mixtureB: polycaprolactone (PCL), polyethylene glycol (PEG), poly(methyl methacrylate) (PMMA) sodium alginate (SA), and hydroxyethyl cellulose (HEC) (mass ratio of 1:1:1:1:1).

Slow evaporation

Slow evaporation experiments are performed under 10 conditions. Briefly, about 15 mg of Type A sample (807302-25-A) is dissolved in about 1.0 mL of corresponding solvent in a 3-mL glass vial. The visually clear solutions are subjected to evaporation at about RT to induce precipitation. The solids are isolated for XRPD analysis, and XRPD patterns are displayed in **FIG. 42** and **FIG. 43**. Results are summarized in **Table 16**, which indicate that a mixture of Type A and B is produced.

Table 16. Summary of slow evaporation experiments

Experiment ID	Solvent (v/v)	Solid Form
807302-35-A1	MeOH	Type A
807302-35-A2	IPA	Type A
807302-35-A3	Acetone	Type A
807302-35-A4	EtOAc	Type A
807302-35-A5	MTBE	Type A
807302-35-A6	ACN	Type A
807302-35-A7	acetic acid/H_2O (4:1)	Type A
807302-35-A8	EtOH/H_2O (4:1)	Type A
807302-35-A9	$CHCl_3$/n-heptane (4:1)	Type A
807302-35-A10	THF/n-heptane(4:1)	Mixture of Type A and B

Grinding

Grinding experiments are performed in two conditions with/without additive. About 15 mg of Type A sample (807302-25-A) is weighed into a mortar and then ground manually using a

pestle for about 5 minutes with or without addition of water. The solid is analyzed by XRPD and, as shown in **FIG. 44**, only Type A is obtained.

Summary of polymorph screening experiments

Polymorph screening experiments are performed using different solution crystallization or solid transition methods. The methods utilized and crystal forms identified are summarized in

Table 17.

Table 17. Summary of polymorph screening experiments

Method	No. of Experiments	Isolated Crystal Forms
Anti-solvent addition	20	Type A
Reverse Anti-solvent Addition	10	Type A
Slow Cooling	9	Type A
Slurry Conversion at about RT/5°C	22	Type A
Solid Vapor Diffusion	14	Type A
Solution Vapor Diffusion	10	Type A
Polymer-induced Crystallization	10	Type A, mixture of Type A and B
Slow Evaporation	10	Type A, mixture of Type A and B
Grinding	2	Type A
Total	107	Type A, mixture of Type A and B

EXAMPLE 31 - Intranasal administration of PRV-002 to evaluate toxicology in a dog model

Objective

The objective of this study is to evaluate the toxicity and toxicokinetics of PRV-002, when administered intranasally at doses three times a day, approximately 4 hours apart, for 14 days at concentrations of about 0 mg/mL, about 3 mg/mL, about 10 mg/mL or about 23 mg/mL

at a volume of about 1 mL/nostril.[1] See **Table 18**. Reversibility of toxicity is evaluated during a 14-day recovery period following the final dose of test article, and systemic exposure is evaluated.

Observations and conclusions

PRV-002 does not affect ophthalmology, body weights or food consumption.

Increased salivation is observed in all combined male and female groups that are treated with PRV-002. Incidence increases with concentration and is considered PRV-002-related, but not an adverse effect.

At Day 15, there are no alterations in hematology, clinical chemistry, coagulation, or urinalysis parameters attributable to the administration of PRV-002. Similarly, there are no changes in organ weights and no macroscopic observations related to administration of PRV-002 at the Day 15 time point.

At Day 15, microscopic observations in both vehicle-only-treated and test article-treated animals are noted in the nasal turbinates and lungs. Purulent exudates involving the nasal turbinates are present in the majority of animals and subacute alveolar inflammation is present in the lungs of only a few animals. These findings are of limited intensity (minimal to mild) and the overall patterns of incidence and severity of these alterations are consistent with these microscopic observations being attributable to the vehicle, 2-hydroxypropyl-ß-cyclodextrin (about 45% w/v aqueous solution).

Table 18. Study Design: 40 (20/sex) dogs

Treatment	Main Study		Recovery	
	No. of Males	No. of Females	No. of Males	No. of Females
Control	3	3	2	2
Low dose	3	3	2	2
Mid dose	3	3	2	2
High dose	3	3	2	2

[1] The present invention contemplates a dosage concentration range and dosage concentrations for PRV-002 (*ent*-19-norprogesterone) in the formulations of the present invention to include as follows: > 0 mg/mL to about 50 mg/mL, 3mg/mL, 10 mg/mL, and 23 mg/mL.

Procedures:

Dose Preparation:	Weekly (if supported by stability data). Dose concentration is confirmed by analytical methods on Days 1, 14.
Dose Regimen:	TID treatment for 14 consecutive days by Intranasal delivery
Observations:	Twice daily (mortality/moribundity)
Clinical Observations:	Once weekly
Physical Examinations:	Prior to first dose. Conducted by staff veterinarian
Food Consumption:	Weekly quantitative assessment
Body Weight:	Measured prior to the study, weekly thereafter, and just prior to necropsy.
Ophthalmic Exams:	Complete ophthalmic exams will be performed on all available study animals prior to the study and just prior to all scheduled necropsies.
Electrocardiograms:	Standard 6-lead ECG to be performed on all available animals prior to the study and post dose on Day 1 and post dose one day prior to study termination.
Clinical Pathology:	Routine panels of serum chemistry, hematology, and coagulation parameters, and urinalysis will be evaluated for all available animals on samples collected pre-dose and at study termination.
TK Samples:	Blood samples (about 1.0 mL) will be collected (in sodium heparin; processed to plasma) from all animals Pre-dose and at 6 time points post dose on Days 1 and 14.
Necropsy:	All animals.
Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries with oviducts, pituitary, prostate, salivary glands, spleen, thyroid with parathyroid, thymus, testes, uterus.
Histopathology:	All animals. A full tissue list (approximately 66 tissue sections) and gross lesions from all animals
Statistics:	Standard as appropriate
Analytical:	Assay development and validation will be conducted under a separate study.

Bioanalytical: Assay development and validation will be conducted under a separate study.

EXAMPLE 32 - Intranasal administration of PRV-002 to evaluate toxicology in a rat model

Objective

The objective of this study is to evaluate the toxicity of PRV-002, when it is administered as three times a day doses, approximately 4 hours apart, for 14 days at concentrations of about 0 mg/mL, about 3 mg/mL, about 10 mg/mL or about 23 mg/mL at a volume of about 50 µL/nostril. Reversibility of toxicity is evaluated during a 14-day recovery period following the final dose of test article, and systemic exposure is evaluated.

Observations and conclusions

During the dosing phase, nose/nares staining/crusty is observed in four, eleven and twenty-two PRV-002 treated animals at concentrations of about 3 mg/mL, about 10 mg/mL, and 23 mg/mL, respectively. See **Table 19**. The combined finding of nose/nares staining/crusty is considered PRV-002-related, but not adverse, as it did not affect the overall well-being of the animals. Mouth staining is observed at a higher incidence at about 23 mg/mL and is considered PRV-002-related. No PRV-002 animals are remarkable for these findings during the recovery phase.

PRV-002 affected body weight in males at about 23 mg/mL. On Day 14, group mean male body weight is lower (about -9%) at about 23 mg/mL, as when compared to the control group mean. On Days 1-14, group mean body weight gain is lower (about -2.1 grams) at about 23 mg/mL, as when compared to the control group mean (about +26.4 grams). These differences are not considered adverse. Recovery group mean values in the about 23 mg/mL male group are generally similar to the control group means.

Group mean food consumption is slightly lower in males at about 23 mg/mL during the dosing and recovery phases, as when compared to the control group means. This difference is not considered adverse.

The nasal turbinates and lungs from all animals in the middle dose group are examined as a possible target tissue.

Table 19. Experimental design

Group	Treatment	Number of Animals					
		Main Study Rats		Recovery Rats		TK Rats	
		M	F	M	F	M	F
1	Control	10	10	5	5	0	0
2	Low	10	10	5	5	9	9
3	Mid	10	10	5	5	9	9
4	High	10	10	5	5	9	9

- Preparation of test material formulation on Study Days 1 and 7 and subsequent sampling on Study Days 1 and 14. Formulation samples are stored at about -70°C prior to dispatch to the Sponsor.
- TID treatment by Intranasal delivery for 14 consecutive days is followed by a 14 day recovery.
- Clinical signs: daily. Signs recorded at about 1 and about 4 hours after each dose.
- Body weights: prior to dose administration, weekly thereafter and at termination.
- Food consumption: prior to dose administration and weekly thereafter.
- Ophthalmoscopic examination of all main and recovery rats pretreatment and prior to termination.
- Toxicokinetics (Toxicokinetic study animals only.
    - Groups 2 – 4: Days 1 and 14; 3 rats/sex/timepoint
    - Timepoints: 10
    - Blood samples centrifuged, plasma/serum harvested and stored at about -70°C prior to dispatch to the Sponsor.
    - Toxicokinetic animals euthanized without further investigation after the last bleed.
- Number of samples = 360
- Hematology: Pretrial, prior to first dose and at termination.
- Coagulation, clinical chemistry, and urinalysis: Main and recovery phase animals prior to first dose and at termination.

- Necropsy examination of all Main study animals one day after the last dose and recovery rats on Day 15 of the recovery period, including bone marrow preparation and preservation of a full tissue list.
- Selected organ weights of all rats (full list).
- Histopathological examination of a full tissue list from all main study rats of Control and High dose, all macroscopic abnormalities and target organs from intermediate groups. Examination of target organs, if any.
- Full audited report of all in-life results and findings.

Archiving of all specimens and data for about 10 years

EXAMPLE 33 - Intranasal administration of PRV-002 effects in a dog model

Evaluation of cardiovascular (hemodynamic) function
and qtc in conscious telemetered male beagles

The purpose of this study is to determine the potential acute effects of PRV-002 on cardiac functions and electrocardiograms (ECG) of conscious telemetered beagle dogs. See **Tables 20-28**. The test article, PRV-002 (Pharmaron: PH-PRV-1302-OB Lot/Batch No.: PH-PRV-1302-GLP-OB-A-1), is supplied by the Sponsor as a powder and is prepared as an about 23 mg/mL Cyclodextrin (about 45% hydroxypropyl-ß-cyclodextrin (HP-ß-CD) – Trappsol ®Hydroxypropyl Beta Cyclodextrin) solution on the day of treatment. There is one treatment group of 4 dogs each receiving vehicle and three doses of PRV-002 (intended doses of about 3 mg/mL, about 10 mg/mL and about 23 mg/mL). On each day of dose administration, the vehicle or test article is dosed on three occasions, approximately 4 hours apart, by administering about 1 mL to each nostril. Animals are observed once daily and on days of dose administration, prior to dose administration and at the completion of the data collection. A washout period of at least three days is allowed between doses. One-minute means of hemodynamic parameters as well as ECG parameters are measured for a period of at least 22 hours following each dose. The following parameters are analyzed using the D.I.S.S. CA Recorder Systems Version 3.0.1: Body temperature, Systolic Arterial Pressure (SAP), Diastolic Arterial Pressure (DAP), Mean Arterial Pressure (MAP), Heart Rate (HR), P duration, PR Interval, QRS Interval, R amplitude, and QT Interval. A board-certified veterinary cardiologist

examined one-minute tracings of the ECGs at about 15 minutes prior to dosing and at about 30 minutes post dose and at about 1, about 2, about 4, about 8, about 12 and about 22 hours post dose.

Administration of PRV-002 is not associated with any clinical signs of toxicity in this study.

Intranasal administration of PRV-002 at doses of about 3 mg/mL, about 10 mg/mL or about 23 mg/mL does not induce any effects on heart rate or blood pressure in conscious telemetered beagle dogs nor does it have any toxicologic effects on cardiac rhythm or ECG morphology in this study.

Bioanalysis of the plasma samples that are collected on each day of dosing at about 10 minutes following the third dose confirms exposure to PRV-002 in a dose-dependent manner. Intranasal administration of PRV-002 three times daily at doses of about 3 mg/mL, about 10 mg/mL or about 23 mg/mL to conscious telemetered dogs does not have any effects on heart rate, arterial blood pressure, cardiac rhythm or ECG morphology.

The effects of intranasal administration of about 3 mg/mL PRV-002 upon Certain cardiovascular parameters are depicted in **FIGS. 45A-45D**.

The effects of intranasal administration of about 10 mg/mL PRV-002 upon certain cardiovascular parameters are depicted in **FIGS. 46A-46D**.

The effects of nitranasal administration of about 23 mg/mL PRV-002 upon certain cardiovascular parameters are depicted in **FIGS. 47A-47D**.

Table 20 – Clinical Observations

Day 1

Animal #	Dose (mL/nostril 3x daily)	Pre Dose	Completion
1	1 (Vehicle)	-	-
2	1 (Low-dose)	-	-
3	1 (Mid-dose)	-	-
4*	1 (High-dose)	-	-

Day 4

Animal #	Dose (mL/nostril 3x daily)	Pre Dose	Completion
1	1 (High-dose)	-	-

2	1 (Vehicle)	-	-
3	1 (Low-dose)	-	-
4	1 (Mid-dose)	-	-

Day 8

Animal #	Dose (mL/nostril 3x daily)	Pre Dose	Completion
1	1 (Mid-dose)	-	-
2	1 (High-dose)	-	-
3	1 (Vehicle)	-	-
4[#]	1 (Low-dose)	-	-

Day 11

Animal #	Dose (mL/nostril 3x daily)	Per Dose	Completion
1	1 (Low-dose)	-	-
2	1 (Mid-dose)	-	-
3	1 (High-dose)	-	-
4	1 (Vehicle)	-	-

- animal appeared normal, * minimal amount of leakage from right nostril following the 3rd daily dose, # minimal amount of leakage from left nostril following the 1st daily dose

All animals appeared normal on Days 2, 3, 5, 6, 7, 9, 10, 12.

Table 21. - Effects of Intranasal Administration of Vehicle Control Upon Cardiovascular Parameters Mean Values (n=4)

TREATMENT	TIME	HR	DAP	MAP	SAP	Temp	QTc
Vehicle Control	0[a]	115	106	127	148	37.4	0.252
0 mg/mL	15 min[b]	108	101	120	139	37.8	0.254
	30 min	95	97	116	133	37.6	0.254
	45 min	96	92	112	128	37.4	0.253
	60 min	82	89	106	124	37.4	0.256
	75 min	91	94	112	130	37.3	0.260
	90 min	88	93	112	131	37.1	0.257
	105 min	88	93	113	133	37.0	0.258
	120 min	80	91	110	130	37.1	0.257
	135 min	96	100	119	141	37.3	0.256
	150 min	85	94	112	132	37.2	0.256
	165 min	91	93	113	134	37.2	0.257
	180 min	97	98	118	138	37.1	0.254
	195 min	100	99	118	137	37.1	0.255
	210 min	96	95	117	136	37.0	0.253
	225 min	90	97	117	137	37.0	0.255
	240 min	109	105	125	144	37.3	0.254
	5 hr[c]	98	96	115	133	37.1	0.255
	6 hr	94	96	114	135	37.3	0.257
	7 hr	96	98	118	138	37.4	0.254

8 hr	113	100	123	143	37.3	0.252
9 hr	96	98	116	134	37.4	0.249
10 hr	86	96	113	133	37.3	0.251
11 hr	87	95	115	135	37.8	0.253
12 hr	77	96	115	137	37.3	0.250
13 hr	74	97	115	139	37.0	0.248
14 hr	78	102	121	142	37.5	0.253
15 hr	94	102	122	142	37.4	0.254
16 hr	72	97	115	138	37.3	0.247
17 hr	80	100	120	142	37.4	0.252
18 hr	89	99	119	142	37.4	0.250
19 hr	82	102	122	146	37.1	0.252
20 hr	95	103	125	148	37.3	0.255
21 hr	106	105	126	148	37.3	0.252
22 hr	132	108	130	153	37.9	0.244

HR - heart rate (beats/min) DAP - diastolic arterial pressure (mmHg)

MAP - mean arterial pressure (mmHg) SAP - systolic arterial pressure (mmHg)

[a]Represents the mean value for 15 minutes prior to dosing.

[b]Represents the mean value for 15 minutes prior to the indicated time.

[c]Represents the mean value for 60 minutes prior to the indicated time.

Table 22.- Effects of Intranasal Administration of about 3 mg/mL PRV-002 Upon Cardiovascular Parameters Mean Values (n=4)

TREATMENT	TIME	HR	% Δ	DAP	% Δ	MAP	% Δ	SAP	% Δ	Temp	% Δ	QTc	% Δ
PRV-002	0[a]	113	-2	106	0	128	1	150	1	37.6	0	0.251	0
3 mg/mL	15 min[b]	101	-6	100	0	120	-1	139	0	37.8	0	0.251	-1
	30 min	97	3	97	1	117	1	136	2	37.6	0	0.252	-1
	45 min	89	-7	94	2	112	0	131	3	37.5	0	0.253	0
	60 min	74	-11	91	2	109	3	128	3	37.4	0	0.247	-3
	75 min	88	-3	95	1	110	-2	134	3	37.3	0	0.255	-2
	90 min	85	-3	93	0	112	0	134	2	37.2	0	0.249	-3
	105 min	76	-14	91	-3	110	-3	131	-1	37.2	1	0.249	-4
	120 min	84	5	94	3	113	2	133	2	37.0	0	0.255	-1
	135 min	89	-7	97	-3	115	-4	137	-3	37.0	-1	0.255	0
	150 min	88	4	96	2	116	4	138	4	36.8	-1	0.252	-1
	165 min	90	0	100	8	121	7	144	7	36.9	-1	0.254	-1
	180 min	80	-17	96	-2	116	-2	139	0	36.9	-1	0.253	0
	195 min	81	-19	98	-1	118	0	141	3	37.0	0	0.255	0
	210 min	87	-10	99	5	119	2	142	4	37.2	0	0.254	0
	225 min	87	-3	96	-1	115	-1	138	0	37.2	1	0.257	1
	240 min	102	-6	108	3	129	3	151	5	37.2	0	0.254	0
	5 hr[c]	86	-13	96	0	115	0	137	3	37.2	0	0.255	0
	6 hr	87	-8	96	-1	116	1	138	2	37.4	0	0.252	-2
	7 hr	87	-9	97	-1	116	-2	138	0	37.4	0	0.250	-2
	8 hr	94	-17	100	0	120	-2	143	0	37.6	1	0.249	-1
	9 hr	92	-4	98	0	118	2	139	3	37.7	1	0.249	0

10 hr	78	-9	96	0	115	2	136	3	37.4	0	0.249	-1
11 hr	86	-1	98	4	117	2	138	3	37.4	-1	0.252	-1
12 hr	83	7	99	2	118	3	140	2	37.2	0	0.251	0
13 hr	81	9	96	-1	114	-1	135	-3	37.5	1	0.252	2
14 hr	80	3	95	-7	114	-5	135	-5	37.0	-1	0.251	-1
15 hr	75	-20	93	-8	113	-7	135	-5	37.2	-1	0.247	-3
16 hr	81	13	97	1	118	2	139	1	37.3	0	0.253	2
17 hr	77	-3	93	-7	113	-5	134	-6	37.1	-1	0.249	-1
18 hr	73	-18	96	-2	115	-3	138	-3	37.3	0	0.249	-1
19 hr	71	-13	98	-4	117	-4	140	-4	37.4	1	0.249	-1
20 hr	81	-14	98	-5	119	-5	141	-4	37.3	0	0.251	-1
21 hr	109	2	105	0	128	2	151	2	37.2	0	0.248	-2
22 hr	134	2	109	1	131	1	154	0	37.8	0	0.245	0

HR - heart rate (beats/min)
DAP - diastolic arterial pressure (mmHg)
MAP - mean arterial pressure (mmHg)
SAP - systolic arterial pressure (mmHg)
% Δ - percent change from corresponding vehicle value
[a]Represents the mean value for 15 minutes prior to dosing.
[b]Represents the mean value for 15 minutes prior to the indicated time.
[c]Represents the mean value for 60 minutes prior to the indicated time.

Table 23.- Effects of Intranasal Administration of about 10 mg/mL PRV-002 Upon Cardiovascular Parameters Mean Values (n=4)

TREATMENT	TIME	HR	% Δ	DAP	% Δ	MAP	% Δ	SAP	% Δ	Temp	% Δ	QTc	% Δ
PRV-002	0[a]	126	10	106	0	127	0	148	0	37.9	1	0.250	-1
10 mg/mL	15 min[b]	113	5	102	1	121	0	140	1	37.9	0	0.252	0
	30 min	102	8	95	-2	114	-1	134	0	37.7	0	0.250	-1
	45 min	100	5	97	6	117	4	137	7	37.7	1	0.246	-3
	60 min	97	18	94	6	114	7	133	7	37.6	1	0.249	-2
	75 min	98	7	97	3	117	4	137	5	37.4	0	0.251	-4
	90 min	87	-2	93	0	112	0	133	1	37.4	1	0.251	-2
	105 min	85	-3	92	-1	110	-3	130	-2	37.4	1	0.256	-1
	120 min	96	19	95	4	116	5	136	5	37.3	0	0.258	0
	135 min	93	-3	94	-6	115	-4	135	-4	37.1	0	0.253	-1
	150 min	88	4	92	-2	112	0	133	1	37.0	-1	0.252	-2
	165 min	93	2	97	4	117	4	140	4	37.0	-1	0.252	-2
	180 min	101	-3	99	19	119	17	141	14	37.0	0	0.252	7
	195 min	92	-8	98	-1	119	1	141	3	37.1	0	0.254	-1
	210 min	89	-7	94	0	114	-2	135	-1	37.2	0	0.250	-1
	225 min	90	0	97	0	117	0	138	1	37.2	0	0.249	-2
	240 min	106	-9	107	-1	128	0	149	0	37.3	0	0.251	-1
	5 hr[c]	97	-2	97	1	118	2	139	5	37.4	1	0.248	-2
	6 hr	94	0	95	-1	115	1	137	1	37.3	0	0.251	-2
	7 hr	92	-4	94	-5	115	-3	136	-1	37.1	-1	0.252	-1
	8 hr	101	-11	100	0	122	-1	143	0	37.4	0	0.250	-1
	9 hr	102	7	97	0	117	1	137	2	37.6	1	0.250	0
	10 hr	88	3	94	-1	114	0	135	2	37.4	0	0.254	1

11 hr	86	-1	96	1	116	0	137	2	37.3	-1	0.253	0
12 hr	83	8	95	-1	116	1	138	1	37.4	0	0.253	1
13 hr	84	13	97	1	116	1	138	-1	37.2	1	0.252	2
14 hr	75	-4	94	-8	113	-6	135	-5	37.1	-1	0.250	-1
15 hr	78	-17	98	-4	118	-3	140	-1	37.1	-1	0.248	-2
16 hr	72	0	95	-1	115	0	139	1	37.5	1	0.248	0
17 hr	84	6	101	1	122	2	145	2	37.4	0	0.249	-1
18 hr	83	-7	101	2	124	3	149	5	37.5	0	0.244	-3
19 hr	89	9	98	-4	121	0	147	1	37.9	2	0.244	-3
20 hr	91	-4	97	-6	122	-3	146	-1	37.7	1	0.250	-2
21 hr	102	-4	104	-1	126	0	149	1	37.2	0	0.250	-1
22 hr	125	-5	108	0	131	0	153	0	37.5	-1	0.250	3

HR - heart rate (beats/min)
DAP - diastolic arterial pressure (mmHg)
MAP - mean arterial pressure (mmHg)
SAP - systolic arterial pressure (mmHg)
% Δ - percent change from corresponding vehicle value
[a]Represents the mean value for 15 minutes prior to dosing.
[b]Represents the mean value for 15 minutes prior to the indicated time.
[c]Represents the mean value for 60 minutes prior to the indicated time.

