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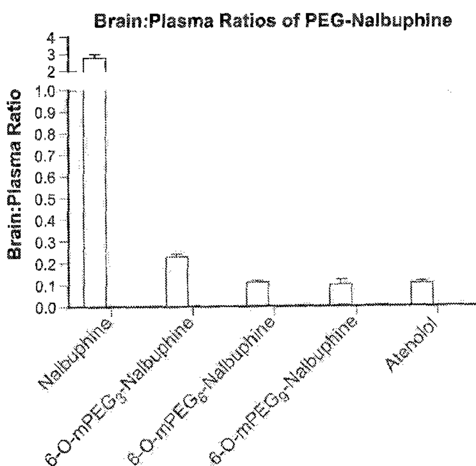

Molecule	Brain:Plasma Ratios
Nalbuphine	2.86
6-O-mPEG$_3$-Nalbuphine	0.23
6-O-mPEG$_6$-Nalbuphine	0.11
6-O-mPEG$_9$-Nalbuphine	0.10
Atenolol	0.11

FIG. 1

(57) Abstract: Provided are methods for reducing the addiction potential and/or reducing one or more CNS-side effects related to the administration of an opioid analgesic drug by administering the opioid analgesic drug in the form of an oligomeric polyethylene glycol conjugate compound. The compounds provided demonstrate notably reduced potential for substance abuse, and possess altered pharmacokinetic profiles relative to the opioid agonists alone, but are not subject to the risk of physical tampering that allows for the recovery and abuse of the opioid agonist associated with certain alternative delivery formulations.

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PEGYLATED OPIOIDS WITH LOW POTENTIAL FOR ABUSE AND SIDE EFFECTS

[0001] This application claims the benefit of priority to the following: U.S. Provisional Patent Application No. 61/294,457 filed January 12, 2010, U.S. Provisional Patent Application No. 61/320,299 filed April 1, 2010, and U.S. Provisional Patent Application No. 61/334,559 filed May 13, 2010, the contents each of which is incorporated by reference in its entirety.

[0002] Among other things, the present invention relates to opioid agonists that are covalently bound to a water-soluble oligomer (i.e., opioid agonist oligomer conjugates), the conjugates having reduced potential for substance abuse and central nervous system (CNS) side-effects, among other features and advantages, and related uses thereof.

[0003] Opioid agonists, such as morphine, have long been used to treat patients suffering from pain. Opioid agonists exert their analgesic and other pharmacological effects through interactions with opioid receptors, of which there are three main classes: mu (μ) receptors, kappa (κ) receptors, and delta (δ) receptors. Many of the clinically used opioid agonists are relatively selective for mu receptors, although opioid agonists typically have agonist activity at other opioid receptors (particularly at increased concentrations).

[0004] Opioids exert their effects, at least in part, by selectively inhibiting the release of neurotransmitters, such as acetylcholine, norepinephrine, dopamine, serotonin, and substance P.

[0005] Pharmacologically, opioid agonists represent an important class of agents employed in the management of pain. Opioid agonists currently used in analgesia, however, possess considerable addictive properties that complicate and limit their use in therapeutic practice. The medical, social and financial complications arising from opioid abuse impose severe constraints on the ability of physicians to prescribe opioids for use in chronic pain. The U.S. Food and Drug Administration has recently described prescription opioid analgesics as being at the center of a major public health crisis of addiction, misuse, abuse, overdose, and death (FDA/Center for Drug Evaluation and Research, Joint Meeting of the Anesthetic and Life Support Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee, Meeting Transcript, July 23-4, 2010).

[0006] Typical opioids pass rapidly through the blood-brain-barrier (BBB) and rapidly reach peak concentrations that relate to the "highs" experienced by opioid abusers. Evidence indicates that reduced addictive properties can be achieved through an altered pharmacokinetic profile, which would deliver opioids at a constant low concentration to the brain, avoiding the concentration peaks of traditional modes of delivery that underlie the addictive potential of opioid agonists. Balster and Schuster, *J Exp Anal Behav* 20:119–129 (1973); Panlilio and Schindler, *Psychopharmacology* 150:61–66 (2000); Winger *et al.*, *J Pharmacol Exp Ther* 301:690–697 (2002); Ko et al., *J Pharmacol Exp Ther* 301:698-704; Abreu et al., *Psychopharmacologia* 154:76–84 (2001). Development efforts in this regard have focused primarily on alternative delivery strategies, such as orally administered delayed release tablets and transdermal patches. These aim to supply a constant low concentration of drug to the circulation, but are complicated by the fact that they can be physically disrupted by crushing or cutting up, enabling the drug to be accessed and then injected directly into the circulation to give the desired pharmacokinetic profile for addictive behavior.

[0007] Thus, there exists a need in the art for opioid agonists with low addiction properties and concomitant low abuse potential over currently available opioids used in analgesia. Preferably, such modified opioid agonists will also exhibit reduced central nervous system side effects, thereby making the prescription and use of such compounds of greater desirability to both physicians as well as patients. In particular, there exists a need for modifications to opioid agonists that alter the molecule itself and slow the penetration of the blood-brain-barrier such that direct injection of the drug does not provide the immediate central nervous system penetration that underlies the addictive "rush." The present disclosure seeks to address these and other needs by providing opioid agonists covalently bound to a water-soluble oligomer that retain their analgesic properties but have a reduced potential for substance abuse and/or reduced CNS-side effects.

[0008] Accordingly, in one aspect, provided herein is a compound of the formula OP-X-POLY, wherein OP is an opioid compound, X is a linker, and POLY is a small water-soluble oligomer.

[0009] In a related embodiment, provided is a composition comprising a compound of the formula OP-X-POLY (where OP, X and POLY are as defined above) and a pharmaceutically acceptable excipient or carrier.

[0010] In another aspect, provided is a method of treating a patient in need of opioid therapy comprising administering an effective amount of a compound of the formula OP-X-POLY.

[0011] In yet another aspect, the provided is a method of reducing the abuse potential of an opioid compound comprising conjugating the compound to a small water-soluble oligomer.

[0012] In a further aspect, provided is a method of reducing the addictive properties of an opioid agonist comprising conjugating the opioid agonist to a small water-soluble oligomer.

[0013] In another aspect, provided is a method of reducing, but not substantially eliminating, the rate of crossing the blood brain barrier of an opioid compound comprising conjugating the compound to a small water-soluble oligomer.

[0014] In yet another aspect, provided is a prodrug comprising a mu, kappa, or delta opioid agonist reversibly attached via a covalent bond to a releasable water soluble oligomeric moiety, wherein a given molar amount of the prodrug administered to a patient exhibits a rate of accumulation and a C_{max} of the mu, kappa, or delta opioid agonist in the central nervous system in the mammal that is less than the rate of accumulation and the C_{max} of an equal molar amount of the mu, kappa, or delta opioid agonist had the mu, kappa, or delta opioid agonist not been administered as part of a prodrug.

[0015] Also provided herein is a method for reducing the addiction potential and reducing one or more central nervous system (CNS) side-effects related to administration of an opioid analgesic drug (OP). The method comprises administering to a mammalian subject suffering from pain a therapeutically effective amount of an opioid compound having the formula: $OP—X—(CH_2CH_2O)_nY$, or a pharmaceutically acceptable salt form thereof, wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group, whereby as a result of the administering, a degree of pain relief is

experienced by the subject, and when evaluated in a suitable animal model, the opioid compound exhibits (i) a measurable reduction in addiction potential over the opioid analgesic drug in unconjugated form, and (ii) a ten-fold or greater reduction of at least one CNS-related side effect when compared to administration of the opioid analgesic drug in unconjugated form.

[0016] In yet another aspect, provided is a method for reducing one or more central-nervous system side-effects related to administration of an opioid analgesic drug (OP) by administering the opioid analgesic drug to a mammalian subject in the following form: $OP—X—(CH_2CH_2O)_nY$, wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group.

[0017] In yet another aspect, provided is the use of an opioid compound having the formula: $OP—X—(CH_2CH_2O)_nY$, wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group, for simultaneously reducing the addiction potential and one or more central nervous system (CNS) side-effects related to administration of the opioid analgesic drug (OP) in unconjugated form.

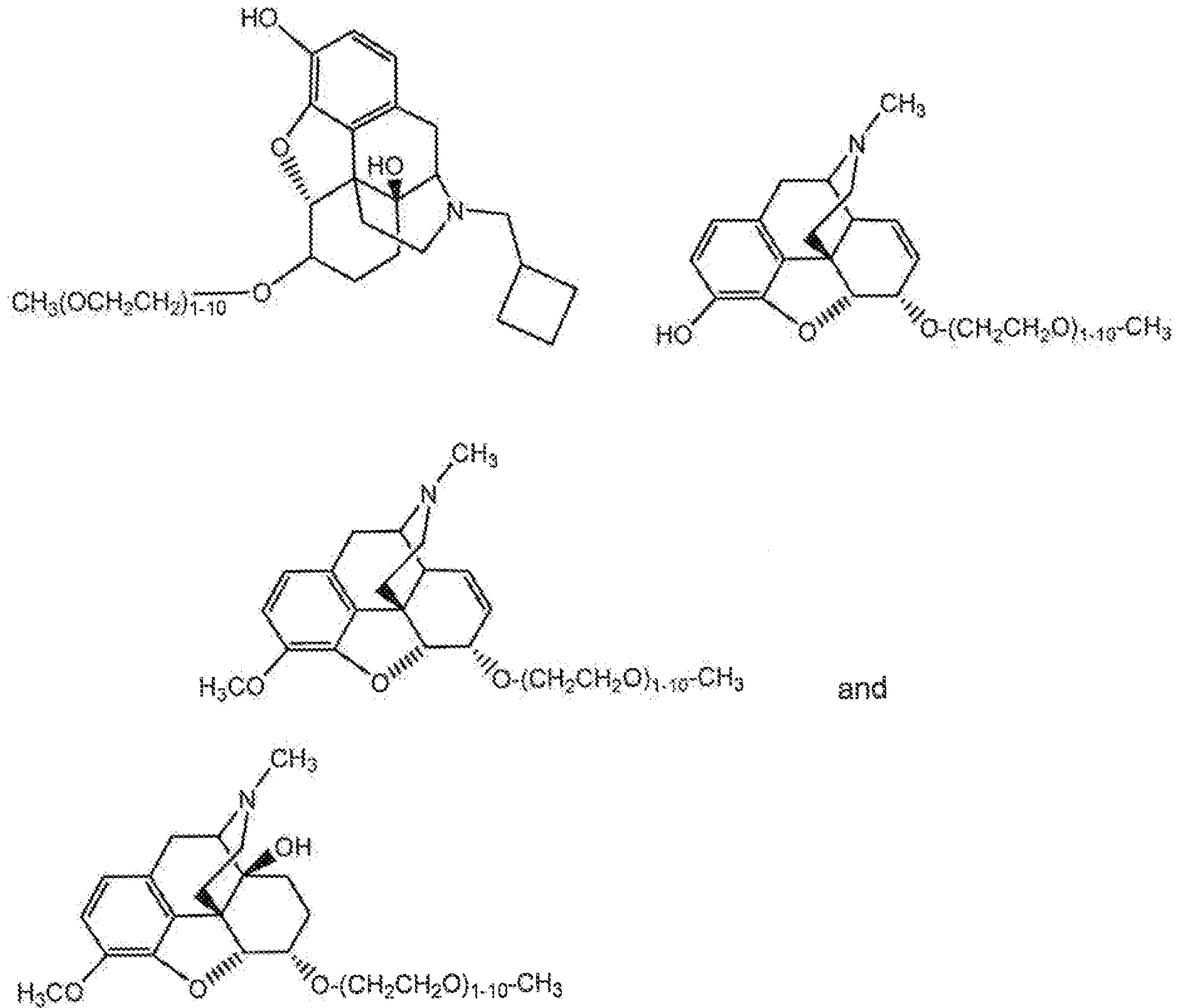
[0018] In yet another aspect, provided is the use of an opioid compound having the formula: $OP—X—(CH_2CH_2O)_nY$, wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group, for the manufacture of a medicament for reducing the addiction potential and reducing one or more central nervous system (CNS) side-effects related to administration of an opioid analgesic drug (OP).

[0019] In one or more embodiments related to the foregoing, the opioid analgesic drug is a mu-opioid analgesic.

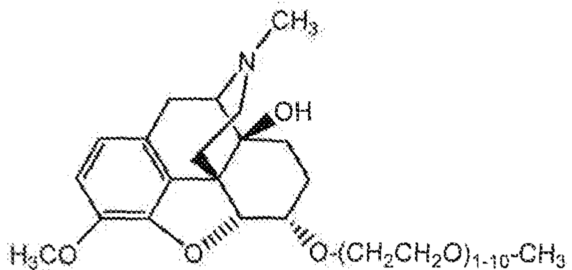
[0020] In yet one or more embodiments related to the foregoing aspects, the opioid analgesic drug is selected from fentanyl, nalbuphine, hydromorphone, methadone, morphine, codeine, oxycodone, and oxymorphone.

[0021] In yet one or more embodiments related to the foregoing aspects, the physiologically stable linker, X, is oxygen.

[0022] In yet one or more further embodiments related to the foregoing aspects, the opioid compound has a structure selected from:



[0023] In one or more particular embodiments, the opioid compound has the structure:



[0024] In one or more embodiments related to the foregoing methods and/or uses, the opioid compound is administered orally.

[0025] In one or more additional embodiments related to the foregoing methods and/or uses, the opioid compound is administered parenterally.

[0026] In yet one or more further embodiments related to the foregoing methods and/or uses, the opioid compound exhibits a measurable reduction in addiction potential over the opioid analgesic drug in unconjugated form when evaluated in an in-vivo self-administration model in rodents or primates.

[0027] In yet one or more additional embodiments related to the foregoing aspects, in particular, the methods and/or uses described above, the opioid compound exhibits a ten-fold or greater reduction in at least one CNS-related side effect associated with administration of the opioid analgesic drug in unconjugated form when evaluated in a mouse model, wherein the one or more CNS-related side effects is selected from straub tail response, locomotor ataxia, tremor, hyperactivity, hypoactivity, convulsions, hindlimb splay, muscle rigidity, pinna reflex, righting reflex and placing.

[0028] In yet one or more additional embodiments related to the foregoing, the opioid compound exhibits a ten-fold or greater reduction in at least one CNS-related side effect associated with administration of the opioid analgesic drug in unconjugated form when evaluated in a mouse model, wherein the one or more CNS-related side effects is selected from straub tail response, muscle rigidity, and pinna reflex.

[0029] In yet one or more additional embodiments, the method and/or use of an opioid compound as provided herein is effective to reduce one or more central nervous system (CNS)

side-effects associated with administration of the opioid analgesic drug in unconjugated form in a mammalian subject, wherein the CNS-side effect is selected from respiratory depression, sedation, myoclonus, and delirium.

[0030] In yet one or more further embodiments of one or more of the methods and/or uses provided herein, the amount of opioid compound administered results in both an analgesic effect and a reduction of one or more central nervous system side effects associated with administration of the opioid analgesic drug in unconjugated form in a mammalian subject.

[0031] In yet or more additional embodiments, the method or use further comprises monitoring the patient over the course of treatment for abuse/addiction potential and/or the existence (or absence) of one or more CNS-side effects associated with administration of the opioid analgesic.

[0032] In yet another one or more additional related embodiments, in the event abuse/addiction potential and/or the existence of one or more CNS-side effects is observed, the monitoring further comprises an assessment of the degree of such abuse/addiction potential and/or CNS-side effect.

[0033] Additional embodiments of the present method, compositions, and the like will be apparent from the following description, drawings, examples, and claims. As can be appreciated from the foregoing and following description, each and every feature or steps described herein, and each and every combination of two or more of such features or steps, is included within the scope of the present disclosure provided that the features/steps included in such a combination are not mutually inconsistent. In addition, any feature or combination of features or steps may be specifically excluded from any embodiment of the present invention. Additional aspects and advantages of the present invention are set forth in the following description and claims, particularly when considered in conjunction with the accompanying examples and drawings.

[0034] These and other objects, aspects, embodiments and features of the invention will become more fully apparent when read in conjunction with the following detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0035] **FIG. 1** is a graph showing brain:plasma ratios of various PEG_{oligo}-nalbuphine conjugates, as described in greater detail in Example 8. The plot demonstrates that PEG conjugation results in a decrease in the brain:plasma ratios of nalbuphine.

[0036] **FIG. 2** is a graph showing percent writhing per total number of mice, n, in the study group, versus dose of $mPEG_n$-O-morphine conjugate administered in an analgesic assay for evaluating the extent of reduction or prevention of visceral pain in mice as described in detail in Example 13. Morphine was used as a control; unconjugated parent molecule, morphine sulfate, was also administered to provide an additional point of reference. Conjugates belonging to the following conjugate series: $mPEG_{2-7,9}$-O-morphine were evaluated.

[0037] **FIG. 3** is a graph showing percent writhing per total number of mice, n, in the study group, versus dose of $mPEG_n$-O-hydroxycodone conjugate administered in an analgesic assay for evaluating the extent of reduction or prevention of visceral pain in mice as described in detail in Example 13. Morphine was used as a control; unconjugated parent molecule, oxycodone, was also administered to provide an additional point of reference. Conjugates belonging to the following conjugate series: $mPEG_{1-4, 6, 7, , 9}$ -O-hydroxycodone were evaluated.

[0038] **FIG. 4** is a graph showing percent writhing per total number of mice, n, in the study group, versus dose of $mPEG_n$-O-codeine conjugate administered in an analgesic assay for evaluating the extent of reduction or prevention of visceral pain in mice as described in detail in Example 13. Morphine was used as a control; unconjugated parent molecule, codeine, was also administered to provide an additional point of reference. Conjugates belonging to the following conjugate series: $mPEG_{3-7, , 9}$ -O-codeine were evaluated.

[0039] **FIGS. 5 - 7** are plots indicating the results of a hot plate latency analgesic assay in mice as described in detail in Example 14. Specifically, the figures correspond to graphs showing latency (time to lick hindpaw), in seconds versus dose of compound. **FIG. 5** provides results for $mPEG_{1-5}$-O-hydroxycodone conjugates as well as for unconjugated parent molecule; **FIG. 6** provides results for $mPEG_{1-5}$-O-morphine conjugates as well for unconjugated parent molecule; and **FIG. 7** provides results for $mPEG_{2-5, 9}$-O-codeine conjugates as well as for the parent molecule. The presence of an asterisk by a data point indicates $p < 0.05$ versus saline by ANOVA/Dunnett's.

[0040] **FIG. 8** shows the mean (+SD) plasma concentration-time profiles for the compounds, oxycodone ($mPEG_0$-oxycodone), $mPEG_1$-O-hydroxycodone, $mPEG_2$-O-hydroxycodone, $mPEG_3$-O-hydroxycodone, $mPEG_4$-O-hydroxycodone, $mPEG_5$-O-hydroxycodone, $mPEG_6$-O-hydroxycodone, $mPEG_7$-O-hydroxycodone, and $mPEG_9$-O-hydroxycodone, following 1.0 mg/kg intravenous administration to rats as described in Example 16.

[0041] **FIG. 9** shows the mean (+SD) plasma concentration-time profiles for the compounds, oxycodone ($mPEG_0$-oxycodone), $mPEG_1$-O-hydroxycodone, $mPEG_2$-O-hydroxycodone, $mPEG_3$-O-hydroxycodone, $mPEG_4$-O-hydroxycodone, $mPEG_5$-O-hydroxycodone, $mPEG_6$-O-hydroxycodone, $mPEG_7$-O-hydroxycodone, and $mPEG_9$-O-hydroxycodone, following 5.0 mg/kg oral administration to rats as described in Example 16.

[0042] **FIG. 10** shows the mean (+SD) plasma concentration-time profiles for the compounds, morphine ($mPEG_0$-morphine), and $mPEG_{1-7, 9}$-O-morphine conjugates, following 1.0 mg/kg intravenous administration to rats as described in detail in Example 17.

[0043] **FIG. 11** shows the mean (+SD) plasma concentration-time profiles for the compounds, morphine ($mPEG_0$-morphine), and $mPEG_{1-7, 9}$-O-morphine conjugates, following 5.0 mg/kg oral administration to rats as described in Example 17.

[0044] **FIG. 12** shows the mean (+SD) plasma concentration-time profiles for the compounds, codeine ($mPEG_0$-codeine), and $mPEG_{1-7, 9}$-O-codeine conjugates, following 1.0 mg/kg intravenous administration to rats as described in detail in Example 18.

[0045] **FIG. 13** shows the mean (+SD) plasma concentration-time profiles for the compounds, codeine ($mPEG_0$-codeine), and $mPEG_{1-7, 9}$-O-codeine conjugates, following 5.0 mg/kg oral administration to rats as described in Example 18.

[0046] **FIGS. 14A, 14B** and **14C** illustrate the brain:plasma ratios of various oligomeric $mPEG_n$-O-morphine, $mPEG_n$-O-codeine and $mPEG_n$-O-hydroxycodone conjugates, respectively, following IV administration to rats as described in Example 21. The brain:plasma ratio of atenolol is provided in each figure as a basis for comparison.

[0047] **FIGS. 15A-H** illustrate brain and plasma concentrations of morphine and various mPEG$_n$-O-morphine conjugates over time following IV administration to rats as described in Example 22. FIG. 15A (morphine, n=0); FIG. 15B (n=1); FIG. 15C (n=2); FIG. 15D (n=3); FIG. 15E (n=4); FIG. 15F (n=5); FIG. 15G (n=6); FIG. 15H (n=7).

[0048] **FIGS. 16A-H** illustrate brain and plasma concentrations of codeine and various mPEG$_n$-O-codeine conjugates over time following IV administration to rats as described in Example 22. FIG. 16A (codeine, n=0); FIG. 16B (n=1); FIG. 16C (n=2); FIG. 16D (n=3); FIG. 16E (n=4); FIG. 16F (n=5); FIG. 16G (n=6); FIG. 16H (n=7).

[0049] **FIGS. 17A-H** illustrate brain and plasma concentrations of oxycodone and various mPEG$_n$-O-hydroxycodone conjugates over time following IV administration to rats as described in Example 22. FIG. 17A (oxycodone, n=0); FIG. 17B (n=1); FIG. 17C (n=2); FIG. 17D (n=3); FIG. 17E (n=4); FIG. 17F (n=5); FIG. 17G (n=6); FIG. 17H (n=7).

[0050] **FIGS. 18A-C** illustrate the rate of brain penetration (Kin values) of certain exemplary PEG$_{olig}$-opioid conjugates in comparison to control compounds, antipyrine and unconjugated opioid, as described in detail in Example 3. Specifically, FIG. 18A illustrates the results for mPEG$_n$-O-morphine conjugates (where n= 1, 2, 3, and 7) in comparison to the control compounds, morphine and antipyrine. FIG. 18B illustrates the results for mPEG$_n$-O-codeine conjugates (where n= 2, 3, and 7) in comparison to the control compounds, codeine and antipyrine. FIG. 18C illustrates the results for mPEG$_n$-O-hydroxycodone conjugates (where n= 1, 2, 3, and 7) in comparison to the control compounds, oxycodone and antipyrine.

[0051] **FIG. 19** provides a graph illustrating rate of brain penetration, Kin, versus PEG oligomer size for mPEGn-O-morphine, mPEGn-O-codeine, and mPEGn-O-hydroxycodone conjugates as described in detail in Example 3.

[0052] **FIG. 20** is a graph illustrating reduced abuse liability in a primate model, as described in Example 7.

[0053] **FIG. 21** is a plot showing the results of an acetic acid writhing assay for mPEG$_n$-hydroxycodone (n=1-7) as described in detail in Example 23 (vertical axis = number of writhes;

horizontal axis demonstrates dose, mg/kg). Saline was used as a control; also shown are results for the unmodified drugs, oxycodone and hydroxycodone.

[0054] **FIG. 22** is a plot showing the results of an acetic acid writhing assay for mPEG$_n$-O-morphine (n=3,4,5,7) as described in detail in Example 23 (vertical axis = number of writhes; horizontal axis demonstrates dose, mg/kg). Saline was used as a control; also shown are results for unmodified parent compound, morphine.

[0055] **FIGs. 23A and 23B** are plots demonstrating reinforcing behavior observed in rats taught to self-administer cocaine in a study designed to investigate the abuse liability associated with various test compounds as described in detail in Example 24. Fig. 23A shows the reinforcing behavior associated with administration of the training dose of cocaine, while Fig. 23B shows the lack of reinforcing behavior for rats administered α -6-mPEG$_6$-O-hydroxycodone.

[0056] **FIGS. 24A and 24B** are plots demonstrating progressive ratio breakpoints in rats taught to self-administer cocaine in a study designed to investigate the abuse liability associated with various test compounds as described in detail in Example 24. Fig. 24A illustrates the results for rats administered saline (used as a negative control), cocaine, hydrocodone, and oxycodone at the doses indicated. Fig. 24B demonstrates the results for test compound, α -6-mPEG$_6$-O-hydroxycodone. From Fig. 24B, it can be seen that no reinforcing behaviour was exhibited by rats administered test compound, α -6-mPEG$_6$-O-hydroxycodone, at the doses indicated.

[0057] **FIG. 25** is a graph illustrating the results of a study evaluating the effects of saline (negative control), an exemplary oligomeric PEG-opioid, α-6-mPEG$_6$-O-hydroxycodone, and oxycodone (unmodified parent opioid) on motor coordination in rats using the rat rotarod treadmill to assess sedation as described in detail in Example 26.

[0058] **FIG. 26** is a graph illustrating the results of a study evaluating respiratory depression in mice administered eqi-efficacious doses of either α-6-mPEG$_6$-O-hydroxycodone or oxycodone when compared to saline as the negative control as described in detail in Example 27.

[0059] As used in this specification, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0060] In describing and claiming the present invention, the following terminology will be used in accordance with the definitions described below.

[0061] The terms "opioid compound" and "opioid agonist" are broadly used herein to refer to an organic, inorganic, or organometallic compound typically having a molecular weight of less than about 1000 Daltons (and typically less than 500 Daltons) and having some degree of activity as a mu, delta and/or kappa agonist. Opioid agonists encompass oligopeptides and other biomolecules having a molecular weight of less than about 1500.

[0062] The terms "spacer moiety," "linkage" and "linker" are used herein to refer to an atom or a collection of atoms optionally used to link interconnecting moieties such as a terminus of a polymer segment and an opioid compound or an electrophile or nucleophile of an opioid compound. The spacer moiety may be hydrolytically stable or may include a physiologically hydrolyzable or enzymatically degradable linkage. Unless the context clearly dictates otherwise, a spacer moiety optionally exists between any two elements of a compound (*e.g.*, the provided conjugates comprising an opioid compound and a water-soluble oligomer that can be attached directly or indirectly through a spacer moiety).

[0063] "Water soluble oligomer" indicates a non-peptidic oligomer that is at least 35% (by weight) soluble, in certain embodiments greater than 70% (by weight), and in certain embodiments greater than 95% (by weight) soluble, in water at room temperature. Typically, an unfiltered aqueous preparation of a "water-soluble" oligomer transmits at least 75%, and in certain embodiments at least 95%, of the amount of light transmitted by the same solution after filtering. In certain embodiments the water-soluble oligomer is at least 95% (by weight) soluble in water or completely soluble in water. With respect to being "non-peptidic," an oligomer is non-peptidic when it has less than 35% (by weight) of amino acid residues.

[0064] The terms "monomer," "monomeric subunit" and "monomeric unit" are used interchangeably herein and refer to one of the basic structural units of a polymer or oligomer. In the case of a homo-oligomer, a single repeating structural unit forms the oligomer. In the case of a co-oligomer, two or more structural units are repeated -- either in a pattern or randomly -- to form the oligomer. In certain embodiments oligomers used in connection with the present invention are homo-oligomers. The water-soluble oligomer typically comprises one or more

monomers serially attached to form a chain of monomers. The oligomer can be formed from a single monomer type (*i.e.*, is homo-oligomeric) or two or three monomer types (*i.e.*, is co-oligomeric).

[0065] An "oligomer" is a molecule possessing from about 2 to about 50 monomers, in certain embodiments from about 2 to about 30 monomers. The architecture of an oligomer can vary. Specific oligomers for use in the invention include those having a variety of geometries such as linear, branched, or forked, to be described in greater detail below.