Table 22. - Effects of Intranasal Administration of about 23 mg/mL PRV-002 upon Cardiovascular Parameters Mean Values (n=4)

TREATMENT	TIME	HR	% Δ	DAP	% Δ	MAP	% Δ	SAP	% Δ	Temp	% Δ	QTc	% Δ
PRV-002	0[a]	123	7	104	-2	125	-1	145	-2	37.7	1	0.253	0
23 mg/mL	15 min[b]	119	10	104	3	123	2	141	2	38.1	1	0.251	-1
	30 min	110	16	95	-2	113	-2	131	-2	37.8	0	0.251	-1
	45 min	101	5	90	-2	111	-1	128	0	37.2	0	0.252	0
	60 min	93	13	88	-1	107	1	126	1	37.2	0	0.253	-1
	75 min	106	16	96	3	116	3	136	4	37.1	-1	0.256	-2
	90 min	93	5	93	-1	111	-1	130	-1	37.3	0	0.253	-1
	105 min	95	9	93	0	113	0	132	0	37.1	0	0.258	0
	120 min	95	18	93	1	112	1	131	1	37.1	0	0.258	0
	135 min	100	4	95	-5	116	-3	135	-4	37.1	-1	0.248	-3
	150 min	90	5	92	-2	113	1	133	0	37.2	0	0.253	-1
	165 min	91	0	92	-1	112	-1	133	-1	37.2	0	0.255	-1
	180 min	92	-4	94	-4	113	-4	134	-3	37.2	0	0.256	1
	195 min	99	-1	96	-4	116	-2	136	-1	37.2	0	0.257	1
	210 min	89	-8	89	-6	113	-3	132	-3	37.2	0	0.253	0
	225 min	85	-5	95	-2	115	-1	137	0	37.4	1	0.254	0
	240 min	120	10	112	7	135	9	157	9	37.3	0	0.251	-1
	5 hr[c]	104	6	98	3	119	4	139	5	37.1	0	0.250	-2
	6 hr	99	5	97	0	117	3	138	2	37.0	-1	0.253	-2
	7 hr	100	4	98	0	119	0	141	2	37.2	0	0.254	0
	8 hr	105	-8	99	-1	122	-1	143	0	37.8	1	0.251	0
	9 hr	98	3	94	-3	115	0	135	0	37.9	1	0.249	0
	10 hr	90	4	95	-1	114	1	135	2	37.4	0	0.250	-1
	11 hr	87	-1	95	0	115	-1	136	1	37.7	0	0.253	0
	12 hr	81	4	94	-2	114	-1	137	0	37.2	0	0.252	1

13 hr	80	7	95	-2	116	0	137	-1	37.3	1	0.248	0
14 hr	77	-1	95	-7	114	-5	138	-3	37.6	0	0.251	-1
15 hr	74	-22	94	-7	114	-7	138	-3	37.2	-1	0.249	-2
16 hr	74	3	96	-1	115	0	139	1	37.3	0	0.249	1
17 hr	74	-7	97	-4	117	-3	141	-1	37.2	0	0.250	-1
18 hr	76	-15	99	0	119	0	143	1	37.3	0	0.251	0
19 hr	79	-4	100	-2	121	-1	146	0	37.2	0	0.249	-1
20 hr	85	-10	103	-1	124	-1	149	1	37.2	0	0.247	-3
21 hr	115	8	109	4	133	5	156	6	37.5	0	0.248	-1
22 hr	132	0	107	-1	132	1	155	1	37.9	0	0.246	1

HR - heart rate (beats/min)
DAP - diastolic arterial pressure (mmHg)
MAP - mean arterial pressure (mmHg)
SAP - systolic arterial pressure (mmHg)
% Δ - percent change from corresponding vehicle value
[a]Represents the mean value for 15 minutes prior to dosing.
[b]Represents the mean value for 15 minutes prior to the indicated time.
[c]Represents the mean value for 60 minutes prior to the indicated time.

Table 25. - Effects of Intranasal Administration of Vehicle Control Upon ECG Parameters Mean

Values (n=4)

TREATMENT	TIME	PR Interval	P Duration	QRS Interval	QT	R Amplitude	QTc
Vehicle Control	0[a]	0.082	0.054	0.043	0.211	2.733	0.252
0 mg/mL	15 min[b]	0.083	0.053	0.043	0.216	2.728	0.254
	30 min	0.084	0.054	0.042	0.222	3.146	0.254
	45 min	0.082	0.052	0.043	0.222	2.808	0.253
	60 min	0.084	0.052	0.042	0.234	2.875	0.256
	75 min	0.084	0.053	0.042	0.232	2.740	0.260
	90 min	0.083	0.054	0.043	0.230	2.842	0.257
	105 min	0.082	0.052	0.042	0.232	3.126	0.258
	120 min	0.083	0.052	0.042	0.239	2.848	0.257
	135 min	0.082	0.052	0.042	0.223	2.762	0.256
	150 min	0.080	0.051	0.042	0.231	2.913	0.256
	165 min	0.081	0.052	0.043	0.229	2.674	0.257
	180 min	0.084	0.054	0.042	0.222	2.821	0.254
	195 min	0.081	0.051	0.042	0.221	2.776	0.255
	210 min	0.081	0.051	0.042	0.222	2.730	0.253
	225 min	0.085	0.056	0.042	0.228	2.695	0.255
	240 min	0.085	0.056	0.043	0.216	2.510	0.254
	5 hr[c]	0.086	0.056	0.043	0.221	2.755	0.255
	6 hr	0.084	0.054	0.043	0.227	2.667	0.257
	7 hr	0.081	0.053	0.043	0.222	2.749	0.254
	8 hr	0.080	0.051	0.042	0.214	2.759	0.252
	9 hr	0.077	0.049	0.042	0.217	2.768	0.249
	10 hr	0.079	0.051	0.042	0.226	2.593	0.251
	11 hr	0.082	0.052	0.042	0.227	2.313	0.253
	12 hr	0.078	0.051	0.041	0.232	2.715	0.250
	13 hr	0.075	0.048	0.041	0.232	2.958	0.248
	14 hr	0.081	0.051	0.042	0.234	2.292	0.253
	15 hr	0.080	0.052	0.042	0.223	2.418	0.254
	16 hr	0.078	0.050	0.042	0.234	2.713	0.247

17 hr	0.077	0.050	0.042	0.232	2.606	0.252
18 hr	0.079	0.052	0.042	0.228	2.612	0.250
19 hr	0.088	0.062	0.043	0.236	2.779	0.252
20 hr	0.082	0.055	0.043	0.230	2.522	0.255
21 hr	0.077	0.051	0.044	0.217	2.578	0.252
22 hr	0.075	0.051	0.044	0.200	2.513	0.244

[a]Represents the mean value for 15 minutes prior to dosing.

[b]Represents the mean value for 15 minutes prior to the indicated time.

[c]Represents the mean value for 60 minutes prior to the indicated time.

Table 26. - Effects of Intranasal Administration of about 3 mg/mL PRV-002 Upon ECG Parameters Mean Values (n=4)

TREATMENT	TIME	PR Interval	P Duration	QRS Interval	QT	R Amplitude	QTc
PRV-002	0[a]	0.084	0.057	0.043	0.212	2.703	0.251
3 mg/mL	15 min[b]	0.086	0.057	0.043	0.216	2.821	0.251
	30 min	0.083	0.055	0.043	0.221	2.855	0.252
	45 min	0.083	0.053	0.043	0.226	2.758	0.253
	60 min	0.086	0.056	0.043	0.233	2.924	0.247
	75 min	0.086	0.055	0.042	0.229	2.913	0.255
	90 min	0.086	0.056	0.042	0.226	3.071	0.249
	105 min	0.086	0.056	0.042	0.233	2.925	0.249
	120 min	0.088	0.057	0.043	0.230	2.852	0.255
	135 min	0.089	0.060	0.043	0.227	2.746	0.255
	150 min	0.081	0.054	0.043	0.225	2.945	0.252
	165 min	0.085	0.056	0.042	0.226	3.069	0.254
	180 min	0.085	0.055	0.042	0.233	3.195	0.253
	195 min	0.086	0.057	0.043	0.234	2.800	0.255
	210 min	0.084	0.054	0.042	0.228	2.883	0.254
	225 min	0.085	0.054	0.042	0.232	2.791	0.257
	240 min	0.087	0.056	0.043	0.219	2.648	0.254
	5 hr[c]	0.085	0.055	0.042	0.229	2.897	0.255
	6 hr	0.083	0.054	0.042	0.225	2.885	0.252
	7 hr	0.081	0.054	0.042	0.223	2.825	0.250
	8 hr	0.079	0.053	0.042	0.219	2.621	0.249
	9 hr	0.080	0.052	0.042	0.220	2.659	0.249
	10 hr	0.080	0.053	0.042	0.229	2.459	0.249
	11 hr	0.077	0.050	0.042	0.225	2.669	0.252
	12 hr	0.080	0.052	0.042	0.228	2.598	0.251
	13 hr	0.085	0.055	0.041	0.230	2.337	0.252
	14 hr	0.078	0.050	0.041	0.229	2.831	0.251
	15 hr	0.079	0.051	0.042	0.230	2.725	0.247
	16 hr	0.085	0.056	0.042	0.231	2.635	0.253
	17 hr	0.080	0.052	0.041	0.230	2.830	0.249
	18 hr	0.079	0.051	0.042	0.233	2.561	0.249
	19 hr	0.079	0.051	0.042	0.236	2.488	0.249
	20 hr	0.080	0.052	0.042	0.230	2.695	0.251
	21 hr	0.079	0.054	0.043	0.211	2.686	0.248
	22 hr	0.080	0.057	0.044	0.201	2.439	0.245

[a]Represents the mean value for 15 minutes prior to dosing.

[b]Represents the mean value for 15 minutes prior to the indicated time.

[c]Represents the mean value for 60 minutes prior to the indicated time.

Table 27. - Effects of Intranasal Administration of about 10 mg/mL PRV-002 Upon ECG Parameters Mean Values (n=4)

TREATMENT	TIME	PR Interval	P Duration	QRS Interval	QT	R Amplitude	QTc
PRV-002	0[a]	0.082	0.057	0.042	0.206	2.717	0.250
10 mg/mL	15 min[b]	0.085	0.056	0.042	0.212	2.762	0.252
	30 min	0.082	0.054	0.042	0.217	2.857	0.250
	45 min	0.084	0.056	0.042	0.214	3.025	0.246
	60 min	0.083	0.055	0.042	0.217	2.961	0.249
	75 min	0.083	0.055	0.042	0.218	3.107	0.251
	90 min	0.080	0.052	0.043	0.224	3.013	0.251
	105 min	0.083	0.052	0.043	0.233	2.828	0.256
	120 min	0.085	0.057	0.043	0.227	2.893	0.258
	135 min	0.085	0.058	0.043	0.224	2.937	0.253
	150 min	0.082	0.056	0.042	0.225	3.164	0.252
	165 min	0.085	0.057	0.042	0.223	3.046	0.252
	180 min	0.082	0.055	0.043	0.218	3.056	0.252
	195 min	0.080	0.052	0.042	0.226	3.033	0.254
	210 min	0.080	0.053	0.042	0.222	3.090	0.250
	225 min	0.080	0.052	0.042	0.225	3.070	0.249
	240 min	0.082	0.053	0.042	0.215	2.847	0.251
	5 hr[c]	0.080	0.053	0.042	0.217	2.896	0.248
	6 hr	0.078	0.052	0.042	0.220	2.816	0.251
	7 hr	0.081	0.054	0.042	0.222	2.887	0.252
	8 hr	0.081	0.053	0.042	0.215	2.610	0.250
	9 hr	0.080	0.053	0.042	0.215	2.613	0.250
	10 hr	0.080	0.053	0.041	0.226	2.735	0.254
	11 hr	0.080	0.054	0.042	0.227	2.644	0.253
	12 hr	0.077	0.050	0.041	0.229	2.618	0.253
	13 hr	0.086	0.058	0.042	0.227	2.546	0.252
	14 hr	0.075	0.049	0.041	0.233	2.841	0.250
	15 hr	0.074	0.049	0.042	0.229	2.735	0.248
	16 hr	0.076	0.050	0.042	0.234	2.514	0.248
	17 hr	0.077	0.052	0.042	0.225	2.587	0.249
	18 hr	0.075	0.050	0.043	0.227	2.683	0.244
	19 hr	0.076	0.050	0.043	0.224	2.562	0.244
	20 hr	0.086	0.059	0.043	0.227	2.589	0.250
	21 hr	0.077	0.053	0.043	0.217	2.791	0.250
	22 hr	0.081	0.058	0.043	0.208	2.660	0.250

[a]Represents the mean value for 15 minutes prior to dosing.

[b]Represents the mean value for 15 minutes prior to the indicated time.

[c]Represents the mean value for 60 minutes prior to the indicated time.

Table 28. - Effects of Intranasal Administration of about 23 mg/mL PRV-002 Upon ECG Parameters Mean Values (n=4)

TREATMENT	TIME	PR Interval	P Duration	QRS Interval	QT	R Amplitude	QTc
PRV-002	0[a]	0.082	0.055	0.043	0.209	2.688	0.253
23 mg/mL	15 min[b]	0.086	0.055	0.042	0.208	2.638	0.251
	30 min	0.085	0.054	0.043	0.212	2.810	0.251
	45 min	0.084	0.054	0.043	0.218	3.013	0.252
	60 min	0.084	0.055	0.042	0.223	3.008	0.253
	75 min	0.084	0.055	0.042	0.218	2.793	0.256

90 min	0.084	0.055	0.042	0.223	3.127	0.253
105 min	0.083	0.054	0.042	0.226	3.001	0.258
120 min	0.082	0.053	0.042	0.227	2.897	0.258
135 min	0.083	0.054	0.042	0.215	2.715	0.248
150 min	0.084	0.054	0.042	0.226	2.821	0.253
165 min	0.084	0.055	0.042	0.227	2.786	0.255
180 min	0.081	0.052	0.042	0.226	2.957	0.256
195 min	0.080	0.052	0.042	0.223	2.882	0.257
210 min	0.083	0.054	0.041	0.226	2.970	0.253
225 min	0.086	0.056	0.042	0.231	3.004	0.254
240 min	0.082	0.054	0.043	0.209	2.671	0.251
5 hr[c]	0.081	0.053	0.043	0.214	2.818	0.250
6 hr	0.079	0.052	0.042	0.220	2.788	0.253
7 hr	0.077	0.051	0.043	0.220	2.789	0.254
8 hr	0.079	0.052	0.042	0.217	2.638	0.251
9 hr	0.078	0.050	0.042	0.216	2.426	0.249
10 hr	0.082	0.054	0.042	0.221	2.514	0.250
11 hr	0.079	0.051	0.042	0.227	2.527	0.253
12 hr	0.074	0.048	0.042	0.231	2.985	0.252
13 hr	0.079	0.051	0.042	0.227	2.452	0.248
14 hr	0.077	0.050	0.042	0.233	2.635	0.251
15 hr	0.076	0.050	0.042	0.234	2.777	0.249
16 hr	0.076	0.049	0.042	0.234	2.637	0.249
17 hr	0.078	0.052	0.042	0.235	2.738	0.250
18 hr	0.081	0.053	0.042	0.236	2.588	0.251
19 hr	0.077	0.051	0.043	0.232	2.885	0.249
20 hr	0.080	0.053	0.043	0.228	2.590	0.247
21 hr	0.076	0.052	0.043	0.211	2.639	0.248
22 hr	0.078	0.055	0.044	0.203	2.618	0.246

[a]Represents the mean value for 15 minutes prior to dosing.

[b]Represents the mean value for 15 minutes prior to the indicated time.

[c]Represents the mean value for 60 minutes prior to the indicated time.

EXAMPLE 34 - Pharmacokinetic evaluation of PRV-002 in brain tissue and cerebral spinal fluid (CSF)

In a study conducted at BASi in Evansville, Indiana, Beagle dogs are treated with PRV-002 intranasal dose formulations in Cyclodextrin or a nano-particle suspension. The purpose of the study is to evaluate the pharmacokinetics of PRV-002 in plasma and concentration of PRV-002 in brain tissue and cerebral spinal fluid (CSF) of two different formulations of PRV-002 when they are administered on a single day, TID (three times per day) with approximately 4 hour intervals.

Experimental design

Numbers represent PRV-002 levels in various parts of the brain in dogs that are dosed IN 3 times at about 4 hour intervals in one day. Dogs are sacrificed and tissues are harvested at

about 30 minutes after the last dose. Dogs are given about 1 mL/nostril at each dosing interval for a total dose of about 46 mg/dog per dosing interval. See **Table 29**.

Table 29. Dog Brain Bio-Distribution: Numbers represent brain PTV-002 levels

Brain tissue section	Mean Drug Conc. (ng/g of brain tissue), N=3	
	Cyclodextrin formulation	Nano-particle formulation
Frontal lobe	1,459	2,403
Occipital lobe	1,474	2,332
Olfactory lobe	1,370	2,049
Parietal lobe	1,529	2,386
Temporal lobe	1,352	2,368
Whole brain	1,374	1,888

Formulation specifications

The nano-formulation is a nanosuspension of compound PRV-1302 in about 1% Kolliphor P338/P188. The final PRV-1302 formulation had an API concentration of about 23.4 mg/mL (PRV-1302) with a particle size of approximately 450 nm[2]. Quantitative determination of compound PRV-1302 is accomplished using the HPLC method provided to us. A final average concentration of about 23.4 mg/mL is measured for the PRV-1302 formulated suspension. See **Table 30**.

Table 30. Nanosuspension formulation specifications

- PRV-002 Concentration (PRV-1302 content by HPLC): about 23.5 mg/mL
- PRV-002 (Particle size of formulated PRV-1302): about 450 nm
- Volume: 1 vial with about 26 mL
- Manufacture date (MFD): December 4, 2014
- Storage conditions: about 25 ºC

Table 31. Cyclodextrin formulation specifications: (Prepared on-site at BASi)

- PRV-002 (Pharmaron: PH-PRV-1302-0B) / PH-PRV-1302-GLP-0B-A-1
- PRV-002 Concentration: about 23 mg/mL

[2] The present invention contemplates a nanoparticle size range for PRV-002 (*ent*-19-norprogesterone) of about 400 nm to about 450 nm.

- Vehicle**:** 2-hydroxypropyl-β-cyclodextrin (45% aqueous solution)

Dog CSF bio-distribution

Numbers represent PRV-002 levels in CSF of dogs that are dosed IN 3 times at about 4 hour intervals in one day. Dogs are anesthetized and CSF is harvested at about 30 minutes after the last dose. Dogs are given about 1mL/nostril at each dosing interval for a total dose of about 46 mg/dog per dosing interval. See **Table 32**.

Table 32. Dog PRV-002 (*ent*-19-norprogesterone) CSF Bio-Distribution

Sample ID	Mean Drug Conc. (ng/mL of CSF, approximate), N=3	
	Cyclodextrin formulation	Nano-particle formulation
Cerebral Spinal Fluid (CSF) samples	22.1	33.2

Dog plasma concentrations

Numbers represent PRV-002 (*ent*-19-norprogesterone) levels in plasma of dogs that are dosed IN 3 times at 4 hour intervals in one day. Dogs are given about 1mL/nostril at each dosing interval for a total dose of about 46 mg/dog per dosing interval. See **Table 33** and **FIG.48**.

Table 33. PRV-002 (*ent*-19-norprogesterone) Dog Plasma Concentrations

Plasma sample time	Mean Drug Conc. (ng/mL of plasma, approximate), N=3	
	Cyclodextrin formulation	Nano-particle formulation
Pre dose	BLQ	BLQ
10 minutes post dose	270	395
30 minutes post dose	185	268
1 hour post dose	135	231
2 hours post dose	125	289
4 hours post dose (just prior to 2nd dose	76	217
8 hours post dose (30 minutes after last dose	428	607

Discussion

The purpose of this study is to determine and compare the bio-availability of the prototype nano-particle formulation to that of the originally tested cyclodextrin formulation. The results clearly show that the nano-particle formulation is more bio-available than the cylcodextrin formulation.

Furthermore this data provides data about the disappearance of PRV-002 in the circulating plasma and of the amount that is present in the brain and CSF.

EXAMPLE 35 - Summary of the Toxicology, Brain and CSF Bio-Distribution and Plasma Concentration Studies of PRV-002 (*ent*-19-norprogesterone) in Rodent and Non-Rodent (Canine) of Examples 31-34

The IND enabling toxicology program was designed in accordance with ICH M3 (R2) and was comprised of 14 day GLP toxicology studies in rodent (rat) and non-rodent (dog) species using formulation administered by the intranasal route. The GLP repeat dose toxicology studies included safety pharmacology elements (CNS in rat, CV/respiratory in dog). A dog brain bio-availability study was conducted in dogs to demonstrate if the drug reaches the brain (the target organ) and if minimal drug amounts circulate systemically when given by IN route. In addition, Prevacus plans to conduct in vitro hERG testing and genotoxicity testing using Ames test, chromosomal damage. The proposed Phase 1 trial will be conducted in healthy volunteers

to evaluate the safety and tolerability profile of single and multiple doses of clinical formulation administered via intranasal route.

The GLP toxicology program was conducted at BASi in Evansville, IN as follows: A 14-day rat toxicology study was done. The objective of this study was to evaluate the toxicity of PRV-002, when administered three times a day, approximately 4 hours apart, for 14 days at concentrations of 0, 3, 10 or 23 mg/mL at a volume of 50 µL/nostril. Reversibility of toxicity was evaluated during a 14-day recovery period following the final dose of test article, and systemic exposure was evaluated. During the dosing phase, nose/nares staining/crusty was observed in four, eleven and twenty-two PRV-002 treated animals at concentrations of 3, 10, and 23 mg/mL, respectively. The combined finding of nose/nares staining/crusty was considered PRV-002-related, but not adverse, as it did not affect the overall well-being of the animals. Mouth staining was observed at a higher incidence at 23 mg/mL and was considered PRV-002-related. No PRV-002 animals were remarkable for these findings during the recovery phase. PRV-002 affected body weight in males at 23 mg/mL. On Day 14, group mean male body weight was lower (-9%) at 23 mg/mL, compared to the control group mean. On Days 1-14, group mean body weight gain was lower (-2.1 grams) at 23 mg/mL, compared to the control group mean (+26.4 grams). These differences were not considered adverse. Recovery group mean values in the 23 mg/mL male group were generally similar to the control group means. Group mean food consumption was slightly lower in males at 23 mg/mL during the dosing and recovery phases, compared to the control group means. This difference was not considered adverse. The report will be updated with clinical pathology and microscopic data, when available. The nasal turbinates and lungs from all animals in the middle dose group were examined as a possible target tissue. This data demonstrates that PRV-002 is well tolerated at doses up to 34.5 mg/kg.

A 14-day dog toxicology study was conducted. The objective of this study was to evaluate the toxicity of PRV-002, when administered three times a day, approximately 4 hours apart, for 14 days at concentrations of 0, 3, 10 or 23 mg/mL at a volume of 1 mL/nostril. Reversibility of toxicity was evaluated during a 14-day recovery period following the final dose of test article, and systemic exposure was evaluated. PRV-002 did not affect ophthalmology, body weights or food consumption. Increased salivation was observed in all combined male and female PRV-002 treated groups, with incidence increased with concentration

and is considered PRV-002-related, but not an adverse effect. At Day 15, there were no alterations in hematology, clinical chemistry, coagulation, or urinalysis parameters attributable to the administration of PRV-002. Similarly, there were no changes in organ weights and no macroscopic observations related to administration of PRV-002 at the Day 15 time point. At Day 15, microscopic observations in both vehicle-only-treated and test article-treated animals were noted in the nasal turbinates and lungs. Purulent exudates involving the nasal turbinates were present in the majority of animals and subacute alveolar inflammation was present in the lungs of only a few animals. These findings were of limited intensity (minimal to mild) and the overall patterns of incidence and severity of these alterations were consistent with these microscopic observations being attributable to the vehicle. This data demonstrates that PRV-002 (*ent*-19-norprogesterone) is well tolerated at doses up to about 28 mg/kg.

A dog brain bio-availability study was conducted as follows: A nanoparticle formulation of PRV-002 was tested in dogs to determine if it is absorbed into the target organ (the brain) after intranasal administration. Three dogs each weighing approximately 6 kg were dosed intranasally. The dogs were given 46 mg of drug at 4-hour intervals three times in one day. Blood samples were taken to determine levels of the drug at 10, 30, 60 minutes and 2, 4, and 8 hours post dose. Cerebral spinal fluid (CSF) samples were taken along with the brain for bioanalysis at necropsy approximately 30 minutes after the last dose. The results showed excellent absorption and distribution into all regions of the brain. In addition, measurable amounts of the drug were found in the CSF. Ninety-three (93)% of the recovered drug was found in the brain with only 5% found in the circulating plasma. Approximately 2% of the recovered drug was found in the CSF. See **Table 29** above and **Table 34**.

Table 34. PRV-002 (*ent*-19-norprogesterone) Dog Brain Bio-distribution: Numbers represent brain PRV-002 (*ent*-19-norprogesterone) levels

Brain tissue section	Mean Drug Conc. (ng/g of brain tissue), N=3
	Nano-particle formulation
Frontal lobe	2,403
Occipital lobe	2,332
Olfactory lobe	2,049
Parietal lobe	2,386
Temporal lobe	2,368
Whole brain	1,888

See **Table 32** above and **Table 35**.

Table 35. PRV-002 (*ent*-19-norprogesterone) Dog CSF bio-distribution. Numbers represent PRV-002 (*ent*-19-norprogesterone) levels in CSF

Sample ID	Mean Drug Conc. (ng/mL of CSF), N=3
	Nano-particle formulation
Cerebral Spinal Fluid (CSF) samples	33.2

See **Table 33** above and **Table 36**.

Table 36. PRV-002 (*ent*-19-norprogesterone) Dog plasma concentrations. Numbers represent PRV-002 (*ent*-19-norprogesterone) plasma levels

Plasma sample time	Mean Drug Conc. (ng/mL of plasma), N=3
	Nano-particle formulation
Pre dose	BLQ
10 minutes post dose	395
30 minutes post dose	268
1 hour post dose	231
2 hours post dose	289
4 hours post dose (just prior to 2nd dose	217
8 hours post dose (30 minutes after last dose	607

With this confirmatory data Prevacus is continuing with development using a dry powder nano-particle formulation for IN delivery with an intranasal delivery device. A dry powder nasal

formulation will facilitate ease of administration in the field of play or the field of battle. The capability to administer this product both prophylactically and following injury gives its use and marketing ability a significant advantage. A dry powder formulation is a practical, stable, field-deliverable compound—suitable for application in a sports field, or for military use.

Summary Product Profile

Criteria	Description of Target Profile
Route of administration	Intranasal using a stable dry powder formulation preloaded into a single use intranasal delivery device
Indication	Treatment of e.g., mTBI
Dosing Regimen	e.g., BID for 14 days
Formulation Description	A dry powder nano-formulation that is stable at high temperatures for, e.g., at least 3 years
Delivery System	Single use intranasal delivery device
Stability/Shelf-life	Phase 1 and 2 formulation - Stable at room temperature for, e.g., at least 2 years Phase 3 and beyond – stable at, e.g., 114 degrees F for at least 3 years

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18. US Patent Application No. 13/645,925, invented by VanLandingham et al. and entitled "Prophylactic and Post-Acute Use of Progesterone in Conjunction with its Enantiomer for Use in Treatment of Mild Traumatic Brain Injuries"

19. International PCT Patent Application No. PCT/US2012/59030, invented by VanLandingham et al. and entitled "Prophylactic and Post-Acute Use of Progesterone to Better Outcomes Associated with Concussion"

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Incorporation By Reference

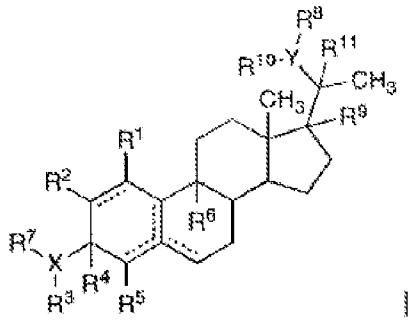
The entire contents of all patents, published patent applications and other references, including articles available at websites, cited herein are hereby expressly incorporated herein in their entireties by reference.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

What is claimed is:

1. A compound of Formula I:



or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof,

wherein, X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^1, R^2, R^5, and R^6 are independently H, C1-C6 alkyl, halogen, OR^{12}, $NR^{13}R^{14}$, SR^{15}, SOR^{16} or SO_2R^{17};

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

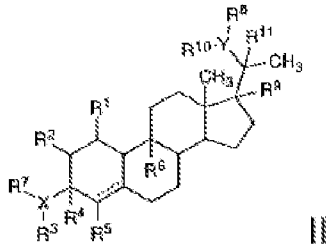
R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16} and R^{17} are independently H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

2. The compound of claim 1 represented by Formula II:



II

wherein, X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^1, R^2, R^5, and R^6 are independently H, C_1-C_6 alkyl, halogen, OR^{12}, $NR^{13}R^{14}$, SR^{15}, SOR^{16} or SO_2R^{17};

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

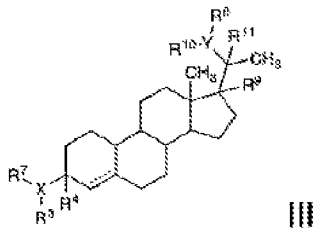
R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16} and R^{17} are independently H, C(O)-C_1-C_6 alkyl or C_1-C_6 alkyl; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

3. The compound of claim 1 represented by Formula Ill:



Ill

wherein;

X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond,

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

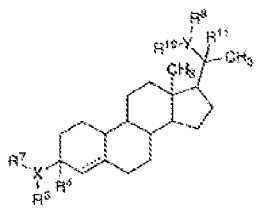
R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond; and

the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

4. The compound of claim 1 represented by Formula IV:



IV

wherein;

Y is O, N or S; or, YR^8R^{10} is absent;

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^9 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl;

R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond; and

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond; and

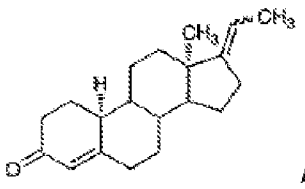
the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

5. The compound of claim 1 wherein, the composition of Formula I possesses the stereochemical configuration of natural steroids.

6. The compound of claim 1 wherein, the composition of Formula I is racemic.

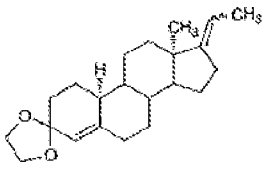
7. The compound of claim 1 wherein, the composition of Formula I possesses a stereochemical configuration that is opposite to that of natural steroids.

8. The compound of claim 1 represented by Structure A



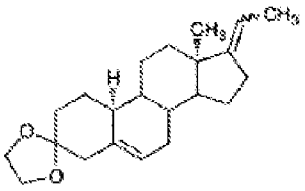
A.

9. The compound of claim 1 represented by Structure B



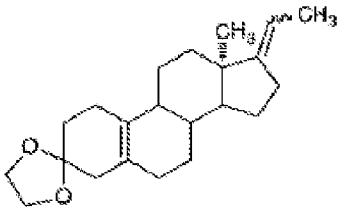
B.

10. The compound of claim 1 represented by Structure C



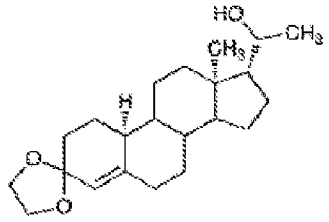
C.

11. The compound of claim 1 represented by Structure D



D.

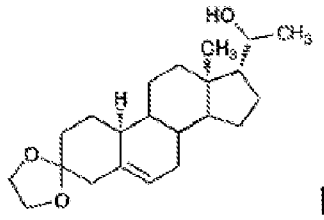
12. The compound of claim 1 represented by Structure E



E.

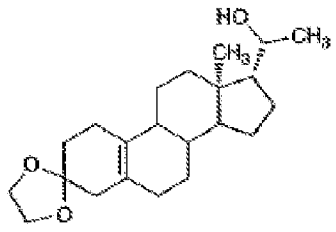
E.

13. The compound of claim 1 represented by Structure F



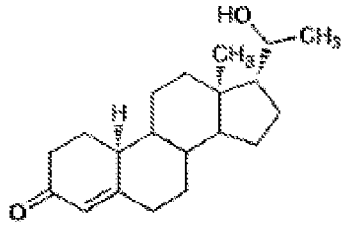
F.

14. The compound of claim 1 represented by Structure G



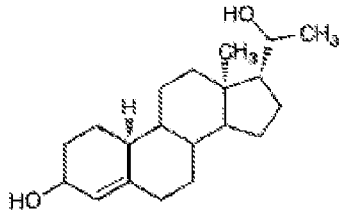
G.

15. The compound of claim 1 represented by Structure H



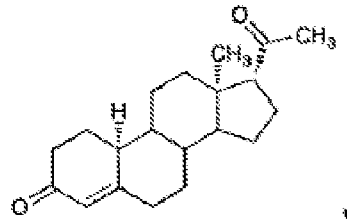
H.

16. The compound of claim 1 represented by Structure I



I.

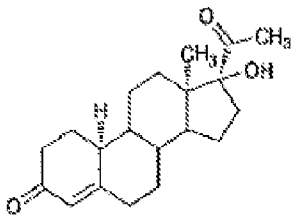
17. The compound of claim 1 represented by Structure J



J.

18. The compound of claim 17, wherein the compound represented by the Structure J is a salt.

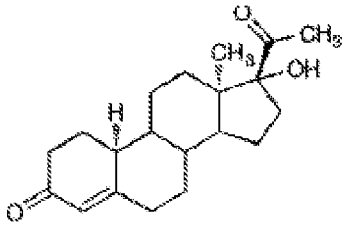
19. The compound of claim 1 represented by Structure K



K.

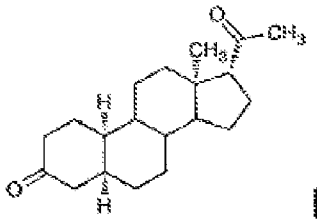
100

20. The compound of claim 1 represented by Structure L



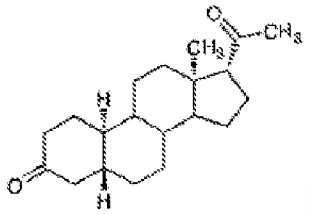
L.

21. The compound of claim 1 represented by Structure M



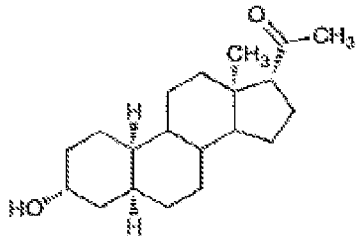
M.

22. The compound of claim 1 represented by Structure N.



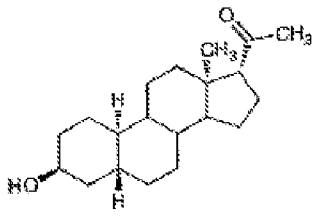
N.

23. The compound of claim 1 represented by Structure O



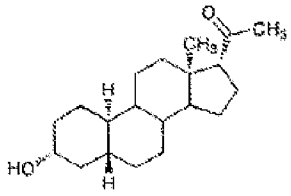
O.

24. The compound of claim 1 represented by Structure P



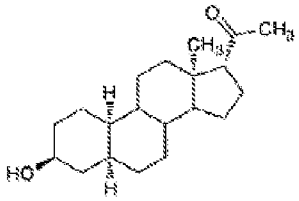
P.

25. The compound of claim 1 represented by Structure Q



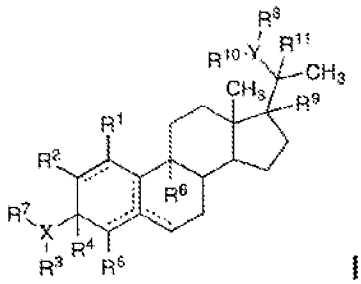
Q.

26. The compound of claim 1 represented by Structure R



R.

27. A pharmaceutical composition comprising a therapeutically useful amount of a compound of Formula I



I

or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug or co-crystal thereof, wherein X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^1, R^2, R^5, and R^6 are independently H, C_1-C_6 alkyl, halogen, OR^{12}, $NR^{13}R^{14}$, SR^{15},

SOR^{16} or SO_2R^{17};

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or

R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)- C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

R^{10} is absent, H, C(O)- C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16} and R^{17} are independently H, C(O)-C_1-C_6 alkyl or C_1-C_6 alkyl; and

the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

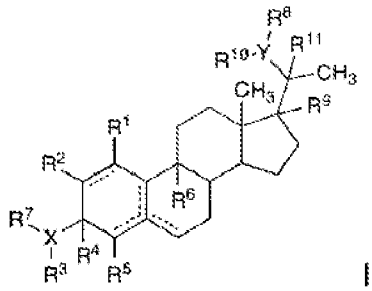
28. The pharmaceutical composition of claim 27 wherein said pharmaceutical composition further comprises an additional therapeutic agent selected from the classes comprising small molecules, antibodies, proteins and enzymes.

29. The pharmaceutical composition of claim 28 wherein said additional therapeutic agent is a neuroprotective agent, an anti-inflammatory agent, an anti-amyloid agent or an anti-Tau agent.

30. The pharmaceutical composition of claim 27, wherein said pharmaceutical composition is a formulation selected from the list comprising a tablet, capsule, gelcap, caplet, powder, solution, suspension, eyedrop, cream, lotion, gel and suppository.

31. The pharmaceutical composition of claim 30 wherein said formulation is a powder, a gel or a solution.

32. A method for treating, minimizing or preventing TBI in an animal in need of TBI treatment, said method comprising administering to a the animal, an effective amount of a compound of Formula I



I

or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof,

wherein X is O, N or S;

Y is O, N or S; or, YR^8R^{10} is absent;

R^1, R^2, R^5, and R^6 are independently H, C_1-C_6 alkyl, halogen, OR^{12}, $NR^{13}R^{14}$, SR^{15}, SOR^{16} or SO_2R^{17};

R^4 is H or C_1-C_6 alkyl; R^4 together with R^3 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; or

R^4 and R^7 together form a double bond;

R^3 is H or C_1-C_6 alkyl; R^3 together with R^4 and X forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^3 is absent;

R^7 is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^7 and R^4 together form a double bond;

R^8 is H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; R^8 together with R^9 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms, or R^8 is absent;

R^9 is H or C_1-C_6 alkyl; R^9 together with R^8 and Y forms an optionally substituted 5-6 membered heterocycle containing 1-2 nitrogen, oxygen or sulfur atoms; R^9 and R^{11} together form a double bond;

R^{10} is absent, H, C(O)-C_1-C_6 alkyl, C_1-C_6 alkyl; or R^{10} and R^{11} together form a double bond;

R^{11} is H or C_1-C_6 alkyl; or R^{11} and R^{10} together form a double bond; R^{11} and R^9 together form a double bond;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16} and R^{17} are independently H, C(O)- C_1-C_6 alkyl or C_1-C_6 alkyl; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens.

33. The method of claim 32, wherein said animal is a human.

34. The method of claim 32-33, wherein said injury or disease is severe or moderate TBI.

35. The method of claim 32-33, wherein said injury or disease is mild traumatic brain injury (MTBI).

35. The method of claim 32-33, wherein said injury or disease is a concussion.

36. A method for treating, minimizing or preventing injury or disease in an animal, the method comprising:

administering to the animal, an effective amount of a compound or composition of any one of claims 1 through 31.

37. The method of claim 37 wherein the animal has suffered a traumatic brain injury.

38. The method of claim 37 wherein the animal has suffered a moderate traumatic brain injury.

39. The method of claim 37 wherein the animal has suffered a severe traumatic brain injury.

40. The method of claim 37 wherein the animal has suffered a mild traumatic brain injury (MTBI).

41. The method of claim 37 wherein the animal has suffered a concussion.

42. The method of any one of claims 36 through 41 wherein the animal has been identified as in need of treatment for the injury or disease and the compound or composition is administered to the identified animal.

43. The method of claim 36 wherein the animal has been identified as having suffered a traumatic brain injury and the compound or composition is administered to the identified animal.

44. The method of claim 36 wherein the animal has been identified as having suffered a concussion and the compound or composition is administered to the identified animal.

45. The method of any one of claims 36 through 44 wherein the animal is a human.

46. The method of any one of claims 36 through 45 wherein the compound or composition is administered to the animal in a formulation having an amount of the compound or composition up to about 50 mg/mL.

47. ent-19-norprogestrone in crystal form or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof.

48. Amorphous ent-19-norprogesterone or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof.

49. ent-19-norprogesterone polymorph or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof.

50. ent-19-norprogesterone Type A polymorph or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof.

51. ent-19-norprogesterone Type B polymorph or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof.

52. ent-19-norprogesterone pseudopolymorph, or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof.

53. A method of treating a subject for TBI, said method comprising administering a therapeutically effective amount of ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof, to the subject to treat the TBI.

54. A method of claim 53, wherein the TBI is severe TBI.

55. A method of claim 53, wherein the TBI is moderate TBI.

56. A method of claim 53, wherein the TBI is mild TBI.

57. A method of treating a subject for a concussion, said method comprising administering a therapeutically effective amount of ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof, to the subject to treat the concussion.

58. A method of treating a subject for TBI, said method comprising administering an effective amount of ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof, to the subject to treat the TBI.

59. A method of claim 53, wherein the TBI is severe TBI.

60. A method of claim 53, wherein the TBI is moderate TBI.

61. A method of claim 53, wherein the TBI is mild TBI.

62. A method of treating a subject for a concussion, said method comprising administering an effective amount of ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof, to the subject to treat the concussion.

63. A substantially pure ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug or co-crystal thereof.

64. The use of ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, prodrug, crystal or co-crystal thereof, in the manufacture of a medicament for the treatment of traumatic brain injury (TBI), wherein the ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof, is in a therapeutically effective

amount, wherein the medicament is to be administered nasally, and wherein the treatment is to be daily and continued in accordance with a prescribed treatment regimen.

65. The use of claim 64, wherein the TBI is severe TBI.

66. The use of claim 64, wherein the TBI is moderate TBI.

67. The use of claim 64, wherein the TBI is mild TBI.

68. The use of claim 64, wherein the TBI is a concussion.

69. ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, prodrug, crystal or co-crystal thereof, for use in the treatment of traumatic brain injury (TBI), wherein the ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, hydrate, solvate prodrug, crystal or co-crystal thereof, is to be administered nasally, and wherein the treatment is to be daily and continued in accordance with a prescribed treatment regimen.

70. ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, prodrug, crystal or co-crystal thereof, of claim 69, wherein the TBI is severe TBI.

71. ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, prodrug, crystal or co-crystal thereof, of claim 69, wherein the TBI is moderate TBI.

72. ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, prodrug, crystal or co-crystal thereof, of claim 69, wherein the TBI is mild TBI.

73. ent-19-norprogesterone, or a pharmaceutically acceptable salt, ester, prodrug, crystal or co-crystal thereof, of claim 69, wherein the TBI is a concussion.

FIG. 1

	Thinking/ Remembering	Difficulty thinking clearly	Feeling slowed down	Difficulty concentrating	Difficulty remembering new information
	Physical	Headache	Nausea or vomiting (early on)	Sensitivity to noise or light	Feeling tired, having no energy
		Fuzzy or blurry vision	Dizziness	Balance problems	
	Emotional/Mood	Irritability	Sadness	More emotional	Nervousness or anxiety
	Sleep	Sleeping more than usual	Sleep less than usual	Trouble falling asleep	

FIG. 2

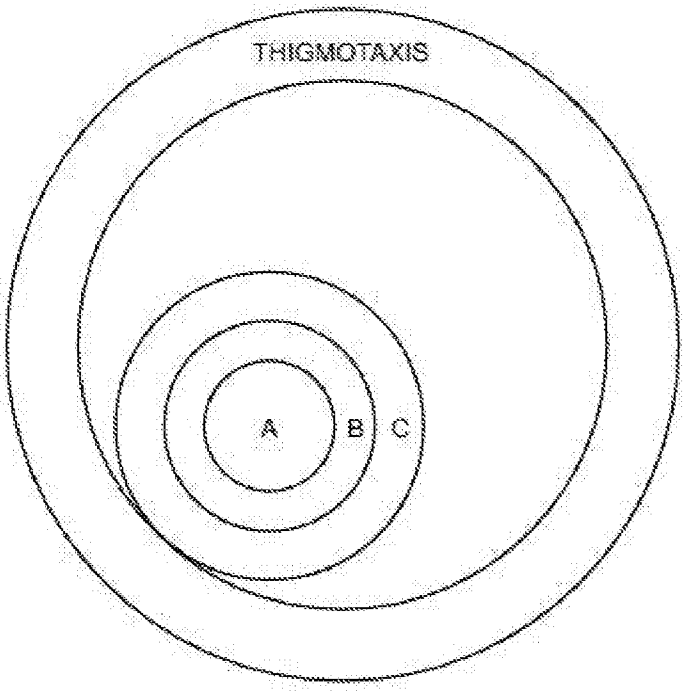


FIG. 3

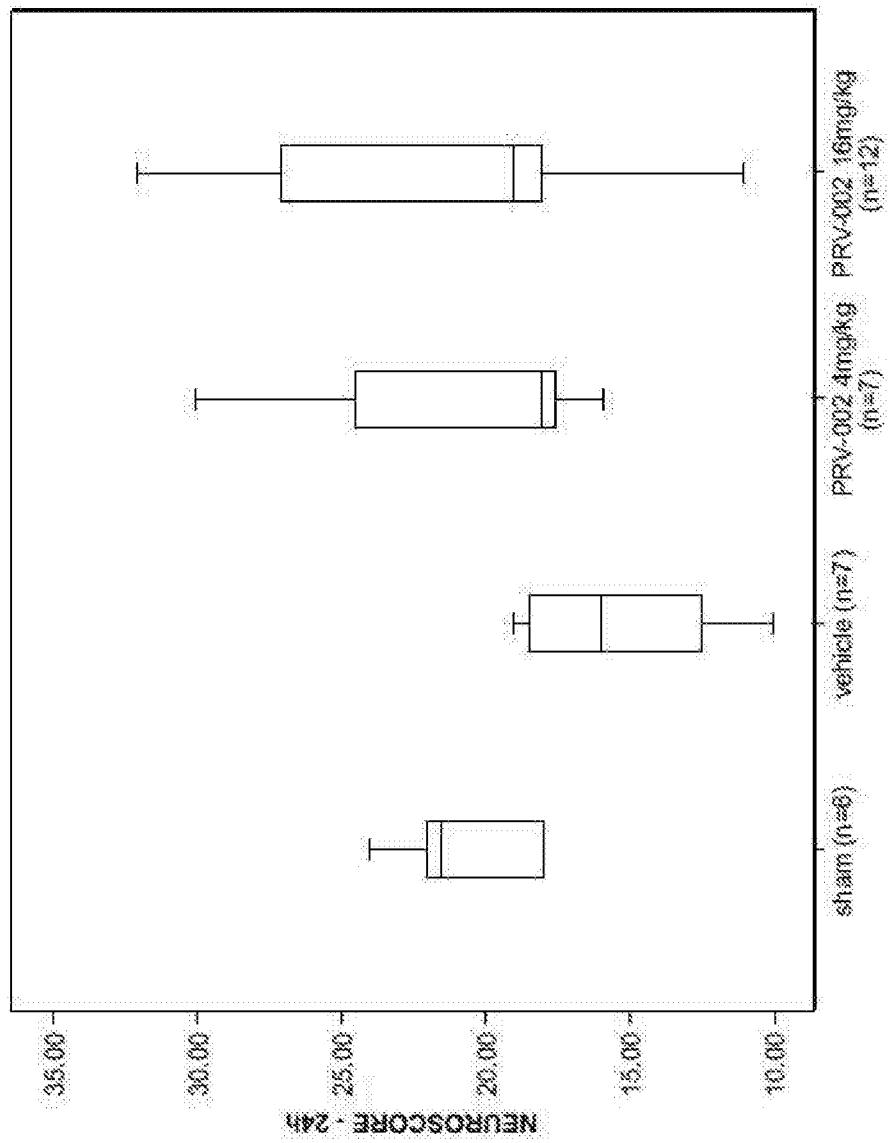

NEUROSCORE - 24h
35.00
30.00
25.00
20.00
15.00
10.00
sham (n=8)
vehicle (n=7)
PRV-002 4mg/kg (n=7)
PRV-002 16mg/kg (n=12)

FIG. 4

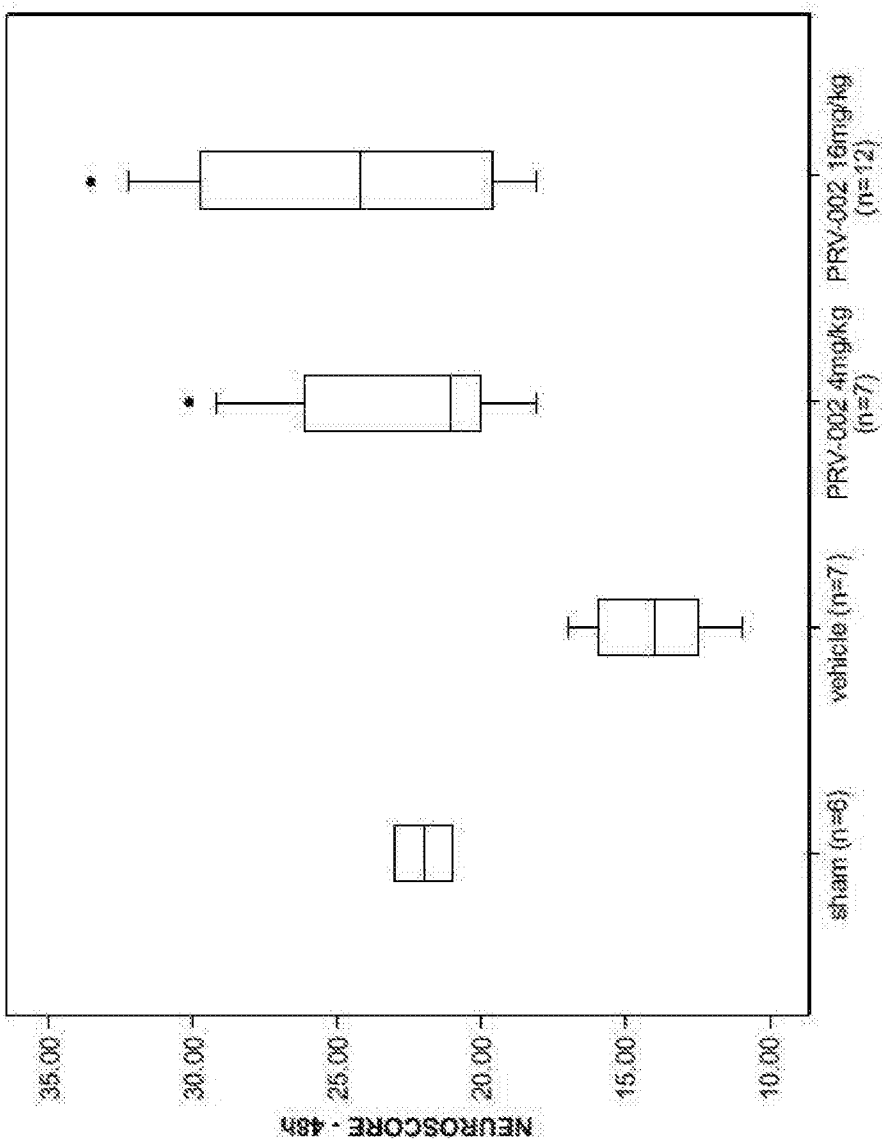

NEUROSCORE - 48h
35.00
30.00
25.00
20.00
15.00
10.00
sham (n=6)
vehicle (n=7)
PRV-002 4mg/kg (n=7)
PRV-002 16mg/kg (n=12)