[0066] "PEG" or "polyethylene glycol," as used herein, is meant to encompass any water-soluble poly(ethylene oxide). Unless otherwise indicated, a "PEG oligomer" (also called an oligoethylene glycol) is one in which substantially all (and in certain embodiments all) monomeric subunits are ethylene oxide subunits. The oligomer may, however, contain distinct end capping moieties or functional groups, *e.g.*, for conjugation. Typically, PEG oligomers for use in the present invention will comprise one of the two following structures: "$-(CH_2CH_2O)_n-$" or "$-(CH_2CH_2O)_{n-1}CH_2CH_2-$," depending upon whether the terminal oxygen(s) has been displaced, *e.g.*, during a synthetic transformation. For PEG oligomers, "n" varies from about 2 to 50, in certain embodiments from about 2 to about 30, and the terminal groups and architecture of the overall PEG can vary. When PEG further comprises a functional group, A, for linking to, *e.g.*, an opioid compound, the functional group when covalently attached to a PEG oligomer does not result in formation of (i) an oxygen-oxygen bond (-O-O-, a peroxide linkage), or (ii) a nitrogen-oxygen bond (N-O, O-N).

[0067] An "end capping group" is generally a non-reactive carbon-containing group attached to a terminal oxygen of a PEG oligomer. Exemplary end capping groups comprise a C_{1-5} alkyl group, such as methyl, ethyl and benzyl), as well as aryl, heteroaryl, cyclo, heterocyclo, and the like. Iin certain embodiments thecapping groups have relatively low molecular weights such as methyl or ethyl. The end-capping group can also comprise a detectable label. Such labels include, without limitation, fluorescers, chemiluminescers, moieties used in enzyme labeling, colorimetric labels (*e.g.*, dyes), metal ions, and radioactive moieties.

[0068] "Branched", in reference to the geometry or overall structure of an oligomer, refers to an oligomer having two or more polymers representing distinct "arms" that extend from a branch point.

[0069] "Forked" in reference to the geometry or overall structure of an oligomer, refers to an oligomer having two or more functional groups (typically through one or more atoms) extending from a branch point.

[0070] A "branch point" refers to a bifurcation point comprising one or more atoms at which an oligomer branches or forks from a linear structure into one or more additional arms.

[0071] The term "reactive" or "activated" refers to a functional group that reacts readily or at a practical rate under conventional conditions of organic synthesis. This is in contrast to those groups that either do not react or require strong catalysts or impractical reaction conditions in order to react (*i.e.*, a "nonreactive" or "inert" group).

[0072] "Not readily reactive," with reference to a functional group present on a molecule in a reaction mixture, indicates that the group remains largely intact under conditions that are effective to produce a desired reaction in the reaction mixture.

[0073] A "protecting group" is a moiety that prevents or blocks reaction of a particular chemically reactive functional group in a molecule under certain reaction conditions. The protecting group will vary depending upon the type of chemically reactive group being protected as well as the reaction conditions to be employed and the presence of additional reactive or protecting groups in the molecule. Functional groups which may be protected include, by way of example, carboxylic acid groups, amino groups, hydroxyl groups, thiol groups, carbonyl groups and the like. Representative protecting groups for carboxylic acids include esters (such as a *p*-methoxybenzyl ester), amides and hydrazides; for amino groups, carbamates (such as *tert*-butoxycarbonyl) and amides; for hydroxyl groups, ethers and esters; for thiol groups, thioethers and thioesters; for carbonyl groups, acetals and ketals; and the like. Such protecting groups are well-known to those skilled in the art and are described, for example, in T.W. Greene and G.M. Wuts, *Protecting Groups in Organic Synthesis*, Third Edition, Wiley, New York, 1999, and references cited therein.

[0074] A functional group in "protected form" refers to a functional group bearing a protecting group. As used herein, the term "functional group" or any synonym thereof encompasses protected forms thereof.

[0075] A "physiologically cleavable" bond is a hydrolyzable bond or an enzymatically degradable linkage. A "hydrolyzable" or "degradable" bond is a relatively labile bond that reacts with water (*i.e.*, is hydrolyzed) under ordinary physiological conditions. The tendency of a bond to hydrolyze in water under ordinary physiological conditions will depend not only on the general type of linkage connecting two central atoms but also on the substituents attached to these central atoms. Such bonds are generally recognizable by those of ordinary skill in the art. Appropriate hydrolytically unstable or weak linkages include but are not limited to carboxylate ester, phosphate ester, anhydrides, acetals, ketals, acyloxyalkyl ether, imines, orthoesters, peptides, oligonucleotides, thioesters, and carbonates.

[0076] An "enzymatically degradable linkage" means a linkage that is subject to degradation by one or more enzymes under ordinary physiological conditions.

[0077] "Releasably attached," *e.g.*, in reference to an opioid compound releasably attached to a water-soluble oligomer, refers to an opioid compound that is covalently attached via a linker that includes a physiologically cleavable or degradable (including enzymatically) linkage as disclosed herein, wherein upon degradation (*e.g.*, by hydrolysis), the opioid compound is released. The opioid compound thus released will typically correspond to the unmodified opioid compound, or may be slightly altered, *e.g.*, possessing a short organic tag of about 8 atoms, *e.g.*, typically resulting from cleavage of a part of the water-soluble oligomer linker not immediately adjacent to the opioid compound. In certain embodiments, the unmodified opioid compound is released.

[0078] A "stable" linkage or bond refers to a chemical moiety or bond, typically a covalent bond, that is substantially stable in water, that is to say, does not undergo hydrolysis under ordinary physiological conditions to any appreciable extent over an extended period of time. Examples of hydrolytically stable linkages include but are not limited to the following: carbon-carbon bonds (*e.g.*, in aliphatic chains), ethers, amides, urethanes, amines, and the like. Generally, a stable linkage is one that exhibits a rate of hydrolysis of less than about 1-2% per

day under ordinary physiological conditions. Hydrolysis rates of representative chemical bonds can be found in most standard chemistry textbooks.

[0079] In the context of describing the consistency of oligomers in a given composition, "substantially" or "essentially" means nearly totally or completely, for instance, 95% or greater, in certain embodiments 97% or greater, in certain embodiments 98% or greater, in certain embodiments 99% or greater, and in certain embodiments 99.9% or greater.

[0080] "Monodisperse" refers to an oligomer composition wherein substantially all of the oligomers in the composition have a well-defined, single molecular weight and defined number of monomers, as determined by chromatography or mass spectrometry. Monodisperse oligomer compositions are in one sense pure, that is, substantially comprising molecules having a single and definable number of monomers rather than several different numbers of monomers (*i.e.*, an oligomer composition having three or more different oligomer sizes). In certain embodiments, a monodisperse oligomer composition possesses a MW/Mn value of 1.0005 or less, and in certain embodiments, a MW/Mn value of 1.0000. By extension, a composition comprised of monodisperse conjugates means that substantially all oligomers of all conjugates in the composition have a single and definable number (as a whole number) of monomers rather than a distribution and would possess a MW/Mn value of 1.0005, and in certain embodiments, a MW/Mn value of 1.0000 if the oligomer were not attached to the residue of the opioid agonist. A composition comprised of monodisperse conjugates can include, however, one or more nonconjugate substances such as solvents, reagents, excipients, and so forth.

[0081] "Bimodal," in reference to an oligomer composition, refers to an oligomer composition wherein substantially all oligomers in the composition have one of two definable and different numbers (as whole numbers) of monomers rather than a distribution, and whose distribution of molecular weights, when plotted as a number fraction versus molecular weight, appears as two separate identifiable peaks. In certain embodiments, for a bimodal oligomer composition as described herein, each peak is generally symmetric about its mean, although the size of the two peaks may differ. Ideally, the polydispersity index of each peak in the bimodal distribution, Mw/Mn, is 1.01 or less, in certain embodiments 1.001 or less, in certain embodiments 1.0005 or less, and in certain embodiments a MW/Mn value of 1.0000. By

extension, a composition comprised of bimodal conjugates means that substantially all oligomers of all conjugates in the composition have one of two definable and different numbers (as whole numbers) of monomers rather than a large distribution and would possess a MW/Mn value of 1.01 or less, in certain embodiments 1.001 or less, in certain embodiments 1.0005 or less, and in certain embodiments a MW/Mn value of 1.0000 if the oligomer were not attached to the residue of the opioid agonist. A composition comprised of bimodal conjugates can include, however, one or more nonconjugate substances such as solvents, reagents, excipients, and so forth.

[0082] A "biological membrane" is any membrane, typically made from specialized cells or tissues, that serves as a barrier to at least some foreign entities or otherwise undesirable materials. As used herein a "biological membrane" includes those membranes that are associated with physiological protective barriers including, for example: the blood-brain barrier (BBB); the blood-cerebrospinal fluid barrier; the blood-placental barrier; the blood-milk barrier; the blood-testes barrier; and mucosal barriers including the vaginal mucosa, urethral mucosa, anal mucosa, buccal mucosa, sublingual mucosa, rectal mucosa, and so forth. In certain contexts the term "biological membrane" does not include those membranes associated with the middle gastro-intestinal tract (*e.g.*, stomach and small intestines) For example, in some instances it may be desirable for a compound of the invention to have a limited ability to cross the blood-brain barrier, yet be desirable that the same compound cross the middle gastro-intestinal tract.

[0083] A "biological membrane crossing rate," as used herein, provides a measure of a compound's ability to cross a biological membrane (such as the membrane associated with the blood-brain barrier). A variety of methods can be used to assess transport of a molecule across any given biological membrane. Methods to assess the biological membrane crossing rate associated with any given biological barrier (*e.g.*, the blood-cerebrospinal fluid barrier, the blood-placental barrier, the blood-milk barrier, the intestinal barrier, and so forth), are known in the art, described herein and/or in the relevant literature, and/or can be determined by one of ordinary skill in the art.

[0084] "Alkyl" refers to a hydrocarbon chain, typically ranging from about 1 to 20 atoms in length. Such hydrocarbon chains are preferably but not necessarily saturated and may be branched or straight chain. In certain embodiments the hydrocarbon chain is a straight chain.

Exemplary alkyl groups include methyl, ethyl, propyl, butyl, pentyl, 2-methylbutyl, 2-ethylpropyl, 3-methylpentyl, and the like. As used herein, "alkyl" includes cycloalkyl when three or more carbon atoms are referenced. An "alkenyl" group is an alkyl of 2 to 20 carbon atoms with at least one carbon-carbon double bond.

[0085] The terms "substituted alkyl" or "substituted C_{q-r} alkyl" where q and r are integers identifying the range of carbon atoms contained in the alkyl group, denotes the above alkyl groups that are substituted by one, two or three halo (*e.g.*, F, Cl, Br, I), trifluoromethyl, hydroxy, C_{1-7} alkyl (*e.g.*, methyl, ethyl, n-propyl, isopropyl, butyl, t-butyl, and so forth), C_{1-7} alkoxy, C_{1-7} acyloxy, C_{3-7} heterocyclic, amino, phenoxy, nitro, carboxy, carboxy, acyl, cyano. The substituted alkyl groups may be substituted once, twice or three times with the same or with different substituents.

[0086] "Lower alkyl" refers to an alkyl group containing from 1 to 6 carbon atoms, and may be straight chain or branched, as exemplified by methyl, ethyl, n-butyl, i-butyl, t-butyl. "Lower alkenyl" refers to a lower alkyl group of 2 to 6 carbon atoms having at least one carbon-carbon double bond.

[0087] "Non-interfering substituents" are those groups that, when present in a molecule, are typically non-reactive with other functional groups contained within the molecule.

[0088] "Alkoxy" refers to an -O-R group, wherein R is alkyl or substituted alkyl, in certain embodiments C_1-C_{20} alkyl (*e.g.*, methoxy, ethoxy, propyloxy, benzyl, etc.), and in certain embodiments C_1-C_7.

[0089] "Pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" refers to component that can be included in the compositions of the invention in order to provide for a composition that has an advantage (*e.g.*, more suited for administration to a patient) over a composition lacking the component and that is recognized as not causing significant adverse toxicological effects to a patient.

[0090] The term "aryl" means an aromatic group having up to 14 carbon atoms. Aryl groups include phenyl, naphthyl, biphenyl, phenanthrenyl, naphthacenyl, and the like. "Substituted phenyl" and "substituted aryl" denote a phenyl group and aryl group, respectively,

substituted with one, two, three, four or five (*e.g.* 1-2, 1-3 or 1-4 substituents) chosen from halo (F, Cl, Br, I), hydroxy, hydroxy, cyano, nitro, alkyl (*e.g.*, C_{1-6} alkyl), alkoxy (*e.g.*, C_{1-6} alkoxy), benzyloxy, carboxy, aryl, and so forth.

[0091] An "aromatic-containing moiety" is a collection of atoms containing at least aryl and optionally one or more atoms. Suitable aromatic-containing moieties are described herein.

[0092] For simplicity, chemical moieties are defined and referred to throughout primarily as univalent chemical moieties (*e.g.*, alkyl, aryl, etc.). Nevertheless, such terms are also used to convey corresponding multivalent moieties under the appropriate structural circumstances clear to those skilled in the art. For example, while an "alkyl" moiety generally refers to a monovalent radical (*e.g.*, CH_3-CH_2-), in certain circumstances a bivalent linking moiety can be "alkyl," in which case those skilled in the art will understand the alkyl to be a divalent radical (*e.g.*, -CH_2-CH_2-), which is equivalent to the term "alkylene." (Similarly, in circumstances in which a divalent moiety is required and is stated as being "aryl," those skilled in the art will understand that the term "aryl" refers to the corresponding divalent moiety, arylene). All atoms are understood to have their normal number of valences for bond formation (*i.e.*, 4 for carbon, 3 for N, 2 for O, and 2, 4, or 6 for S, depending on the oxidation state of the S).

[0093] "Pharmacologically effective amount," "physiologically effective amount," and "therapeutically effective amount" are used interchangeably herein to mean the amount of a water-soluble oligomer-opioid compound conjugate present in a composition that is needed to provide a threshold level of active agent and/or conjugate in the bloodstream or in the target tissue. The precise amount will depend upon numerous factors, *e.g.*, the particular active agent, the components and physical characteristics of the composition, intended patient population, patient considerations, and the like, and can readily be determined by one skilled in the art, based upon the information provided herein and available in the relevant literature.

[0094] A "difunctional" oligomer is an oligomer having two functional groups contained therein, typically at its termini. When the functional groups are the same, the oligomer is said to be homodifunctional. When the functional groups are different, the oligomer is said to be heterobifunctional.

[0095] A basic reactant or an acidic reactant described herein include neutral, charged, and any corresponding salt forms thereof.

[0096] The term "patient," refers to a living organism suffering from or prone to a condition that can be prevented or treated by administration of a conjugate as described herein, typically, but not necessarily, in the form of a water-soluble oligomer-opioid compound conjugate, and includes both humans and animals.

[0097] "Optional" or "optionally" means that the subsequently described circumstance may but need not necessarily occur, so that the description includes instances where the circumstance occurs and instances where it does not.

[0098] Unless the context clearly dictates otherwise, when the term "about" precedes a numerical value, the numerical value is understood to mean the stated numerical value and also ± 10% of the stated numerical value.

[0099] As indicated above, the present disclosure is directed to (among other things) compounds of the formula:

OP-X-POLY

wherein OP is an opioid compound, X is a linker, and POLY is a small water-soluble oligomer. In preparing and characterizing the subject conjugates, the inventors have discovered that derivatization of an opioid compound with a small water-soluble oligomer reduces the speed of delivery of the opioid compound to the brain. Based on the covalent modification of the opioid agonist molecule itself, the conjugates described herein represent an improvement over the anti-abuse opioid agonist formulations of the prior art. That is to say, opioid compounds conjugated with small water-oligomers possess altered pharmacokinetic profiles, but are not subject to the risk of physical tampering that allows for the recovery and abuse of the rapid acting opioid compound associated with certain alternative delivery formulations such as transdermal patches. The opioid compounds provided herein are useful for eliminating the euphoric high associated with administration of opioids while still maintaining an analgesic effect comparable to that of unmodified opioid. The present compounds are also useful in reducing or eliminating CNS-side

effects associated with opioid use, as well as in reducing the associated addiction and/or abuse potential associated therewith.

[00100] Accordingly, OP can be any opioid compound, including any compound interacting with mu (μ), kappa (κ), or delta (δ) opioid receptors, or any combination thereof. In one embodiment, the opioid is selective for the mu (μ) opioid receptor. In another embodiment, the opioid is selective for the kappa (κ) opioid receptor. In a further embodiment, the opioid is selective for the delta (δ) opioid receptor. Opioids suitable for use can be naturally occurring, semi-synthetic or synthetic molecules.

[00101] Opioid compounds that may be used include, but are not limited to, acetorphine, acetyldihydrocodeine, acetyldihydrocodeinone, acetylmorphinone, alfentanil, allylprodine, alphaprodine, anileridine, benzylmorphine, bezitramide, biphalin, buprenorphine, butorphanol, clonitazene, codeine, desomorphine, dextromoramide, dezocine, diampromide, diamorphone, dihydrocodeine, dihydromorphine, dimenoxadol, dimepheptanol, dimethylthiambutene, dioxaphetyl butyrate, dipipanone, dynorphins (including dynorphin A and dynorphin B), endorphins (including beta-endorphin and α/β-neo-endorphin), enkephalins (including Met-enkephalin and Leu-enkephalin), eptazocine, ethoheptazine, ethylmethylthiambutene, ethylmorphine, etonitazene, etorphine, dihydroetorphine, fentanyl and derivatives, heroin, hydrocodone, hydromorphone, hydroxypethidine, isomethadone, ketobemidone, levorphanol, levophenacylmorphan, lofentanil, meperidine, meptazinol, metazocine, methadone, metopon, morphine, myrophine, narceine, nicomorphine, norlevorphanol, normethadone, nalorphine, nalbuphine, normorphine, norpipanone, opium, oxycodone, oxymorphone, papaveretum, pentazocine, phenadoxone, phenomorphan, phenazocine, phenoperidine, piminodine, piritramide, propheptazine, promedol, properidine, propoxyphene, sufentanil, tilidine, and tramadol.

[00102] In certain embodiments, the opioid agonist is selected from the group consisting of hydrocodone, morphine, hydromorphone, oxycodone, codeine, levorphanol, meperidine, methadone, oxymorphone, buprenorphine, fentanyl, dipipanone, heroin, tramadol, nalbuphine, etorphine, dihydroetorphine, butorphanol, and levorphanol.

[00103] In other embodiments, the opioid agonist is selected from the group consisting of fentanyl, hydromorphone, methadone, morphine, codeine, oxycodone, and oxymorphone.

[00104] Any other opioid compound having opioid agonist activity may also be used. Assays for determining whether a given compound (regardless of whether the compound is in conjugated form or not) can act as an agonist on an opioid receptor are described herein and are known in the art.

[00105] In some instances, opioid agonists can be obtained from commercial sources. In addition, opioid agonists can be synthesized using standard techniques of synthetic organic chemistry. Synthetic approaches for preparing opioid agonists are described in the literature and in, for example, U.S. Patent Nos.: 2,628,962, 2,654,756, 2,649,454, and 2,806,033.

[00106] Each of these (and other) opioid agonists can be covalently attached (either directly or through one or more atoms) to a water-soluble oligomer.

[00107] Opioid compounds useful in the invention generally have a molecular weight of less than about 1500 Da (Daltons), and even more typically less than about 1000 Da. Exemplary molecular weights of opioid compounds include molecular weights of: less than about 950 Da; less than about 900 Da; less than about 850 Da; less than about 800 Da; less than about 750 Da; less than about 700 Da; less than about 650 Da; less than about 600 Da; less than about 550 Da; less than about 500 Da; less than about 450 Da; less than about 400 Da; less than about 350 Da; and less than about 300 Da.

[00108] The opioid compounds used in the invention, if chiral, may be in a racemic mixture, or an optically active form, for example, a single optically active enantiomer, or any combination or ratio of enantiomers (*i.e.*, scalemic mixture). In addition, the opioid compound may possess one or more geometric isomers. With respect to geometric isomers, a composition can comprise a single geometric isomer or a mixture of two or more geometric isomers. An opioid compound for use in the present invention can be in its customary active form, or may possess some degree of modification. For example, an opioid compound may have a targeting agent, tag, or transporter attached thereto, prior to or after covalent attachment of a water-soluble oligomer. Alternatively, the opioid compound may possess a lipophilic moiety attached thereto, such as a phospholipid (*e.g.*, distearoylphosphatidylethanolamine or "DSPE,"

dipalmitoylphosphatidylethanolamine or "DPPE," and so forth) or a small fatty acid. In some instances, however, it is preferred that the opioid compound does not include attachment to a lipophilic moiety.

[00109] The opioid agonist for coupling to a water-soluble oligomer possesses a free hydroxyl, carboxyl, carbonyl, thio, amino group, or the like (*i.e.*, "handle") suitable for covalent attachment to the oligomer. In addition, the opioid agonist can be modified by introduction of a reactive group, for example,by conversion of one of its existing functional groups to a functional group suitable for formation of a stable covalent linkage between the oligomer and the opioid compound.

[00110] Accordingly, each oligomer is composed of up to three different monomer types selected from the group consisting of: alkylene oxide, such as ethylene oxide or propylene oxide; olefinic alcohol, such as vinyl alcohol, 1-propenol or 2-propenol; vinyl pyrrolidone; hydroxyalkyl methacrylamide or hydroxyalkyl methacrylate, where in certain embodiments, alkyl is methyl; α-hydroxy acid, such as lactic acid or glycolic acid; phosphazene, oxazoline, amino acids, carbohydrates such as monosaccharides, saccharide or mannitol; and N-acryloylmorpholine. In certain embodiments, monomer types include alkylene oxide, olefinic alcohol, hydroxyalkyl methacrylamide or methacrylate, N-acryloylmorpholine, and α-hydroxy acid. In certain embodiments, each oligomer is, independently, a co-oligomer of two monomer types selected from this group, or, in certain embodiments, is a homo-oligomer of one monomer type selected from this group.

[00111] The two monomer types in a co-oligomer may be of the same monomer type, for example, two alkylene oxides, such as ethylene oxide and propylene oxide. In certain embodiments, the oligomer is a homo-oligomer of ethylene oxide. Usually, although not necessarily, the terminus (or termini) of the oligomer that is not covalently attached to an opioid compound is capped to render it unreactive. Alternatively, the terminus may include a reactive group. When the terminus is a reactive group, the reactive group is either selected such that it is unreactive under the conditions of formation of the final oligomer or during covalent attachment of the oligomer to an opioid compound, or it is protected as necessary. One common end-functional group is hydroxyl or –OH, particularly for oligoethylene oxides.

[00112] The water-soluble oligomer (*e.g.*, "POLY" in the structures provided herein) can have any of a number of different geometries. For example, it can be linear, branched, or forked. Most typically, the water-soluble oligomer is linear or is branched, for example, having one branch point. Although much of the discussion herein is focused upon poly(ethylene oxide) as an illustrative oligomer, the discussion and structures presented herein can be readily extended to encompass any of the water-soluble oligomers described above.

[00113] The molecular weight of the water-soluble oligomer, excluding the linker portion, in certain embodiments is generally relatively low. For example, the molecular weight of the water-soluble oligomer is typically below about 2200 Daltons, and more typically at around 1500 Daltons or below. In certain other embodiments, the molecular weight of the water-soluble oligomer may be below 800 Daltons.

[00114] In certain embodiments, exemplary values of the molecular weight of the water-soluble oligomer include less than or equal to about 500 Daltons, or less than or equal to about 420 Daltons, or less than or equal to about 370 Daltons, or less than or equal to about 370 Daltons, or less than or equal to about 325 Daltons, less than or equal to about 280 Daltons, less than or equal to about 235 Daltons, or less than or equal to about 200 Daltons, less than or equal to about 175 Daltons, or less than or equal to about 150 Daltons, or less than or equal to about 135 Daltons, less than or equal to about 90 Daltons, or less than or equal to about 60 Daltons, or evan less than or equal to about 45 Daltons.

[00115] In certain embodiments, exemplary values of the molecular weight of the water-soluble oligomer, excluding the linker portion, include: below about 1500 Daltons; below about 1450 Daltons; below about 1400 Daltons; below about 1350 Daltons; below about 1300 Daltons; below about 1250 Daltons; below about 1200 Daltons; below about 1150 Daltons; below about 1100 Daltons; below about 1050 Daltons; below about 1000 Daltons; below about 950 Daltons; below about 900 Daltons; below about 850 Daltons; below about 800 Daltons; below about 750 Daltons; below about 700 Daltons; below about 650 Daltons; below about 600 Daltons; below about 550 Daltons; below about 500 Daltons; below about 450 Daltons; below about 400 Daltons; and below about 350 Daltons; but in each case above about 250 Daltons.

[00116] In certain embodiments, rather than being bound to an oligomer, the opioid is covalently attached to a water-soluble polymer, i.e., a moiety having a more than 50 repeating subunits. For instance, the molecular weight of the water-soluble polymer, excluding the linker portion, may be below about 80,000 Daltons; below about 70,000 Daltons; below about 60,000 Daltons; below about 50,000 Daltons; below about 40,000 Daltons; below about 30,000 Daltons; below about 20,000 Daltons; below about 10,000 Daltons; below about 8,000 Daltons; below about 6,000 Daltons; below about 4,000 Daltons; below about 3,000 Daltons; and below about 2,000 Daltons; but in each case above about 250 Daltons.

[00117] In certain embodiments, exemplary ranges of molecular weights of the water-soluble, oligomer (excluding the linker) include: from about 45 to about 225 Daltons; from about 45 to about 175 Daltons; from about 45 to about 135 Daltons; from about 45 to about 90 Daltons; from about 90 to about 225 Daltons; from about 90 to about 175 Daltons; from about 90 to about 135 Daltons; from about 135 to about 225 Daltons; from about 135 to about 175 Daltons; and from about 175 to about 225 Daltons.

[00118] In other alternative embodiments, exemplary ranges of molecular weights of the water-soluble oligomer (excluding the linker) include: from about 250 to about 1500 Daltons; from about 250 to about 1200 Daltons; from about 250 to about 800 Daltons; from about 250 to about 500 Daltons; from about 250 to about 400 Daltons; from about 250 to about 500 Daltons; from about 250 to about 1000 Daltons; and from about 250 to about 500 Daltons.

[00119] In other embodiments related to water-soluble polymer bound opioids, exemplary ranges of molecular weights of the water-soluble polymer (excluding the linker) include: from about 2,000 to about 80,000 Daltons; from about 2,000 to about 70,000 Daltons; from about 2,000 to about 60,000 Daltons; from about 2,000 to about 50,000 Daltons; from about 2,000 to about 40,000 Daltons; from about 2,000 to about 30,000 Daltons; from about 2,000 to about 20,000 Daltons; from about 2,000 to about 10,000 Daltons; from about 2,000 to about 8,000 Daltons; from about 2,000 to about 6,000 Daltons; from about 2,000 to about 4,000 Daltons; from about 2,000 to about 3,000 Daltons; from about 10,000 to about 80,000 Daltons; from about 10,000 to about 60,000 Daltons; from about 10,000 to about 40,000 Daltons; from about

30,000 to about 80,000 Daltons; from about 30,000 to about 60,000 Daltons; from about 40,000 to about 80,000 Daltons; and from about 60,000 to about 80,000 Daltons.

[00120] The number of monomers in the water-soluble oligomer may be between about 1 and about 1825 (inclusive), including all integer values within this range.

[00121] In certain embodiments, the number of monomers in the water-soluble oligomer falls within one or more of the following inclusive ranges: between 1 and 5 (i.e., is selected from 1, 2, 3, 4, and 5); between 1 and 4 (i.e., can be 1, 2, 3, or 4); between 1 and 3 (i.e., selected from 1, 2, or 3); between 1 and 2 (i.e., can be 1 or 2); between 2 and 5 (i.e., can be selected from 2, 3, 4, and 5); between 2 and 4 (i.e., is selected from 2, 3, and 4); between 2 and 3 (i.e., is either 2 or 3); between 3 and 5 (i.e., is either 3, 4 or 5); between 3 and 4 (i.e., is 3 or 4); and between 4 and 5 (i.e., is 4 or 5). In a specific instance, the number of monomers in series in the oligomer (and the corresponding conjugate) is selected from 1, 2, 3, 4, or 5. Thus, for example, when the water-soluble oligomer includes CH_3-$(OCH_2CH_2)_n$-, "n" is an integer that can be 1, 2, 3, 4, or 5.