FIG. 5A

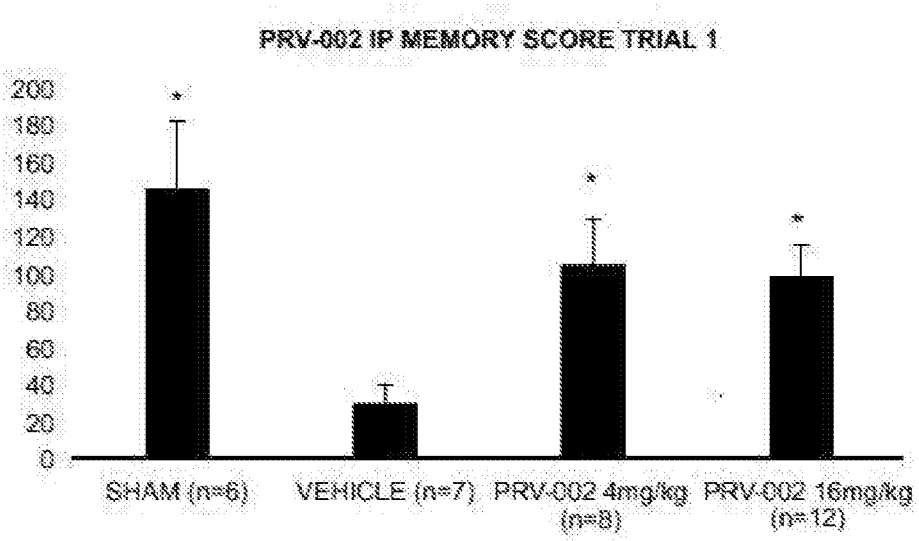

PRV-002 IP MEMORY SCORE TRIAL 1
200
180
160
140
120
100
80
60
40
20
0
*
*
*
SHAM (n=6)
VEHICLE (n=7)
PRV-002 4mg/kg (n=8)
PRV-002 16mg/kg (n=12)

FIG. 5B

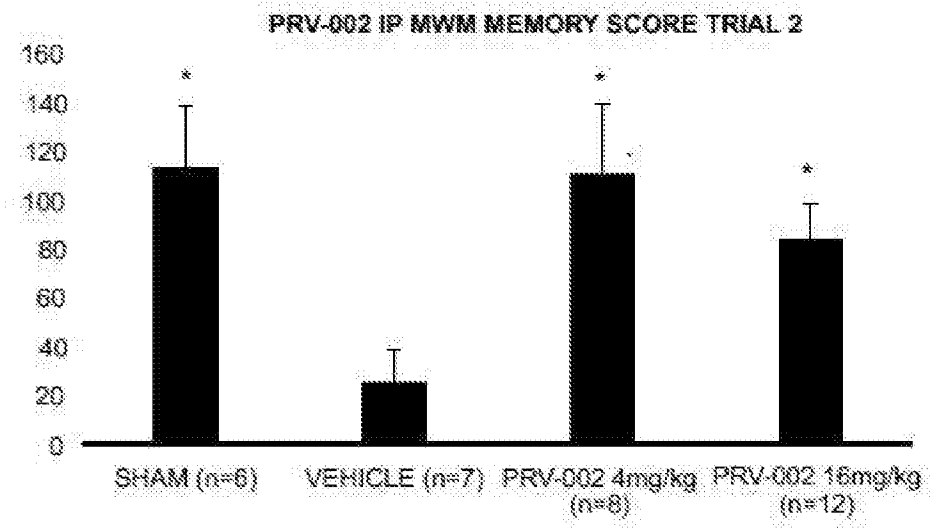

PRV-002 IP MWM MEMORY SCORE TRIAL 2
160
140
120
100
80
60
40
20
0
*
*
*
SHAM (n=6)
VEHICLE (n=7)
PRV-002 4mg/kg (n=8)
PRV-002 16mg/kg (n=12)

FIG. 6A

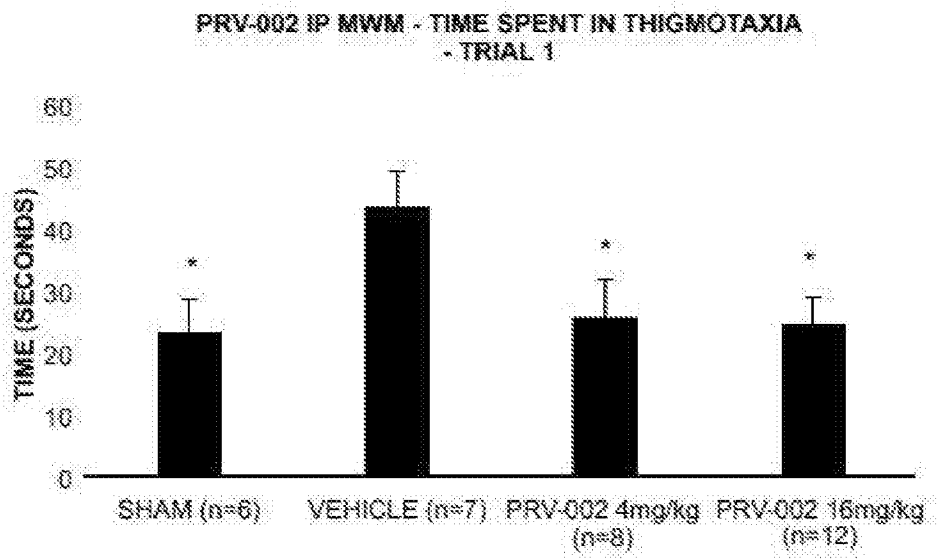

PRV-002 IP MWM - TIME SPENT IN THIGMOTAXIA
- TRIAL 1
TIME (SECONDS)
60
50
40
30
20
10
0
*
*
*
SHAM (n=6)
VEHICLE (n=7)
PRV-002 4mg/kg (n=8)
PRV-002 16mg/kg (n=12)

FIG. 6B

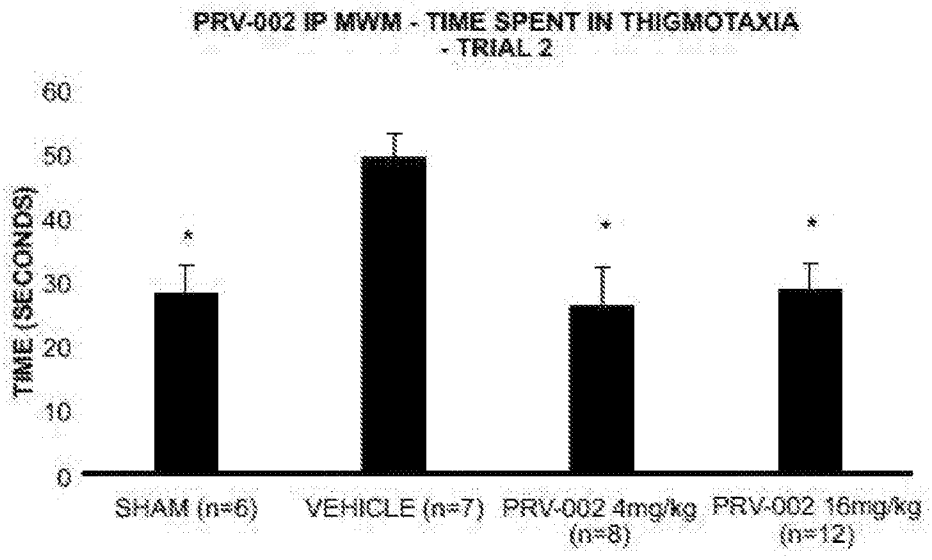

PRV-002 IP MWM - TIME SPENT IN THIGMOTAXIA
- TRIAL 2
TIME (SECONDS)
60
50
40
30
20
10
0
*
*
*
SHAM (n=6)
VEHICLE (n=7)
PRV-002 4mg/kg (n=8)
PRV-002 16mg/kg (n=12)

FIG 7C

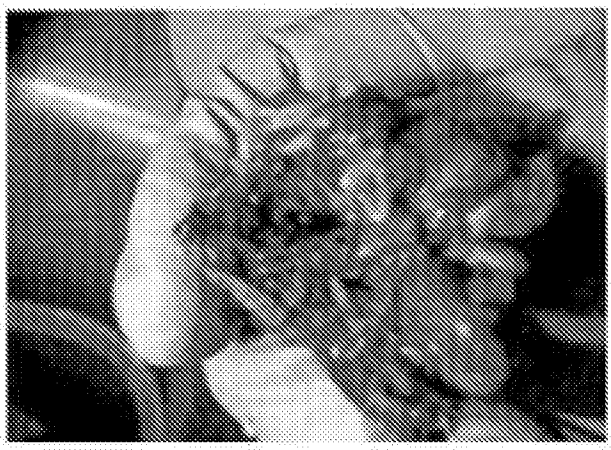


FIG 7B

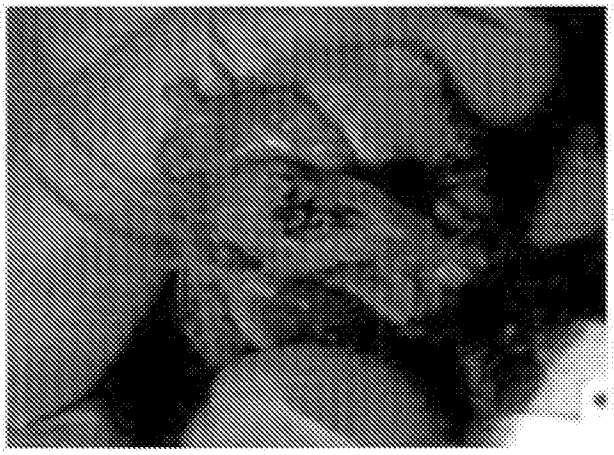


FIG 7A

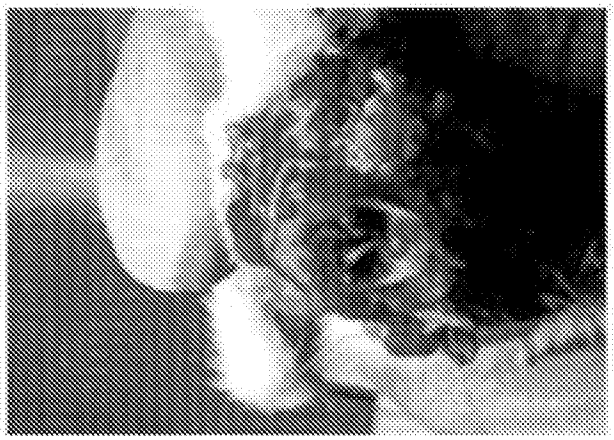


FIG. 8A

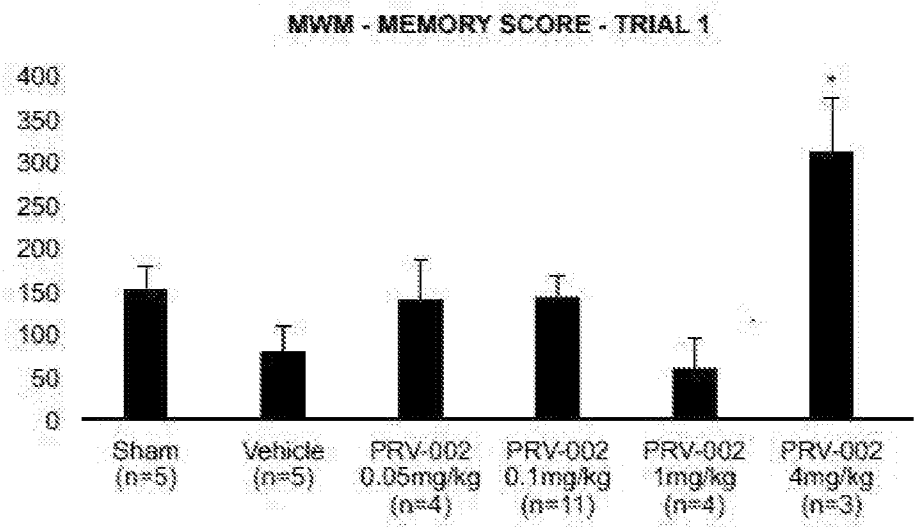

MWM - MEMORY SCORE - TRIAL 1
400
350
300
250
200
150
100
50
0
*
Sham (n=5)
Vehicle (n=5)
PRV-002 0.05mg/kg (n=4)
PRV-002 0.1mg/kg (n=11)
PRV-002 1mg/kg (n=4)
PRV-002 4mg/kg (n=3)

FIG. 8B

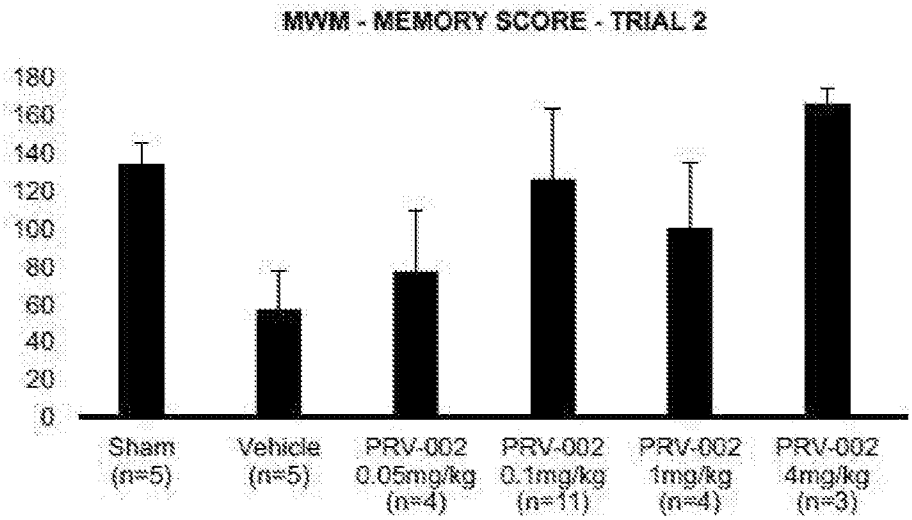

MWM - MEMORY SCORE - TRIAL 2
180
160
140
120
100
80
60
40
20
0
Sham (n=5)
Vehicle (n=5)
PRV-002 0.05mg/kg (n=4)
PRV-002 0.1mg/kg (n=11)
PRV-002 1mg/kg (n=4)
PRV-002 4mg/kg (n=3)

FIG. 9A

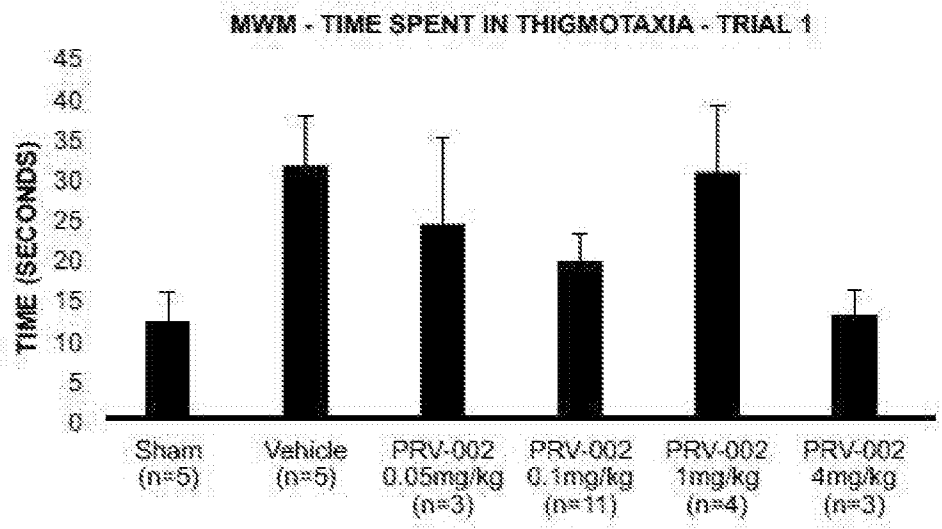

MWM - TIME SPENT IN THIGMOTAXIA - TRIAL 1
TIME (SECONDS)
45
40
35
30
25
20
15
10
5
0
Sham (n=5)
Vehicle (n=5)
PRV-002 0.05mg/kg (n=3)
PRV-002 0.1mg/kg (n=11)
PRV-002 1mg/kg (n=4)
PRV-002 4mg/kg (n=3)

FIG. 9B

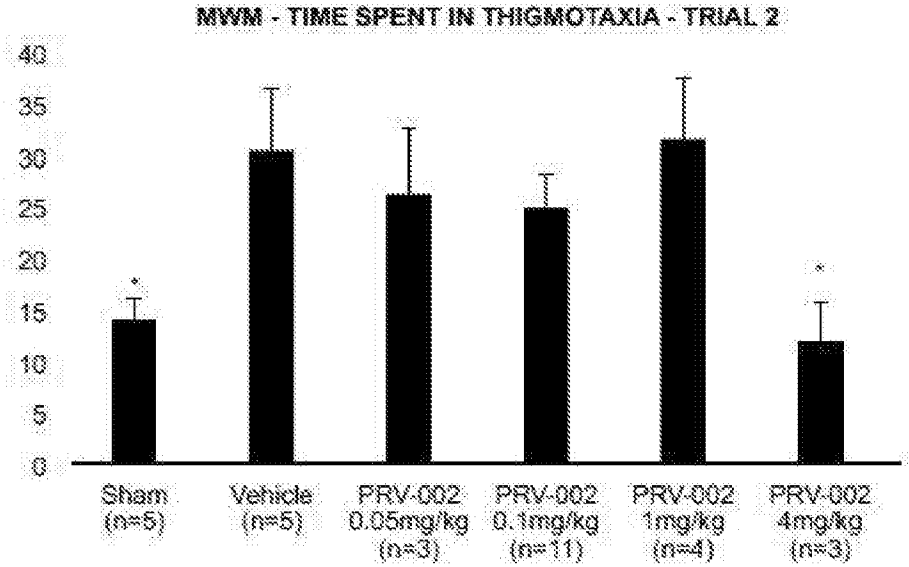

MWM - TIME SPENT IN THIGMOTAXIA - TRIAL 2
40
35
30
25
20
15
10
5
0
*
*
Sham (n=5)
Vehicle (n=5)
PRV-002 0.05mg/kg (n=3)
PRV-002 0.1mg/kg (n=11)
PRV-002 1mg/kg (n=4)
PRV-002 4mg/kg (n=3)

FIG. 10

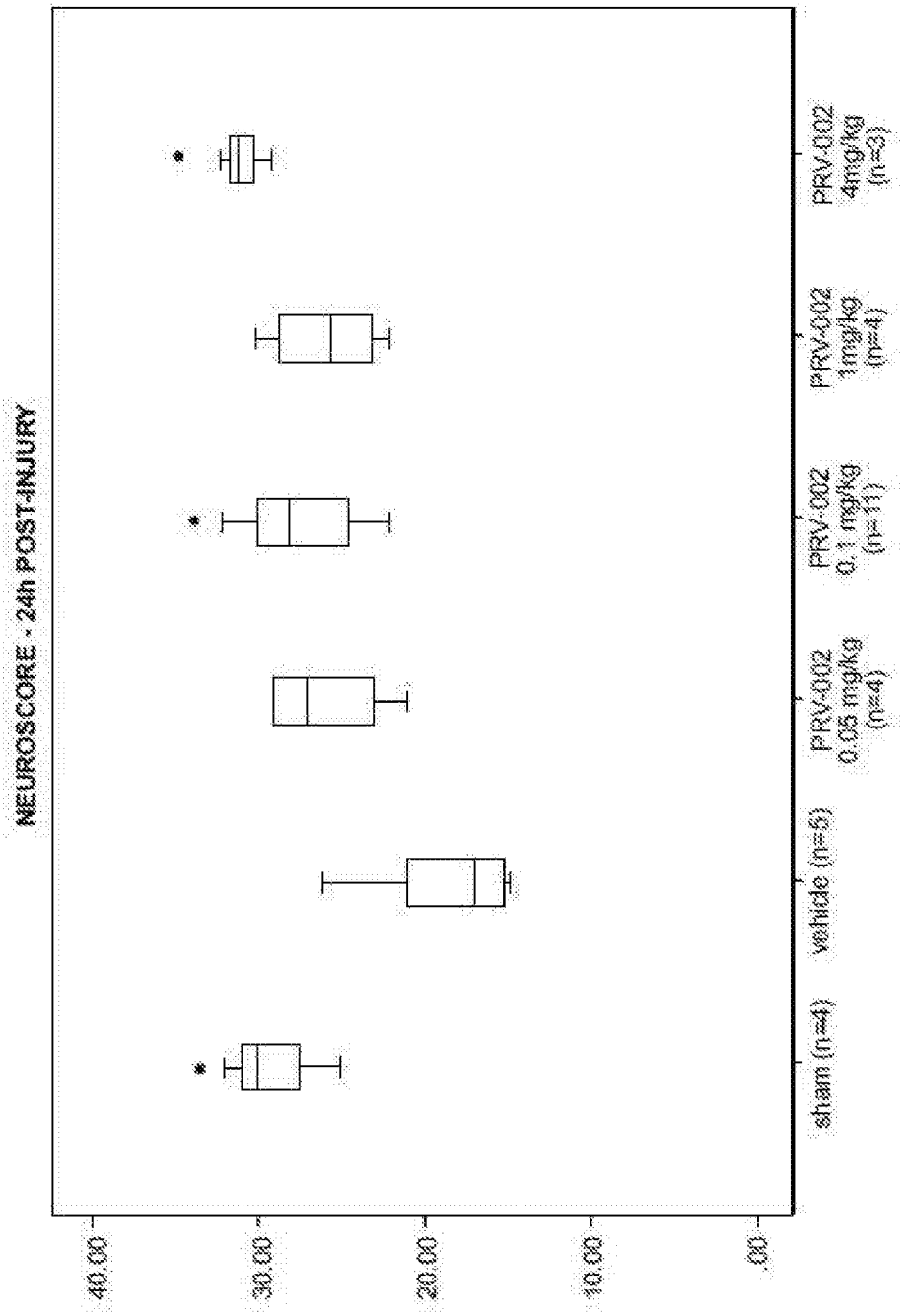

NEUROSCORE - 24h POST-INJURY
40.00
30.00
20.00
10.00
.00
sham (n=4)
vehicle (n=6)
PRV-002 0.05 mg/kg (n=4)
PRV-002 0.1 mg/kg (n=11)
PRV-002 1mg/kg (n=4)
PRV-002 4mg/kg (n=3)

FIG. 11

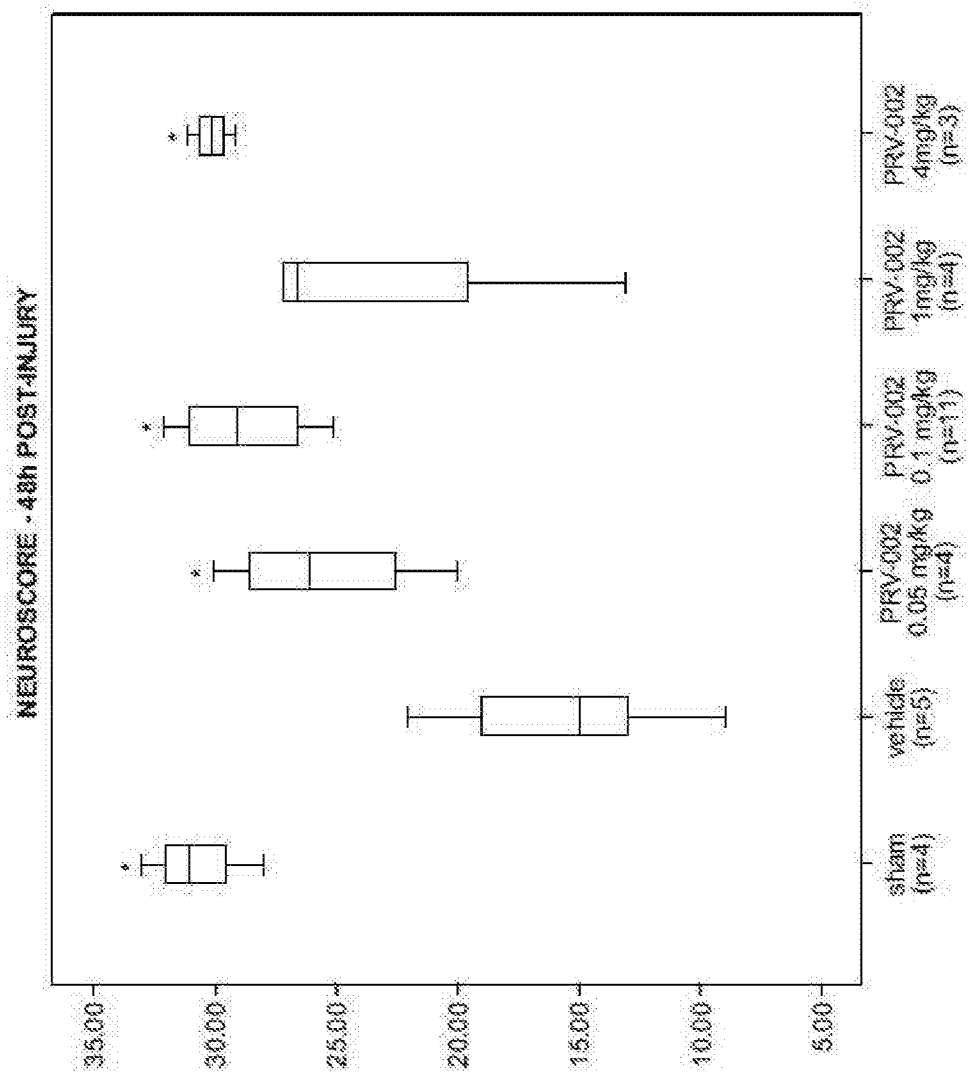

NEUROSCORE - 48h POST-INJURY
35.00
30.00
25.00
20.00
15.00
10.00
5.00
sham (n=4)
vehicle (n=5)
PRV-002 0.05 mg/kg (n=4)
PRV-002 0.1 mg/kg (n=11)
PRV-002 1mg/kg (n=4)
PRV-002 4mg/kg (n=3)

FIG. 12

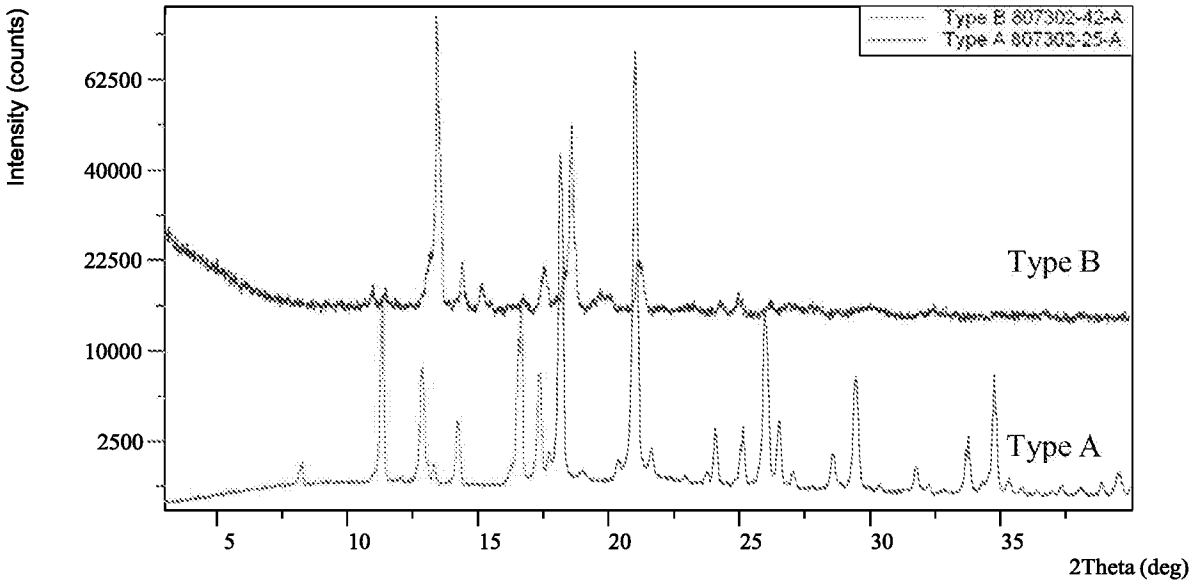

Type B 807302-42-A
Type A 807302-25-A
Intensity (counts)
62500
40000
22500
10000
2500
Type B
Type A
5
10
15
20
25
30
35
2Theta (deg)

FIG. 13

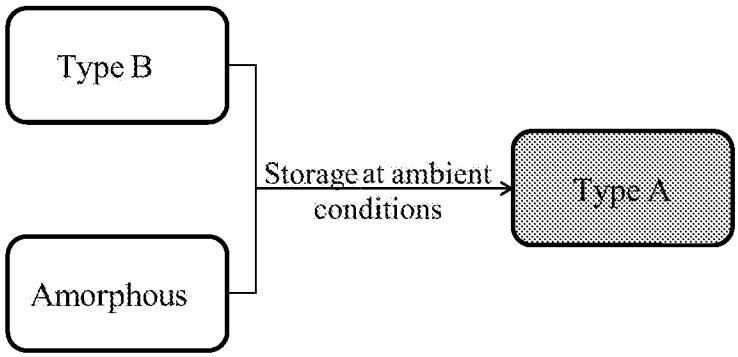


FIG. 14

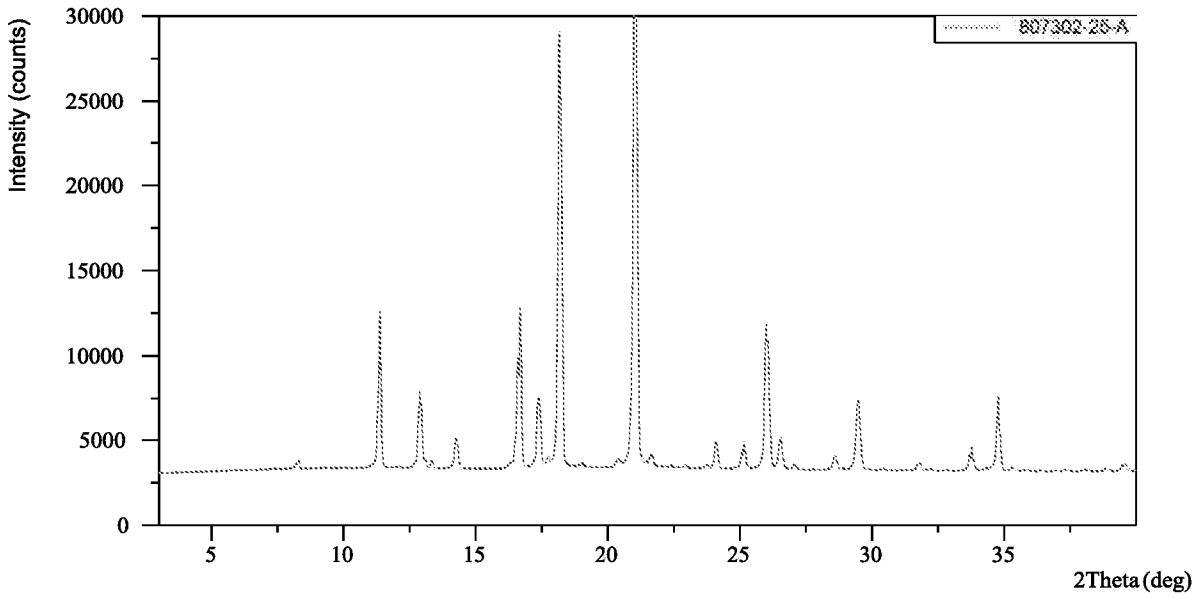

807302-28-A
Intensity (counts)
30000
25000
20000
15000
10000
5000
0
5
10
15
20
25
30
35
2Theta (deg)

FIG. 15

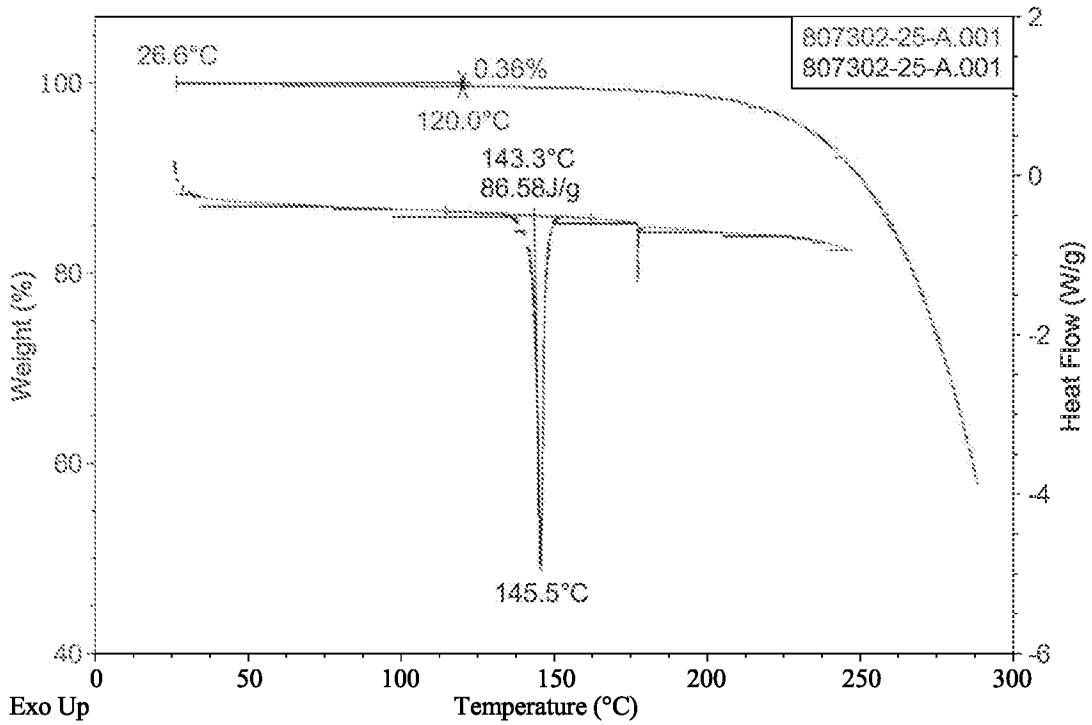

807302-25-A.001
807302-25-A.001
26.6°C
0.36%
120.0°C
143.3°C
86.58J/g
145.5°C
Weight (%)
Heat Flow (W/g)
Exo Up
Temperature (°C)
100
80
60
40
2
0
-2
-4
-6
0
50
100
150
200
250
300

FIG. 16

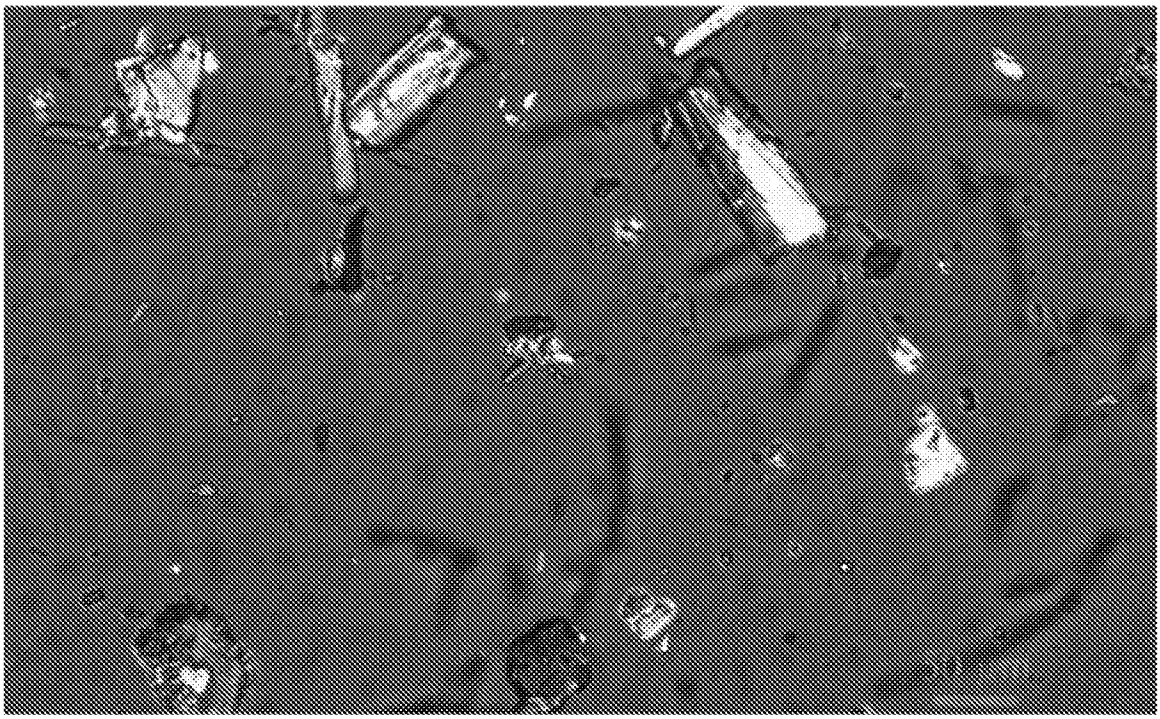


FIG 17A

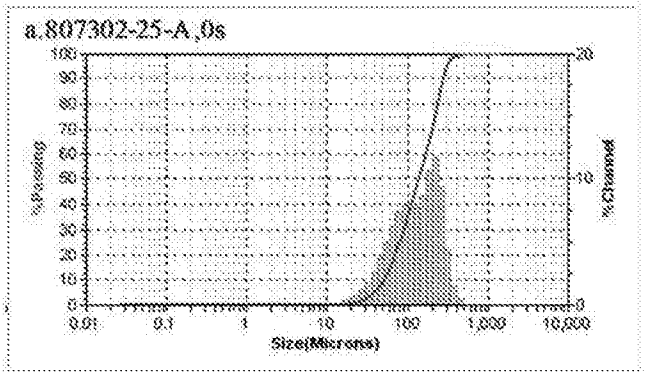


FIG 17B

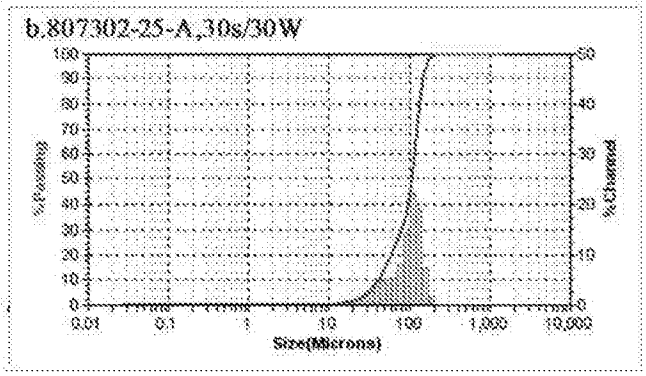


FIG. 18A

FIG. 18B

FIG. 18C

FIG. 18D

FIG. 19

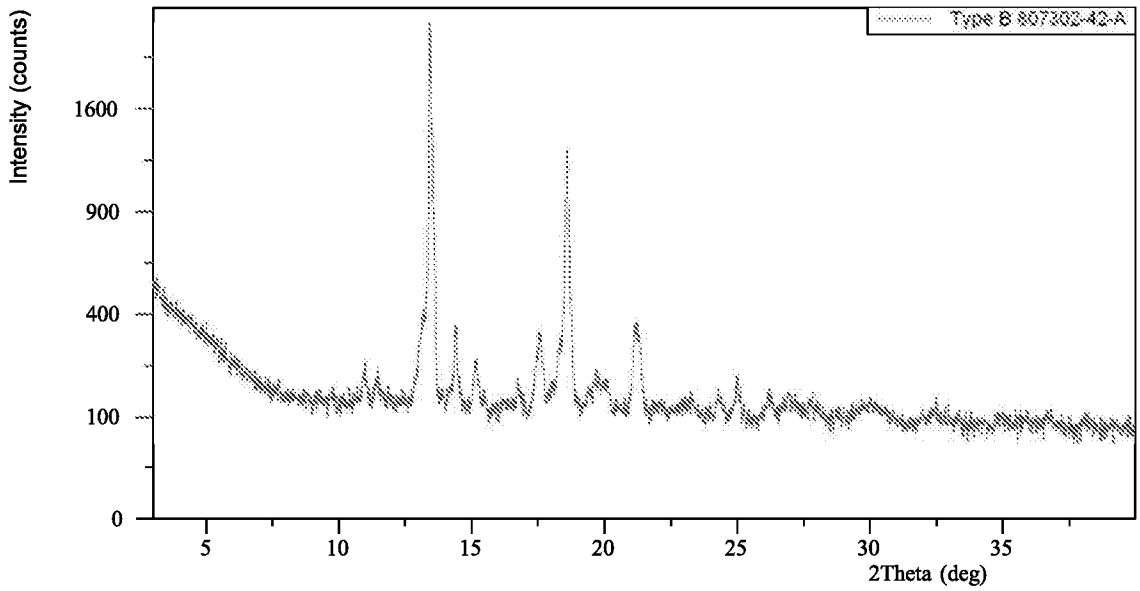

Type B 807302-42-A
Intensity (counts)
1600
900
400
100
0
5
10
15
20
25
30
35
2Theta (deg)

FIG. 20

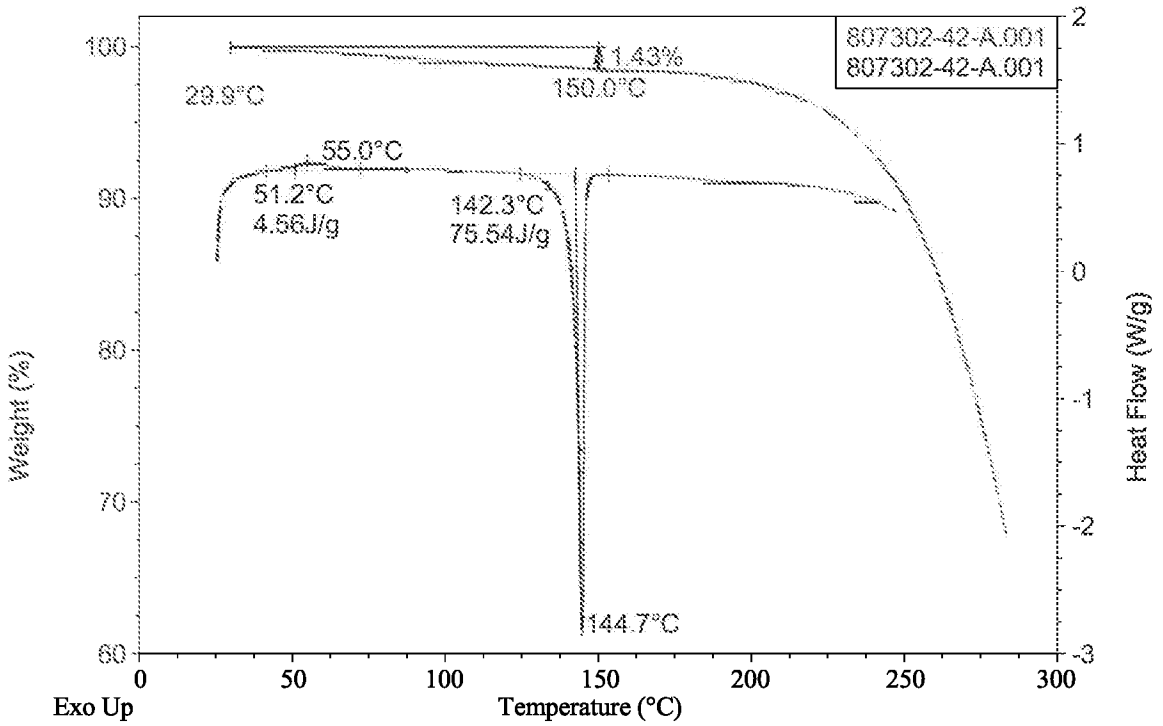

807302-42-A.001
807302-42-A.001
1.43%
29.9°C
150.0°C
55.0°C
51.2°C
4.56J/g
142.3°C
75.54J/g
144.7°C
Weight (%)
Heat Flow (W/g)
Exo Up
Temperature (°C)
100
90
80
70
60
2
1
0
-1
-2
-3
0
50
100
150
200
250
300

FIG. 21

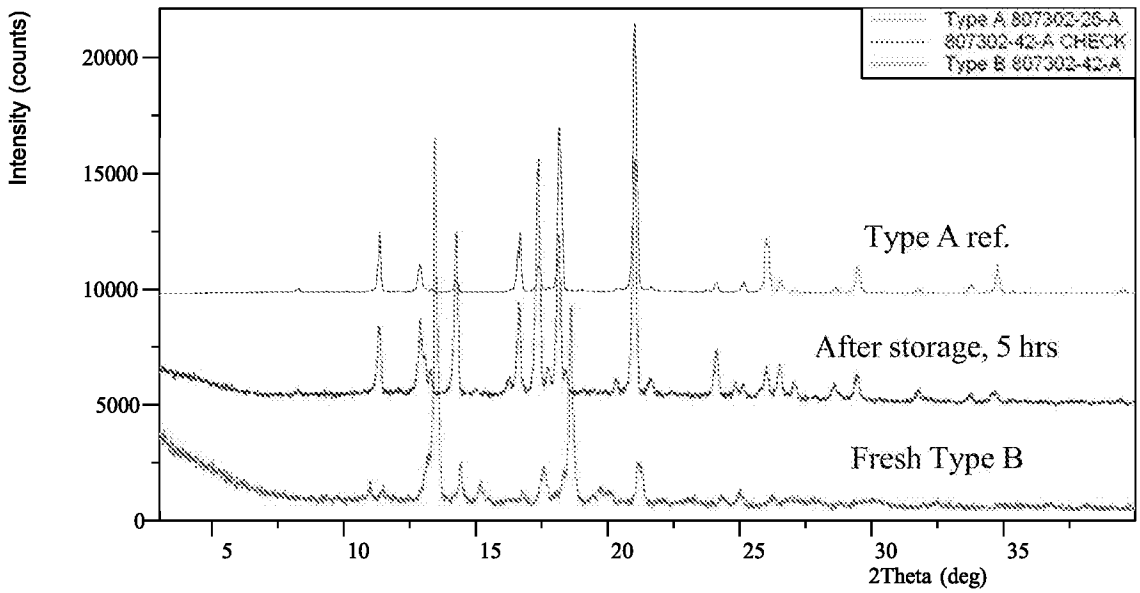

Type A 607302-26-A
607302-42-A CHECK
Type B 607302-42-A
Intensity (counts)
20000
15000
10000
5000
0
Type A ref.
After storage, 5 hrs
Fresh Type B
5
10
15
20
25
30
35
2Theta (deg)

FIG. 22

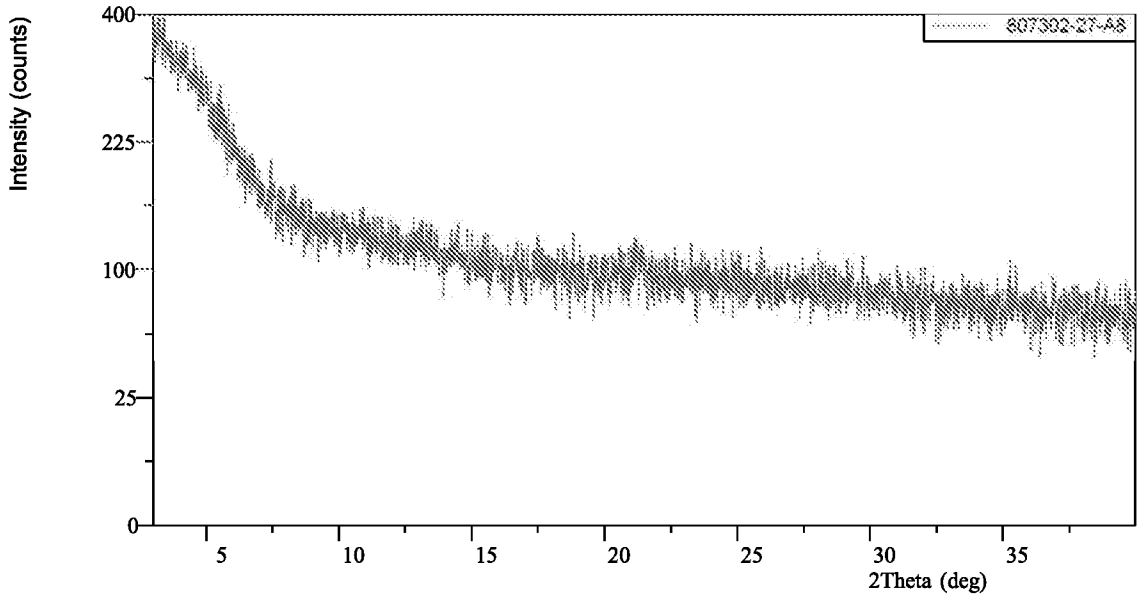

807302-27-A8
Intensity (counts)
400
225
100
25
0
5
10
15
20
25
30
35
2Theta (deg)