[00122] In certain embodiments , the number of monomers in the water-soluble oligomer falls within one or more of the following inclusive ranges: between 6 and 30 (i.e., is selected from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30); between 6 and 25 (i.e., is selected from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25); between 6 and 20 (i.e., is selected from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20); between 6 and 15 (is selected from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15); between 6 and 10 (i.e., is selected from 6, 7, 8, 9, and 10); between 10 and 25 (i.e., is selected from 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25); and between 15 and 20 (i.e., is selected from 15, 16, 17, 18, 19, and 20). In certain instances, the number of monomers in series in the oligomer (and the corresponding conjugate) is one of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25. Thus, for example, when the water-soluble oligomer includes CH_3-$(OCH_2CH_2)_n$-, "n" is an integer that can be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25.

[00123] In yet another embodiment, the number of monomers in the water-soluble oligomer falls within the following inclusive range: between 1 and 10, i.e., is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

[00124] In certain other embodiments, the number of monomers in the water-soluble oligomer falls within one or more of the following inclusive ranges: between 35 and 1825; between 100 and 1800; between 200 and 1600; between 400 and 1400; between 600 and 1200; between 800 and 1000; between 35 and 1000; between 35 and 600; between 35 and 400; between 35 and 200; between 35 and 100; between 1000 and 1825; between 1200 and 1825; between 1400 and 1825; and between 1600 and 1825.

[00125] When the water-soluble oligomer has 1, 2, 3, 4, or 5 monomers, these values correspond to a methoxy end-capped oligo(ethylene oxide) having a molecular weight of about 75, 119, 163, 207, and 251 Daltons, respectively. When the oligomer has 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 monomers, these values correspond to a methoxy end-capped oligo(ethylene oxide) having a molecular weight of about 295, 339, 383, 427, 471, 515, 559, 603, 647, and 691 Daltons, respectively.

[00126] When the water-soluble oligomer is attached to the opioid agonist (in contrast to the step-wise addition of one or more monomers to effectively "grow" the oligomer onto the opioid agonist), the composition containing an activated form of the water-soluble oligomer may be monodispersed. In those instances, however, where a bimodal composition is employed, the composition will possess a bimodal distribution centering around any two of the above numbers of monomers. Ideally, the polydispersity index of each peak in the bimodal distribution, Mw/Mn, is 1.01 or less, and in certain embodiments, is 1.001 or less, and in certain embodimentsis 1.0005 or less. In certain embodiments, each peak possesses a MW/Mn value of 1.0000. For instance, a bimodal oligomer may have any one of the following exemplary combinations of monomer subunits: 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, and so forth; 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, and so forth; 3-4, 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, and so forth; 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, and so forth; 5-6, 5-7, 5-8, 5-9, 5-10, and so forth; 6-7, 6-8, 6-9, 6-10, and so forth; 7-8, 7-9, 7-10, and so forth; and 8-9, 8-10, and so forth.

[00127] In some instances, the composition containing an activated form of the water-soluble oligomer will be trimodal or even tetramodal, possessing a range of monomers units as previously described. Oligomer compositions possessing a well-defined mixture of oligomers (*i.e.*, being bimodal, trimodal, tetramodal, and so forth) can be prepared by mixing purified

monodisperse oligomers to obtain a desired profile of oligomers (a mixture of two oligomers differing only in the number of monomers is bimodal; a mixture of three oligomers differing only in the number of monomers is trimodal; a mixture of four oligomers differing only in the number of monomers is tetramodal), or alternatively, can be obtained from column chromatography of a polydisperse oligomer by recovering the "center cut", to obtain a mixture of oligomers in a desired and defined molecular weight range.

[00128] In certain embodimentsthe water-soluble oligomer is obtained from a composition that is unimolecular or monodisperse. That is, the oligomers in the composition possess the same discrete molecular weight value rather than a distribution of molecular weights. Some monodisperse oligomers can be purchased from commercial sources such as those available from Sigma-Aldrich, or alternatively, can be prepared directly from commercially available starting materials such as Sigma-Aldrich. Water-soluble oligomers can be prepared as described in Chen and Baker, *J. Org. Chem.* 6870-6873 (1999), WO 02/098949, and U.S. Patent Application Publication 2005/0136031.

[00129] When present, the spacer moiety (through which the water-soluble oligomer is attached to the opioid agonist) may be a single bond, a single atom, such as an oxygen atom or a sulfur atom, two atoms, or a number of atoms. In particular, "X" may represent a covalent bond between OP and POLY, or alternatively it may represent a chemical moiety not present on OP and/or POLY alone. A spacer moiety is typically but is not necessarily linear in nature. In certain embodiments, the spacer moiety, "X" is hydrolytically stable, and is in certain embodiments also enzymatically stable. In certain embodiments, the spacer moiety, "X" is physiologically cleavable, *i.e.* hydrolytically cleavable or enzymatically degradable. In certain embodiments, the spacer moiety "X" is one having a chain length of less than about 12 atoms, and in certain embodiments less than about 10 atoms, in certain embodimentsless than about 8 atoms and in certain embodimentsless than about 5 atoms, whereby length is meant the number of atoms in a single chain, not counting substituents. For instance, a urea linkage such as this, $R_{oligomer}$-NH-(C=O)-NH-R'_{OP}, is considered to have a chain length of 3 atoms (-$\underline{N}$H-$\underline{C}$(O)-$\underline{N}$H-). In certain embodiments, the spacer moiety linkage does not comprise further spacer groups.

[00130] In some instances, the spacer moiety "X" comprises an ether, amide, urethane, amine, thioether, urea, or a carbon-carbon bond. Functional groups are typically used for forming the linkages. The spacer moiety may also comprise (or be adjacent to or flanked by) spacer groups, as described further below.

[00131] More specifically, in certain embodiments, a spacer moiety, X, may be any of the following: "-" (*i.e.*, a covalent bond, that may be stable or degradable, between the residue of the opioid agonist and the water-soluble oligomer), -O-, -NH-, -S-, -C(O)-, -C(O)O-, -OC(O)-, $-CH_2-C(O)O-$, $-CH_2-OC(O)-$, $-C(O)O-CH_2-$, $-OC(O)-CH_2-$, C(O)-NH, NH-C(O)-NH, O-C(O)-NH, -C(S)-, $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-$, $-O-CH_2-$, $-CH_2-O-$, $-O-CH_2-CH_2-$, $-CH_2-O-CH_2-$, $-CH_2-CH_2-O-$, $-O-CH_2-CH_2-CH_2-$, $-CH_2-O-CH_2-CH_2-$, $-CH_2-CH_2-O-CH_2-$, $-CH_2-CH_2-CH_2-O-$, $-O-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-O-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-O-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-O-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-O-$, $-C(O)-NH-CH_2-$, $-C(O)-NH-CH_2-CH_2-$, $-CH_2-C(O)-NH-CH_2-$, $-CH_2-CH_2-C(O)-NH-$, $-C(O)-NH-CH_2-CH_2-CH_2-$, $-CH_2-C(O)-NH-CH_2-CH_2-$, $-CH_2-CH_2-C(O)-NH-CH_2-$, $-CH_2-CH_2-CH_2-C(O)-NH-$, $-C(O)-NH-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-C(O)-NH-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-C(O)-NH-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-C(O)-NH-CH_2-$, $-CH_2-CH_2-CH_2-C(O)-NH-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-C(O)-NH-$, $-NH-C(O)-CH_2-$, $-CH_2-NH-C(O)-CH_2-$, $-CH_2-CH_2-NH-C(O)-CH_2-$, $-NH-C(O)-CH_2-CH_2-$, $-CH_2-NH-C(O)-CH_2-CH_2$, $-CH_2-CH_2-NH-C(O)-CH_2-CH_2$, $-C(O)-NH-CH_2-$, $-C(O)-NH-CH_2-CH_2-$, $-O-C(O)-NH-CH_2-$, $-O-C(O)-NH-CH_2-CH_2-$, $-NH-CH_2-$, $-NH-CH_2-CH_2-$, $-CH_2-NH-CH_2-$, $-CH_2-CH_2-NH-CH_2-$, $-C(O)-CH_2-$, $-C(O)-CH_2-CH_2-$, $-CH_2-C(O)-CH_2-$, $-CH_2-CH_2-C(O)-CH_2-$, $-CH_2-CH_2-C(O)-CH_2-CH_2-$, $-CH_2-CH_2-C(O)-$, $-CH_2-CH_2-CH_2-C(O)-NH-CH_2-CH_2-NH-$, $-CH_2-CH_2-CH_2-C(O)-NH-CH_2-CH_2-NH-C(O)-$, $-CH_2-CH_2-CH_2-C(O)-NH-CH_2-CH_2-NH-C(O)-CH_2-$, bivalent cycloalkyl group, $-N(R^6)-$, where R^6 is H or an organic radical selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl and substituted aryl. An exemplary linker is oxygen.

[00132] For purposes of the present disclosure, however, a group of atoms is not considered a spacer moiety when it is immediately adjacent to an oligomer segment, and the group of atoms is the same as a monomer of the oligomer such that the group would represent a mere extension of the oligomer chain.

[00133] The linkage "X" between the water-soluble oligomer and the opioid compound is typically formed by reaction of a functional group on a terminus of the oligomer (or one or more monomers when it is desired to "grow" the oligomer onto the opioid agonist) with a corresponding functional group within the opioid agonist. For example, an amino group on an oligomer may be reacted with a carboxylic acid or an activated carboxylic acid derivative on the opioid compound, or vice versa, to produce an amide linkage. Alternatively, reaction of an amine on an oligomer with an activated carbonate (*e.g.* succinimidyl or benzotriazyl carbonate) on the opioid compound, or vice versa, forms a carbamate linkage. Reaction of an amine on an oligomer with an isocyanate (R-N=C=O) on an opioid compound, or vice versa, forms a urea linkage (R-NH-(C=O)-NH-R'). Further, reaction of an alcohol (alkoxide) group on an oligomer with an alkyl halide, or halide group within an opioid compound, or vice versa, forms an ether linkage. In yet another coupling approach, an opioid compound having an aldehyde function is coupled to an oligomer amino group by reductive amination, resulting in formation of a secondary amine linkage between the oligomer and the opioid compound.

[00134] In certain embodiments, the water-soluble oligomer is an oligomer bearing an aldehyde functional group. In this regard, the oligomer will have the following structure: $CH_3O\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}(CH_2)_p\text{-}C(O)H$, wherein (n) is one of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 and (p) is one of 1, 2, 3, 4, 5, 6 and 7. In certain embodiments (n) values include 1, 2, 3, 4, 7, 8, 9, and 10 and (p) values 2, 3 and 4. In addition, the carbon atom alpha to the -C(O)H moiety can optionally be substituted with alkyl.

[00135] Typically, the terminus of the water-soluble oligomer not bearing a functional group is capped to render it unreactive. When the oligomer does include a further functional group at a terminus other than that intended for formation of a conjugate, that group is either selected such that it is unreactive under the conditions of formation of the linkage "X," or it is protected during the formation of the linkage "X." Such exemplary oligomeric termini include hydroxyl, alkoxy, and or a protecting group.

[00136] As stated above, the water-soluble oligomer includes at least one functional group prior to conjugation. The functional group typically comprises an electrophilic or nucleophilic group for covalent attachment to an opioid compound, depending upon the reactive group

contained within or introduced into the opioid compound. Examples of nucleophilic groups that may be present in either the oligomer or the opioid compound include hydroxyl, amine, hydrazine ($-NHNH_2$), hydrazide ($-C(O)NHNH_2$), and thiol. Preferred nucleophiles include amine, hydrazine, hydrazide, and thiol, particularly amine. Most opioid compounds for covalent attachment to an oligomer will possess a free hydroxyl, amino, thio, aldehyde, ketone, or carboxyl group.

[00137] Examples of electrophilic functional groups that may be present in either the oligomer or the opioid compound include carboxylic acid, carboxylic ester, particularly imide esters, orthoester, carbonate, isocyanate, isothiocyanate, aldehyde, ketone, thione, alkenyl, acrylate, methacrylate, acrylamide, sulfone, maleimide, disulfide, iodo, epoxy, sulfonate, thiosulfonate, silane, alkoxysilane, and halosilane. More specific examples of these groups include succinimidyl ester or carbonate, imidazoyl ester or carbonate, benzotriazole ester or carbonate, vinyl sulfone, chloroethylsulfone, vinylpyridine, pyridyl disulfide, iodoacetamide, glyoxal, dione, mesylate, tosylate, and tresylate (2,2,2-trifluoroethanesulfonate).

[00138] Also included are sulfur analogs of several of these groups, such as thione, thione hydrate, thioketal, is 2-thiazolidine thione, etc., as well as hydrates or protected derivatives of any of the above moieties (*e.g.* aldehyde hydrate, hemiacetal, acetal, ketone hydrate, hemiketal, ketal, thioketal, thioacetal).

[00139] An "activated derivative" of a carboxylic acid refers to a carboxylic acid derivative which reacts readily with nucleophiles, generally much more readily than the underivatized carboxylic acid. Activated carboxylic acids include, for example, acid halides (such as acid chlorides), anhydrides, carbonates, and esters. Such esters include imide esters, of the general form $-(CO)O-N[(CO)-]_2$; for example, N-hydroxysuccinimidyl (NHS) esters or N-hydroxyphthalimidyl esters. Also included are imidazolyl esters and benzotriazole esters. Particularly preferred are activated propionic acid or butanoic acid esters, as described in co-owned U.S. Patent No. 5,672,662. These include groups of the form $-(CH_2)_{2-3}C(=O)O-Q$, where Q is selected from N-succinimide, N-sulfosuccinimide, N-phthalimide, N-glutarimide, N-tetrahydrophthalimide, N-norbornene-2,3-dicarboximide, benzotriazole, 7-azabenzotriazole, and imidazole.

[00140] Other electrophilic groups include succinimidyl carbonate, maleimide, benzotriazole carbonate, glycidyl ether, imidazoyl carbonate, p-nitrophenyl carbonate, acrylate, tresylate, aldehyde, and orthopyridyl disulfide.

[00141] These electrophilic groups are subject to reaction with nucleophiles, *e.g.* hydroxy, thio, or amino groups, to produce various bond types. Several of the electrophilic functional groups include electrophilic double bonds to which nucleophilic groups, such as thiols, can be added, to form, for example, thioether bonds. These groups include maleimides, vinyl sulfones, vinyl pyridine, acrylates, methacrylates, and acrylamides. Other groups comprise leaving groups that can be displaced by a nucleophile; these include chloroethyl sulfone, pyridyl disulfides (which include a cleavable S-S bond), iodoacetamide, mesylate, tosylate, thiosulfonate, and tresylate. Epoxides react by ring opening by a nucleophile, to form, for example, an ether or amine bond. Reactions involving complementary reactive groups such as those noted above on the oligomer and the opioid compound are utilized to prepare the conjugates of the invention.

[00142] In certain embodiments of the invention, reactions favor formation of a hydrolytically stable linkage. For example, carboxylic acids and activated derivatives thereof, which include orthoesters, succinimidyl esters, imidazolyl esters, and benzotriazole esters, react with the above types of nucleophiles to form esters, thioesters, and amides, respectively, of which amides are the most hydrolytically stable. Carbonates, including succinimidyl, imidazolyl, and benzotriazole carbonates, react with amino groups to form carbamates. Isocyanates (R-N=C=O) react with hydroxyl or amino groups to form, respectively, carbamate (RNH-C(O)-OR') or urea (RNH-C(O)-NHR') linkages. Aldehydes, ketones, glyoxals, diones and their hydrates or alcohol adducts (*i.e.* aldehyde hydrate, hemiacetal, acetal, ketone hydrate, hemiketal, and ketal) are reacted with amines, followed by reduction of the resulting imine, if desired, to provide an amine linkage (reductive amination).

[00143] In certain embodiments of the invention, reactions avor formation of a physiologically cleavable linkage. The releasable linkages may, but do not necessarily, result in the water-soluble oligomer (and any spacer moiety) detaching from the opioid compound *in vivo* (and in some cases *in vitro*) without leaving any fragment of the water-soluble oligomer (and/or any spacer moiety or linker) attached to the opioid compound. Exemplary releasable linkages

include carbonate, carboxylate ester, phosphate ester, thiolester, anhydrides, acetals, ketals, acyloxyalkyl ether, imines, certain carbamates, and orthoesters. Such linkages can be readily formed by reaction of the opioid compound and/or the polymeric reagent using coupling methods commonly employed in the art. Hydrolyzable linkages are often readily formed by reaction of a suitably activated oligomer with a non-modified functional group contained within the opioid compound.

[00144] In some instances the opioid agonist may not have a functional group suited for conjugation. In this instance, it is possible to modify the "original" opioid agonist so that it does have the desired functional group. For example, if the opioid agonist has an amide group, but an amine group is desired, it is possible to modify the amide group to an amine group by way of a Hofmann rearrangement, Curtius rearrangement (once the amide is converted to an azide) or Lossen rearrangement (once amide is concerted to hydroxamide followed by treatment with tolyene-2-sulfonyl chloride/base).

[00145] It is possible to prepare a conjugate of an opioid agonist bearing a carboxyl group wherein the carboxyl group-bearing opioid agonist is coupled to an amino-terminated oligomeric ethylene glycol, to provide a conjugate having an amide group covalently linking the opioid agonist to the oligomer. This can be performed, for example, by combining the carboxyl group-bearing opioid agonist with the amino-terminated oligomeric ethylene glycol in the presence of a coupling reagent, (such as dicyclohexylcarbodiimide or "DCC") in an anhydrous organic solvent.

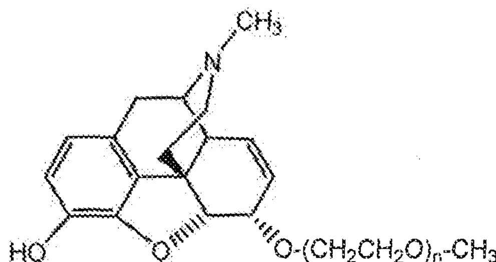
[00146] Further, it is possible to prepare a conjugate of an opioid agonist bearing a hydroxyl group wherein the hydroxyl group-bearing opioid agonist is coupled to an oligomeric ethylene glycol halide to result in an ether (-O-) linked opioid compound conjugate. This can be performed, for example, by using sodium hydride to deprotonate the hydroxyl group followed by reaction with a halide-terminated oligomeric ethylene glycol.

[00147] In another example, it is possible to prepare a conjugate of an opioid agonist bearing a ketone group by first reducing the ketone group to form the corresponding hydroxyl group. Thereafter, the opioid agonist now bearing a hydroxyl group can be coupled as described herein.

[00148] In still another instance, it is possible to prepare a conjugate of an opioid agonist bearing an amine group. In one approach, the amine group-bearing opioid agonist and an aldehyde-bearing oligomer are dissolved in a suitable buffer after which a suitable reducing agent (*e.g.*, $NaCNBH_3$) is added. Following reduction, the result is an amine linkage formed between the amine group of the amine group-containing opioid agonist and the carbonyl carbon of the aldehyde-bearing oligomer.

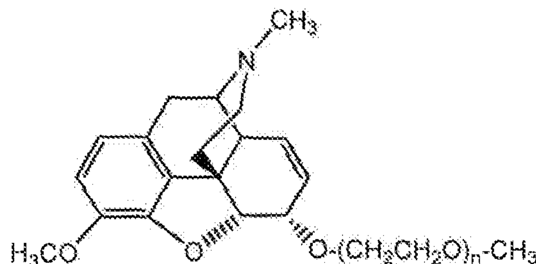
[00149] In another approach for preparing a conjugate of an opioid agonist bearing an amine group, a carboxylic acid-bearing oligomer and the amine group-bearing opioid agonist are combined, typically in the presence of a coupling reagent (*e.g.*, DCC). The result is an amide linkage formed between the amine group of the amine group-containing opioid agonist and the carbonyl of the carboxylic acid-bearing oligomer.

[00150] The synthesis of exemplary opioid compounds (i.e., conjugates) is described in detail Example 10, Example 11, and Example 12. Example 10 describes the synthesis of oligomeric $mPEG_n$-morphine conjugates. Since morphine has two hydroxyl functions, in the synthesis employed, the non-target hydroxyl group (i.e., the aromatic hydroxyl) is first protected with a suitable protecting group such as β-methoxyethoxymethyl ether, MEM, followed by reaction of the MEM-protected morphine with oligomeric PEG-mesylate (PEG_n-OMs) in the presence of the strong base, sodium hydride, to introduce the oligomeric polyethylene glycol moiety. The MEM protecting group is then removed by treatment with acid, e.g., hydrochloric acid, to provide the desired 6-$mPEG_n$-O-morphine conjugates (n=1, 2, 3, 4, 5, 6, 7, 9) having the generalized structure shown below:

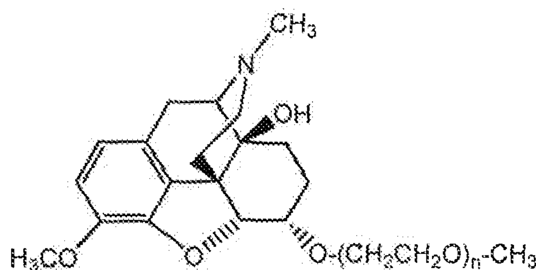


[00151] The synthesis of illustrative oligomeric PEG codeine conjugates is described in detail in Example 11. In the approach employed, codeine, having a single target hydroxyl

function, is reacted with $mPEG_n$ mesylate in the presence of a strong base, e.g., sodium hydride, to provide the desired compound. The products can be purified, for example, using high performance liquid chromatography (HPLC). The oliogomeric $mPEG_n$-O-codeine conjugates (n=1, 2, 3, 4, 5, 6, 7, 9) prepared have the generalized structure shown below:



[00152] In a similar fashion, oligomeric PEG hydroxycodone conjugates were prepared as described in detail in Example 12 (n=1, 2, 3, 4, 5, 6, 7,9). The conjugates possess the following generalized structure:



[00153] Additional compounds may be similarly prepared.

[00154] In certain embodiments of the invention, X is a stable linker. In accordance with the invention, it has been found that certain opioid compounds bound to small water-soluble oligomers via a stable linkage, while retaining the ability to cross the blood-brain barrier, do so at a reduced BBB crossing rate relative to the unconjugated opioid compound. Without wishing to be bound by any particular theory, it is believed that the reduced BBB membrane crossing rate is a direct function of changes in the intrinsic BBB permeability properties of the molecule relative to the unconjugated opioid compound. Again not wishing to be bound by any particular theory, it is presumed that such opioid conjugates possess low addictive properties due to a slow crossing of the BBB, avoiding the rapid peak concentrations associated with unconjugated opioid

agonists and underlying addictive highs. Additionally, the compounds of the present invention may exhibit an improved side effect profile relative to the unconjugated opioid due to an altered tissue distribution of the opioid *in vivo* or decreased activity at peripheral opioid receptors.

[00155] Thus, in accordance with these embodiments of the invention, any combination of opioid compound, linker, and water-soluble oligomer may be used, provided that the conjugate is able to cross the BBB. In certain embodiments, the conjugate crosses the BBB at a reduced rate relative to the unconjugated opioid agonist. In certain embodiments, the water-soluble oligomer is a PEG moiety. Typically, the PEG moiety is a small monomeric PEG consisting of 1-3 (*i.e.* 1, 2, or 3) polyethylene glycol units. In certain embodiments the PEG moiety may be 4 or 5 or 6 polyethylene glycol units.

[00156] With respect to the blood-brain barrier ("BBB"), this barrier restricts the transport of drugs from the blood to the brain. This barrier consists of a continuous layer of unique endothelial cells joined by tight junctions. The cerebral capillaries, which comprise more than 95% of the total surface area of the BBB, represent the principal route for the entry of most solutes and drugs into the central nervous system.

[00157] As will be understood by one of skill in the art, molecular size, lipophilicity, and PgP interaction are among the primary parameters affecting the intrinsic BBB permeability properties of a given molecule. That is to say, these factors, when taken in combination, control whether a given molecule passes through the BBB, and if so, at what rate.

[00158] Due to the small pore size within the BBB, molecular size plays a significant role in determining whether a given molecule will pass through the BBB. Very large molecules, for example a molecule having a molecular weight of 5,000 Daltons, will not cross the BBB, whereas small molecules are more likely to permeate the BBB. Other factors, however, also play a role in BBB crossing. Antipyrine and atenolol are both small molecule drugs; antipyrine readily crosses the BBB, whereas passage of atenolol is very limited, or effectively non-existent. Antipyrine is an industry standard for a high BBB permeation; atenolol is an industry standard for low permeation of the BBB. *See, e.g.*, Summerfield et al., *J Pharmacol Exp Ther* 322:205-213 (2007). Therefore, in accordance with the invention, where X is a stable linker, opioid conjugates having 1-3 polyethylene glycol units can generally be expected to cross the BBB. In

certain circumstances, where the intrinsic BBB permeability properties as a whole are suitable, particular opioid conjugates having 4 or 5 polyethylene glycol units may also cross the BBB.

[00159] Lipophilicity is also a factor in BBB permeation. Lipophilicity may be expressed as logP (partition coefficient) or in some instances logD (distribution coefficient). The logP (or logD) for a given molecule can be readily assessed by one of skill in the art. The value for logP may be a negative number (more hydrophilic molecules) or a positive number (more hydrophobic molecules). As used herein when referring to logP, "more negative" means moving in the direction, on the logP scale, from positive to negative logP (*e.g.*, a logP of 2.0 is "more negative" than a logP of 4.0, a logP of -2.0 is "more negative" than a logP of -1.0). Molecules having a negative logP (hydrophilic molecules) generally do not permeate the BBB. In certain embodiments, the opioid conjugates of the invention have a logP between about 0 and about 4.0. In certain embodiments, the opioid conjugates of the invention have a logP between about 1.0 and about 3.5. In certain embodiments, the conjugates of the invention have a logP of about 4.0, of about 3.5, of about 3.0, of about 2.5, of about 2.0, of about 1.5, of about 1.0, of about 0.5, or of about 0, or they may have a logP in the range of about 0 to about 3.5, of about 0 to about 3.0, of about 0 to about 2.0, of about 0 to about 1.0, of about 1.0 to about 4.0, of about 1.0 to about 3.0, of about 1.0 to about 2.0, of about 2.0 to about 4.0, of about 2.0 to about 3.5, of about 2.0 to about 3.0, of about 3.0 to about 4.0, or of about 3.0 to about 3.5.

[00160] Permeability across the BBB is also dependent on P-glycoprotein, or PgP, an ATP-dependent efflux transporter highly expressed at the BBB. One of skill in the art can readily determine whether a compound is a substrate for PgP using *in vitro* methods. Compounds which are substrates for PgP *in vitro* likely will not permeate the BBB *in vivo*. Conversely, poor substrates for PgP, as assessed *in vitro*, are generally likely to display *in vivo* permeability of the BBB, provided the compound meets other criteria as discussed herein and as known to one of skill in the art. *See, e.g.*, Tsuji, *NeuroRx* 2:54-62 (2005) and Rubin and Staddon, *Annu. Rev. Neurosci.* 22:11-28 (1999).

[00161] In certain embodiments, the water-soluble oligomer may be selected in accordance with the desired pharmacokinetic profile of the opioid conjugate. In other words, conjugation of the opioid compound to a water-soluble oligomer will result in a net reduction in

BBB membrane crossing rate, however the reduction in rate may vary depending on the size of the oligomer used. Generally, where a minimal reduction in BBB crossing rate is desired, a smaller oligomer may be used; where a more extensive reduction in BBB crossing rate is desired, a larger oligomer may be used. In certain embodiments, a combination of two or more different opioid conjugates may be administered simultaneously, wherein each conjugate has a differently sized water-soluble oligomer portion, and wherein the rate of BBB crossing for each conjugate is different due to the different oligomer sizes. In this manner, the rate and duration of BBB crossing of the opioid compound can be specifically controlled through the simultaneous administration of multiple conjugates with varying pharmacokinetic profiles.

[00162] For compounds whose degree of blood-brain barrier crossing ability is not readily known, such ability can be determined using a suitable animal model such as an *in situ* rat brain perfusion ("RBP") model. Briefly, the RBP technique involves cannulation of the carotid artery followed by perfusion with a compound solution under controlled conditions, followed by a wash out phase to remove compound remaining in the vascular space. (Such analyses can be conducted, for example, by contract research organizations such as Absorption Systems, Exton, PA). More specifically, in the RBP model, a cannula is placed in the left carotid artery and the side branches are tied off. A physiologic buffer containing the analyte (typically but not necessarily at a 5 micromolar concentration level) is perfused at a flow rate of about 10 mL/minute in a single pass perfusion experiment. After 30 seconds, the perfusion is stopped and the brain vascular contents are washed out with compound-free buffer for an additional 30 seconds. The brain tissue is then removed and analyzed for compound concentrations via liquid chromatograph with tandem mass spectrometry detection (LC/MS/MS). Alternatively, blood-brain barrier permeability can be estimated based upon a calculation of the compound's molecular polar surface area ("PSA"), which is defined as the sum of surface contributions of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. The PSA has been shown to correlate with compound transport properties such as blood-brain barrier transport. Methods for determining a compound's PSA can be found, *e.g.*, in, Ertl, P., *et al.*, *J. Med. Chem.* 2000, **43**, 3714-3717; and Kelder, J., *et al.*, *Pharm. Res.* 1999, **16**, 1514-1519.