FIG. 23

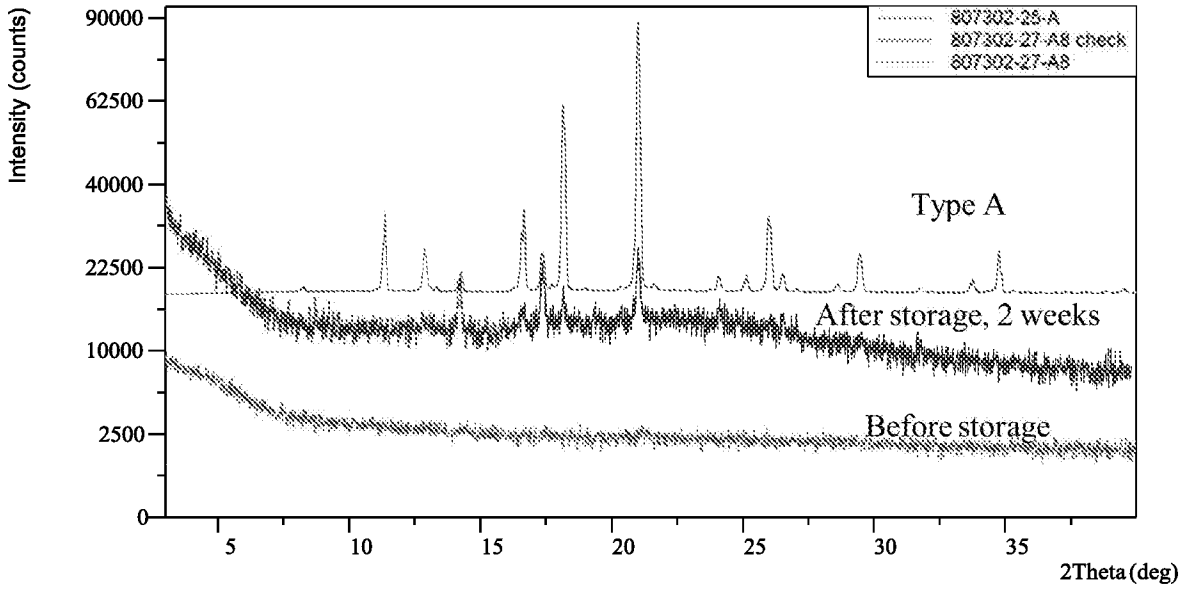

807302-25-A
807302-27-A8 check
807302-27-A8
Intensity (counts)
90000
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
Type A
After storage, 2 weeks
Before storage

FIG. 24

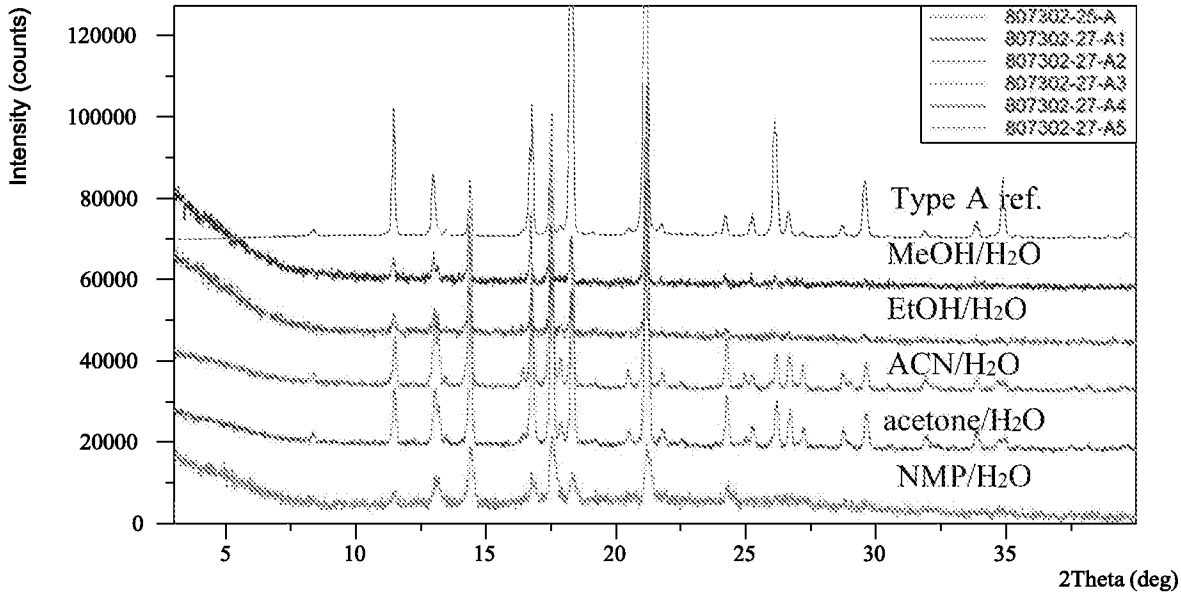

Intensity (counts)
120000
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-27-A1
807302-27-A2
807302-27-A3
807302-27-A4
807302-27-A5
Type A ref.
MeOH/H2O
EtOH/H2O
ACN/H2O
acetone/H2O
NMP/H2O

FIG. 25

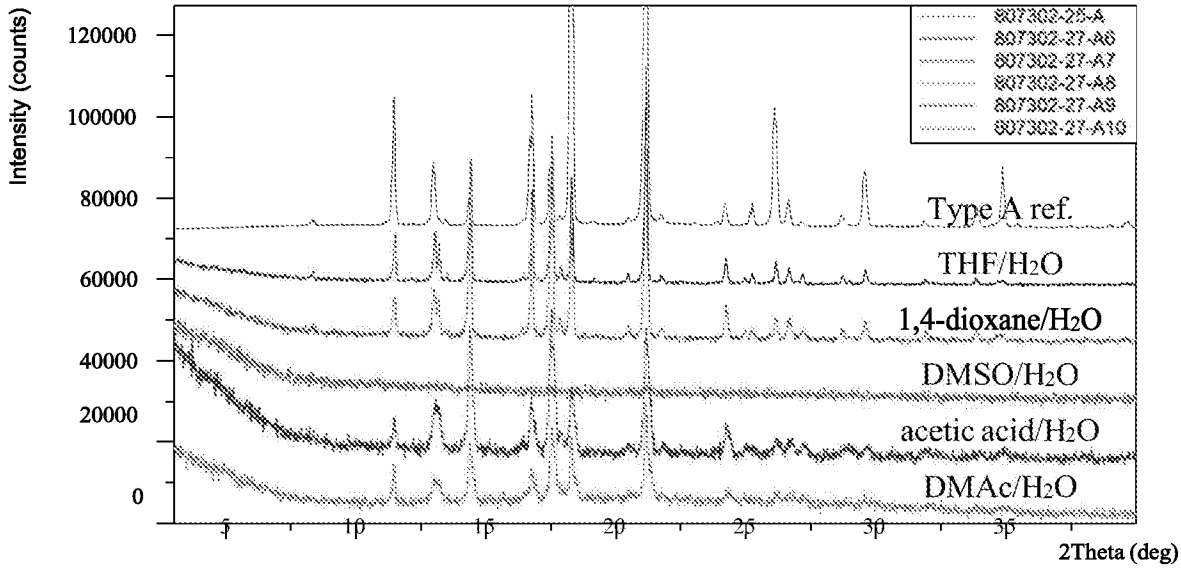

Intensity (counts)
120000
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-27-A6
807302-27-A7
807302-27-A8
807302-27-A9
807302-27-A10
Type A ref.
THF/H2O
1,4-dioxane/H2O
DMSO/H2O
acetic acid/H2O
DMAc/H2O

FIG. 26

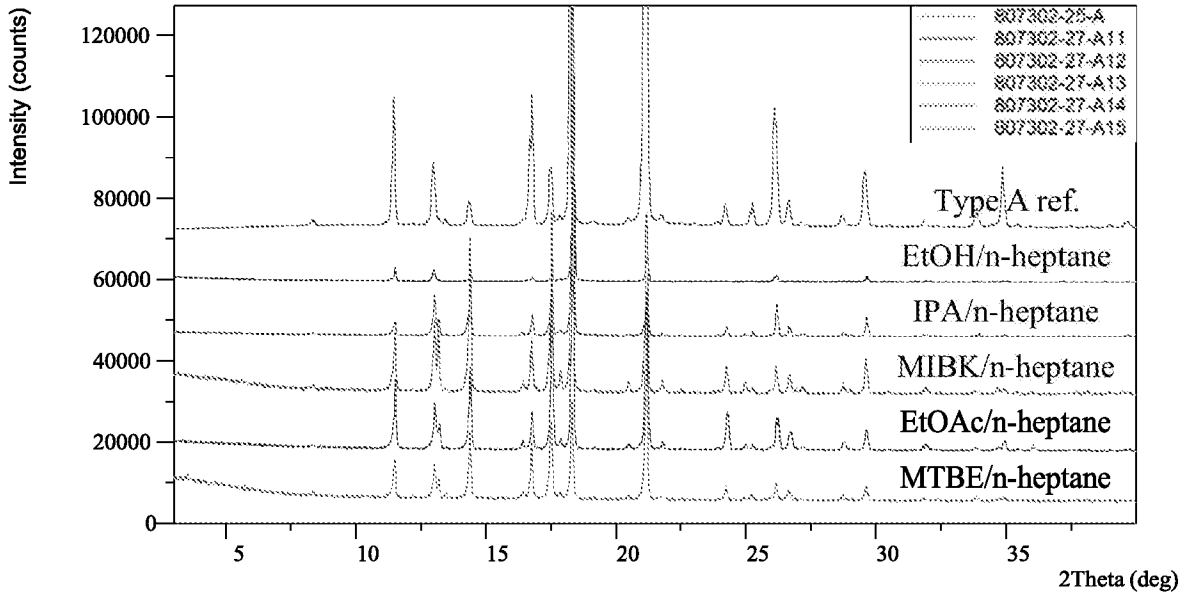

Intensity (counts)
120000
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-27-A11
807302-27-A12
807302-27-A13
807302-27-A14
807302-27-A15
Type A ref.
EtOH/n-heptane
IPA/n-heptane
MIBK/n-heptane
EtOAc/n-heptane
MTBE/n-heptane

FIG. 27

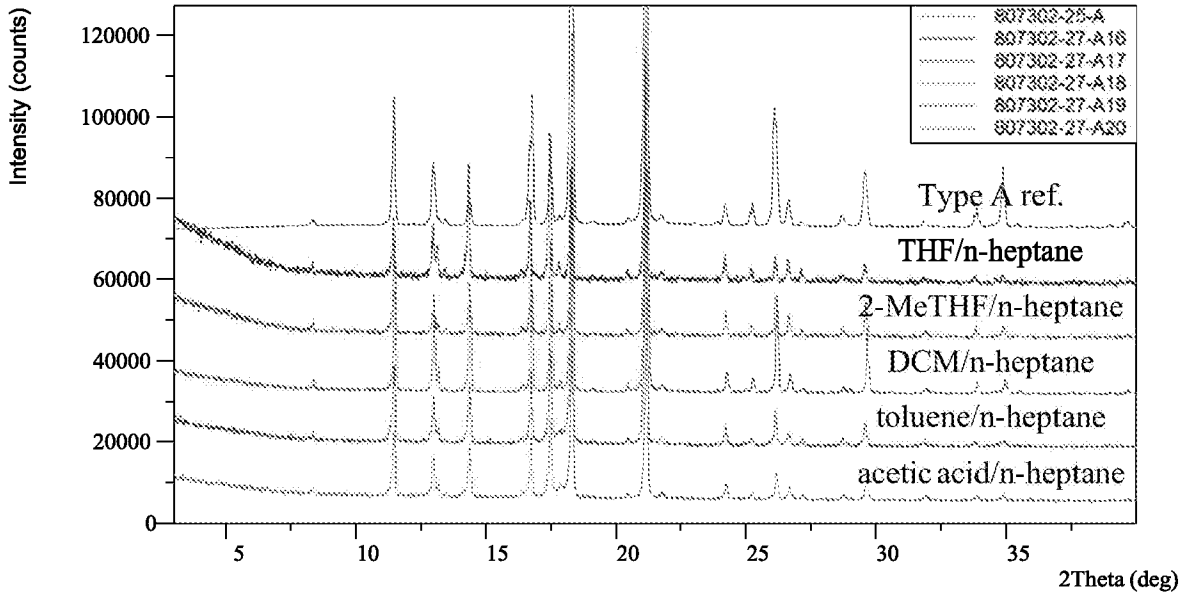

Intensity (counts)
120000
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-27-A16
807302-27-A17
807302-27-A18
807302-27-A19
807302-27-A20
Type A ref.
THF/n-heptane
2-MeTHF/n-heptane
DCM/n-heptane
toluene/n-heptane
acetic acid/n-heptane

FIG. 28

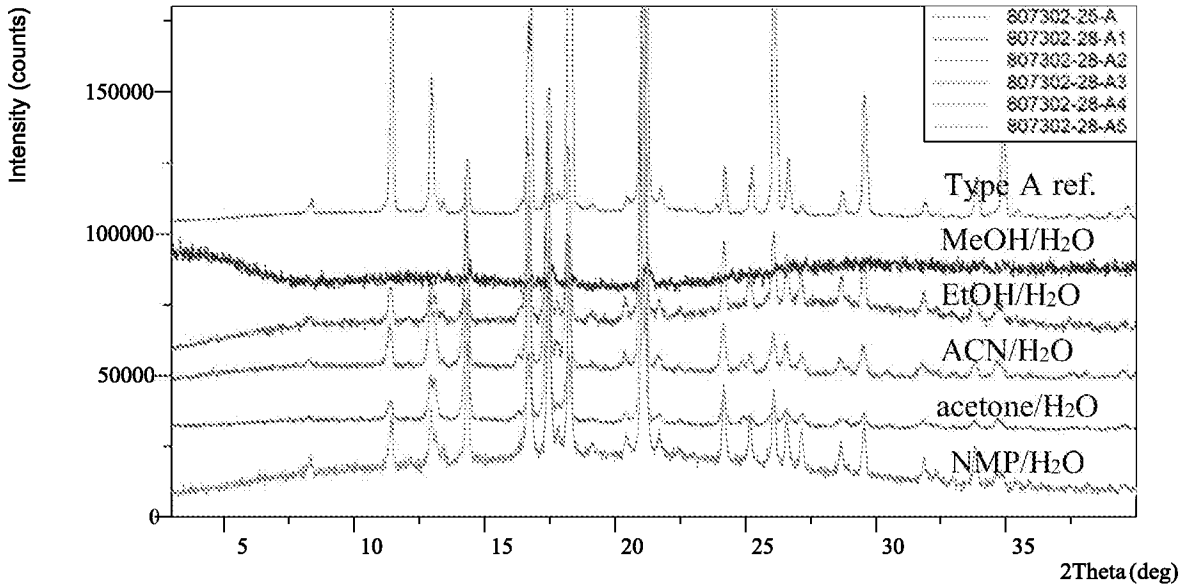

Intensity (counts)
150000
100000
50000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-28-A
807302-28-A1
807302-28-A2
807302-28-A3
807302-28-A4
807302-28-A5
Type A ref.
MeOH/H2O
EtOH/H2O
ACN/H2O
acetone/H2O
NMP/H2O

FIG. 29

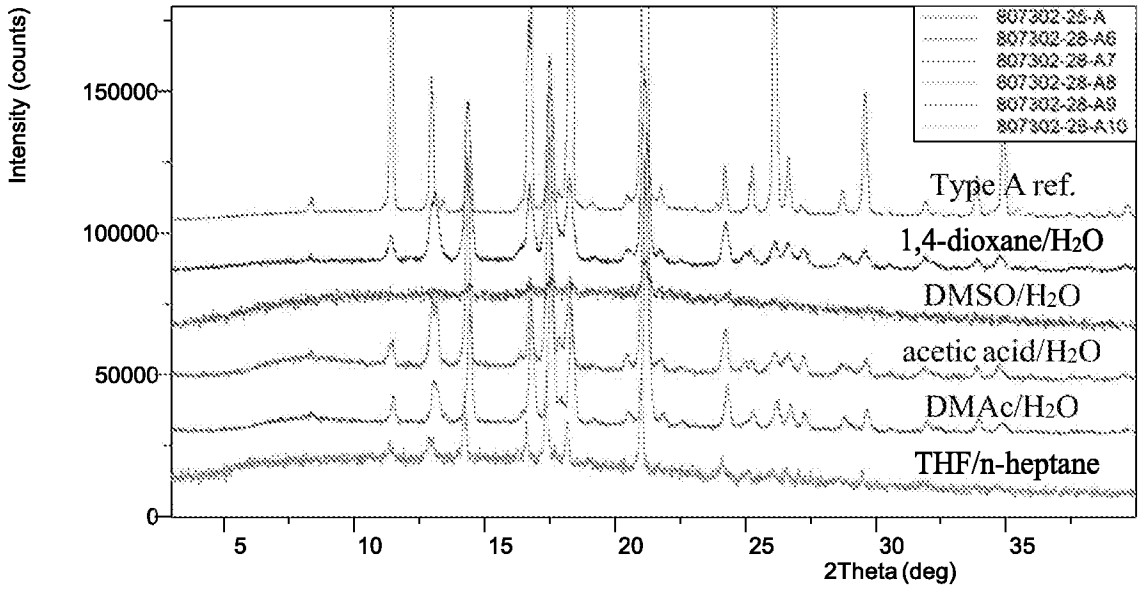

Intensity (counts)
150000
100000
50000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-28-A6
807302-28-A7
807302-28-A8
807302-28-A9
807302-28-A10
Type A ref.
1,4-dioxane/H2O
DMSO/H2O
acetic acid/H2O
DMAc/H2O
THF/n-heptane

FIG. 30

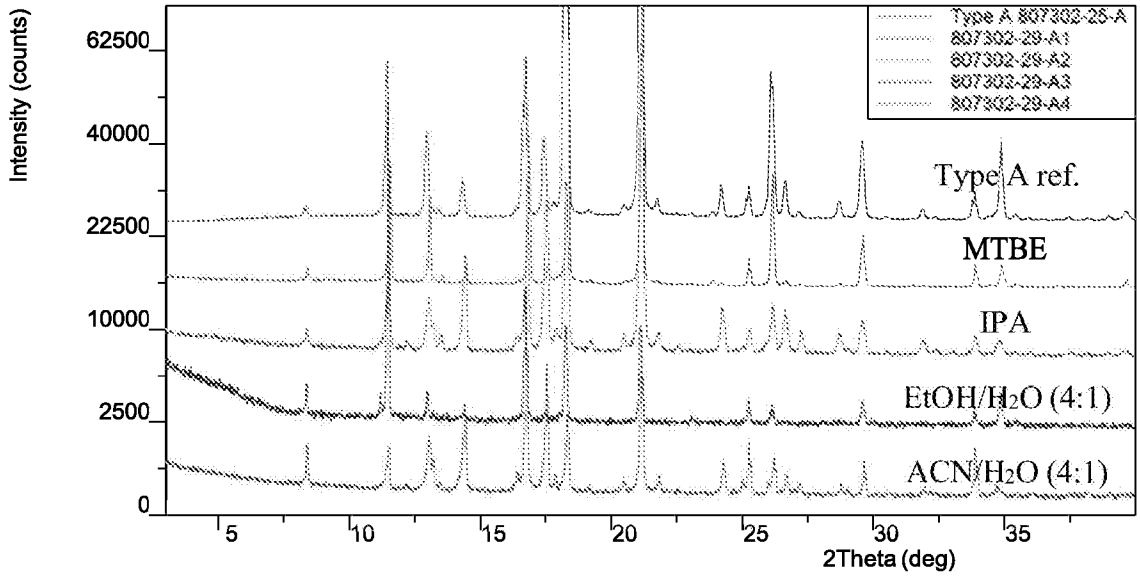

Intensity (counts)
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
Type A 807302-25-A
807302-29-A1
807302-29-A2
807302-29-A3
807302-29-A4
Type A ref.
MTBE
IPA
EtOH/H2O (4:1)
ACN/H2O (4:1)

FIG. 31

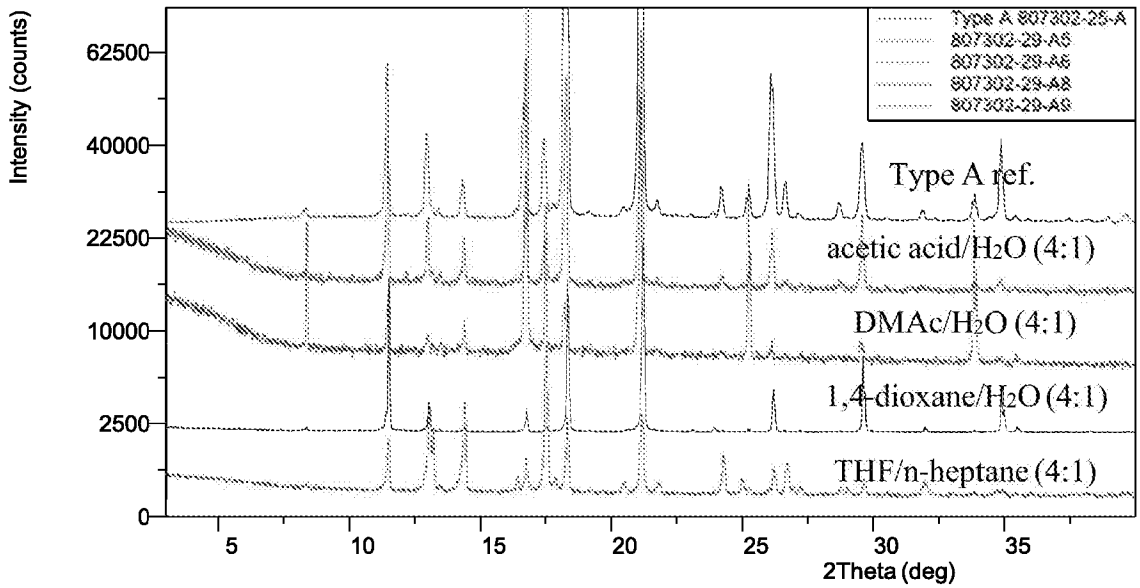

Intensity (counts)
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
Type A ref.
acetic acid/H2O (4:1)
DMAc/H2O (4:1)
1,4-dioxane/H2O (4:1)
THF/n-heptane (4:1)

FIG. 32

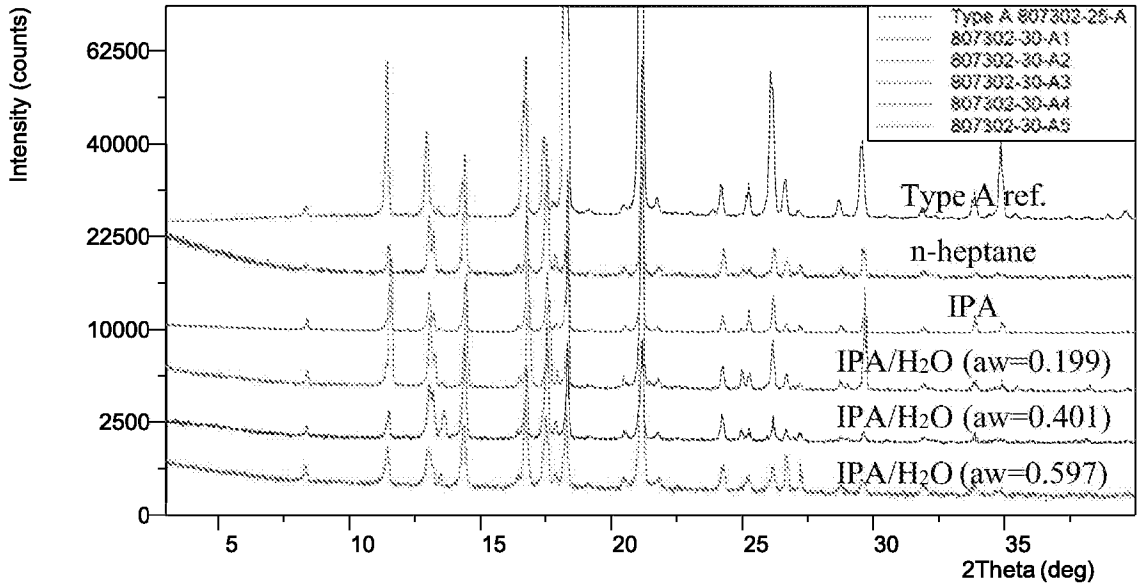

Intensity (counts)
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
Type A 807302-25-A
807302-30-A1
807302-30-A2
807302-30-A3
807302-30-A4
807302-30-A5
Type A ref.
n-heptane
IPA
IPA/H2O (aw=0.199)
IPA/H2O (aw=0.401)
IPA/H2O (aw=0.597)

FIG. 33

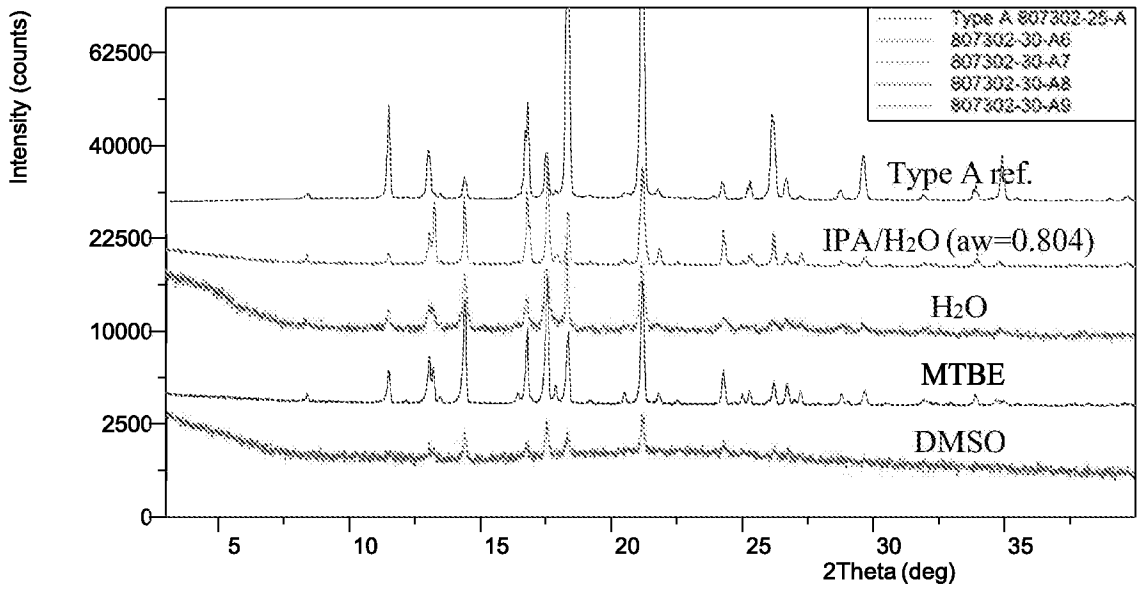

Intensity (counts)
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
Type A ref.
IPA/H2O (aw=0.804)
H2O
MTBE
DMSO

FIG. 34

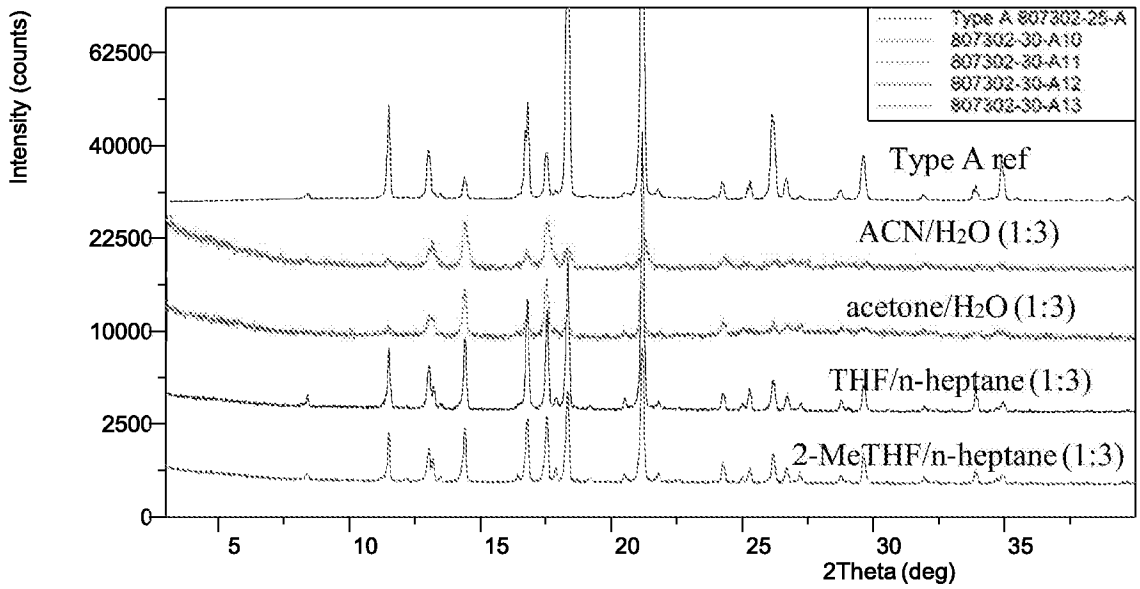

Intensity (counts)
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
Type A 807302-25-A
807302-30-A10
807302-30-A11
807302-30-A12
807302-30-A13
Type A ref
ACN/H2O (1:3)
acetone/H2O (1:3)
THF/n-heptane (1:3)
2-MeTHF/n-heptane (1:3)

FIG. 35

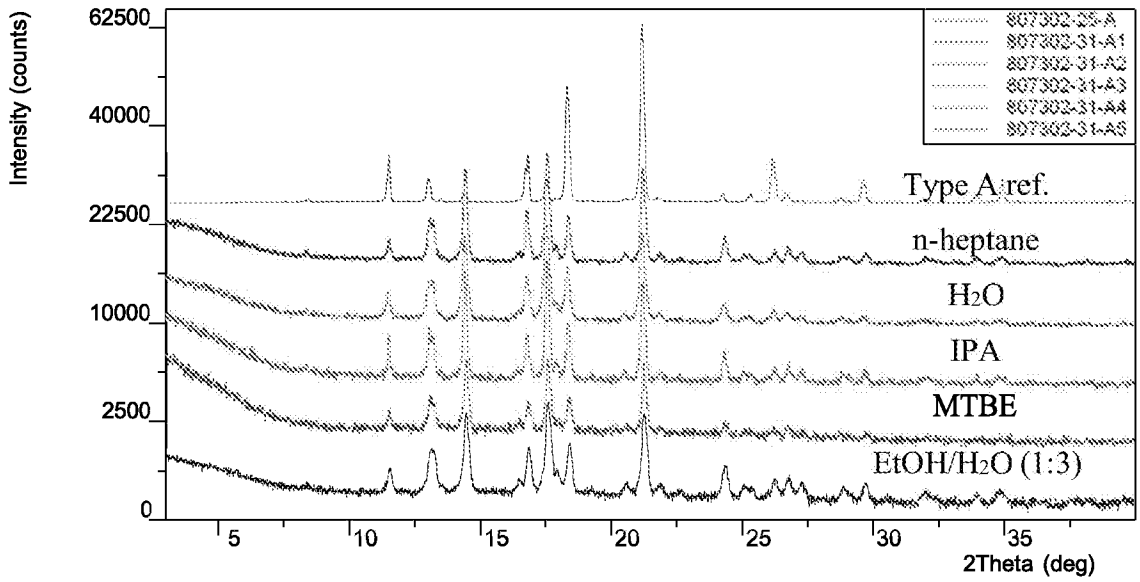

Intensity (counts)
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
807302-28-A
807302-31-A1
807302-31-A2
807302-31-A3
807302-31-A4
807302-31-A5
Type A ref.
n-heptane
H2O
IPA
MTBE
EtOH/H2O (1:3)

FIG. 36

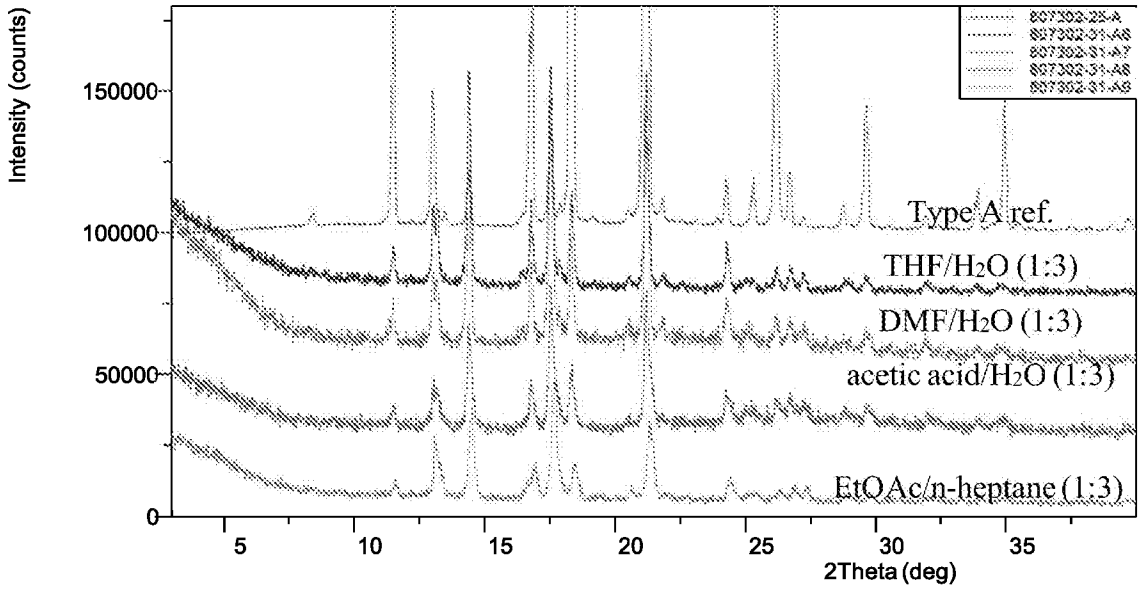

Intensity (counts)
150000
100000
50000
0
5
10
15
20
25
30
35
2Theta (deg)
Type A ref.
THF/H2O (1:3)
DMF/H2O (1:3)
acetic acid/H2O (1:3)
EtOAc/n-heptane (1:3)

FIG. 37

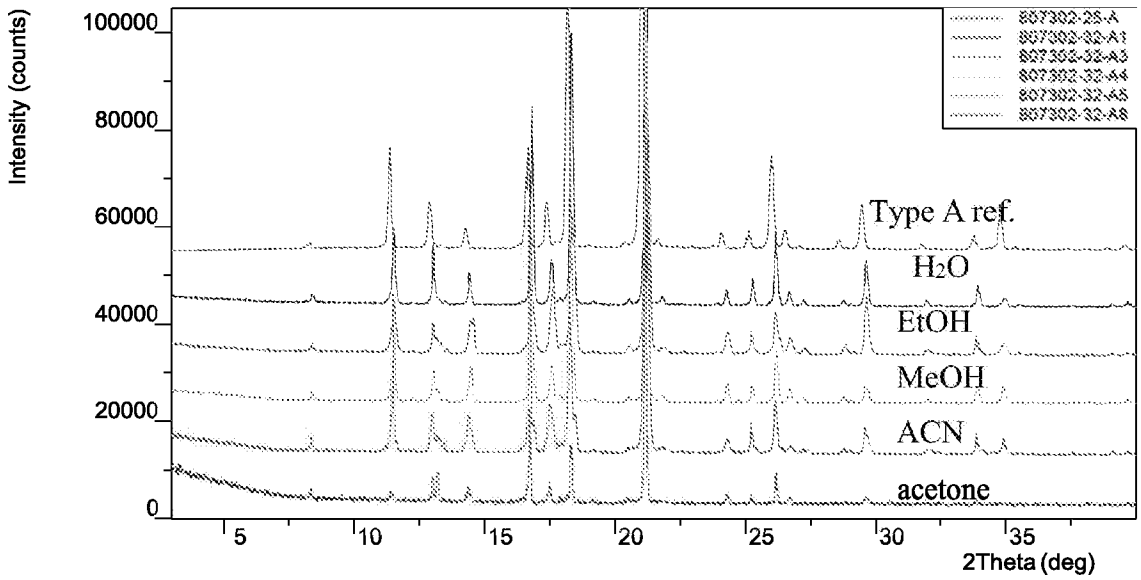

Intensity (counts)
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
Type A ref.
H2O
EtOH
MeOH
ACN
acetone

FIG. 38

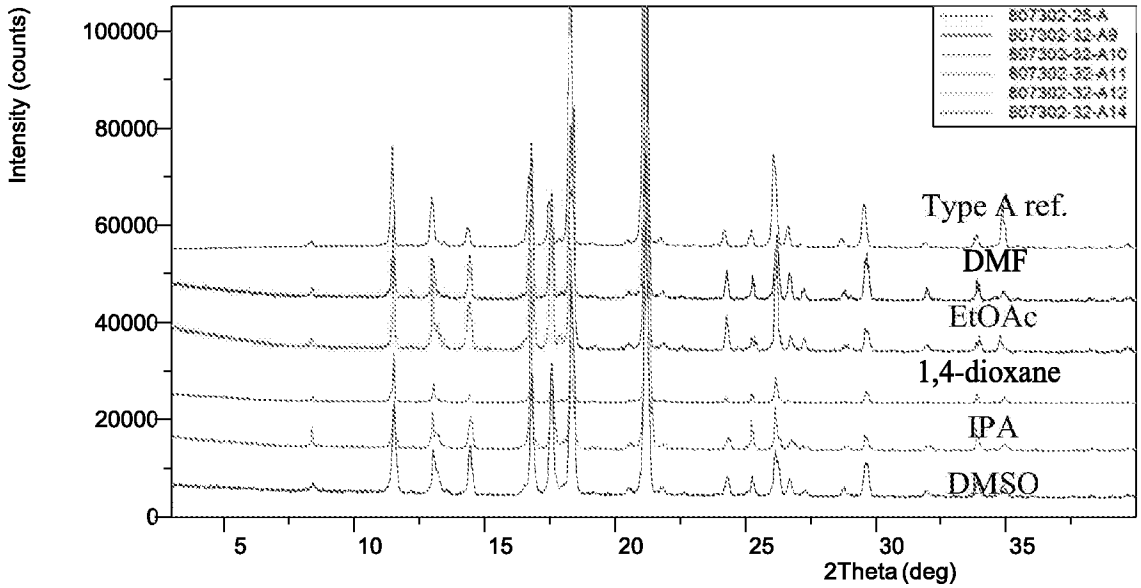

Intensity (counts)
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
Type A ref.
DMF
EtOAc
1,4-dioxane
IPA
DMSO

FIG. 39

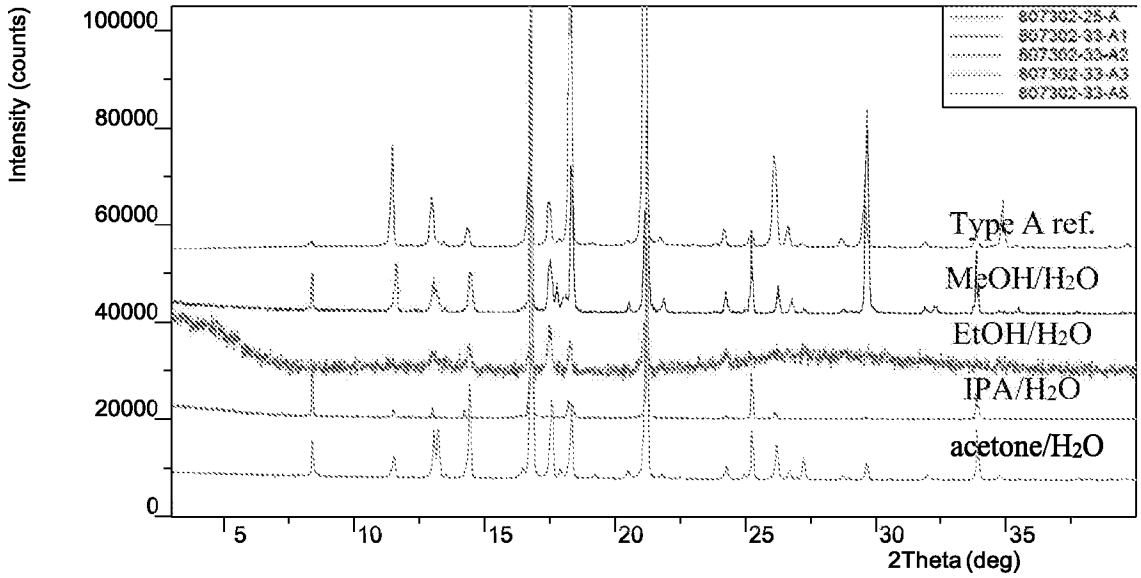

Intensity (counts)
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
Type A ref.
MeOH/H2O
EtOH/H2O
IPA/H2O
acetone/H2O

FIG. 40

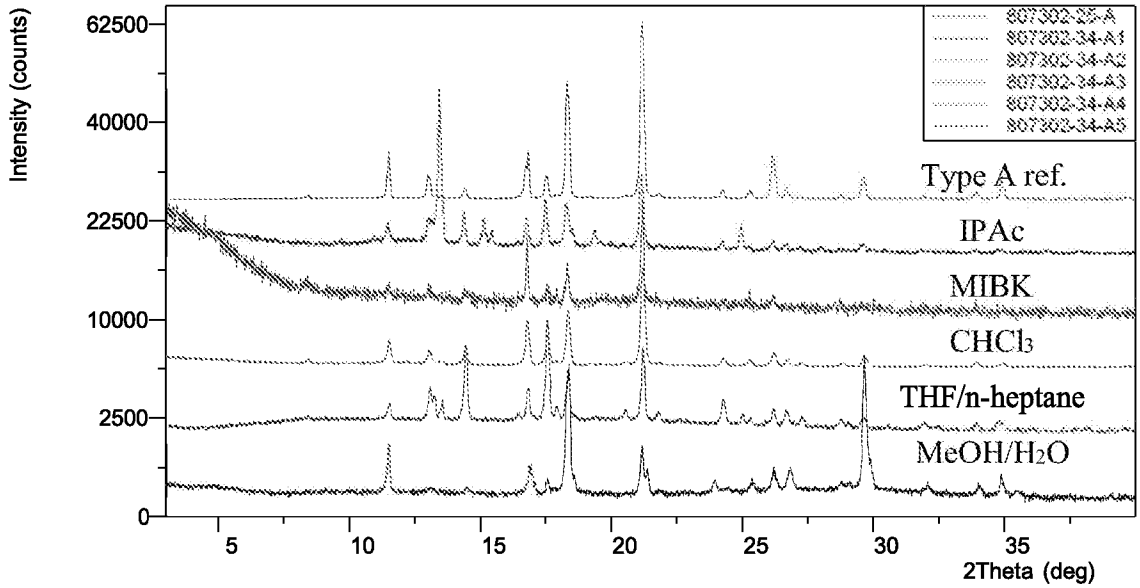
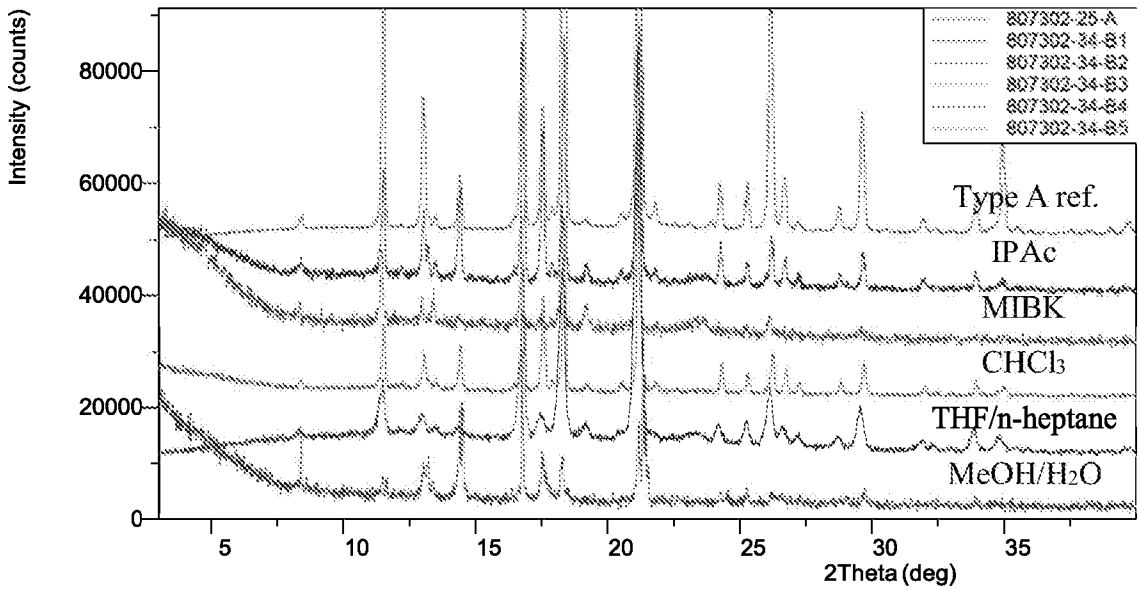

Intensity (counts)
62500
40000
22500
10000
2500
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-34-A1
807302-34-A2
807302-34-A3
807302-34-A4
807302-34-A5
Type A ref.
IPAc
MIBK
CHCl3
THF/n-heptane
MeOH/H2O

FIG. 41


Intensity (counts)
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-34-B1
807302-34-B2
807302-34-B3
807302-34-B4
807302-34-B5
Type A ref.
IPAc
MIBK
CHCl3
THF/n-heptane
MeOH/H2O

B

FIG. 42

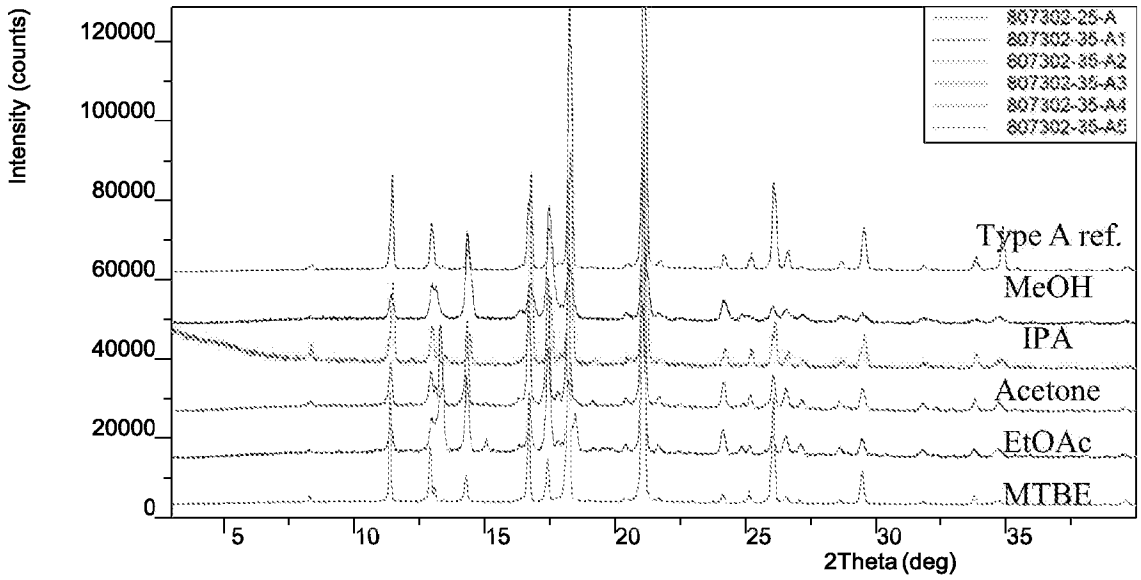

Intensity (counts)
120000
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-25-A
807302-35-A1
807302-35-A2
807302-35-A3
807302-35-A4
807302-35-A5
Type A ref.
MeOH
IPA
Acetone
EtOAc
MTBE

FIG. 43

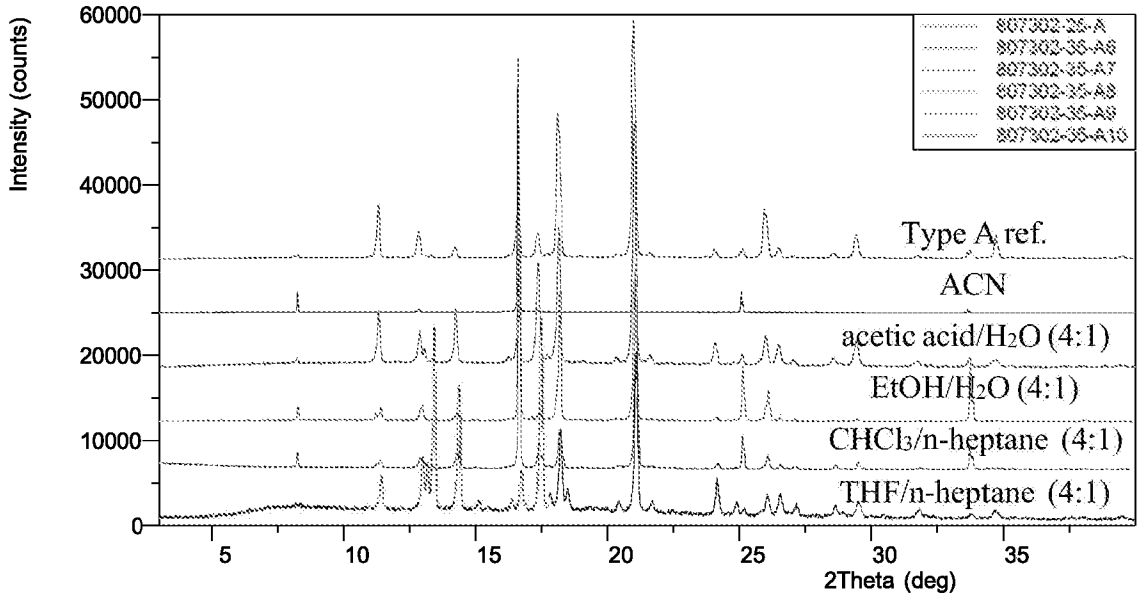

Intensity (counts)
60000
50000
40000
30000
20000
10000
0
5
10
15
20
25
30
35
2Theta (deg)
807302-35-A
807302-35-A6
807302-35-A7
807302-35-A8
807302-35-A9
807302-35-A10
Type A ref.
ACN
acetic acid/H2O (4:1)
EtOH/H2O (4:1)
CHCl3/n-heptane (4:1)
THF/n-heptane (4:1)