[00163] In certain embodiments, where X is a stable linker, the molecular weight of the opioid conjugate is less than 2000 Daltons, and in certain embodiments less than 1000 Daltons.

In certain embodiments, the molecular weight of the conjugate is less than 950 Daltons, less than 900 Daltons, less than 850 Daltons, less than 800 Daltons, less than 750 Daltons, less than 700 Daltons, less than 650 Daltons, less than 600 Daltons, less than 550 Daltons, less than 500 Daltons, less than 450 Daltons, or less than 400 Daltons.

[00164] In certain embodiments, where X is a stable linker, the molecular weight of X-POLY (*i.e.* the water soluble oligomer in combination with the linker, where present) isless than 2000 Daltons. In certain embodiments, the molecular weight of the X-POLY is less than 1000 Daltons. In certain embodiments, the molecular weight of X-POLY is less than 950 Daltons, less than 900 Daltons, less than 850 Daltons, less than 800 Daltons, less than 750 Daltons, less than 700 Daltons, less than 650 Daltons, less than 600 Daltons, less than 550 Daltons, less than 500 Daltons, less than 450 Daltons, less than 400 Daltons, less than 350 Daltons, less than 300 Daltons, less than 250 Daltons, less than 200 Daltons, less than 150 Daltons, less than 100 Daltons, or less than 50 Daltons.

[00165] In cetain embodiments, where X is a stable linker, the conjugate (*i.e.* OP-X-POLY) is less hydrophobic than the unconjugated opioid compound (*i.e.* OP). In other words, the logP of the conjugate is more negative than the logP of the unconjugated opioid compound. In certain embodiments, the logP of the conjugate is about 0.5 units more negative than that of the unconjugated opioid compound. In certain embodiments, the log P of the conjugate is about 4.0 units more negative, about 3.5 units more negative, about 3.0 units more negative, about 2.5 units more negative, about 2.0 units more negative, about 1.5 units more negative, about 1.0 units more negative, about 0.9 units more negative, about 0.8 units more negative, about 0.7 units more negative, about 0.6 units more negative, about 0.4 units more negative, about 0.3 units more negative, about 0.2 units more negative or about 0.1 units more negative than the unconjugated opioid compound. In certain embodiments, the logP of the conjugate is about 0.1 units to about 4.0 units more negative, about 0.1 units to about 3.5 units more negative, about 0.1 units to about 3.0 units more negative, about 0.1 units to about 2.5 units more negative, about 0.1 units to about 2.0 units more negative, about 0.1 units to about 1.5 units more negative, about 0.1 units to about 1.0 units more negative, about 0.1 units to about 0.5 units more negative, about 0.5 units to about 4.0 units more negative, about 0.5 units to about 3.5 units more negative, about 0.5 units to about 3.0 units more negative, about 0.5 units to about 2.5 units more negative, about 0.5

units to about 2.0 units more negative, about 0.5 units to about 1.5 units more negative, about 0.5 units to about 1.0 units more negative, about 1.0 units to about 4.0 units more negative, about 1.0 units to about 3.5 units more negative, about 1.0 units to about 3.0 units more negative, about 1.0 units to about 2.5 units more negative, about 1.0 units to about 2.0 units more negative, about 1.0 units to about 1.5 units more negative, about 1.5 units to about 4.0 units more negative, about 1.5 units to about 3.5 units more negative, about 1.5 units to about 3.0 units more negative, about 1.5 units to about 2.5 units more negative, about 1.5 units to about 2.0 units more negative, about 2.0 units to about 4.0 units more negative, about 2.0 units to about 3.5 units more negative, about 2.0 units to about 3.0 units more negative, about 2.0 units to about 2.5 units more negative, about 2.5 units to about 4.0 units more negative, about 2.5 units to about 3.5 units more negative, about 2.5 units to about 3.0 units more negative, about 3.0 units to about 4.0 units more negative, about 3.0 units to about 3.5 units more negative, or about 3.5 units to about 4.0 units more negative than the unconjugated opioid compound. In some mbodiments, the logP of the conjugate is the same as, or is more positive than, the logP of the unconjugated opioid compound.

[00166] Example 3 provided herein describes an *in situ* rat brain perfusion study in which the relative permeability of illustrative opioid compounds across a model of the blood-brain barrier is examined. Results are shown in Figs. 18A-C and Fig. 19. As shown therein, a size dependent decrease in the rate of brain entry was observed for oligomeric PEG conjugates. For instance, the rates of brain entry of PEG-7-codeine and PEG-7-oxycodone were less than one percent of their respective parent compounds. Example 21 provided herein describes the results of a study to assess the brain:plasma ratios in rats following intravenous administration of oligomeric PEG-opioid compounds. Figs. 14 A, 16B, and 16C show the brain:plasma ratios of various oligomeric $mPEG_n$-O-morphine, $mPEG_n$-O-codeine, and $mPEG_n$-O-hydroxycodone conjugates, respectively. With the exception of $mPEG_1$-O-morphine, conjugation of oligomeric PEG results in a decrease in the brain:plasma ratio of all conjugates in comparison to their respective unconjugated parent opioid molecule. Example 22 provides the concentrations of various oligomeric PEG-opioid conjugates in the brain and plasma following intravenous administration in rats. Results are provided in Figs. 15A-H (morphine-based compounds), Figs. 16A-H (codeine series) and Figs. 17A-H (oxycodone/hydroxycodone series). The data appear to demonstrate that a maximal increase in brain concentrations for both the parent and oligomeric

conjugates occurs at the earliest time point following administration, e.g., ten minutes. Conjugation of oligomeric PEG appears to result in a significant reduction in brain concentrations; with the larger PEG oligomeric conjugates, e.g., with n greater than or equal to 4, the brain concentrations appear to remain relatively low and steady over time.

[00167] In certain embodiments, where X is a stable linker, the conjugate of the invention retains a suitable affinity for its target receptor(s), and by extension a suitable concentration and potency within the brain. In certain embodiments the water-soluble oligomer is conjugated to the opioid in a manner such that the conjugated opioid binds, at least in part, to the same receptor(s) to which the unconjugated opioid compound binds. To determine whether the opioid agonist or the conjugate of an opioid agonist and a water-soluble oligomer has activity as mu, kappa, or delta opioid receptor agonist, for example, it is possible to test such a compound. For example, a radioligand binding assay in CHO cells that heterologously express the recombinant human mu, kappa, or delta opioid receptor can be used. Briefly, cells are plated in 24 well plates and washed with assay buffer. Competition binding assays are conducted on adherent whole cells incubated with increasing concentrations of opioid conjugates in the presence of an appropriate concentration of radioligand. [^{3}H]naloxone, [^{3}H]diprenorphine and [^{3}H]DPDPE are used as the competing radioligands for mu, kappa and delta receptors respectively. Following incubation, cells are washed, solubilized with NaOH and bound radioactivity is measured using a scintillation counter.

[00168] In certain embodiments, the Ki values of the conjugates of the invention fall within the range of 0.1 to 900 nM, in certain embodiments within the range of 0.1 and 300 nM, and in certain embodimentswithin the range of 0.1 and 50 nM. In certain embodiments, where X is a stable linker, there is no loss of affinity of the conjugated opioid compound (*i.e.* the OP of OP-X-POLY) relative to the affinity of OP to its target receptor(s), and in certain embodiments the affinity of the conjugated opioid compound may be greater than the affinity of OP to its target receptor(s). In certain embodiments, where X is a stable linker, the affinity of the conjugated opioid compound (*i.e.* the OP of OP-X-POLY) is reduced minimally relative to the affinity of OP to its target receptor(s), and in some cases may even show an increase in affinity or no change in affinity. In certain embodiments, there is less than about a 2-fold loss of affinity of the conjugated opioid compound relative to the affinity of the unconjugated opioid compound

for its target receptor(s). In certain embodiments, there is less than about a 5-fold loss, less than about a 10-fold loss, less than about a 20-fold loss, less than about a 30-fold loss, less than about a 40-fold loss, less than about a 50-fold loss, less than about a 60-fold loss, less than about a 70-fold loss, less than about an 80-fold loss, less than about a 90-fold loss, or less than about a 100-fold loss of affinity of the conjugated opioid compound relative to the affinity of the unconjugated opioid compound for its target receptor(s)

[00169] In certain other embodiments where X is a stable linker, the reduction in affinity of the conjugated opioid compound relative to the affinity of the unconjugated opioid compound for its target receptor(s) is less than 20%. In certain embodiments, the reduction in affinity of the conjugated opioid compound relative to the unconjugated opioid compound is less than 10%, less than 30%, less than 40%, less than 50%, less than 60%, less than 70%, less than 80%, less than 90%, or less than 95%.

[00170] Example 19 describes in-vitro studies in which the binding affinities of exemplary oligomeric PEG-opioid conjugates were measured. The binding affinities were measured in vitro in membrane preparations prepared from CHO cells that heterologously express the cloned human mu, kappa, or delta opioid receptors. The conjugates evaluated each displayed measurable binding to the mu-opioid receptor, consistent with the pharmacology of the unmodified parent molecules. Binding affinities are provided in Table 11. The illustrative compounds act as mu-selective agonists when tested in binding and functional studies at human recombinant receptors heterologously expressed in CHO cells

[00171] Example 20 describes a study to examine the in-vitro efficacy of exemplary oligomeric PEG-opioid conjugates by exploring their ability to inhibit cAMP formation following receptor activation. The overall results of the receptor binding and functional activity indicate that the PEG-opioids are mu agonists in vitro.

[00172] In certain embodiments where X is a stable linker, the rate of crossing the BBB, or the permeability of the conjugate is less than the rate of crossing of OP alone. In certain embodiments, the rate of crossing is at least about 50% less than the rate of OP alone. In certain embodiments, there is at least about a 10% reduction, at least about a 15% reduction, at least about a 20% reduction, at least about a 25% reduction, at least about a 30% reduction, at least

about a 35% reduction, at least about a 40% reduction, at least about a 45% reduction, at least about a 55% reduction, at least about a 60% reduction, at least about a 65% reduction, at least about a 70% reduction, at least about a 75% reduction, at least about an 80% reduction, at least about an 85% reduction, at least about a 90% reduction at least about a 95% reduction, or at least about a 99% reduction in the BBB crossing rate of the conjugate relative to the rate of crossing of OP alone. In other embodiments, the conjugates of the invention may exhibit a 10-99% reduction, a 10-50% reduction, a 50-99% reduction, a 50-60% reduction, a 60-70% reduction, a 70-80% reduction, an 80-90% reduction, or a 90-99% reduction in the BBB crossing rate of the conjugate relative to the rate of crossing of OP alone.

[00173] The conjugates of the invention, where X is a stable linker, may exhibit a 1 to 100 fold reduction in the BBB crossing rate relative to the rate of crossing of the OP alone. In certain embodiments, there may be at least about a 2-fold loss, at least about a 5-fold loss, at least about a 10-fold loss, at least about a 20-fold loss, at least about a 30-fold loss, at least about a 40-fold loss, at least about a 50-fold loss, at least about a 60-fold loss, at least about a 70-fold loss, at least about an 80-fold loss, at least about a 90-fold loss, or at least about a 100-fold loss in the BBB crossing rate of the conjugated opioid compound relative to the BBB crossing rate of the unconjugated opioid compound.

[00174] The rate of BBB crossing of the conjugates of the invention, where X is a stable linker, may also be viewed relative to the BBB crossing rate of antipyrine (high permeation standard) and/or atenolol (low permeation standard). It will be understood by one of skill in the art that implied in any reference to BBB crossing rates of the conjugates of the invention relative to the BBB crossing rate of antipyrine and/or atenolol is that the rates were evaluated in the same assay, under the same conditions. Thus, in certain embodiments the conjugates of the invention may exhibit at least about a 2-fold lower, at least about a 5-fold lower, at least about a 10-fold lower, at least about a 20-fold lower, at least about a 30-fold lower, at least about a 40-fold lower, at least about a 50-fold lower, at least about a 60-fold lower, at least about a 70-fold lower, at least about an 80-fold lower, at least about a 90-fold lower, or at least about a 100-fold lower rate of BBB crossing rate relative to the BBB crossing rate of antipyrine. In other embodiments, the conjugates of the invention, the conjugates of the invention may exhibit at least about a 2-fold greater, at least about a 5-fold greater, at least about a 10-fold greater, at least

about a 20-fold greater, at least about a 30-fold greater, at least about a 40-fold greater, at least about a 50-fold greater, at least about a 60-fold greater, at least about a 70-fold greater, at least about an 80-fold greater, at least about a 90-fold greater, or at least about a 100-fold greater rate of BBB crossing rate relative to the BBB crossing rate of atenolol.

[00175] In certain embodiments, where X is a stable linker, the conjugate (*i.e.* OP-X-POLY) may retain all or some of the opioid agonist bioactivity relative to the unconjugated opioid compound (*i.e.* OP). In certain embodiments, the conjugate retains all the opioid agonist bioactivity relative to the unconjugated opioid compounds, or in some circumstances, is even more active than the unconjugated opioid compounds. In certain emodiments, the conjugates of the invention exhibit less than about a 2-fold decrease, less than about a 5-fold decrease, less than about a 10-fold decrease, less than about a 20-fold decrease, less than about a 30-fold decrease, less than about a 40-fold decrease, less than about a 50-fold decrease, less than about a 60-fold decrease, less than about a 70-fold decrease, less than about an 80-fold decrease, less than about a 90-fold decrease, or less than about a 100-fold decrease in bioactivity relative to the unconjugated opioid compounds. In some embodiments, the conjugated opioid compound retains at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the opioid agonist bioactivity relative to the unconjugated opioid compound.

[00176] It will be understood by one of skill in the art that the values recited herein are exemplary and non-limiting, and that certain conjugates of an opioid agonist and a water-soluble oligomer may fall outside the ranges recited herein yet remain within the spirit and scope of the invention. Conjugates may be prepared and tested as a matter of routine experimentation for one of skill in the art. In particular, opioid agonists, bound to a water-soluble oligomer via a stable linkage, may be tested for penetration of the blood brain barrier as described above. Thus one of skill in the art can readily ascertain whether a conjugate is able to cross the BBB.

[00177] While it is believed that the full scope of the conjugates of these embodiments of the invention has been described, an optimally sized oligomer can be determined as follows.

[00178] First, an oligomer obtained from a monodisperse or bimodal water-soluble oligomer is conjugated to the opioid agonist through a stable linkage. Next, *in vitro* retention of activity is analyzed. The ability of the conjugate to cross the blood-brain barrier is then determined using an appropriate model and compared to that of the unmodified parent opioid compound. If the results are favorable, that is to say, if, for example, the rate of crossing is reduced to an appropriate degree, then the bioactivity of conjugate is further evaluated. In certain embodiments, the compounds according to the invention maintain a significant degree of bioactivity relative to the parent opioid compound, *i.e.*, greater than about 30% of the bioactivity of the parent opioid compound, or greater than about 50% of the bioactivity of the parent opioid compound. In certain embodiments, the opioid agonist is orally bioavailable.

[00179] The above steps are repeated one or more times using oligomers of the same monomer type but having a different number of subunits and the results are compared.

[00180] For each conjugate whose ability to cross the blood-brain barrier is appropriately reduced in comparison to the non-conjugated opioid agonist, its oral bioavailability is then assessed. Based upon these results, that is to say, based upon the comparison of conjugates of oligomers of varying size to a given opioid agonist at a given position or location within the opioid agonist, it is possible to determine the size of the oligomer most effective in providing a conjugate having an optimal balance between appropriate reduction in biological membrane crossing, oral bioavailability, and bioactivity. The small size of the oligomers makes such screenings feasible, and allows one to effectively tailor the properties of the resulting conjugate. By making small, incremental changes in oligomer size, and utilizing an experimental design approach, one can effectively identify a conjugate having a favorable balance of reduction in biological membrane crossing rate, bioactivity, and oral bioavailability. In some instances, attachment of an oligomer as described herein is effective to actually increase oral bioavailability of the opioid agonist.

[00181] For example, one of ordinary skill in the art, using routine experimentation, can determine a best suited molecular size and linkage for improving oral bioavailability by first preparing a series of oligomers with different weights and functional groups and then obtaining the necessary clearance profiles by administering the conjugates to a patient and taking periodic

blood and/or urine sampling. Once a series of clearance profiles have been obtained for each tested conjugate, a suitable conjugate can be identified.

[00182] Animal models (rodents and dogs) can also be used to study oral drug transport. In addition, non-*in vivo* methods include rodent everted gut excised tissue and Caco-2 cell monolayer tissue-culture models. These models are useful in predicting oral drug bioavailability.

[00183] In certain embodiments of the invention, X is a physiologically cleavable linker. In accordance with the invention, it has been found that certain opioid compounds bound to small water-soluble oligomers via a cleavable linkage are unable to cross the BBB in their conjugated form, and therefore exhibit a net reduced BBB membrane crossing rate due to slow physiological cleavage of the opioid compound from the water-soluble oligomer. In particular, X may be selected in accordance with the desired pharmacokinetic profile of the unconjugated opioid compound. In other words, conjugation of the opioid compound to a water-soluble oligomer will result in a net reduction in BBB membrane crossing rate, however the reduction in rate may vary depending on the linker used. Where a minimal reduction in BBB crossing rate is desired, X may be a rapidly degraded linker; where an extensive reduction in BBB crossing rate is desired, X may be a more slowly degraded linker. In certain embodiments, a combination of two or more different opioid conjugates may be administered simultaneously, wherein each conjugate has a different linker X, and wherein the rate of degradation of each X is different. In other words, for each different conjugate, the opioid compound will be cleaved from the water-soluble oligomer at a different rate, resulting in different net BBB membrane crossing rates. A similar effect may be achieved through the use of multifunctional water-soluble oligomers having two or more sites of opioid attachment, with each opioid linked to the water-soluble oligomer through linkers having varying rates of degradation. In this manner, the rate and duration of BBB crossing of the opioid compound can be specifically controlled through the simultaneous administration of multiple conjugates with varying pharmacokinetic profiles.

[00184] Not wishing to be bound by any particular theory, it is presumed that such opioid conjugates possess low addictive properties due to the net slow crossing of the BBB (due to slow physiological cleavage following administration of the conjugate), avoiding the rapid peak concentrations associated with unconjugated opioid agonists and underlying addictive highs.

Again, not wishing to be bound by any particular theory, it is believed that the opioid conjugates of the invention circulate in the plasma, and are cleaved *in vivo* at a rate dependant upon the specific cleavable linker used (and, for enzymatically degradable linkers, enzyme concentration and affinity), such that the concentration of unconjugated opioid circulating in the periphery is generally very low due to the slow rate of cleavage. Once cleavage has occurred, the unconjugated opioid may travel to the brain to cross the BBB; the slow release of the unconjugated opioid through cleavage results in a net slow delivery of the unconjugated opioid to the brain. Additionally, the compounds of the present invention exhibit an improved side effect profile relative to the unconjugated opioid dues to an altered tissue distribution of the opioid *in vivo* and altered receptor interaction at the periphery.

[00185] Moreover, in accordance with these embodiments of the invention, any combination of opioid compound, linker, and water-soluble oligomer may be used, provided that the conjugate is not able to cross the BBB or only a small fraction of the conjugate, in certain embodiments less than 5% of that administered, is able to cross the BBB. In certain embodiments, the conjugate is not able to cross the BBB. In certain embodiments, the opioid portion of the molecule, due to physiological cleavage of the conjugate, crosses the BBB at a net reduced rate relative to the unconjugated opioid agonist. In certain embodiments, the water-soluble oligomer is a PEG moiety. In certain embodiments, the PEG moiety is a small monomeric PEG consisting of at least 6 polyethylene glycol units, preferably 6-35 polyethylene glycol units. In certain embodiments, the PEG moiety may be 6-1825 polyethylene glycol units.

[00186] In certain embodiments, where X is a physiologically cleavable linker, the conjugate (*i.e.* OP-X-POLY) may or may not be bioactive. In certain embodiments, the conjugate is not bioactive. Such a conjugate is nevertheless effective when administered *in vivo* to a mammalian subject in need thereof, due to release of the opioid compound from the conjugate subsequent to administration. In certain embodiments, the conjugates of the invention exhibit greater than about a 10-fold decrease, greater than about a 20-fold decrease, greater than about a 30-fold decrease, greater than about a 40-fold decrease, greater than about a 50-fold decrease, greater than about a 60-fold decrease, greater than about a 70-fold decrease, greater than about an 80-fold decrease, greater than about a 90-fold decrease, greater than about a 95-fold decrease, greater than about a 97-fold decrease, or greater than about a 100-fold decrease in

bioactivity relative to the unconjugated opioid compounds. In some embodiments, the conjugated opioid compound retains less than 1%, less than 2%, less than 3%, less than 4%, less than 5%, less than 10%, less than 15%, less than 20%, less than 25%, less than 30%, less than 35%, less than 40%, less than 50%, less than 60%, less than 70%, less than 80% or less than 90% of the opioid agonist bioactivity relative to the unconjugated opioid compound.

[00187] In certain embodiments where X is a physiologically cleavable linker, the affinity of OP-X-POLY for the OP target receptor is substantially reduced relative to the affinity of OP to its target receptor. In certain embodiments, there is at least about a 2-fold loss of affinity of the conjugated opioid compound relative to the affinity of the unconjugated opioid compound for its target receptor(s). In certain embodiments, there is at least about a 5-fold loss, at least about a 10-fold loss, at least about a 20-fold loss, at least about a 30-fold loss, at least about a 40-fold loss, at least about a 50-fold loss, at least about a 60-fold loss, at least about a 70-fold loss, at least about an 80-fold loss, at least about a 90-fold loss, or at least about a 100-fold loss of affinity of the conjugated opioid compound relative to the affinity of the unconjugated opioid compound for its target receptor(s).

[00188] In certain embodiments where X is a physiologically cleavable linker, the reduction in affinity of the conjugated opioid compound relative to the affinity of the unconjugated opioid compound for its target receptor(s) is at least 20%. In certain embodiments, the reduction in affinity of the conjugated opioid compound relative to the unconjugated opioid compound is at least 10%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%.

[00189] As previously noted, in certain embodiments where X is a physiologically cleavable linker, the conjugate is not bioactive. Such a conjugate represents a prodrug, where the compound as administered is inactive, and is made active subsequent to administration through physiological processes. Thus, in certain embodiments, the invention provides a prodrug comprising an opioid agonist reversibly attached via a covalent bond to a releasable moiety, wherein a given molar amount of the prodrug administered to a patient exhibits a rate of accumulation and a C_{max} of the opioid agonist in the central nervous system in the mammal that is less than the rate of accumulation and the C_{max} of an equal molar amount of the opioid agonist had the opioid agonist not been administered as part of a prodrug. The releasable moiety may be

a water-soluble oligomer, and in certain embodiments is a polyethylene glycol oligomer. The agonist may be a mu, kappa, or delta opioid agonist.

[00190] In certain embodiments of the invention, X is a physiologically cleavable linker and POLY is a small monomeric PEG consisting of 1-5 (*i.e.* 1, 2, 3, 4, or 5) polyethylene glycol units, and in certain embodiments, 1-3 (*i.e.* 1, 2, or 3) polyethylene glycol units. Such compounds are small enough to cross the blood-brain barrier, but do so at a reduced membrane crossing rate relative to the unconjugated opioid compound, and as such possess low addictive properties as previously discussed. In certain embodiments, X is selected to provide for cleavage of the linker and release of the opioid compound subsequent to crossing the BBB. Alternatively, cleavage of the linker may happen both prior to, and after, crossing the BBB; in this manner the rate and duration of BBB crossing of the opioid compound can be specifically controlled.

[00191] Under the World Health Organization nomenclature, dependence syndrome (also referred to as withdrawal syndrome) is defined as a state, psychic and sometimes also physical, resulting from the interaction between a living organism and a drug, characterized by behavioral and other responses that always include a compulsion to take the drug on a continuous or periodic basis in order to experience its psychic effects, and sometimes to avoid the discomfort of its absence (WHO Expert Committee on Drug Dependence. 28th Report. Geneva, Switzerland: WHO 1993). The International Classification of Diseases or ICD-10 uses a slightly different standard to assess dependence syndrome (WHO. The ICD-10 Classification of Mental and Behavioral Disorders: Clinical Descriptions and Diagnostic Guidelines. Geneva, Switzerland: WHO, 1992). The ICD-10 uses the term "dependence syndrome" when at least 3 of the 6 features are identified with dependence syndrome. Of the six criteria, four relate to compulsivity: i) a persistent, strong desire to take a drug; ii) difficulty controlling drug use; iii) impairment of function, including neglect of pleasures and interests; and iv) harm to self. The remaining two factors relate to evidence of withdrawal symptoms and tolerance.

[00192] Studies to assess potential opioid compound misuse in humans may be carried out using, for example, one or more screening questionnaires designed to screen for such risk of opioid medication misuse. A number of screening tests have been developed to assess a patients' susceptibility to drug misuse or current misuse, abuse, or addition to opioid drugs. An overview

of such screening tests is provided in *Manchikanti, L., et al., Pain Physician 2008*; Opioids Special Issue: 11:S155-S180. Any one or more of the screening tests described therein may be useful in evaluating a patient's tendency towards or current abuse of opioid drugs in the management and treatment of pain. One particularly useful tool to predict potential substance misuse in pain patients is described in Atluri and Sudarshan (Atluri SL, Sudarshan, G. Pain Physician 2004; 7:333-338). Another example of a useful screening tool is the Pain Medication Questionnaire or PMQ (Adams, L., 27 *et al., J. Pain and Symptom Management,* (5), 440-459 (2004)), among others. Commonly used criteria for evaluation of drug abuse include an evaluation of excessive opioid needs (e.g., multiple dose escalations, multiple emergency room visits, multiple calls to obtain more opiates, and the like), deception or lying to obtain controlled substances, current or prior doctor shopping, etc. Also indicative of a potential for addiction or abuse is the exaggeration of pain by the subject, or an unclear etiology of the pain.

[00193] One biological method for screening or monitoring opioid use is urine analysis. Although opioid testing may be carried out on urine, serum, or for example, hair, urine analysis is typically carried out due to its relatively good specificity, sensitivity, ease of administration, and cost. Such screening can be carried out at the beginning of treatment to establish a baseline, and/or to detect the presence of opioids and/or other drugs, and during the course of treatment to ensure compliance (i.e., to detect the prescribed medication), or misuse (i.e., overuse) of the prescribed medication, and to identify substances that are not to be expected in the urine. Two illustrative urine drug tests that may be used include immunoassay drug testing ("dipstick testing") and laboratory-based specific drug identification using gas chromatography/mass spectrometry and high performance liquid chromatography. Any of a variety of acceptable monitoring methods may be used to assess the potential for abuse/addiction potential of the subject opioid compounds.

[00194] In turning now to the features of the subject opioid compounds, in addition to demonstrating analgesic activity, the compounds provided herein advantageously display very low abuse potential in preclinical studies in monkeys and in rats using self-administration and drug discrimination protocols as described in detail in Examples 7 (monkey) and 24 (rat). Briefly, in the monkey study, squirrel monkeys with indwelling intravenous (IV) catheters were trained in standard lever-press methods using morphine prior to testing with test articles using a

schedule of reinforcement in daily sessions of 90 minutes. Dose-related effects of the test articles were examined, using two or more doses of each drug in 3-4 subjects in a double alternation schedule in which each unit dose (or vehicle) was used in two consecutive sessions before a change in unit dose. In the self-administration studies in monkeys, the illustrative oligomeric PEG opioid compound, mPEG$_6$-O-hydroxycodone, displayed significantly lower potency than oxycodone and morphine, and showed a marked reduction in reinforcing strength at the highest doses tested of 3.2 mg/kg/injection. Specifically, morphine and oxycodone produced 100 % injection lever responses (%ILR) at doses of 0.03 mg/kg/injection and 0.1 mg/kg/injection, respectively. By contrast, the oligomeric mPEG-opioid compound produced exclusive injection lever responding in only two subjects at the highest dose tested, 3.2 mg/kg/injection. The compound produced 22%, 39% and 50% ILR at 0.32, 1.0 and 3.2 mg/kg, respectively.