FIG. 44

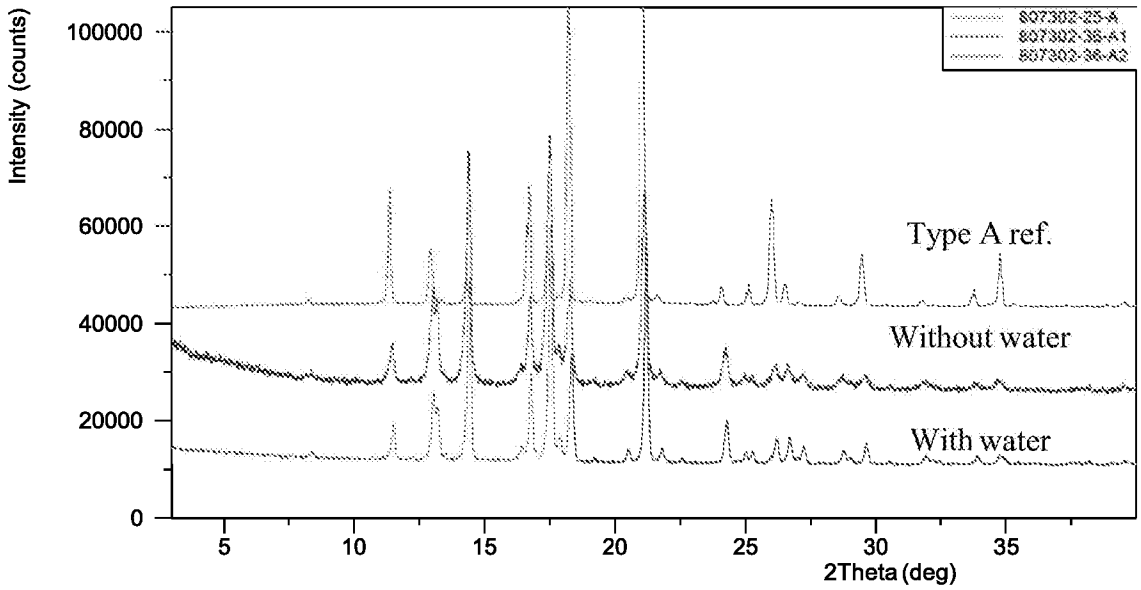

Intensity (counts)
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
35
2Theta (deg)
Type A ref.
Without water
With water

FIG. 45A

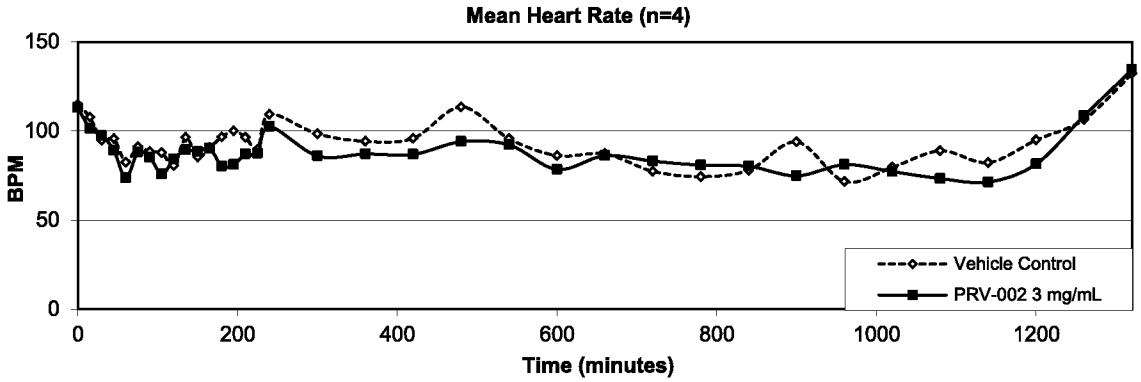


FIG. 45B

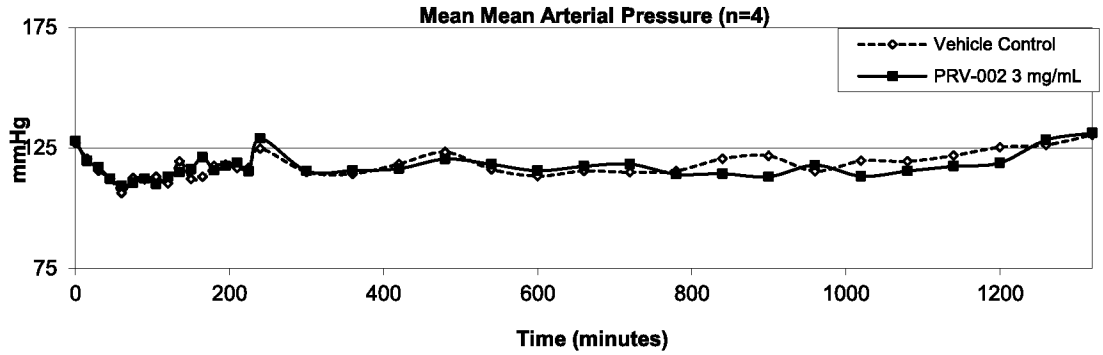


FIG. 45C

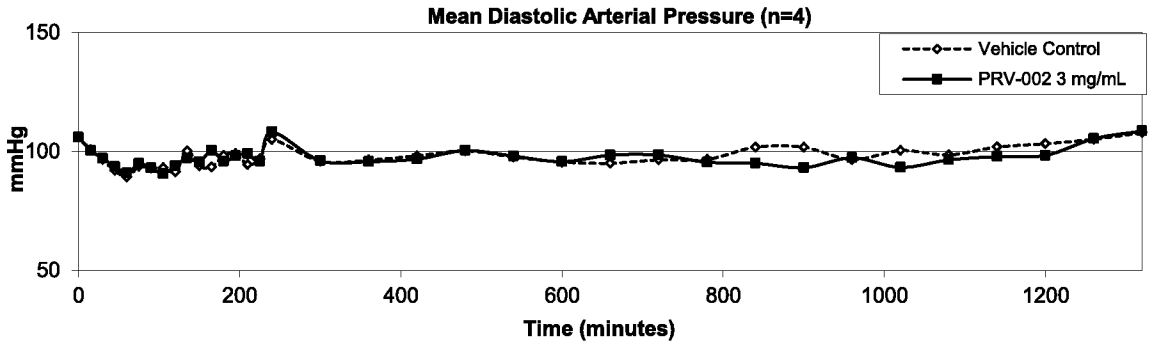


FIG. 45D

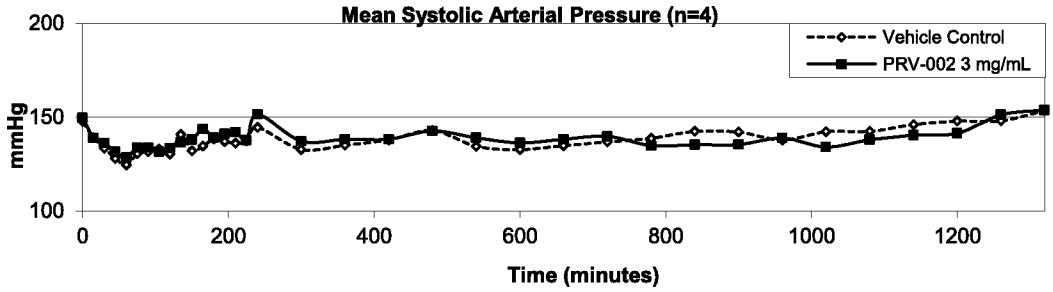


FIG. 46A

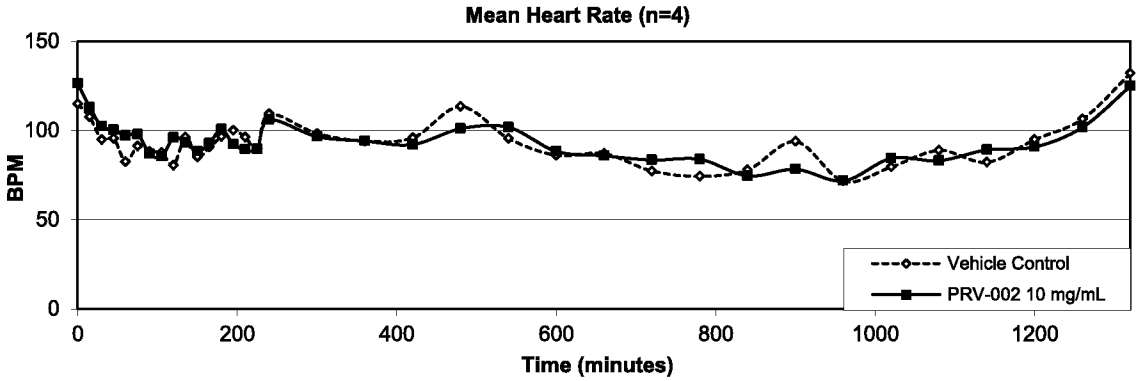


FIG. 46B

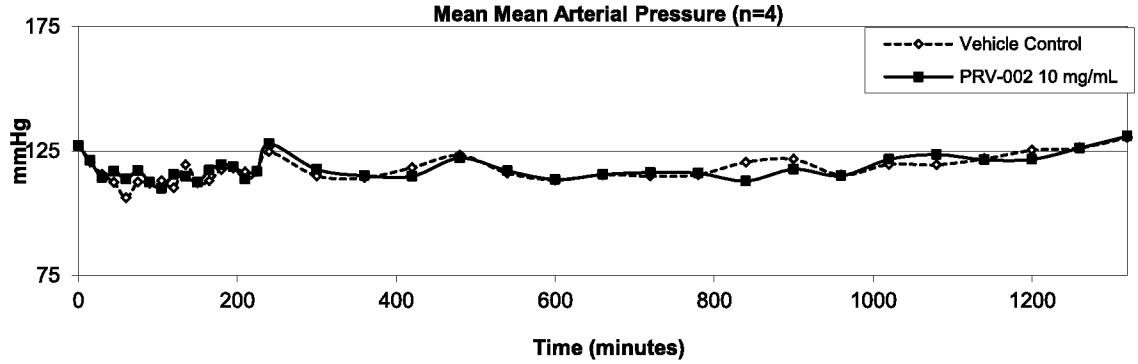


FIG. 46C

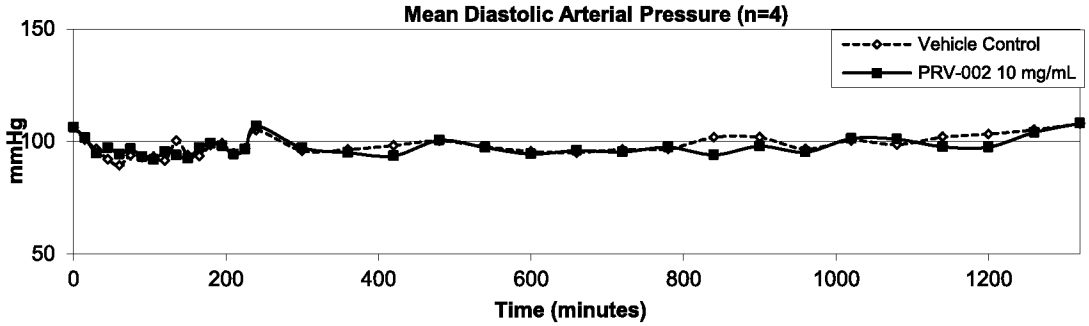


FIG. 46D

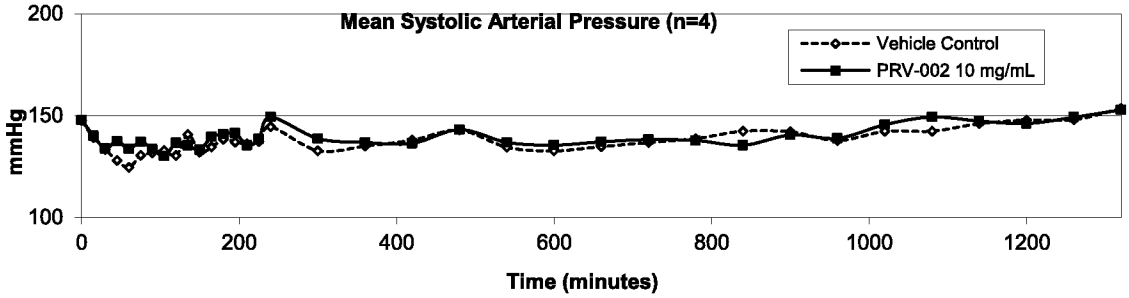


FIG. 47A

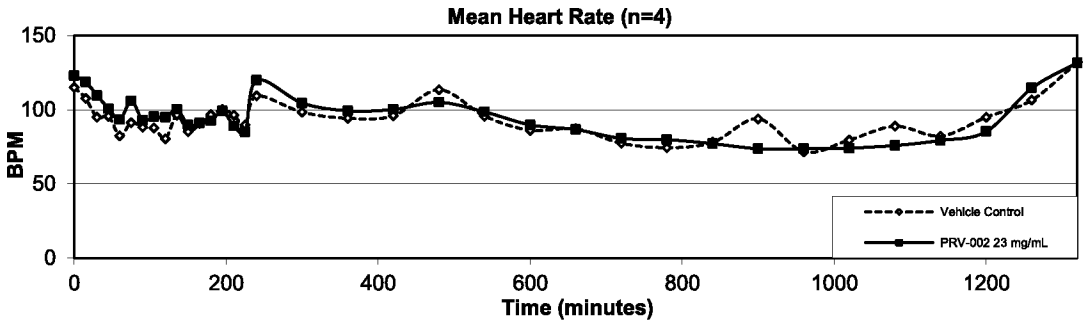


FIG. 47B

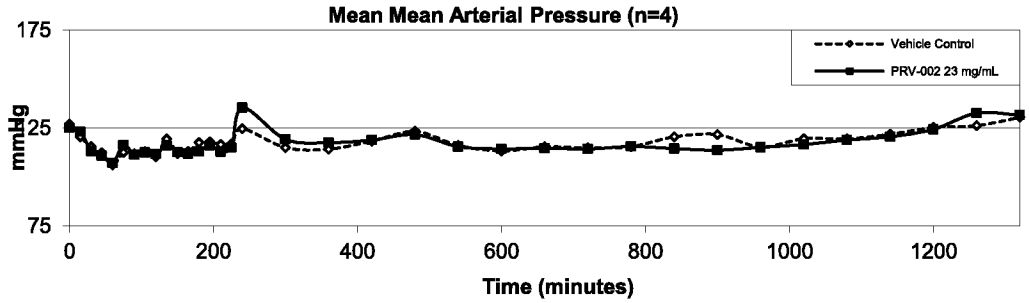


FIG. 47C

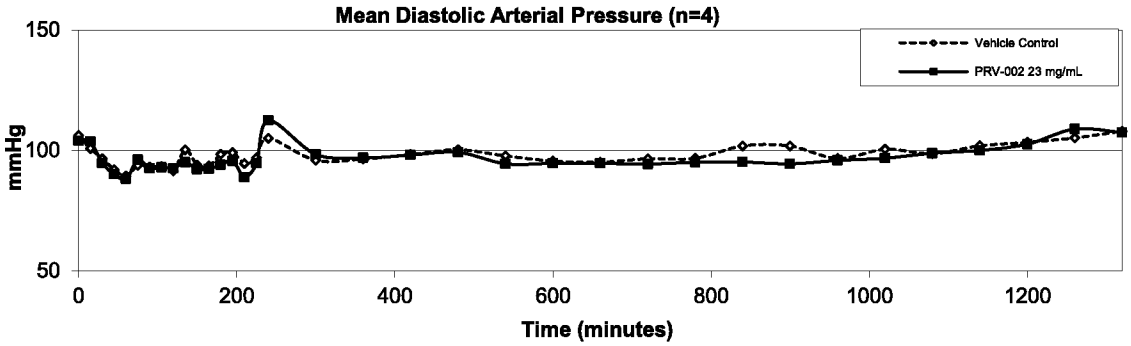


FIG. 47D

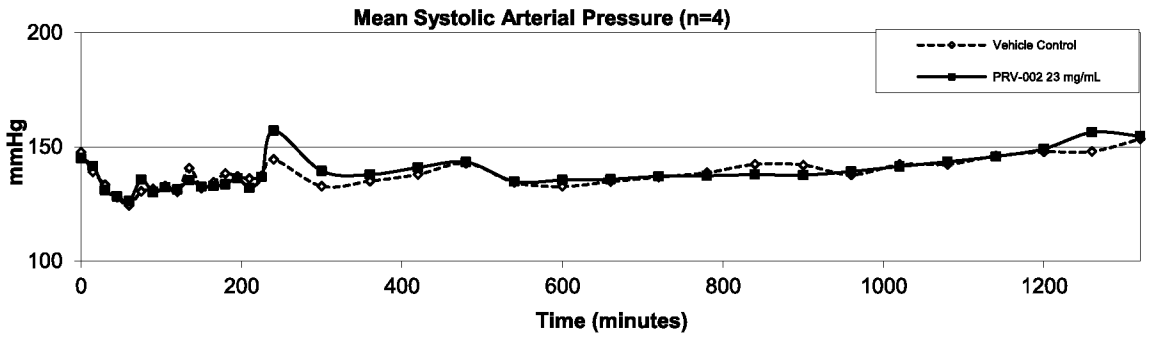
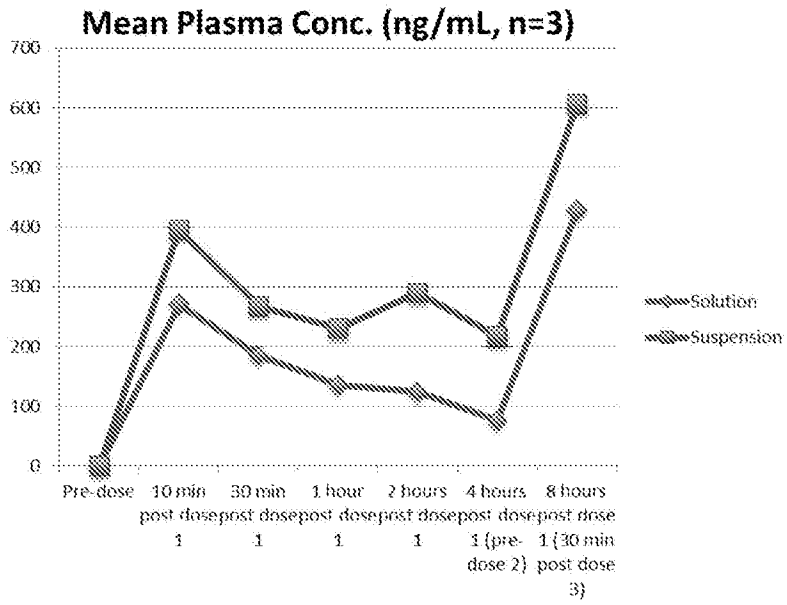


FIG. 48


Mean Plasma Conc. (ng/mL, n=3)
700
600
500
400
300
200
100
0
Pre-dose
10 min post dose 1
30 min post dose 1
1 hour post dose 1
2 hours post dose 1
4 hours post dose 1 (pre-dose 2)
8 hours post dose 1 (30 min post dose 3)
Solution
Suspension

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/38582

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/58, C07J 1/00 (2016.01)
CPC - A61K 31/566, C07J 5/0015, A61K 45/06, A61K 31/58
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)
IPC(8): A61K 31/58, C07J 1/00 (2016.01)
CPC: A61K 31/566, C07J 5/0015, A61K 45/06, A61K 31/58

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)
PatBase; Keyword limited: traumatic brain injury/TBI; severe/moderate/mild traumatic brain injury/TBI/concussion; progesterone; norprogesterone; ent-19-norprogesteron; nor-19-progesterone;19-nor-pregnene-3,20-dione

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	PubChem-CID-54056177, Create Date: 04 December 2011 (04.12.2011), pg 3, Fig.	1-4 --- 5-7,16
Y	SEELEY et al. "Molecular Interactions of Progesterone Analogues with Rabbit Uterine Cytoplasmic Receptor", The Journal of Biological Chemistry. 1982. Vol. 257 (22), pp 13359-13366, entire document, especially: pg 13360, Figure 1, progesterone; pg 13362, Table II, 19-Nor-4-pregnene-3,20-dione; pg 13363, col 2, para 2.	5-7,16
A	US 2013/116217 A1 (SITRUK-WARE et al.) 09 May 2013 (09.05.2013), entire document.	1-7,16
A	US 2003/0069217 A1 (SIMPKINS et al.) 10 April 2003 (10.04.2003), entire document.	1-7,16

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
02 November 2016	01 DEC 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/38582

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 34, 35A, 35B, 42, 45-46
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
--See attached extra sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7 and 16

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/38582

-- Continuation of Box III - Lack of Unity--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-31, 47-52 and 63 directed to a compound of Formula (I) or a pharmaceutically acceptable salt, ester, hydrate, solvate, prodrug, crystal or co-crystal thereof. The compound of Formula (I) will be searched to the extent that it encompasses the first species of claim 1, wherein X is O; Y is O; R1, R2, R5, and R6 are H; R4 is H; R3 is H; R8 is H; R9 is H; R10 is absent; R11 is H; R12, R13, R14, R15, R16 and R17 are H; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens. It is believed that claims 1-7 and 16 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass the first species of claim 1. Applicant is invited to elect additional compounds of Formula (I), wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a compound of claim 1, wherein X is O; Y is O; R1, R2, R5, and R6 are H; R4 is H; R3 is H; R8 is H; R10 is absent; R12, R13, R14, R15, R16 and R17 are H; R11 and R9 together form a double bond; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens. (i.e. claims 1-7 and 23-26).

Group II: Claims 32-33, 36-41, 43-44, 53-56, 58-61 and 64-73 drawn to a method for treating, minimizing or preventing TBI comprising administering a compound of Formula I.

Group III: Claims 57 and 62 drawn to a method of treating a subject for a concussion, said method comprising administering ent-19-norprogesterone.

Special Technical Features
The inventions listed as Groups I+ and II-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I+ does not require the compounds of any other invention of group I+.

Group II requires a method for treating, minimizing or preventing TBI comprising administering a compound of Formula I, not required by groups I+ or III

Group III requires a method of treating a subject for a concussion, said method comprising administering ent-19-norprogesterone, not required by groups I+ or II

Shared Common Features
Groups I+, II and III share the technical feature of a compound of formula (I) listed in instant claim 1.However, this shared technical feature does not represent a contribution over prior art, because the shared technical features is anticipated by the article entitled "Molecular Interactions of Progesterone Analogues with Rabbit Uterine Cytoplasmic Receptor" by Seeley et al ('Seeley'). Seeley teaches a compound of formula I wherein X is O; Y is O; R1, R2, R5, and R6 are H; R3 is H; R8 is H; R9 is H; R10 is absent; R12, R13, R14, R15, R16 and R17 are H; R7 and R4 together form a double bond; R11 and R9 together form a double bond; and the dotted line indicates the presence of either a single or a double bond wherein the valences of a single bond are completed by hydrogens (pg 13360, Figure 1, progesterone).

Groups I+, II-III share the technical feature of technical feature of ent-19-norprogestrone which is the enantiomer of 19-norprogestrone (see instant specification pg 13, para 2, chemical names for for ent-19-norprogesterone include ent-19-norpregn-4-ene-3,20-dione). However, this shared technical features do not represent a contribution over prior art, because the shared technical features is obvious over Seeley. Seeley determined the binding energy of a wide range of progesterone derivatives (pg 13359, col 1, para 1, determined systematically the relative binding energies of progesterone analogues which differ from progesterone by a single substituent or by another simple modification) and specifically tested 19-norprogestrone (pg 13362, Table II, Compound 19-Nor-4-pregnene-3,20-dione; see also instant specification pg 13, para 2, chemical names for for ent-19-norprogesterone include ent-19-norpregn-4-ene-3,20-dione), but Seeley did not specify which enanatiomer was tested. However, it would have been obvious to a person having ordinary skill in the art to isolate and test both enantiomers of 19-norprogesterone in order to identify the preferred enantiomer for treating TBI.

As the shared technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups. Therefore, groups I+ and II--III lack unity under PCT Rule 13.

Note:
claims 34, 35a, 35b, 42, 45-46 are determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Claim 37 is a self dependent claim and claim 38-41 are improperly depending from claim 37. For the purpose of completing this opinion, claims 37-41 will be interpretted as if they are depending from claim 36