[00195] In the three-day rat substitution tests, rats trained to self-administer cocaine were exposed to saline or test article via intravenous bolus infusions for one hour sessions on three consecutive days. A compound was considered to exhibit reinforcing properties if animals maintained lever press responding with less than 20% variability over three consecutive sessions. Progressive ratio studies were performed by progressively increasing the number of lever presses needed to result in drug delivery and the break point is defined as the number of lever-presses at which the animal no longer presses in order to achieve the drug reward.

[00196] In self-administration studies in rats, the representative compound, mPEG$_6$-O-hydroxycodone, produced no behavioral evidence of positive reinforcement when tested at doses of up to 3.2 mg/kg/injection, using three-day substitution tests and progressive ratio tests on cocaine-trained animals. The PEG-opioid compound showed no reinforcing properties and behaved like saline in progressive ratio tests in rats. Five out of six tested doses of the compound generated progressive ratio breakpoints lower than that produced by saline. By contrast, the maintenance dose of cocaine (0.56 mg/kg/infusion) produced a breakpoint of 128 responses for the delivery of a single bolus of drug. Likewise, hydrocodone, at a dose of 0.18 mg/kg/infusion, produced a breakpoint of 114, whereas oxycodone at test doses of 0.01 and 0.032 mg/kg/infusion produced mean breakpoints respectively of 56 and 79.

[00197] Thus, the opioid compounds provided herein, in addition to demonstrating antinociceptive properties, demonstrate a marked reduction in self-administration in primates, which is a key indicator of abuse liability for drugs. In one or more of the methods provided herein, an opioid compound is characterized as producing a measurable reduction in addiction potential over the opioid analgesic drug in unconjugated form when valuated in an in-vivo self-administration model in rodents or primates as described in Examples 7 and 24 herein. For example, as a guideline, an opioid compound when evaluated in a self administration model in primates such as monkeys, will display a reduction in reinforcing strength at a particular dose (mg/kg/injection or unit dose) of at least 25% over the unmodified parent compound. For example, if a parent opioid produces 100% injection lever responses (ILR) at a given dose, then the corresponding oligomeric PEG-opioid, if considered to demonstrate a reduction in abuse or addiction potential, will produce 75% ILR or less when evaluated in the same model at an equivalent dose. Similarly, when evaluated in a rat substitution test as described herein, an oligomeric PEG opioid compound is considered to demonstrate reduced addiction/abuse potential if, at an equivalent dose, the compound generates a mean breakpoint that is at least 25% lower in value than the mean breakpoint of the opioid compound itself. In certain embodiments, the oligomeric PEG-opioid compound shows no or minimal reinforcing properties when studied in rats.

[00198] The instant compounds, in addition to possessing analgesic properties (see, e.g., Examples 13, 14, and 23), and having the ability to reduce addiction/abuse potential associated with administration of opioids (see the foregoing section), have been discovered to also reduce one or more CNS side-effects typically associated with administration of opioid drugs. Thus, provided herein is a method for reducing one or more CNS-side effects related to the administration of an opioid analgesic drug by administering an opioid compound as provided herein. Also provided herein is a method for reducing the addiction potential and simultanetously reducing one or more CNS-side effects related to administration of an opioid analgesis drug by administering to a subject suffering from pain a therapeutically effective amount of an opioid compound as provided herein.

[00199] In one or more embodiments of the method(s), an opioid compound as provided herein is considered to be effective in reducing one or more CNS-related side effects related to

administration of the opioid analgesic drug if the opioid compound exhibits a ten-fold or greater reduction in at least one CNS-related side effect associated with administration of the opioid analgesic drug in unconjugated form when evaluated in a mouse or other suitable animal model at an equivalent dose, wherein the one or more CNS-related side effects/elicited behaviors is selected from straub tail response, locomotor ataxia, tremor, hyperactivity, hypoactivity, convulsions, hindlimb splay, muscle rigidity, pinna reflex, righting reflex and placing. One particularly useful indicator for CNS activity is the straub-tail response, although any of the other herein described indicators may be used as well. In certain embodiments, compounds will exhibit a 10- to 100-fold decrease in CNS activity for a given behavior, e.g., will exhibit at least a 15-fold, or at least a 20-fold, or at least a 25-fold, or at least a 30-fold, or at least a 40-fold, or at least a 50-fold, or at least a 60-fold, or at least a 70-fold, or at least an 80-fold, or at least a 90-fold, or a 100-fold or greater decrease in CNS activity for one of the indicative behaviors observed. See, e.g., Table 18, which provides a summary of reduction of CNS activity related to a given behavior for the particular oligomeric-PEG opioid compounds investigated. As can be seen from the data presented in Table 18, significant reductions in CNS-related behaviors were observed for each of the oligomeric-PEG opioids.

[00200] As an illustration, Example 25 demonstrates a reduction in CNS-side effects for a representative oligomeric opioid conjugate when compared to its unmodified parent opioid drug and administered in mice. As an example, referring to Table 15 therein, the lowest response at which the illustrative oligomeric mPEG-opioid compound caused a detectable response in the straub test was the highest dose tested. At oral doses up to 100 mg/kg, where maximal analgesia was obtained with oral doses of 14 mg/kg for oxycodone, 20 mg/kg for morphine, and 100 mg/kg for 6-mPEG6-O-hydroxycodone, the straub tail response was observed in 100 percent of mice treated with morphine and oxycodone, but in none of the mice treated with 6-mPEG6-O-hydroxycodone. Thus, the illustrative oligomeric PEG-opioid compound evaluated demonstrates striking advantages in terms of significantly reduced CNS side effects, even when administered at a dose correlated with maximal analgesic effect.

[00201] CNS side effects that may accompany administration of opioids include cognitive failure, organic hallucinations, respiratory depression, sedation, myoclonus (involuntary twitching), and delirium, among others. When assessing one or more of the foregoing side-

effects, the physician should ideally evaluate the patient to exclude other underlying etiologies. As suggested by the preclinical results provided herein, in one aspect, provided herein is a method for reducing one or more CNS side-effects related to administration of an opioid analgesic by administering the opioid in the form of an oligomeric PEG-opioid drug as described herein. In one embodiment of the method, the amount of opioid compound administered results in both an analgesic effect and a reduction of one or more central nervous system side effects associated with administration of the opioid analgesic drug in unconjugated form in a mammalian subject. In one or more related embodiments, the method further comprises monitoring the patient over the course of treatment for the existence and or absence of one or more CNS-side effects associated with administration of the opioid analgesic. In the event the existence of one or more CNS-side effects is observed, the monitoring may further comprise an assessment of the degree of the CNS-side effect. The monitoring may then further comprise a comparison of the degree or magnitude of the reduced CNS-side effect relative to the degree or magnitude of such CNS-side effect associated with the administration of the unmodified opioid compound.

[00202] In preliminary *in vivo* preclinical studies, an illustrative oligomeric PEG-opioid, $mPEG_6$-O-hydroxycodone, was found to produce less sedation and less respiratory depression in rodents in comparison to the unmodified opioid compound, oxycodone, when administered at equianalgesic doses, in further support of the foregoing method and its related features. See Figs. 25 and 26, respectively.

[00203] In further embodiments, the invention provides for compositions comprising the OP-X-POLY compounds disclosed herein and a pharmaceutically acceptable excipient or carrier. Generally, the conjugate itself will be in a solid form (*e.g.*, a precipitate), which can be combined with a suitable pharmaceutical excipient that can be in either solid or liquid form.

[00204] Exemplary excipients include, without limitation, those selected from the group consisting of carbohydrates, inorganic salts, antimicrobial agents, antioxidants, surfactants, buffers, acids, bases, and combinations thereof.

[00205] A carbohydrate such as a sugar, a derivatized sugar such as an alditol, aldonic acid, an esterified sugar, and/or a sugar polymer may be present as an excipient. Specific

carbohydrate excipients include, for example: monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol, sorbitol (glucitol), pyranosyl sorbitol, myoinositol, and the like.

[00206] The excipient can also include an inorganic salt or buffer such as citric acid, sodium chloride, potassium chloride, sodium sulfate, potassium nitrate, sodium phosphate monobasic, sodium phosphate dibasic, and combinations thereof.

[00207] The preparation may also include an antimicrobial agent for preventing or deterring microbial growth. Nonlimiting examples of antimicrobial agents suitable for the present invention include benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate, thimersol, and combinations thereof.

[00208] An antioxidant can be present in the preparation as well. Antioxidants are used to prevent oxidation, thereby preventing the deterioration of the conjugate or other components of the preparation. Suitable antioxidants for use in the present invention include, for example, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, and combinations thereof.

[00209] A surfactant may be present as an excipient. Exemplary surfactants include: polysorbates, such as "Tween 20" and "Tween 80," and pluronics such as F68 and F88 (both of which are available from BASF, Mount Olive, New Jersey); sorbitan esters; lipids, such as phospholipids such as lecithin and other phosphatidylcholines, phosphatidylethanolamines (although preferably not in liposomal form), fatty acids and fatty esters; steroids, such as cholesterol; and chelating agents, such as EDTA, zinc and other such suitable cations.

[00210] Pharmaceutically acceptable acids or bases may be present as an excipient in the preparation. Nonlimiting examples of acids that can be used include those acids selected from the group consisting of hydrochloric acid, acetic acid, phosphoric acid, citric acid, malic acid, lactic acid, formic acid, trichloroacetic acid, nitric acid, perchloric acid, phosphoric acid, sulfuric

acid, fumaric acid, and combinations thereof. Examples of suitable bases include, without limitation, bases selected from the group consisting of sodium hydroxide, sodium acetate, ammonium hydroxide, potassium hydroxide, ammonium acetate, potassium acetate, sodium phosphate, potassium phosphate, sodium citrate, sodium formate, sodium sulfate, potassium sulfate, potassium fumerate, and combinations thereof.

[00211] The amount of the conjugate in the composition will vary depending on a number of factors, but will optimally be a therapeutically effective dose when the composition is stored in a unit dose container. A therapeutically effective dose can be determined experimentally by repeated administration of increasing amounts of the conjugate in order to determine which amount produces a clinically desired endpoint.

[00212] The amount of any individual excipient in the composition will vary depending on the activity of the excipient and particular needs of the composition. Typically, the optimal amount of any individual excipient is determined through routine experimentation, *i.e.*, by preparing compositions containing varying amounts of the excipient (ranging from low to high), examining the stability and other parameters, and then determining the range at which optimal performance is attained with no significant adverse effects.

[00213] Generally, however, the excipient will be present in the composition in an amount of about 1% to about 99% by weight, in certain embodiments from about 5%-98% by weight, in certain embodimentsfrom about 15-95% by weight of the excipient, and in certain embodiments concentrations less than 30% by weight.

[00214] These foregoing pharmaceutical excipients along with other excipients and general teachings regarding pharmaceutical compositions are described in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, NJ (1998), and Kibbe, A.H., Handbook of Pharmaceutical Excipients, 3rd Edition, American Pharmaceutical Association, Washington, D.C., 2000.

[00215] The pharmaceutical compositions can take any number of forms and the invention is not limited in this regard. In certain embodiments, preparations are in a form suitable for oral

administration such as a tablet, caplet, capsule, gel cap, troche, dispersion, suspension, solution, elixir, syrup, lozenge, transdermal patch, spray, suppository, and powder.

[00216] Oral dosage forms are preferred for those conjugates that are orally active, and include tablets, caplets, capsules, gel caps, suspensions, solutions, elixirs, and syrups, and can also comprise a plurality of granules, beads, powders or pellets that are optionally encapsulated. Such dosage forms are prepared using conventional methods known to those in the field of pharmaceutical formulation and described in the pertinent texts.

[00217] Tablets and caplets, for example, can be manufactured using standard tablet processing procedures and equipment. Direct compression and granulation techniques are preferred when preparing tablets or caplets containing the conjugates described herein. In addition to the conjugate, the tablets and caplets will generally contain inactive, pharmaceutically acceptable carrier materials such as binders, lubricants, disintegrants, fillers, stabilizers, surfactants, coloring agents, and the like. Binders are used to impart cohesive qualities to a tablet, and thus ensure that the tablet remains intact. Suitable binder materials include, but are not limited to, starch (including corn starch and pregelatinized starch), gelatin, sugars (including sucrose, glucose, dextrose and lactose), polyethylene glycol, waxes, and natural and synthetic gums, *e.g.*, acacia sodium alginate, polyvinylpyrrolidone, cellulosic polymers (including hydroxypropyl cellulose, hydroxypropyl methylcellulose, methyl cellulose, microcrystalline cellulose, ethyl cellulose, hydroxyethyl cellulose, and the like), and Veegum. Lubricants are used to facilitate tablet manufacture, promoting powder flow and preventing particle capping (*i.e.*, particle breakage) when pressure is relieved. Useful lubricants are magnesium stearate, calcium stearate, and stearic acid. Disintegrants are used to facilitate disintegration of the tablet, and are generally starches, clays, celluloses, algins, gums, or crosslinked polymers. Fillers include, for example, materials such as silicon dioxide, titanium dioxide, alumina, talc, kaolin, powdered cellulose, and microcrystalline cellulose, as well as soluble materials such as mannitol, urea, sucrose, lactose, dextrose, sodium chloride, and sorbitol. Stabilizers, as well known in the art, are used to inhibit or retard drug decomposition reactions that include, by way of example, oxidative reactions.

[00218] Capsules are also preferred oral dosage forms, in which case the conjugate-containing composition can be encapsulated in the form of a liquid or gel (*e.g.*, in the case of a gel cap) or solid (including particulates such as granules, beads, powders or pellets). Suitable capsules include hard and soft capsules, and are generally made of gelatin, starch, or a cellulosic material. Two-piece hard gelatin capsules are preferably sealed, such as with gelatin bands or the like.

[00219] Included are parenteral formulations in the substantially dry form (typically as a lyophilizate or precipitate, which can be in the form of a powder or cake), as well as formulations prepared for injection, which are typically liquid and requires the step of reconstituting the dry form of parenteral formulation. Examples of suitable diluents for reconstituting solid compositions prior to injection include bacteriostatic water for injection, dextrose 5% in water, phosphate-buffered saline, Ringer's solution, saline, sterile water, deionized water, and combinations thereof.

[00220] In some cases, compositions intended for parenteral administration can take the form of nonaqueous solutions, suspensions, or emulsions, each typically being sterile. Examples of nonaqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate.

[00221] The parenteral formulations described herein can also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. The formulations are rendered sterile by incorporation of a sterilizing agent, filtration through a bacteria-retaining filter, irradiation, or heat.

[00222] The conjugate can also be administered through the skin using conventional transdermal patch or other transdermal delivery system, wherein the conjugate is contained within a laminated structure that serves as a drug delivery device to be affixed to the skin. In such a structure, the conjugate is contained in a layer, or "reservoir," underlying an upper backing layer. The laminated structure can contain a single reservoir, or it can contain multiple reservoirs.

[00223] The conjugate can also be formulated into a suppository for rectal administration. With respect to suppositories, the conjugate is mixed with a suppository base material which is

(*e.g.*, an excipient that remains solid at room temperature but softens, melts or dissolves at body temperature) such as coca butter (theobroma oil), polyethylene glycols, glycerinated gelatin, fatty acids, and combinations thereof. Suppositories can be prepared by, for example, performing the following steps (not necessarily in the order presented): melting the suppository base material to form a melt; incorporating the conjugate (either before or after melting of the suppository base material); pouring the melt into a mold; cooling the melt (*e.g.*, placing the melt-containing mold in a room temperature environment) to thereby form suppositories; and removing the suppositories from the mold.

[00224] The invention also provides a method for administering an oligomeric PEG opioid conjugate as provided herein to a patient suffering from a condition that is responsive to treatment with the conjugate such as pain. The method comprises administering, generally orally, a therapeutically effective amount of the conjugate (in certain embodiments provided as part of a pharmaceutical preparation). Other modes of administration are also contemplated, such as pulmonary, nasal, buccal, rectal, sublingual, transdermal, and parenteral. As used herein, the term "parenteral" includes subcutaneous, intravenous, intra-arterial, intraperitoneal, intracardiac, intrathecal, and intramuscular injection, as well as infusion injections.

[00225] In instances where parenteral administration is utilized, it may be necessary to employ somewhat bigger oligomers than those described previously (e.g., polymers), with molecular weights ranging from about 500 to 30 kilodaltons (*e.g.*, having molecular weights of about 500 daltons, 1000 daltons, 2000 daltons, 2500 daltons, 3000 daltons, 5000 daltons, 7500 daltons, 10000 daltons, 15000 daltons, 20000 daltons, 25000 daltons, 30000 daltons or even more).

[00226] The method of administering may be used to treat any condition that can be remedied or prevented by administration of the particular conjugate. Most commonly, the conjugates provided herein are administered for the management of chronic pain. Those of ordinary skill in the art appreciate which conditions a specific conjugate can effectively treat. The actual dose to be administered will vary depend upon the age, weight, and general condition of the subject as well as the severity of the condition being treated, the judgment of the health care professional, and conjugate being administered. Therapeutically effective amounts are

known to those skilled in the art and/or are described in the pertinent reference texts and literature. Generally, a therapeutically effective amount will range from about 0.001 mg to 1000 mg, in certain embodiments in doses from 0.01 mg/day to 750 mg/day, and in certain embodiments in doses from 0.10 mg/day to 500 mg/day.

[00227] The unit dosage of any given conjugate (in certain embodimentsprovided as part of a pharmaceutical preparation) can be administered in a variety of dosing schedules depending on the judgment of the clinician, needs of the patient, and so forth. The specific dosing schedule will be known by those of ordinary skill in the art or can be determined experimentally using routine methods. Exemplary dosing schedules include, without limitation, administration five times a day, four times a day, three times a day, twice daily, once daily, three times weekly, twice weekly, once weekly, twice monthly, once monthly, and any combination thereof. Once the clinical endpoint has been achieved, dosing of the composition is halted.

[00228] One advantage of administering the conjugates of the present invention is that a reduction in speed of delivery of the opioid agonist to the brain is achieved, thus avoiding the rapid peak concentrations associated with unconjugated opioid agonists and underlying addictive highs. Moreover, based on the covalent modification of the opioid agonist molecule, the conjugates of the invention are not subject to the risk of physical tampering that allows for the recovery and abuse of the rapid acting opioid compound associated with certain alternative delivery forms intended to provide, *in vivo*, a reduced BBB crossing rate. As such, the compounds of the invention possess low addictive, anti-abuse properties. The desired pharmacokinetic properties of the conjugates may be modulated by selecting the oligomer molecular size, linkage, and position of covalent attachment to the opioid compound. One of ordinary skill in the art can determine the ideal molecular size of the oligomer based upon the teachings herein.

[00229] The compounds provided herein are useful in the treatment of pain. Generally, treatment comprises administering an analgesically effective amount of a compound having a formula $OP\text{-}X\text{-}(CH_2CH_2O)_nY$ as disclosed herein above. Generally, such treatment is for the management of pain (e.g., acute or chronic pain). The compounds provided herein may, for example, be used to treat visceral pain, musculo-skeletal pain, nerve pain, and/or sympathetic

pain. Representative studies demonstrating the ability of the subject compounds to reduce or prevent pain are provided in at least Examples 13, 14, and 23. Administration of an opioid compound as provided herein may, for example, be used in the treatment of chronic pain ranging from moderate to severe, including neuropathic pain. Neuropathic pain is pain due to nerve injury, neurologic disease, or the involvement of nerves due to other disease processes. The oligomeric PEG opioids described herein may be used in the treatment of pain associated with any of a number of conditions such as cancer, fibromyalgia, lower back pain, neck pain, sciatica, osteoarthritis, and the like. The compounds may also be used for relieving breakthrough pain.

[00230] In another aspect, provided is a method of reducing the abuse potential of an opioid compound comprising conjugating the compound to a small water-soluble oligomer. In certain embodiments, the conjugate is of the formula $OP\text{-}X\text{-}(CH_2CH_2O)_nY$ as described herein.

[00231] In a further embodiment, provided is a method of reducing the addictive properties of an opioid agonist comprising conjugating the opioid agonist to a small water-soluble oligomer. In certain embodiments, the conjugate is of the formula $OP\text{-}X\text{-}(CH_2CH_2O)_nY$ as described herein.

[00232] In another embodiment, provided is a method of reducing, but not substantially eliminating, the rate of crossing the blood brain barrier of an opioid compound comprising conjugating the compound to a small water-soluble oligomer to provide a compound as provided herein.

[00233] The compounds described herein may be used for reducing the addiction potential and reducing one or more central nervous system (CNS) side-effects related to administration of an opioid analgesic drug (OP). In practicing the method, a therapeutically effective amount of an opioid compound having the formula: $OP\text{---}X\text{---}(CH_2CH_2O)_nY$, or a pharmaceutically acceptable salt form thereof, is administered to a mammalian subject suffering from pain wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group, whereby as a result of the administering, a degree of pain relief is experienced by the subject, and when evaluated in a suitable animal model, the opioid compound exhibits (i) a measurable reduction in addiction potential over the opioid analgesic drug in unconjugated

form, and (ii) a ten-fold or greater reduction of at least one CNS-related side effect when compared to administration of the opioid analgesic drug in unconjugated form.

[00234] In yet another use, provided is a method for reducing one or more central-nervous system side-effects related to administration of an opioid analgesic drug (OP) by administering the opioid analgesic drug to a mammalian subject in the following form:

$OP—X—(CH_2CH_2O)_nY$, wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group.

[00235] In yet one or more additional embodiments, the method and/or use of an opioid compound as provided herein is effective to reduce one or more central nervous system side-effects associated with administration of the opioid analgesic drug in unconjugated form in a mammalian subject selected from respiratory depression, sedation, myoclonus, and delirium.

[00236] It is to be understood that while the invention has been described in conjunction with certain nd specific embodiments, the foregoing description as well as the examples that follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

EXAMPLES

[00237] All chemical reagents referred to in the appended examples are commercially available unless otherwise indicated. .The preparation of PEG-mers is described in, for example, U.S. Patent Application Publication No. 2005/0136031.

Example 1
Determination of logP Values

[00238] Log P and Log D provide measures of the lipophilicity of a compound, such that a higher or more positive value represents a more hydrophobic compound whereas a lower or more negative value represents a more hydrophilic compound. LogP (Octanol: isopronalol/water partition coefficient) of test compounds is measured using a potentiometric titration method with

a Sirius GLpKa instrument (Sirius Analytical Instruments, Ltd, East Sussex, UK). A 50 μL aliquot of the 0.1 M test compound solution in DMSO is placed into a titration vial. Assays are conducted at 25°C. A measured volume of octanol is added to the sample automatically, after which the instrument adds a measured volume of isopropanol water. The pH of the solution is adjusted to 2 by adding 0.5 M HCl automatically. A titration with 0.5 M KOH is performed automatically until a pH value of 12 is reached. To perform the second and third titrations, an additional octanol volume is delivered automatically to the titration vial. The data sets for the three titrations are combined in RefinementPro to create a Multiset. The Log (D), at various pH values, is automatically calculated by the software.

[00239] Log P and Log D values are used in predicting or evaluating the properties of a molecule that relate to its lipophilicity such as the ability to traverse membranes.

Example 2
Transport assays for PgP

[00240] P-glycoprotein, PgP, is an efflux transporter expressed in various cells in the body, and highly expressed at the blood-brain barrier. Molecules that are substrates for PgP show poor penetration into, or efflux from, tissues where the PgP is expressed.

[00241] The contribution of PgP to net transport is measured in MDCKII cells that over-express PgP (MDR-MDCKII). For transport studies, MDR-MDCKII and MDCKII cells are grown on permeable inserts (3-4 days) until a tight monolayer is formed, as measured by transepithelial measurements. Test compounds in Krebs buffer are added at 10μM to the apical or basolateral sides of the MDCKII cells and allowed to incubate at 37°C. The transport of compounds is measured in two directions: Apical-basolateral (A-B) and basolateral-apical (B-A) in both parent and MDR overexpressing cells. At times 0, 30, 60, 90, 120 and 180 minutes, aliquots are withdrawn from the apical and basolateral compartments. Samples are analyzed for test compounds by LC-MS/MS. The flux is calculated as the slope of the linear portion of the cumulative concentration versus time plot. The apparent permeability is calculated as Papp = Flux/C_0.A, where C_0 is the initial concentration of test compound (10 μM) used and A is the surface area of the insert. The efflux ratio is calculated as the ratio of Papp(B-A)/Papp(A-B).

Involvement of a PgP mediated efflux mechanism is indicated when the ratio of the efflux in MDCKII –MDRI cells to that in parent MDCKII cells is greater than 2, *i.e.* Efflux ratio(MDCKII-MDRI)/Efflux ratio (MDCKII cells) $\geq$ 2.

[00242] PgP interaction data are used in predicting or evaluating the properties of a molecule that relate to its PgP status such as the ability to traverse membranes or enter compartments such as the CNS where PgP is highly expressed.

Example 3
***In situ* Rat Brain Perfusion**

[00243] The *in situ* perfusion experiment measures the relative permeability of compounds across a model of the blood-brain barrier. *In situ* perfusion of opioids into rat brain was performed as described in Summerfield et al., *J Pharmacol Exp Ther* 322: 205-213 (2007).

[00244] Adult male Sprague Dawley rats were used for the study. Rats were anaesthetized and the left common carotid artery was surgically cannulated for perfusion. Test compounds were perfused at concentrations of 5-50 μM in a Krebs Ringer perfusion buffer (pH 7.4). Atenolol and antipyrine were included as low and moderate permeability markers, respectively. At the end of a 30 second perfusion, the brains were removed, the left brain hemisphere was excised and homogenized. Test compounds were extracted and analyzed using LC-MS/MS. The brain permeability of the test compounds is calculated as follows:

$$P = Kin/S,$$

where P is the permeability in cm/s, Kin is the unidirectional transfer constant (ml/min/gram), and S is the luminal area of the brain vascular space.

[00245] The relative permeability as determined in the in situ brain perfusion experiment provides information regarding the rates at which compounds enter the central nervous system from the periphery. It is used to characterize and compare the degree to which conjugation with a water-soluble oligomer slows penetration of the BBB for a given opioid compound.

[00246] The brain penetration potential of morphine, codeine, oxydone and their respective PEG conjugates were evaluated in male Sprague-Dawley rats using an *in-situ* brain

perfusion model. Synthesis of the conjugates is described in Examples 10, 11 and 12. Rats were anesthetized and a cannula was implanted into the left carotid artery. Branch arteries were tied, and the cardiac supply was cut off prior to brain perfusion. Perfusion was performed using the single time-point method. Each animal was co-perfused with a test compound (10 μM) and control compounds (5 μM antipyrine and 50 μM atenolol). The compounds in Kreb's Ringer buffer were infused into the animals via the left external carotid artery for 30 seconds by an infusion pump. Following 30 seconds of perfusion, the pump was stopped, and the brain was immediately removed from the skull. The brain was cut longitudinally in half. Each left hemisphere was placed into a chilled tube, frozen on dry ice, and stored frozen at -60°C to -80°C until analyzed.

[00247] For bioanalysis, each left brain hemisphere was thawed, weighed and homogenized by sonication in 20% methanol. Concentrations of test and control compounds were determined by LC-MS/MS analyses using pre-validated analytical methods.

[00248] Results for the brain penetration of the test and control compounds are presented as the unidirectional brain transfer constants Kin (mL/g/min) using the following equation for the single-point perfusion assay:

Kin = [Cbr/Cpf]/t , where:

Cbr/Cpf is the apparent brain distribution volume (mL/g of brain tissue).

Cbr is the concentration of drug in the brain tissue (pmol of drug per g of brain tissue).

Cpf is the drug concentration in the perfusion fluid (pmol/mL of perfusate).

t is the net perfusion time (minutes).

[00249] To exclude the drug contained in the capillary space from the brain concentration values, the apparent brain distribution volume of atenolol was subtracted from the drug values in each animal. If the concentration of the test compound was a negative value after correcting for the brain distribution volume of atenolol, the Kin value is reported as zero.

[00250] Following perfusion, the vascular space marked by atenolol, a compound that does not penetrate the brain, did not exceed 20 μL/g of brain tissue. These results indicate preserved blood-brain barrier properties during perfusion. The Kin values for morphine, codeine and oxycodone are shown in FIGS. 18A-C and FIG. 19. The Kin values for parent morphine,

codeine and oxycodone compounds were approximately 14%, 40% and 60% of Kin values of antipyrine, the positive control that possess high brain penetration potential. PEG conjugation resulted in a further size-dependent decrease in the rate of brain entry of codeine and oxycodone conjugates. The rates of brain entry of PEG-7 codeine and PEG-7-oxycodone were <1% of their respective parent compounds. However, the Kin values of PEG-1, PEG-2 morphine were greater than parent morphine, and equivalent to parent in the case of PEG-3-morphine. The Kin value of PEG-7-morphine was significantly lower (<4%) than that of parent morphine.

Example 4
Opioid Receptor Binding Assay in Whole Cells

[00251] Receptor binding affinity is used as a measure of the intrinsic bioactivity of the compound. The receptor binding affinity of the opioid conjugates (or opioid alone) is measured using a radioligand binding assay in CHO cells that heterologously express the recombinant human mu, delta or kappa opioid receptor. Cells are plated in 24 well plates at a density of 0.2-0.3 x 10^{-6} cells/well and washed with assay buffer containing 50 mM Tris.HCl and 5 mM $MgCl_2$ (pH 7.4). Competition binding assays are conducted on adherent whole cells incubated with increasing concentrations of opioid conjugates in the presence of an appropriate concentration of radioligand. 0.5 nM [^{3}H]naloxone, 0.5 nM [^{3}H]diprenorphine and 0.5 nM [^{3}H]DPDPE are used as the competing radioligands for mu, kappa and delta receptors respectively. Incubations are carried out for 2 hours at room temperature using triplicate wells at each concentration. At the end of the incubation, cells are washed with 50 mM Tris HCl (pH 8.0), solubilized with NaOH and bound radioactivity is measured using a scintillation counter.

[00252] Specific binding is determined by subtraction of the cpm bound in the presence of 50-100X excess of cold ligand. Binding data assays are analyzed using GraphPad Prism 4.0 and IC50 is generated by non-linear regression from dose-response curves. Ki values are calculated using the Cheng Prusoff equation using the Kd values from saturation isotherms as follows: Ki = IC50/(1+ [Ligand]/Kd).

[00253] The Ki value is used as an indicator of the binding affinity of the compound and may be compared to the binding affinity of other opioid agonists. It also is used as a marker for

potency and permits evaluation of the likelihood of a given compound to provide effective analgesia.

Example 5
cAMP Measurements in Whole Cells

[00254] Inhibition of forskolin-stimulated cAMP production is used as a measure of *in vitro* bioactivity of opioids. CHO cells that heterologously express any one of the recombinant human mu, delta or kappa opioid receptor are plated in 24 well plates at 0.2-0.3x10^{-6} cells/well and washed with PBS + 1 mM IBMX (isobutyl methyl xanthine). Cells in triplicate wells are incubated with increasing concentrations of opioid conjugate followed 10 mins later by the addition of 10 μM forskolin. Following incubation with forskolin for 10 minutes, cells are lysed and cAMP in cells is measured using a commercially available competitive immunoassay kit (Catchpoint®- Molecular Devices). The fluorescence signal is calibrated against a standard curve of cAMP and data are expressed as moles of cAMP/10^6 cells. IC50 values are calculated for each opioid conjugate by analysis of the dose-response curve using non-linear regression (Graph Pad Prism), where "dose" is the concentration of the opioid conjugate used.

[00255] The cAMP assay is used to provide a measure of the ability of an opioid compound to induce a functional response upon receptor binding, and provides a further indication of the analgesic potential of the compound. It also enables comparison with other opioids for relative potency.

Example 6
Rat Model of Analgesia

[00256] The hotplate withdraw assay is used as a measure of *in vivo* bioactivity of opioids. This experiment uses a standard hotplate withdrawal assay in which latency of withdrawal from a heat stimulus is measured following administration of a test compound. Compounds are administered to the animal and 30 minutes later, a thermal stimulus is provided to the hindpaw. Latency for hindpaw withdrawal in the presence of morphine is used as the measure of full analgesia, while latency in the presence of saline is used as a negative control for no analgesia. The agonist effect of the test compound is evaluated by measuring time to withdrawal compared with a negative control (saline).

Example 7
Monkey Model of Addictive Potential

[00257] Addictive potential of opioid compounds and opioid conjugates of the invention may be assessed through the use of squirrel monkey models as known in the art. See Bergman et al. (2006) *Mol Interventions* 6:273-283.

[00258] Briefly, a "self-administration model" was used in which monkeys were first trained to understand that the illumination of a color lamp in their environment indicated that each of two response levers (also in their environment) were operational. Further, the monkeys were trained to understand that one lever was associated with the delivery of food to a receptacle accessible by the monkey while the other lever was associated with the delivery of morphine via intravenous injection through a catheter previously inserted in the monkey.

[00259] The monkey is subjected to a double alternation schedule to test different dosages of the drug (morphine or test compound) once the monkey demonstrates sufficient training, i.e., after lever pressing for response-contingent injections of morphine is reliable under a fixed ratio schedule wherein a certain number of lever presses is understood by the monkey to trigger the delivery of the morphine or test compound. Under the double alternation schedule, each unit dose (or vehicle) was available for intravenous self-administration for two consecutive sessions before the unit dose was changed. In this way, it was possible to establish the function relating unit dose of intravenous drug to (i) number of injections per session, and/or (ii) the percentage of

total responses that occur on the lever leading to self-administration (injection-lever responding, %ILR). Unit doses of drugs ranged from 0.01 to 3.2 mg/kg/injection.

[00260] See Bergman et al. (2006) *Mol Interventions* 6:273-283 and Gasior et al. (2005) *Neuropsychopharmacology* 30:758-764 for further description of the principles of the test and general details concerning monkey preparation and test conditions.

[00261] In the experiment, most monkeys substantially ignored drug (both morphine and test compound) and chose food almost exclusively when drug dose was relatively low. However, as the dose of the drug (morphine or test compound) increases, reinforcing behavior was evidenced where the monkey will typically only press drug lever and ignore food lever. A monkey was deemed to exhibit "addictive behavior" when the drug lever was selected over 95% of the time. In the experiment, all animals almost always either picked food more than 95% of the time or drug more than 95% of the time; there were rarely subjects exhibiting behavior other than at these extremes.

[00262] As shown in FIG. 20, oxycodone triggered "addictive behavior" in 25% of test subjects at the 0.003 and 0.01 mg/kg/injection and 100% of subjects at the 0.03 mg/kg/injection doses. In contrast, α-6-mPEG$_6$-O-hydroxycodone (prepared in accordance with Example 12) caused "addictive behavior" in 25% of subjects at a dose of 1mg/kg/injection and in 50% of subjects at a dose of 3 mg/kg/injection. This demonstrates that even at a 100-fold higher dose, α-6-mPEG$_6$-O-hydroxycodone shows less abuse potential (as a result of less reinforcing strength) than oxycodone.

[00263] Similarly, α-6-mPEG$_3$-O-hydroxycodone exhibited "addictive behavior" in 25% of subjects at 0.1 mg/kg/injection, in 50% of subjects at 0.3 mg/kg/injection, and 100% of subjects at 1 mg/kg/injection. This demonstrates that a 33-fold higher dose of α-6-mPEG$_3$-O-hydroxycodone is required for all animals to display addictive behavior and thus this drug, too, has a lower abuse potential than oxycodone.

[00264] As described above, oxycodone demonstrated reinforcing behavior resulting in 100% ILR at a dose of 0.03 mg/kg/injection. In contrast, α-mPEG-6-O-hydroxycodone and α-mPEG-7-O-hydroxycodone produced only food lever responses at this dose. At doses of 1 and 3.2 mg/kg/injection, α-mPEG-6-O-hydroxycodone produced 38 ± 24% and 50 ± 29% ILR. α-

mPEG-7-O-hydroxycodone produced only food lever responses at doses of 3.2 mg/kg/injection which was the maximal dose tested.

[00265] In a similar study, morphine exhibited "addictive behavior" in 100% of subjects at 0.1 mg/kg/injection, whereas at this same dose, α-6-mPEG$_3$-O-morphine (prepared in accordance with Example 10) caused "addictive behavior" in only 33% of subjects. At 0.3 mg/kg/injection of α-6-mPEG$_3$-O-morphine all animals displayed "addictive behavior."

Example 8
In vivo Brain Penetration of PEG-Nalbuphine Conjugates

[00266] The ability of the PEG-nalbuphine conjugates to cross the blood brain barrier (BBB) and enter the CNS was measured using the brain:plasma ratio in rats. Briefly, rats were injected intravenously with 25 mg/kg of nalbuphine, PEG-nalbuphine conjugate or atenolol. An hour following injection, the animals were sacrificed and plasma and the brain were collected and frozen immediately. Following tissue and plasma extractions, concentrations of the compounds in brain and plasma were measured using LC-MS/MS. The brain:plasma ratio was calculated as the ratio of measured concentrations in the brain and plasma. Atenolol, which does not cross the BBB, was used as a measure of vascular contamination of the brain tissue.

[00267] FIG. 1 shows the ratio of brain:plasma concentrations of PEG-nalbuphine conjugates. The brain:plasma ratio of nalbuphine was 2.86:1, indicating a nearly 3 fold greater concentration of nalbuphine in the brain compared to the plasma compartment. PEG conjugation significantly reduced the entry of nalbuphine into the CNS as evidenced by a lower brain:plasma ratio of the PEG-nalbuphine conjugates. Conjugation with 3 PEG units reduced the brain:plasma ratio to 0.23:1, indicating that the concentration of 6-O-mPEG$_3$-Nalbuphine in the brain was approximately 4 fold less than that in the plasma. 6-O-mPEG$_6$-Nalbuphine and 6-O-mPEG$_9$-Nalbuphine (6 PEG units and 9 PEG units, respectively) were significantly excluded from the CNS, since their brain:plasma ratios were not significantly different from the vascular marker, atenolol.

Table 1. Brain:Plasma Ratios

Molecule	Brain: Plasma Ratios
Nalbuphine	2.86
6-O-mPEG$_3$-Nalbuphine	0.23
6-O-mPEG$_6$-Nalbuphine	0.11
6-O-mPEG$_9$-Nalbuphine	0.10
Atenolol	0.11

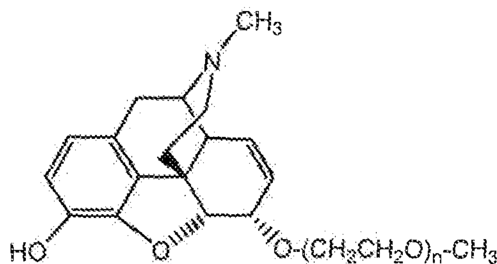
EXAMPLE 9
Preparation of mPEG$_n$-OMs (mPEGn-O-Mesylate)

[00268] In a 40-mL glass vial was mixed HO-$CH_2CH_2OCH_2CH_2$-OH (1.2 ml, 10 mmol) and DIEA (N,N-diisopropylethylamine, 5.2 ml, 30 mmol, 3 eq), the resulting homogeneous colorless mixture was cooled to 0 °C and MsCl (1.55 ml, 20 mmol, 2 eq) was added via syringe slowly, over 4 minutes, with vigorous stirring. A biphasic mixture resulted upon addition: yellow solid on the bottom and clear supernatant. The ice bath was removed and the reaction was allowed to warm to room temperature overnight. At this point it was dissolved in water, extracted with $CHCl_3$(3x50 mL), washed with 0.1M HCl/brine mixture 2x50 mL, followed by brine 50 mL. The organic layer was dried over $MgSO_4$, filtered to give a yellow solution and evaporated to give brown oil (2.14 g). ^{1}H NMR confirms product identity 3.3 (1H NMR δ 3.1 (s, 3H), 3.3 (s, 3H), 3.5-3.55 (m, 2H), 3.6-3.65 (m, 2H), 3.7-3.75 (m, 2H), 4.3-4.35 (m, 2H) ppm).

[00269] All other PEG$_n$-OMs's (n = 3, 4, 5, 6, 7 and 9) were made in similar fashion and afforded final compounds in each case that were isolated as brown oils. Mass spectral and proton NMR data (not shown) confirmed the formation of the desired OMs PEGylated products.

EXAMPLE 10

Preparation of mPEG$_n$-O-Morphine Conjugates

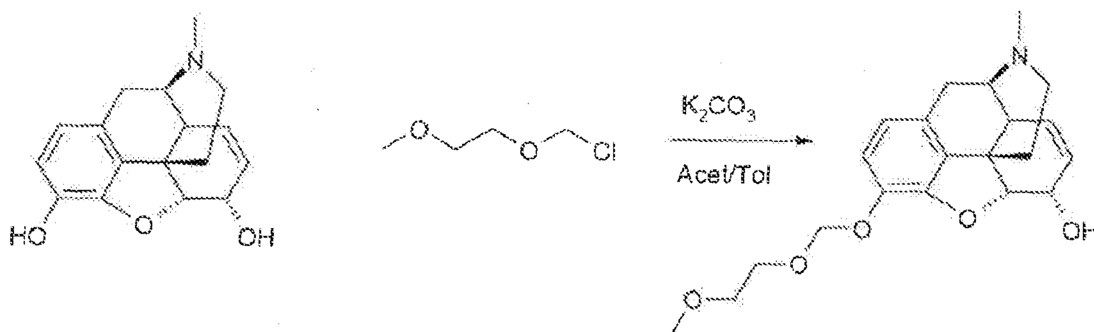


[00270] The following describes the preparation of free base using commercially available morphine sulfate hydrate (generally procedure).

[00271] Morphine sulfate USP from Spectrum (510 mg) was dissolved in water (70 ml). The solution was then basified to pH 10 using aqueous K_2CO_3 to give a white suspension. To the white suspension DCM (dichloromethane, 50 ml) was added, but failed to dissolve the solid. The mixture was made acidic with 1M HCl to result in clear biphasic solution. The organic phase was split off and the aqueous phase was carefully brought to pH 9.30 (monitored by a pH meter) using the same solution of K_2CO_3 as above. A white suspension resulted again. The heterogeneous mixture was extracted with DCM (5x25 ml) and an insoluble white solid contaminated both the organic and aqueous layers. The organic layer was dried with $MgSO_4$, filtered and rotary evaporated to yield 160 mg of morphine free base (56% recovery). No additional product was recovered from the filter cake using MeOH, but another 100 mg was recovered from the aqueous phase by 2x50ml extraction with EtOAc to give a combined yield of 260 mg (68%).

MEM Protection of Morphine free base

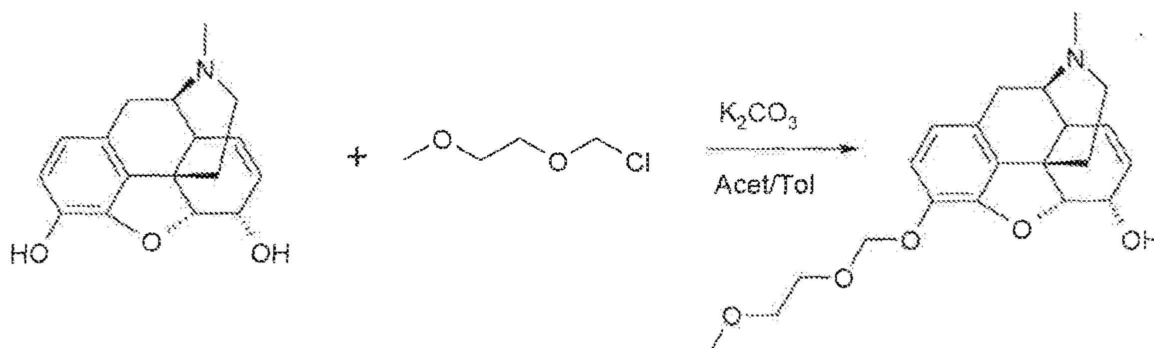
[00272] The general approach for protecting the free base of morphine with the protecting group β-methoxyethoxymethyl ether ("MEM") is schematically shown below.



[00273] Free base morphine (160 mg, 0.56 mmol) was dissolved in 20 ml of Acetone/Toluene (2/1 mixture). To the resulting solution was added K_2CO_3 (209 mg, 1.51 mmol, 2.7 eq) followed by MEMCl (96 µl, 0.84 mmol, 1.5 eq) and the resulting heterogeneous mixture was stirred overnight at room temperature. After five hours at room temperature, the reaction was deemed comlete by LC-MS. Morphine free base retention time under standard six minute gradient run conditions (std 6 min, Onyx Monolyth C18 column, 50x4.6 mm; 0 to 100% Acetonitrile 0.1% TFA in Water 0.1% TFA, 1.5 ml/min; detection: UV254, ELSD, MS; retention times are quoted for UV254 detector, ELSD has about 0.09 min delay and MS has about 0.04 min delay relative to UV) was 1.09 min; retention time for product 1.54 min (std 6 min), major impurity 1.79 min. The reaction mixture was evaporated to dryness, dissolved in water, extracted with EtOAc (3x, combined organic layer washed with brine, dried over $MgSO_4$, filtered and rotary evaporated) to give 160 mg (77%) of the desired product as a colorless oil. Product purity was estimated to be about 80% by UV254.

Direct MEM protection of morphine sulfate (general procedure)

[00274] The general approach for protecting morphine sulfate with the protecting group β-methoxyethoxymethyl ether ("MEM") is schematically shown below. Although not explicitly shown in the scheme below, morphine is actually morphine sulfate hydrate, morphine.0.5 H_2SO_4.2.5 H_2O.



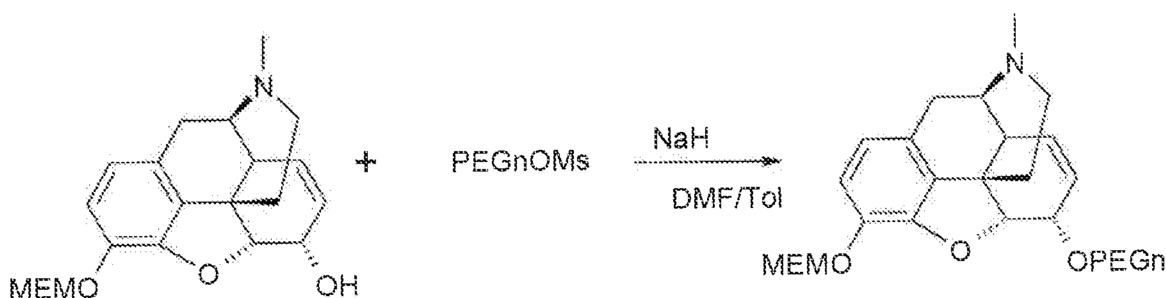
[00275] To a suspension of 103 mg of morphine sulfate hydrate (0.26 mmol) in 10 ml of 2:1 acetone:toluene solvent mixture was added 135 mg (1 mmol, 3.7 eq) of K_2CO_3 and the suspension stirred at room temperature for 25 minutes. To the resulting suspension was added 60 μl (0.52 mmol) of MEMCl and the mixture allowed to react at room temperature. It was sampled after one hour (38% nominal conversion, additional peaks at 1.69 min and 2.28 min), three hours (40% nominal conversion, additional peak at 1.72 min (M+1 =493.2)), four and one-half hours (56% nominal conversion, additional peak at 1.73 min), and twenty-three hours (>99% nominal conversion, additional peak at 1.79 min - about 23% of the product peak by height in UV_{254}); thereafter, the reaction was quenched with MeOH, evaporated, extracted with EtOAc to give 160 mg of clear oil.

[00276] The same reaction was repeated starting with 2 g (5.3 mmol) of morphine sulfate hydrate, 2.2 g (16 mmol, 3 eq) of K_2CO_3, 1.2 ml (10.5 mmol, 2 eq) of MEMCl in 100 ml of solvent mixture. Sampling occurred after two hours (61% nominal conversion, extra peak at 1.72 min (M+1 = 492.8)), after one day (80% nominal conversion, extra peak at 1.73 min), after three days (85% nominal conversion, only small impurities, 12 min gradient run), and after six days (91% conversion); thereafter, the reaction was quenched, evaporated, extracted with EtOAc, purified on combi-flash using a 40 g column, DCM:MeOH 0 to 30% mobile phase. Thre

peaks (instead of two) were identified, wherein the middle peak was collected, 1.15 g (58% yield) of light yellow oil, UV_{254} purity about 87%.

Conjugation of MEM-protected morphine to provide a MEM-protected morphine conjugate

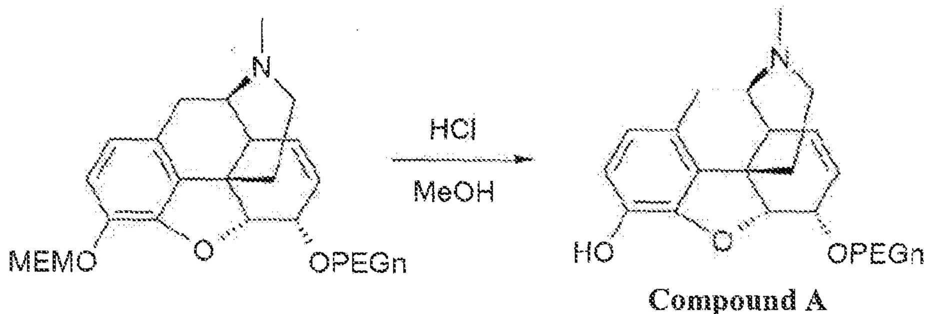
[00277] The general approach for conjugating MEM-protected morphine with a water-soluble oligomer to provide a MEM-protected morphine PEG-oligomer conjugate is schematically shown below.



[00278] To a solution of toluene/DMF (2:1 mixture, 10 volumes total) was charged MEM-morphine free base followed by NaH (4-6 eq) and then PEG_nOMs (1.2-1.4 eq.), previously prepared. The reaction mixture was heated to 55-75 °C and was stirred until reaction completion was confirmed by LC-MS analysis (12-40 hours depending on PEG chain length). The reaction mixture was quenched with methanol (5 volumes) and the reaction mixture was evaporated to dryness *in vacuo*. The residue was redissolved in methanol (3 volumes) and was chromatographed using a Combiflash system (0-40% MeOH/DCM). The fractions containing large amounts of product were collected, combined and evaporated to dryness. This material was then purified by RP-HPLC to give the products as yellow to orange oils.

Deprotection of MEM-protected morphine conjugate to provide a morphine conjugate

[00279] The general approach for deprotecting a MEM-protected morphine conjugate to provide a morphine conjugate is schematically shown below.

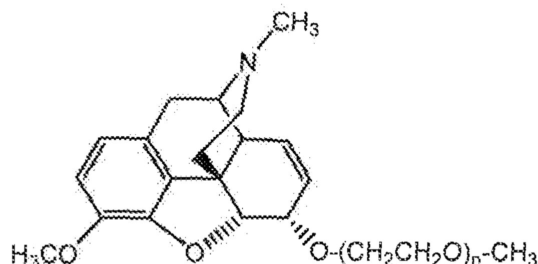


Compound A

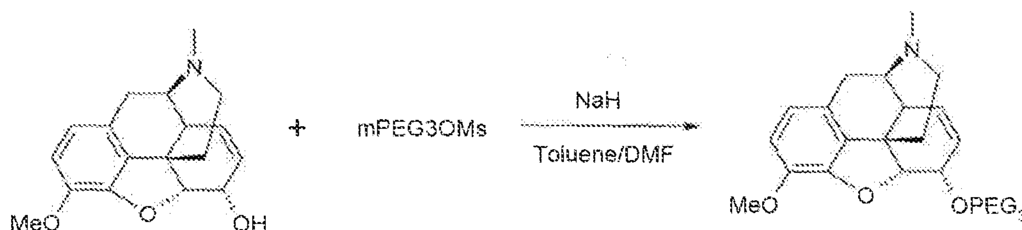
[00280] To a solution of MEM-protected morphine conjugate TFA salt suspended in DCM (8 volumes) was charged 6 volumes of 2M HCl in diethyl ether. The reaction mixture was allowed to stir at room temperature for two hours and was then evaporated to dryness under reduced pressure. The oily residue was dissolved in MeOH (8 volumes), filtered through glass wool and then evaporated under reduced pressure to give a thick orange to yellow oil in quantitative yield. Compounds made by this method include: α-6-mPEG$_3$-O-morphine (Compound A, n=3) 217 mg of HCl salt 97% pure (95% by UV254; 98% by ELSD); α-6-mPEG$_4$-O-morphine (Compound A, n=4) 275 mg of HCl salt 98% pure (97% by UV254; 98% by ELSD); α-6-mPEG$_5$-O-morphine (Compound A, n=5) 177 mg of HCl salt 95% pure (93% by UV254; 98% by ELSD); α-6-mPEG$_6$-O-morphine (Compound A, n=6) 310 mg of HCl salt 98% pure (98% by UV254; 99% by ELSD); α-6-mPEG$_7$-O-morphine (Compound A, n=7)541 mg of HCl salt 96% pure (93% by UV254; 99% by ELSD); and α-6-mPEG-O$_9$-morphine (Compound A, n=9) 466 mg of HCl salt 98% pure (97% by UV254; 99% by ELSD). Additionally, morphine conjugates having a single PEG monomer attached, α-6-mPEG$_1$-O-morphine (Compound A, n=1), 124 mg of HCl salt, 97% pure (95% pure by UV$_{254}$; 98% by ELSD); as well as α-6-mPEG$_2$-O-morphine (Compound A, n=2), 485 mg of HCl salt, 97% pure (95% pure by UV$_{254}$; 98% by ELSD) were similarly prepared.

EXAMPLE 11

Preparation of mPEG$_n$-O-Codeine Conjugates



[00281] The general approach for conjugating codeine with an activated sulfonate ester of a water-soluble oligomer (using mPEG$_3$OMs as a representative oligomer) to provide a codeine conjugate is schematically shown below.



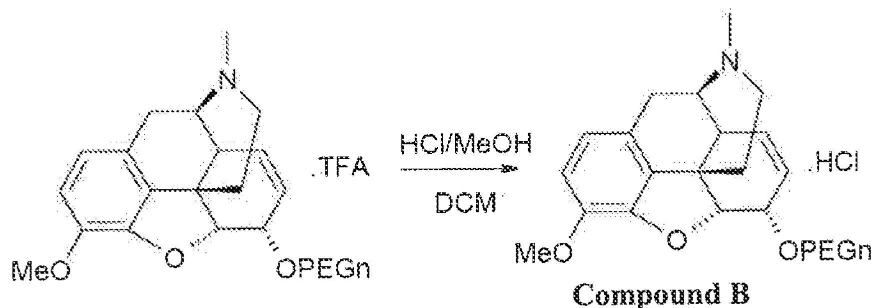
[00282] Codeine (30 mg, 0.1 mmol) was dissolved in toluene/DMF (75:1) solvent mixture followed by addition of HO-$CH_2CH_2OCH_2CH_2OCH_2CH_2$OMs (44 ml, 2eq) and NaH (60% suspension in mineral oil, 24 mg, 6 eq). The resulting homogeneous yellow solution was heated to 45 °C. After one hour, the reaction showed 11% conversion (extra peak at 2.71 min, 12 min run), after eighteen hours, the reaction showed 7% conversion (extra peak at 3.30 min, 12 min run), and after 24 hours, the reaction showed 24% conversion (multitude of extra peaks, two tallest ones are 1.11 min and 2.79 min). At this point, an additional 16 mg of NaH was added and heating continued for six hours, after which, an additional 16 mg of NaH was added followed by continued heating over sixty-six hours. Thereafter, no starting material remained, and analysis revealed many extra peaks, the two tallest ones corresponding to 2.79 min and 3 min (product peak is the second tallest among at least 7 peaks).

[00283] This synthesis was repeated using 10x scale wherein 30 ml of solvent mixture was used. After eighteen hours, analysis revealed 71% nominal conversion with additional peaks in the UV (one tall peak at 3.17 min and many small ones; wherein the desired peak corresponded to 3.43 min in UV). Thereafter, 80 mg (2 mmol) of NaH was added followed by continued heating. After three hours, analysis revealed 85% nominal conversion (several extra peaks, main 3.17 min). Reaction mixture was diluted with water, extracted with EtOAc (3x, combined organic layer washed with brine, dried over $MgSO_4$, filtered and rotary evaporated) to give yellow oil (no sm in LC-MS, 90% pure by ELSD, 50% pure by UV – major impurity at 3.2 min). The crude product was dissolved in DCM, applied to a small cartridge filled with 230-400 mesh SiO_2, dried, eluted on a Combi-flash via a 4g pre-packed column cartridge with solvent A = DCM and solvent B = MeOH, gradient 0 to 30% of B. Analysis revealed two peaks of poor symmetry: a small leading peak and a larger peak with a tail. LC-MS was used to analyze fractions, wherein none were identified as containing pure product. Combined fractions that contained any product (tt#22-30) yielded, following solvent evaporation, 150 mg (34% yield) of impure product (LC-MS purity at 3.35 min by UV254, wherein about 25% represented the main impurities 3.11 min, 3.92 min, 4.32 min, 5.61 min of a 12 min run). A second purification by HPLC (solvent A = water, 0.1% TFA; solvent B = acetonitrile, 0.1% TFA) employing a gradient corresponding to 15-60% B, 70 min, 10 ml/min) resulted in poor separation from adjacent peaks. Only two fractions were clean enough and gave 21 mg of TFA salt (>95% pure, 4.7% yield). Three additional fractions both before and after the desired product-containing fractions (for a total of six additional fractions were combined to give 70 mg of about 50% pure product as TFA salts.

[00284] Using this same approach, other conjugates differing by the number of ethylene oxide units (n= 4, 5, 6, 7, and 9) were made using these NaH conditions outlined above.

Converstion of Codeine-Oligomer Conjugate TFA Salts to Codeine-Oligomer HCl salts.

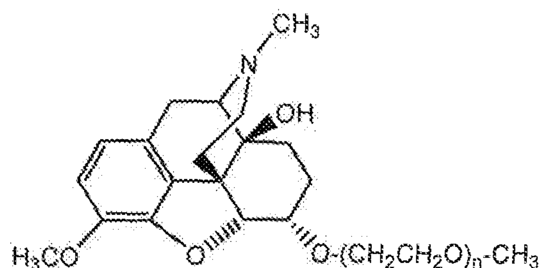
[00285] The general approach for converting codeine-oligomer TFA salts to codeine-oligomer HCl salts is schematically shown below.



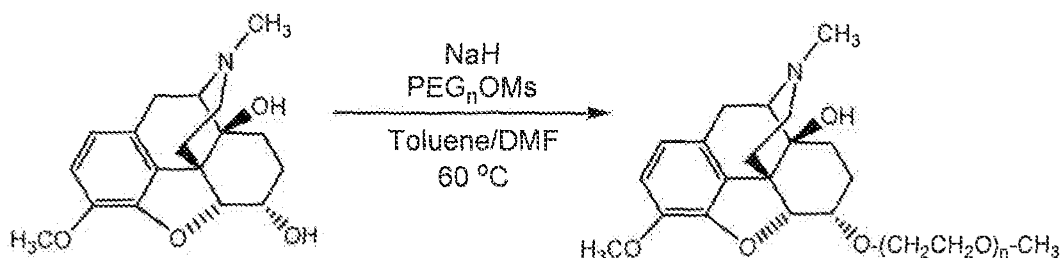
[00286] To a solution of codeine-oligomer conjugate TFA salt suspended in DCM (8 volumes) was charged 6 volumes of 2M HCl in diethyl ether. The reaction mixture was allowed to stir at room temperature for two hours and was then evaporated to dryness under reduced pressure. The oily residue was dissolved in MeOH (8 volumes), filtered through glass wool and then evaporated under reduced pressure to give a thick orange to yellow oil in quantitative yield. Following this general procedure, the following compounds were synthesized: α-6-mPEG$_3$-O-codeine (Compound B, n=3) 235 mg of HCl salt, 98% pure; α-6-mPEG$_4$-O-codeine (Compound B, n=4) 524 mg of HCl salt, 98% pure; α-6-mPEG$_5$-O-codeine (Compound B, n=5) 185 mg of HCl salt, 98% pure + 119 mg of HCl salt 97% pure, α-6-mPEG$_6$-O-codeine (Compound B, n=6) 214 mg of HCl salt, 97% pure; α-6-mPEG$_7$-O-codeine (Compound B, n=7) 182 mg of HCl salt, 98% pure; α-6-mPEG$_9$-O-codeine (Compound B, n=9) 221 mg of HCl salt, 97% pure; α-6-mPEG$_1$-O-codeine (Compound B, n=1) 63 mg of HCl salt, 90% pure; and α-6-mPEG$_2$-O-codeine (Compound B, n=2) 178 mg of HCl salt, 90% pure.

EXAMPLE 12

Preparation of mPEG$_n$-O-Hydroxycodone Conjugates



[00287] The general approach for conjugating hydroxycodone with an activated sulfonate ester of a water-soluble oligomer (using "mPEG$_n$OMs" as a representative oligomer) to provide a hydroxycodone conjugate is schematically shown below.



Compound C

Reduction of Oxycodone to α-6-hydroxycodone:

[00288] To a solution of oxycodone free base in dry THF under nitrogen cooled at -20 °C, was added a 1.0 M THF solution of potassium tri-sec-butylborohydride over 15 minutes. The solution was stirred at -20 °C under nitrogen for 1.5 hours and then water (10 mL) was added slowly. The reaction mixture was stirred another 10 minutes at -20 °C and then allowed to warm to room temperature. All solvents were removed under reduced pressure and CH_2Cl_2 was added to the remaining residue. The CH_2Cl_2 phase was extracted with a 0.1 N HCl/NaCl water solution and the combined 0.1 N HCl solution extracts were washed with CH_2Cl_2, then Na_2CO_3 was added to adjust the pH = 8. The solution was extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the desired α-6-HO-3-hydroxycodone was obtained.

Conjugation of mPEG$_n$OMs to α-6-hydroxycodone:

[00289] To a solution of Toluene/DMF (2:1 mixture, 10 volumes total) was charged hydroxycodone (prepared as set forth in the preceding paragraph) followed by NaH (4 eq) and then mPEG$_n$OMs (1.3 e.). The reaction mixture was heated to 60-80 °C and was stirred until reaction completion was confirmed by LC-MS analysis (12-40 hours depending on PEG chain length). The reaction mixture was quenched with methanol (5 volumes) and the reaction mixture was evaporated to dryness in vacuo. The residue was re-dissolved in methanol (3 volumes) and was chromatographed using Combiflash (0-40% MeOH/DCM). The fractions containing large amounts of product were collected, combined and evaporated to dryness. This material was then purified by RP-HPLC to provide the final products as yellow to orange oils.

Conversion of hydroxycodone conjugate TFA salts to hydroxycodone conjugate HCl salts

[00290] To a solution of hydroxycodone conjugate TFA salt suspended in DCM (8 volumes) was charged 6 volumes of 2M HCl in diethyl ether. The reaction mixture was allowed to stir at room temperature for two hours and was then evaporated to dryness under reduced pressure. The oily residue was dissolved in MeOH (8 volumes), filtered through glass wool and then evaporated under reduced pressure to give a thick orange to yellow oil in quantitative yield. Following this general procedure, the following compounds were synthesized: α-6-mPEG$_3$-O-oxycodone (aka α-6-mPEG$_3$-O-hydroxycodone) (Compound C, n=3) 242 mg of HCl salt, 96% pure; α-6-mPEG$_4$-O-oxycodone (aka α-6-mPEG$_4$-O-hydroxycodone) (Compound C, n=4) 776 mg of HCl salt, 94% pure; α-6-mPEG$_5$-O-oxycodone (aka α-6-mPEG$_5$-O-hydroxycodone) (Compound C, n=5) 172 mg of HCl salt, 93% pure; α-6-mPEG$_6$-O-oxycodone (aka α-6-mPEG$_6$-O-hydroxycodone) (Compound C, n=6) 557 mg of HCl salt, 98% pure; α-6-mPEG$_7$-O-oxycodone (aka α-6-mPEG$_7$-O-hydroxycodone) (Compound C, n=7) 695 mg of HCl salt, 94% pure; and α-6-mPEG$_9$-O-oxycodone (aka α-6-mPEG$_9$-O-hydroxycodone) (Compound C, n=9) 435 mg of HCl salt 95% pure. The following compounds, α-6-mPEG$_1$-O-oxycodone (aka α-6-mPEG$_1$-O-hydroxycodone) (Compound C, n=1) 431 mg of HCl salt 99% pure; and α-6-mPEG$_2$-O-oxycodone (aka α-6-mPEG$_2$-O-hydroxycodone) (Compound C, n=2) 454 mg HCl salt, 98% pure, were similarly prepared.

EXAMPLE 13

In-Vivo Analgesic Assay: Phenylquinone Writhing

[00291] An analgesic assay was used to determine whether exemplary PEG-oligomer-opioid agonist conjugates belonging to the following conjugate series: $mPEG_{2-7,9}$-O-morphine, $mPEG_{3-7,9}$-O-codeine, and $mPEG_{1-4,6,7,9}$-O-hydroxycodone, are effective in reducing and/or preventing visceral pain in mice.

[00292] The assay utilized CD-1 male mice (5-8 mice per group), each mouse being approximately 0.020-0.030 kg on the study day. Mice were treated according to standard protocols. Mice were given a single "pretreatment" dose of a compound lacking covalent attachment of a water-soluble, non-peptidic oligomer (i.e., non-PEG oligomer-modified parent molecule), a corresponding version comprising the compound covalently attached to a water-soluble, non-peptidic oligomer (i.e., the conjugate), or control solution (IV, SC, IP or orally) thirty minutes prior to the administration of the phenylquinone (PQ) solution. Each animal was given an IP injection of an irritant (phenylquinone, PQ) that induces "writhing" which may include: contractions of the abdomen, twisting and turning of the trunk, arching of the back, and the extension of the hindlimbs. Each animal was given an IP injection of PQ (1 mg/kg PQ, 0.1 mL/10 g bodyweight). After the injection, the animals were returned to their observation enclosure and their behavior was observed. Contractions were counted between 35 and 45 minutes after the 'pretreatment". The animals were used once. Each tested article was dosed at a range between 0.1and 100 mg/kg (n=5-10 animals/dose).

[00293] The results are shown in FIG. 2 ($mPEG_{2-7,9}$-O-morphine and control), FIG. 3 ($mPEG_{1-4,6,7,9}$-O-hydroxycodone and control), and FIG. 4 ($mPEG_{3-7,9}$-O-codeine and control). ED50 values are provided in Tables 2 and 3 below.

Table 2. ED_{50} values for $mPEG_n$-O-Morphine Series

	MORPHINE	PEG 2	PEG 3	PEG 4	PEG 5	PEG 6	PEG 7	PEG 9
ED_{50} (mg/kg)	0.3693	2.512	13.58	3.281	13.4	n/a	n/a	n/a

Table 3. ED_{50} values for $mPEG_n$-O-HydroxyCodone Series

	OXYCODONE	PEG 1	PEG 2	PEG 3	PEG 4	PEG 6	PEG 7	PEG 9
ED_{50} (mg/kg)	0.6186	6.064	n/a	n/a	17.31	n/a	n/a	n/a

EXAMPLE 14

In-Vivo Analgesic Assay: Hot Plate Latency Assay

[00294] A hot plate latency analgesic assay was used to determine whether exemplary PEG-oligomer-opioid agonist conjugates belonging to the following conjugate series: $mPEG_{1-5}$-O-morphine, $mPEG_{1-5}$-O-hydroxycodone, and $mPEG_{2-5, 9}$-O-codeine, are effective in reducing and/or preventing visceral pain in mice.

[00295] The assay utilized CD-1 male mice (10 mice per group), each mouse being approximately 0.028-0.031 kg on the study day. Mice were treated according to standard protocols. Mice were given a single "pretreatment" dose of a compound lacking covalent attachment of a water-soluble, non-peptidic oligomer (unmodified parent molecule), a corresponding version comprising the compound covalently attached to a water-soluble, non-peptidic oligomer (i.e., the conjugate), or control solution (SC) thirty minutes prior to the hot plate test. The hot plate temperature was set at 55 ± 1°C, calibrated with a surface thermometer before commencement of the experiment. Thirty minutes after "pretreatment", each mouse was placed on the hot plate, and latency to lick a hindpaw was recorded to the nearest 0.1 second. If no lick occurred within 30 seconds, the mouse was removed. Immediately after hot plate testing, a temperature probe was inserted 17 mm into the rectum, and body temperature was read to the nearest 0.1°C when the meter stabilized (approximately 10 seconds). The animals were used once. Each tested article was dosed at a range between 0.3 and 30 mg/kg (n=5-10 animals/dose).

[00296] Results are shown in FIG. 5 (hydroxycodone series), FIG. 6 (morphine series) and FIG. 7 (codeine). Plots illustrate latency (time to lick hindpaw, in seconds) versus dose of compound administered in mg/kg.

EXAMPLE 15

Pharmacokinetics of PEG_{oligo}-Opioid Compounds Following Intravenous (IV) and Oral (PO) Dosing in Male Sprague-Dawley Rats - Study Design

[00297] One hundred seventy five (175) adult male Sprague-Dawley rats with indwelling jugular vein and carotid artery catheters (JVC/CAC) (Charles River Labs, Hollister, CA) were utilized for the study. There were 3 rats/group. All animals were food fasted overnight. Prior to dosing the rats were weighed, the tails and cage cards were labeled for identification and the doses were calculated. Anesthesia was induced and maintained with 3.0-5.0% isoflurane. The JVC and CAC were externalized, flushed with HEP/saline (10 IU/mL HEP/ mL saline), plugged, and labeled to identify the jugular vein and carotid artery. The predose sample was collected from the JVC. When all of the animals had recovered from anesthesia and the predose samples were processed, the animals for intravenous group were were dosed, intravenously (IV) via the JVC using a 1 mL syringe containing the appropriate test article, the dead volume of the catheter was flushed with 0.9% saline to ensure the animals received the correct dose and oral group animals were treated orally via gavage.

[00298] Following a single IV dose, blood samples were collected at 0 (pre-dose collected as described above), 2, 10, 30, 60, 90, 120, and 240 minutes and following oral dose, blood samples were collected 0 (pre-dose collected as described above), 15, 30, 60, 120, 240 and 480 minutes via the carotid artery catheter and processed as stated in the protocol. Following the last collection point, the animals were euthanized.

[00299] Bioanalytical analysis of the plasma samples was conducted using LC-MS/MS methods.

[00300] <u>Pharmacokinetic Analyses</u>: PK analysis was performed using WinNonlin (Version 5.2, Mountain View, CA-94014). Concentrations in plasma that were below LLOQ were replaced with zeros prior to generating Tables and PK analysis. The following PK parameters were estimated using plasma concentration-time profile of each animal:

C_0	Extrapolated concentration to time "zero"
C_{max}	Maximum (peak) concentration
AUC_{all}	Area under the concentration-time from zero to time of last concentration value

$T_{1/2(Z)}$	Terminal elimination half-life
AUC_{inf}	Area under the concentration-time from zero to time infinity
T_{max}	Time to reach maximum or peak concentration following administration
CL	Total body clearance
V_z	Volume of distribution based on terminal phase
V_{ss}	Volume of distribution at steady state
MRT_{last}	Mean residence time to last observable concentration
F	Bioavailability

[00301] Oral bioavailability was estimated using mean dose-normalized AUCall data for the compounds where one of IV or PO groups with only reported data for <n=3/group.

EXAMPLE 16

IV and PO Pharmacokinetics of $mPEG_n$-O-Hydroxycodone Conjugates

[00302] A pharmacokinetic study was conducted in Sprague-Dawley rats as described in Example 15 above. Compounds administered were $mPEG_n$-O-hydroxycodone conjugates where n=1, 2, 3, 4, 5, 6, 7, and 9, as well as the parent compound, oxycodone. The objective was to determine the pharmacokinetics of the parent compound and its various oligomer conjugates administered both intravenously and orally.

[00303] A summary of plasma PK parameters following IV (1 mg/kg) and PO (5 mg/kg) delivery for oxycodone, $mPEG_0$-oxycodone, $mPEG_1$-O-hydroxycodone, $mPEG_2$-O-hydroxycodone, $mPEG_3$-O-hydroxycodone, $mPEG_4$-O-hydroxycodone, $mPEG_5$-O-hydroxycodone, $mPEG_6$-O-hydroxycodone, $mPEG_7$-O-hydroxycodone, and $mPEG_9$-O-hydroxycodone, are shown in the following tables, Tables 4 and 5.

[00304] Based on the observed data (Table 4) for IV administration, $mPEG_9$-O-hydroxycodone appeared to achieve higher plasma concentration with a mean $t_{1/2}$ value 3 times that of the corresponding mean $t_{1/2}$ value observed after parent oxycodone was given.

[00305] FIG. 8 shows the mean plasma concentration-time profiles for IV-administered mPEGn-O-hydroxycodone compounds as described above, as well as for oxycodone per se, when administered at a concentration of 1.0 mg/kg.

[00306] Based on the observed data (Table 5) for oral administration, $mPEG_5$-O-hydroxycodone, $mPEG_6$-O-hydroxycodone, and $mPEG_7$-O-hydroxycodone appeared to achieve higher mean exposure (approximately 3- to 8-fold) in plasma as compared to parent molecule, oxycodone.

[00307] FIG. 9 shows the mean plasma concentration-time profiles for the $mPEG_n$-O-hydroxycodone compounds described above, as well as for oxycodone, when administered orally to rats at a concentration of 5.0 mg/kg.

Table 4. Comparative PK Parameters of $mPEG_n$-O-hydroxycodone conjugates given intravenously to rats (Mean ± SD)

PEG-length	C_{max} (ng/mL)	$T_{1/2}(z)$ min	AUC_{all} (min.ng /mL)	AUC_{inf} (min.ng /mL)	MRT_{last} min	CL (mL/min/kg)	V_{ss} (L/kg)
0	495±56.0	47.0±3.99	12800±1090	13000±1070	37.0±1.28	77.1±6.26	3.17±0.293
1	425±41.3	47.2±6.37	9890±1320	10100±1440	38.7±4.54	100±13.4	4.31±0.222
2	513±48.8	44.6±1.80	12000±1610	12200±1650	37.0±2.60	83.3±10.8	3.36±0.298
3	746±2.08	48.5±7.83	13800±1050	14000±1010	32.5±1.92	71.7±4.99	2.62±0.206
4	537±31.0	43.6±3.27	11500±783	11600±827	35.6±2.88	86.5±6.36	3.34±0.113
5	622±39.7	62.1±3.85	16900±1800	17700±1990	46.2±1.86	57.0±6.07	3.30±0.184
6	445±83.6	62.2±5.17	12600±2370	13100±2390	47.7±1.41	77.9±14.4	4.68±0.938
7	489±26.5	87.0±3.25	14300±583	15800±728	54.3±0.372	63.3±2.99	5.31±0.139
9	955±149	143±14.3	16600±2190	21000±4230	52.7±4.04	48.9±9.41	6.35±0.349

Table 5. Comparative PK Parameters of mPEG$_n$-O-hydroxycodone conjugates given orally to Sprague Dawley rats (Mean ± SD)

PEG-length	C_{max} (ng/mL)	$T_{1/2(z)}$ min	AUC_{all} (min.ng /mL)	AUC_{inf} (min.ng /mL)	T_{max}* min	MRT_{last} min	F%
0	25.5±1.86	NC	4520 ±1660	NC	15.0	179±17.4	7.1
1	14.3±6.43	57.7*	1050±205	1150*	15.0	66.8±23.8	2.1
2	99.4±47.3	48.5±12.0	5910±2690	5830±2600	15.0	55.4±14.7	9.4
3	44.5±29.4	65.6*	3620±1910	4210*	15.0	84.7±17.0	5.3
4	55.8±4.69	70.3*	6340±1810	5280*	15.0	96.6±33.6	11.0
5	178±14.7	75.8±1.08	32800±2020	33300±2090	15.0	124±4.84	37.6
6	171±76.6	85.4±7.83	35100±10100	36200±10200	120	154±6.46	55.3
7	114±38.0	115±29.2	20400±3670	22200±2900	120	178±6.09	28.1
9	27.6±19.6	106(n=1)	7620±4510	13500 (n=1)	120	203±43.8	9.2

*: n=2, NC: Not calculated. Tmax is reported as median value.

[00308] To summarize the results, intravenous administration of PEGylated hydroxycodone with varying oligomeric PEG-lengths (PEG1 to PEG9) resulted in variable plasma concentrations and exposures as compared to oxycodone. PEGs with chain lengths 3, 5, 7 and 9 showed higher mean exposure (AUC) while PEG6 showed comparable mean exposure (AUC) and PEGs with chain lengths 1, 2 or 4 showed slightly lower mean exposure (AUC). The compounds having a PEG length greater than 5 showed trends of lower clearance, higher volume of distribution at steady state, increase in elimination half life values, with increasing PEG length.

[00309] Oral administration of PEGylated hydroxycodone with varying oligomeric PEG-lengths (PEG1 to PEG9) resulted in improvement in plasma exposure with the exception of hydroxycodone covalently attached to PEG1 and to PEG3. Oral bioavailability was highest for hydroxycodone covalently attached to mPEG6, 55.3%) followed by mPEG5-hydroxycodone and mPEG7-hydroxycodone with 37.6% and 28.1%, respectively. The elimination half-life values showed a trend of increasing with increase in PEG-length.

EXAMPLE 17

IV and PO Pharmacokinetics of mPEG$_n$-O-Morphine Conjugates

[00310] A pharmacokinetic study was conducted in Sprague-Dawley rats as described in Example 15 above. Compounds administered were mPEG$_n$-O-morphine conjugates where n=1, 2, 3, 4, 5, 6, 7, and 9, as well as the parent compound, morphine. The objective was to determine the pharmacokinetics of the parent compound and its various oligomer conjugates administered both intravenously and orally.

[00311] A summary of plasma PK parameters following IV(1 mg/kg) and PO (5 mg/kg) routes for morphine, mPEG$_1$-O-morphine, mPEG$_2$-O-morphine, mPEG$_3$-O-morphine, mPEG$_4$-O-morphine, mPEG$_5$-O-morphine, mPEG$_6$-O-morphine, mPEG$_7$-O-morphine, mPEG$_9$-O-morphine, are shown in Table 6 and Table 7, respectively.

[00312] For the intravenous group: FIG. 10 shows the mean plasma concentration-time profiles for the above mPEG$_n$-O-morphine conjugates after 1.0 mg/kg intravenous administration to rats. There appeared to be one outlier datum in each animal that are inconsistent with plasma profiles of mPEG$_2$-O-morphine, and were excluded from the PK analysis.

[00313] Based on the observed data (Table 6), mPEG$_9$-O-morphine appeared to achieve higher plasma concentration with a mean $t_{1/2}$ value 4 times that of the corresponding $t_{1/2}$ value observed after parent morphine was given.

Table 6. Comparative PK Parameters of mPEG$_n$-O-morphine Conjugates given intravenously to rats

PEG-Length	C_{max} (ng/mL)	$T_{1/2(z)}$ min	AUC_{all} (min.ng /mL)	AUC_{inf} (min.ng /mL)	MRT_{last} min	CL (mL/min/kg)	V_{ss} (L/kg)
0	132 ± 5.86	51.1 ± 20.8	2730±276	2760 ± 218	28.5 ± 6.79	364 ± 27.5	14.9 ± 4.0
1	483 ± 37.1	40.0 ± 2.58	11400±1230	11500 ± 1260	29.8 ± 5.05	87.8 ± 9.40	2.75 ± 0.236
2	378 ± 48.8	38.1±8.03	7510±106	7410±404	26.4±5.90	135±7.60	4.2 ± 0.270
3	483 ± 81.0	45.0 ± 2.73	12700±1950	12900 ± 1990	39.3 ± 1.69	78.5 ± 11.8	3.43 ± 0.616
4	622 ± 72.5	52.9 ± 6.50	14600±1140	15000 ± 1270	40.1 ± 0.962	67.1 ± 5.58	3.17 ± 0.168
5	514 ± 38.6	68.4 ±0.826	13200±998	14000 ± 1050	49.7 ± 1.20	71.6 ± 5.17	4.74 ± 0.347
6	805 ± 30.6	93.7 ± 17.1	19000±1430	21600 ± 2060	56.2 ± 3.84	46.6 ± 4.67	4.39 ± 0.630
7	1110 ± 123	111 ± 32.9	18100±956	21200 ± 1990	49.6 ± 5.20	47.4 ± 4.21	4.76 ± 0.997
9	1840 ± 123	204 ± 28.3	23300±1460	29000 ± 3240	34.2 ± 2.72	34.7 ± 3.64	4.52 ± 0.473

[00314] For the oral group, FIG. 11 the mean plasma concentration-time profiles for the above described mPEG$_n$-O-morphine conjugates after the oral administration (5.0 mg/kg) to rats.

[00315] Based on the observed data (Table 7), mPEG$_4$-O-morphine appeared to achieve highest plasma concentrations among the conjugates tested as compared to parent molecule, morphine.

Table 7. Comparative PK Parameters of mPEG$_n$-O-morphine conjugates given orally to Sprague Dawley rats (Mean ± SD)

PEG-Length	C_{max} (ng/mL)	$T_{1/2(z)}$ min	AUC_{all} (min.ng /mL)	AUC_{inf} (min.ng /mL)	T_{max} min	MRT_{last} min	F%
0	29.8 ± 7.78	144 ± 32.1	5510 ± 667	7230 ± 897	15.0	194 ± 22.0	40.4$^{¥}$
2	3.84*	104*	448*	778*	15.0*	60.7*	0.15
3	30.3 ± 4.42	377*	4250± 2140	8370*	15.0	151 ± 69.4	9.0
4	87.1 ± 53.6	191 ± 104	15600 ± 7690	18200±10300	30.0	149 ± 26.7	22.1
5	35.6 ± 19.8	247*	9190 ± 5650	17400*	120	205 ± 26.2	13.9
6	42.8 ± 31.2	121*	8290 ± 4970	10800 *	120	177 ± 29.4	8.7
7	9.38 ± 0.883	236*	2210 ± 221	2720*	60.0	187 ± 32.0	2.4
9	7.15 ± 3.34	363*	1360 ± 311	2270*	15.0	166 ± 26.0	1.2

No PK parameters were not reported for mPEG$_1$-morphine because all the concentrations were <LLOQ. *n=2.

[00316] In summary, for the IV data, administration of oligomeric PEGylated morphine with varying PEG-lengths (PEG1 to PEG9) resulted in higher plasma concentrations and exposure (AUC) as compared to morphine per se. There was a clear trend of increase in mean AUC with increase in PEG-length of 5 onwards, with 10-fold higher mean AUC for the PEG9-morphine compound as compared to non-conjugated morphine. The mean half-life and mean residence time also increased with increase in PEG-length. The lower mean clearance values were consistent with observed higher mean AUC values.

[00317] Mean volume of distribution estimated for steady state, immediately decreased by 5-fold with the introduction of single PEG, and reached a constant value at PEG-length 5. Overall, PEGylation appeared to increase the elimination $t_{1/2}$ and lower the tissue distribution of morphine.

[00318] Based upon the oral data, administration of PEGylated morphine conjugates with varying PEG-lengths (PEG1 to PEG9) resulted in a reduction in oral bioavailability compared to morphine. The reduction in bioavailability appeared to be related to the absorption component

rather than metabolism component for these PEG-conjugates. Among the PEG-conjugates, the conjugate with PEG-length 4 showed maximum F-value (22.1%) while conjugates with shorter or longer PEG-length showed a clear trend of loss in absorption.

[00319] In this study, morphine F% value was 3-fold higher than literature value of 15% at 7.5 mg/kg (*J. Pharmacokinet. Biopharm.* 1978, 6:505-19). The reasons for this higher exposure are not known.

Example 18

IV and PO Pharmacokinetics of mPEG$_n$-O-Codeine Conjugates

[00320] A pharmacokinetic study was conducted in Sprague-Dawley rats as described in Example 15 above. Compounds administered were mPEG$_n$-O-codeine conjugates where n=1, 2, 3, 4, 5, 6, 7, and 9, as well as the parent compound, codeine (n=0). The objective was to determine the pharmacokinetics of the parent compound, i.e., codeine, and its various oligomer conjugates administered both intravenously and orally.

[00321] A summary of plasma PK parameters following IV(1 mg/kg) and PO (5 mg/kg) routes for codeine, mPEG$_1$-O-codeine, mPEG$_2$-O-codeine, mPEG$_3$-O-codeine, mPEG$_4$-O-codeine, mPEG$_5$-O-codeine, mPEG$_6$-O-codeine, mPEG$_7$-O-codeine, mPEG$_9$-O-codeine, are shown in Table 8 and Table 9, respectively.

[00322] For the IV group: FIG. 12 shows the mean plasma-concentration-time profiles for parent molecule, codeine, as well as for the mPEG$_n$-O-codeine conjugates described above, after intravenous administration.

[00323] Based on the observed data (Table 8), mPEG$_6$-O-codeine appeared to achieve higher plasma concentrations among the tested conjugates with a mean $t_{1/2}$ value approximately 2.5 times that of the corresponding $t_{1/2}$ value observed following administration of the parent molecule, codeine.

Table 8. Comparative PK Parameters of Codeine and its Oligomeric PEG Conjugates Admnistered Intravenously to Rats

PEG-Length	C_{max} (ng/mL)	$T_{1/2(z)}$ min	AUC_{all} (min.ng /mL)	AUC_{inf} (min.ng /mL)	MRT_{last} min	CL (mL/min/kg)	V_{ss} (L/kg)
0	469 ± 20.4	42.1±3.15	11000 ± 1600	11400±2070	40.2 ± 9.08	89.7±15.3	4.14±0.700
1	723 ± 31.2	42.1 ± 4.84	15500±2020	15700 ± 2130	32.2 ± 4.59	64.6 ± 8.75	2.22 ±0.899
2	685 ± 41.0	35.3 ± 2.78	14500±1590	14600 ± 1590	31.5 ± 2.96	69.0 ±7.57	2.25 ± 0.166
3	732 ± 27.1	39.4 ± 1.49	17300±1520	17400 ± 1550	33.8 ± 2.40	57.7 ± 4.89	2.07 ± 0.127
4	746 ± 70.0	57.1 ± 43.8	15200±2160	15400 ± 2240	27.5 ± 4.55	65.9 ± 10.4	2.30 ± 0.720
5	533 ± 38.9	42.7 ± 3.56	11500±878	11700 ± 913	31.8 ± 1.53	86.2 ± 7.04	2.95 ± 0.157
6	1780 ± 149	58.0 ± 4.79	45600±2020	47100±2000	41.7 ± 3.08	21.3 ± 0.876	1.08 ± 0.143
7	443 ± 43.3	74.5 ± 5.76	12700±481	13700 ± 320	50.7 ± 2.07	73.1 ± 1.73	5.20 ± 0.596
9	730 ± 68.0	109 ± 1.80	17800±2310	20800 ± 2840	57.2 ± 2.46	48.6 ± 6.74	5.18 ± 0.538

Tmax is reported as median value. *: n=2.

[00324] For the oral group, FIG. 13 shows the mean plasma concentration-time profiles for parent molecule, codeine, versus mPEG$_n$-codeine conjugates after oral administration to rats (5.0 mg/kg).

[00325] Based on the observed data (Table 9), the PEG-6 compound, mPEG$_6$-codeine, appeared to achieve highest plasma concentrations (52 times higher mean AUCall) among the tested conjugates as parent molecule, codeine.

Table 9. Comparative PK Parameters of Codeine and Various mPEG$_n$-Codeine Conjugates given Orally to Sprague Dawley Rats (Mean ± SD)

PEG-Length	C_{max} (ng/mL)	$T_{1/2(z)}$ min	AUC_{all} (min.ng /mL)	AUC_{inf} (min.ng /mL)	T_{max} min	MRT_{last} min	F%
0	6.24 ± 2.51	80.8$^{\#}$	328 ± 216	431$^{\#}$	15.0	33.2 ± 12.9	0.60
2	3.47 ± 0.606	97.6 ± 28.4	351±195	419 ± 226	15.0	62.0 ± 27.4	0.57
3	25.0 ± 6.59	125 ± 64.6	1920±245	2080 ± 498	15.0	71.0 ± 9.16	2.39
4	31.1 ± 13.1	118 ± 60.0	2530±682	2670 ± 870	15.0	83.8 ± 22.5	3.47
5	48.7 ± 10.8	125 ± 63.7	5510±963	5890 ±1470	15.0	108 ± 35.4	10.1
6	617 ± 56.4	126 ± 54.1	70500±12300	74500±10000	15.0	119 ± 11.1	31.6
7	76.6 ± 12.8	97.6*	17100±4220	16000*	120	171 ± 21.7	26.9
9	31.5 ± 8.43	143*	7320±3330	6840*	15.0	179 ± 21.6	8.22

No PK parameters were not reported for NKT-10479 because the concentrations were LLOQ.
$^{\#}$: n=1, *: n=2. T_{max} is reported as median value.

[00326] In summary, for the IV data, PEGylation of codeine with varying oligomeric PEG-lengths (PEG1 to PEG9) improved exposure (mean AUC) only slightly and moderate improvement (approximately 4-fold) was observed for the PEG-6 conjugate. Both clearance and volume of distribution decreased for this PEG-conjugate by 4-fold. Conjugates with PEG-lengths 7 and 9 showed longer mean $t_{1/2}$ values, however, mean clearance and mean volume of distribution (Vss) were decreased for both for both the PEG7- and PEG9-codeine conjugates.

[00327] For the oral data, oral bioavailability for codeine is very low (F=0.52%). Oral bioavailability appeared to increase with increase in PEG-length from 2 onwards, reaching maximum with 32% bioavailability for the codeine conjugate with PEG-length 6, decreasing thereafter. In general, mean $t_{1/2}$ and mean residence values increased with PEG-length. There was no difference in time to reach peak concentrations (Tmax = 15 min) amongst all the compounds tested, suggesting that absorption was rapid and the absorption rate was not altered.

EXAMPLE 19

In-Vitro Binding of mPEG$_n$-O-Opioid Conjugates to Opioid Receptors

[00328] The binding affinities of the various PEG-opioid conjugates (mPEG$_n$-O-morphine, mPEG$_n$-O-codeine, and mPEG$_n$-O-hydroxycodone) were measured *in vitro* in membrane preparations prepared from CHO cells that heterologously express the cloned human mu, kappa or delta opioid receptors in a manner similar to that described in Example 4. Radioligand displacement was measured using scintillation proximity assays (SPA).

[00329] Briefly, serial dilutions of the test compounds were placed in a 96-well plate to which were added SPA beads, membrane and radioligand. The assay conditions for each opioid receptor subtype are described in Table 10 below. The plates were incubated for 8 hours-overnight at room temperature, spun at 1000 rpm to pellet the SPA beads, and radioactivity was measured using the TopCount® microplate Scintillation counter. Specific binding at each concentration of test compound was calculated by subtracting the non-specific binding measured in the presence of excess cold ligand. IC_{50} values were obtained by non-linear regression of specific binding versus concentration curves and Ki values were calculated using Kd values that were experimentally pre-determined for each lot of membrane preparations.

Table 10. Assay conditions for opioid receptor binding assays.

EXPERIMENTAL VARIABLE	MU OPIOID RECEPTOR	KAPPA OPIOID RECEPTOR	DELTA OPIOID RECEPTOR
SPA beads	PVT-WGA PEI Type A (GE Healthcare, Cat. # RPNQ0003)	PVT-WGA (GE Healthcare, Cat. #RPNQ0001)	PVT-WGA PEI Type B (GE Healthcare, Cat. #RPNQ0004)
Radioligand; Concentration	DAMGO, [Tyrosyl-3,5-3H(N)]- (Perkin Elmer, Cat. # NET-902); 6 nM	U-69,593, [Phenyl-3,4-3H]- (Perkin Elmer, Cat. #NET-952); 10 nM	Naltrindole, [5',7'-3H]- (Perkin Elmer, Cat. #NET-1065); 3 nM
Non-specific binding control	CTAP	*nor*-Binaltorphimine (*nor*-BNI)	SNC80
Buffer	50 mM Tris-HCl, pH 7.5 5 mM MgCl2 ; 1 mM EDTA	50 mM Tris-HCl, pH 7.5 5 mM MgCl2	50 mM Tris-HCl, pH 7.5 5 mM MgCl2
Receptor and source	Recombinant human mu opioid receptor expressed in CHO-K1 host cell membranes (Perkin Elmer, Cat. #ES-542-M)	Recombinant human kappa opioid receptor expressed in Chem-1 host cell membranes (Millipore, Cat. #HTS095M)	Recombinant human delta opioid receptor expressed in Chem-1 host cell membranes (Millipore, Cat. #HTS100M).

[00330] The binding affinities of the oligomeric PEG conjugates of morphine, codeine and hydroxycodone are shown in Table 11. Overall, all of the conjugates displayed measurable binding to the mu-opioid receptor, consistent with the known pharmacology of the parent molecules. For a given PEG size, the rank order of mu-opioid binding affinity was PEG-morphine > PEG-hydroxycodone > PEG-codeine. Increasing PEG size resulted in a progressive decrease in the binding affinity of all PEG conjugates to the mu opioid receptor compared to unconjugated parent molecule. However, the PEG-morphine conjugates still retained a high binding affinity that was within 15X that of parent morphine. The mu-opioid binding affinities of PEG-hydroxycodones were 20-50 fold lower than those of the PEG-morphine conjugates. Codeine and its PEG conjugates bound with very low affinity to the mu opioid receptor. PEG-morphine conjugates also bound to the kappa and delta opioid receptors; the rank order of

selectivity was mu>kappa>delta. Binding affinities of codeine and hydroxycodone conjugates to the kappa and delta opioid receptors were significantly lower than that at the mu-opioid receptor.

Table 11. Binding affinities of the PEG-opioid conjugates to opioid receptors.

COMPOUND	KI (NM)		
	Mu opioid receptor	Kappa opioid receptor	Delta opioid receptor
Morphine	8.44	118.38	4,297
α-6-mPEG$_1$-O-Morphine	15.72	444.54	2,723
α-6-mPEG$_2$-O-Morphine	21.97	404.33	2,601
α-6-mPEG$_3$-O-Morphine	50.66	575.98	6,176
α-6-mPEG$_4$-O-Morphine	23.11	438.88	3,358
α-6-mPEG$_5$-O-Morphine	39.40	557.54	2,763
α-6-mPEG$_6$-O-Morphine	72.98	773.56	2,595
α-6-mPEG$_7$-O-Morphine	56.86	669.56	2,587
α-6-mPEG$_9$-O-Morphine	111.05	1253.71	5,783
Oxycodone	133.48	N/A	N/A
α -6-mPEG$_1$-O-Hydroxycodone	653.90	N/A	N/A
α -6-mPEG$_2$-O-Hydroxycodone	631.76	N/A	N/A
α -6-mPEG$_3$-O-Hydroxycodone	775.19	N/A	N/A
α -6-mPEG$_4$-O-Hydroxycodone	892.70	N/A	N/A
α -6-mPEG$_5$-O-Hydroxycodone	1862.14	N/A	N/A
α -6-mPEG$_6$-O-Hydroxycodone	1898.30	N/A	N/A
α -6-mPEG$_7$-O-Hydroxycodone	1607.19	N/A	N/A
α -6-mPEG$_9$-O-Hydroxycodone	3616.60	N/A	N/A
Codeine	1,953	28,067	N/A
α-6-mPEG$_1$-O-Codeine	1821.51	54669.89	N/A
α-6-mPEG$_2$-O-Codeine	1383.07	22603.05	N/A
α-6-mPEG$_3$-O-Codeine	4260.21	36539.78	N/A
α-6-mPEG$_4$-O-Codeine	2891.36	96978.61	N/A
α-6-mPEG$_5$-O-Codeine	2427.13	59138.22	N/A
α-6-mPEG$_6$-O-Codeine	14202.77	>160,000	N/A
α-6-mPEG$_7$-O-Codeine	9963.93	108317.50	N/A
α-6-mPEG$_9$-O-Codeine	9975.84	72246.23	N/A

[00331] N/A indicates that Ki values could not be calculated since a 50% inhibition of binding was not achieved at the highest concentration of compound tested. Additional studies indicate Ki values for certain compounds that are lower than those recorded in Table 11.

EXAMPLE 20

In-Vitro Efficacy of mPEG$_n$-O-Opioid Conjugates to Inhibit cAMP Formation

[00332] The efficacy of the various PEG-opioid conjugates was measured by their ability to inhibit cAMP formation following receptor activation in a manner similar to that described in Example 5. Studies were conducted in CHO cells heterologously expressing the cloned human mu, kappa or delta opioid receptors. cAMP was measured using a cAMP HiRange homogenous time-resolved fluorescence assay (HTRF Assay), that is based on a competitive immunoassay principle (Cisbio, Cat.#62AM6PEC).

[00333] Briefly, suspensions of cells expressing either the mu, kappa or delta opioid receptors were prepared in buffer containing 0.5 mM isobutyl-methyl xanthine (IBMX). Cells were incubated with varying concentrations of PEG-opioid conjugates and 3 μM forskolin for 30 minutes at room temperature. cAMP was detected following a two-step assay protocol per the manufacturer's instructions and time resolved fluorescence was measured with the following settings: 330 nm excitation; 620 nm and 665 nm emission; 380 nm dichroic mirror. The 665nm/620nm ratio is expressed as Delta F% and test compound-related data is expressed as a percentage of average maximum response in wells without forskolin. EC_{50} values were calculated for each compound from a sigmoidal dose-response plot of concentrations versus maximum response. To determine if the compounds behaved as full or partial agonists in the system, the maximal response at the highest tested concentrations of compounds were compared to that produced by a known full agonist.

[00334] The EC_{50} values for inhibition of cAMP formation in whole cells are shown in Table 12. Oligomeric PEG conjugates of morphine, codeine and hydroxycodone were full agonists at the mu opioid receptor, albeit with varying efficacies. Morphine and its conjugates were the most potent of the three series of opioids tested, while the PEG hydroxycodone and PEG codeine conjugates displayed significantly lower efficacies. A progressive decrease in the efficacy of the PEG-morphine conjugates was observed with increasing PEG size, however the conjugates retained mu-agonist potency to within 40X of parent. In contrast to the effect at the mu opioid receptor, morphine and PEG-morphine conjugates behaved as weak partial agonists at

the kappa opioid receptor, producing 47-87% of the maximal possible response. EC_{50} values could not be calculated for the codeine and hydroxycodone conjugates at the kappa and delta opioid receptors since complete dose-response curves could not be generated with the range of concentrations tested (upto 500 μM).

[00335] Overall, the results of the receptor binding and functional activity indicate that the PEG-opioids are mu agonists *in vitro*.

Table 12. ***In vitro*** **efficacies of PEG-opioid conjugates**

COMPOUND	MU OPIOID RECEPTOR		KAPPA OPIOID RECEPTOR		DELTA OPIOID RECEPTOR
	EC_{50}, nM	% max effect	EC_{50}, nM	% max effect	
Morphine	28.5	102	624	69	N/A
α-6-mPEG$_1$-O-Morphine	85.0	91	1,189	81	N/A
α-6-mPEG$_2$-O-Morphine	93.3	91	641	87	N/A
α-6-mPEG$_3$-O-Morphine	270	100	4,198	82	N/A
α-6-mPEG$_4$-O-Morphine	128	100	3,092	77	N/A
α-6-mPEG$_5$-O-Morphine	157	95	2,295	71	N/A
α-6-mPEG$_6$-O-Morphine	415	98	3,933	62	N/A
α-6-mPEG$_7$-O-Morphine	508	90	4,237	57	N/A
α-6-mPEG$_9$-O-Morphine	1,061	87	4,417	47	N/A
Oxycodone	478	95	N/A	N/A	N/A
Hydroxycodone	3,162		N/A	N/A	
α -6-mPEG$_1$-O-Hydroxycodone	3,841	102	N/A	N/A	N/A
α -6-mPEG$_2$-O-Hydroxycodone	5,005	101	N/A	N/A	N/A
α -6-mPEG$_3$-O-Hydroxycodone	2,827	108	N/A	N/A	N/A
α -6-mPEG$_4$-O-Hydroxycodone	3,715	109	N/A	N/A	N/A
α -6-mPEG$_5$-O-Hydroxycodone	5,037	108	N/A	N/A	N/A
α -6-mPEG$_6$-O-Hydroxycodone	12,519	102	N/A	N/A	N/A
α -6-mPEG$_7$-O-Hydroxycodone	7,448	101	N/A	N/A	N/A
α -6-mPEG$_9$-O-Hydroxycodone	17,948	95	N/A	N/A	N/A
Codeine	10,418	81	N/A	3	N/A
α-6-mPEG$_1$-O-Codeine	8,574	80	N/A	51	N/A
α-6-mPEG$_2$-O-Codeine	5,145	75	40,103	59	N/A
α-6-mPEG$_3$-O-Codeine	19,740	91	N/A	49	N/A
α-6-mPEG$_4$-O-Codeine	22,083	99	N/A	61	N/A
α-6-mPEG$_5$-O-Codeine	23,235	95	N/A	60	N/A
α-6-mPEG$_6$-O-Codeine	97,381	80	N/A	21	N/A
α-6-mPEG$_7$-O-Codeine	44,729	75	N/A	48	N/A
α-6-mPEG$_9$-O-Codeine	48,242	80	N/A	61	N/A

EXAMPLE 21

Brain:Plasma Ratios of mPEG$_n$-O-Opioid Conjugates

[00336] The ability of oligomeric mPEG-O-morphine, mPEG-O-codeine and mPEG-O-hydroxycodone conjugates to cross the blood brain barrier (BBB) and enter the CNS (central nervous system) was assessed by measuring the brain:plasma ratio in rats subsequent to IV administration.

[00337] Briefly, groups of 3 rats were injected intravenously (i.v) with 5 mg/kg each of morphine, mPEG$_n$-O-morphine conjugate, codeine and m-PEG$_n$-O-codeine conjugates. PEG-2,3 and 4-oxycodone conjugates were administered at 10mg/kg i.v. and oxycodone and the other PEG sizes of oxycodone conjugates were administed at 1 mg/kg (i.v). The doses of the oxycodone conjugates had to be adjusted to allow for the detection of sufficient concentrations in brain tissue. Atenolol, which does not cross the BBB, was used as a measure of vascular contamination of the brain tissue and was administered at a concentration of 10 mg/kg to a separate group of rats. An hour following injection, the animals were sacrificed and plasma and the brain were collected and frozen immediately. Following tissue and plasma extractions, concentrations of the compounds in brain and plasma were measured using LC-MS/MS. The brain:plasma ratio was calculated as the ratio of measured concentrations in the brain and plasma. The results are shown in FIGS. 16A-C.

[00338] FIGs 14A, 16B, and 16C show the brain:plasma ratios of various oligomeric mPEG$_n$-O-morphine, mPEG$_n$-O-codeine, and PEG$_n$-O-hydroxycodone conjugates, respectively. The brain:plasma ratio of atenolol is shown in each figure to provide a basis for comparison. PEG-conjugation results in a decrease in the brain:plasma ratio of all conjugates compared to their respective unconjugated parent molecule, which in the case of hydroxycodone is oxycodone. Only PEG-1-morphine displayed a greater brain:plasma ratio than its parent, morphine.

EXAMPLE 22

Time-Course of Brain and Plasma Concentrations of Various Exemplary mPEG$_n$-O-Opioid Conjugates

[00339] Experiments were conducted to determine the concentrations of various oligomeric PEG-opioid conjugates in brain and plasma over time following IV administration.

[00340] The experimental methodology and concentrations used were the same as those used for the single time point experiments described in Example 21, however, the brains and plasma were harvested at various differing time points.

[00341] All PEG-hydroxycodone conjugates were administered at 10 mg/kg iv, while the oxycodone parent was administered at 1 mg/kg iv. The data for the brain and plasma concentrations versus time for the various PEG-opioid conjugates administered is shown in FIGS. 15A-H (morphine series), FIGS. 16A-H (codeine series), and FIGS. 17A-H (oxycodone/hydroxycodone series).

[00342] The data demonstrate that the maximal increase in brain concentrations for all parent molecules and oligomeric PEG-conjugates occurs at the earliest time point, i.e., 10 minutes following iv injection. PEG conjugation results in a significant reduction in the brain concentrations and with the larger PEG conjugates (≥ PEG-4), the brain concentrations remain relatively low and steady over time.

EXAMPLE 23

In Vivo Analgesis Assay: Acetic Acid Writhing in Mice

[00343] The analgesic potencies of oligomeric PEG-opioid conjugates, mPEG$_n$-O-morphine and mPEG$_n$-O-hydroxycodone, were determined using an acetic acid writhing assay in mice.

[00344] Mice were given a single "pretreatment" orally of an analgesic or control solution 30 minutes prior to intraperitoneal administration of 0.5% acetic acid (0.1 mL/10 g bodyweight). Acetic acid induces "writhing" which includes: contractions of the abdomen, twisting and turning of the trunk, arching of the back and the extension of the hindlimbs. After the injection the animals were placed in an observation beaker and their behavior was observed. Contractions were counted in four x five minute segments, between 0 and 20 minutes after the acetic acid injection. The animals were used once and euthanized immediately following the completion of the study. Each compound was tested at dose range of 1-100 mg/kg.

[00345] FIG. 21 and FIG. 22 show the results of the acetic acid writhing assay for mPEG$_n$-hydroxycodone (n=1-7) and mPEG$_n$-O-morphine (n=3,4,5,7) conjugates, respectively. The oligomeric PEG-opioid conjugates were found to exhibit analgesic potencies, as can be seen by their ability to prevent writhing in mice following acetic acid injection.

EXAMPLE 24

Abuse Liability Study: Assessment of the Relative Reinforcing Efficacy of Exemplary Oligomeric PEG Opioid Conjugates Compared to Non-Oligomer Containing Opioids in Rats Trained to Self-Administer Cocaine

[00346] The objective of the study was to assess the relative reinforcing efficacy of various test articles including α-6-mPEG$_6$-O-hydroxycodone, oxycodone, and hydrocodone relative to cocaine (positive control article) and saline (negative control) in Sprague-Dawley rats conditioned to self-administer cocaine during daily access periods. Three-day substitution test sessions were instituted in rats trained to self-administer cocaine, where complete substitution is defined as drug-maintained lever-press responding for three consecutive days at levels similar to that which is maintained by the maintenance dose of cocaine.

[00347] Animals were trained to self-administer 0.56 mg/kg/injection of cocaine in a standard operant single-lever training procedure. To date, the particular history used to establish a drug as a reinforcer has not been shown to control the later behavior maintained by the drug.

(Griffiths RR, et al., T Thompson, PB Dews (Eds), *Advances in Behavioral Pharmacology,* 1979, New York:Plenum Press, 2, 163-208; Johanson CE, Balster RL, *Bulletin Narcotics* , 1979, 30:43-50). The factors controlling a behavioral repertoire prior to establishment of a contingent

relationship between behavior and drug delivery may be more important to the initiation than to the maintenance of drug-reinforced responding. Once contingent cocaine drug delivery has gained control of a behavioral repertoire, the development and maintenance of future behavior appear controlled primarily by prevailing access conditions rather than by the conditions important in initially establishing the drug as a reinforcer.

[00348] Training/Maintenance Session: Animals were trained to operate the lever using a method of successive approximations to shape the animals' behavior to the lever using a food pellet delivery system as a reward. A single lever press response delivered a single food pellet. Initially animals respond on the lever one time to receive a single food pellet. Over successive training sessions, the number of responses required to earn a reinforcer was raised to 10.

[00349] Once the lever press response was learned by the animal at an FR1 (fixed ratio = FR) response contingency, the total number of responses required to earn a reinforcer (food or drug) was raised up to 10 consecutive responses (FR10) over consecutive daily training sessions. When responding for food, the animal was switched over to drug reinforcement by pairing the lever press responding with both food and drug deliveries for a single training session. Once shifted over to drug reinforcers, food deliveries were ceased and the animal responded on the lever solely for the delivery of drug infusions. When an animal was shifted from food to drug reinforcement, the rate at which the fixed ratio requirements was raised was dependent upon the observed behavior of each animal and no pre-set criteria. For this part of training the animals remained attached to an infusion pump via a swivel tether system.

[00350] For initial drug self-administration training each animal could press 10 consecutive times on a lever to deliver a single bolus of 0.56 mg/kg/infusion of cocaine through the catheter system (this was dependent on the actual FR component during training, i.e. from 1 to 10). Cocaine was used because of its' robust reinforcing properties which have been shown within and between operant conditioning laboratories to establish rapid lever-press responding to minimize the initial operant training period. The maximum number of drug deliveries was set at 10 during a one hour access period with a 10 second time out between the end of the infusion and the opportunity to respond for the next injection. Once trained and stable response rates were demonstrated the animals were tested.

[00351] Test Session: Each animal was allowed to press 10 consecutive times on a lever to deliver a single bolus of cocaine or saline (doses as described in the Study Design Table below). There was no maximum number of injections earned during this one hour session conducted for three consecutive days. A 10 second time out was required between the end of the infusion and the opportunity to respond for the next injection.

[00352] Substitution Session: Each animal was allowed to press 10 consecutive times on a lever to deliver a single bolus of a selected dose of test article or its vehicle. There was no maximum number of injections during this one hour session conducted for three consecutive days. A 10 second time out was required between the end of the infusion and the opportunity to respond for the next injection.

[00353] Reinforcer Efficacy Substitution Test Procedures (Progressive Ratio): The rate of self-administration is determined not only by reinforcing effects of drug but also by the direct effects of the drug on motor behavior. To measure reinforcing effects in a quantitative way, a procedure was utilized in which responding was determined by reinforcing effects uncontaminated with other drug effects. Reinforcing efficacy has been used by behavioral pharmacologists to refer to the magnitude of a drug's reinforcing effects. See, e.g., Griffiths RR, Brady JV, Bradford LD. *Advances in Behavioral Pharmacology (Vol 2).* T Thompson, PB Dews (Eds) 1979; New York:Plenum Press, pp163-208.

[00354] For each dose of the test article which maintained self-administration of compound over three consecutive days at levels that were similar to those levels maintained by cocaine, a second substitution test was conducted under a progressive ratio (PR) schedule of drug delivery. Under this schedule, the animal responds on the lever for delivery of test article. For each subsequent drug delivery the total number of responses emitted on the lever required for drug delivery was incremented upward using a logarithmic (base e) scale using the following equation:

$$\text{Response requirement} = 5 * e^{(\text{response increment} * 0.2)} - 5$$

The progressive "break point" is defined as the highest number of responses emitted by the animal to earn a single reinforcer delivery of drug or vehicle. This break point is used as a

behavioral marker of how much work will be expended by an experimental subject to earn a single reinforcer delivery. The amount of work expended to earn a single reinforcer is used to compare the efficacy of drug deliveries with respect to the hedonic valence induced by the drug injection with the assumption that the subjective hedonic valence of a reinforcer determines its abuse liability.

[00355] Criteria for establishment of the test article as a "reinforcer": A test article dose was considered to fully substitute for the maintenance dose of cocaine if the total number of injections of self-administered drug was equivalent to the total number of injections engendered by the maintenance dose (0.56 mg/kg/infusion) of cocaine or maintained a stable number of injections across the three consecutive days of substitution. If the total number of injections declined over the course of the three day substitution period or there was clear "vehicle-like" response topography, then the drug was considered as an ineffective reinforcer.

[00356] Dosing: The dosing regime is outlined in Table 13 and described below.

Table 13. Dosing Summary

TREATMENT	Maintenance DOSE (mg/kg/infusion)*	NUMBER OF ANIMALS
		Males[a]
1	0 (saline, vehicle)	18-24 May be up to 100
	Cocaine Maintenance Drug Dose (mg/kg)	
2	0.032 mg/kg/infusion	≥ 6
3	0.1 mg/kg/infusion	≥ 6
4	0.32 mg/kg/infusion	≥ 6
5	0.56 mg/kg/infusion	18-24 May be up to 100
6	1.0 mg/kg/infusion	≥ 6

[00357] Doses for test articles are provided in Fig. 23. Doses (mg/kg/infusion) evaluated were as follows: 0.032, 0.1, 0.32, and 0.56 cocaine, 0.18 hydrocodone, 0.032 oxycodone, 0.01 oxycodone, 0.032, 0.01, 0.32, and 1.0 α -6-mPEG$_6$-O-hydroxycodone.

[00358] Treatments were given to the same trained animals with appropriate training and washout days between treatments. Animals were conditioned to self-administer cocaine. Once self-administration was established with cocaine, various doses of the maintenance drug and its vehicle as well as the test article and its vehicle were administered during 60 minute access periods over the course of three consecutive days in a pseudorandom order. The first two tests in the series of tests were with 0.56 mg/kg/injection of cocaine and saline to clearly identify that animals were self-administering the maintenance dose of cocaine prior to initiating any other test sessions. The "total dose" administered to the animal is expressed as the total "self-administered" dose delivered in the session.

[00359] Substitution tests were interspersed with cocaine maintenance training sessions. Three consecutive cocaine self-administration sessions with less than 20% variability were imposed between substitution tests (i.e., test article) to ensure stable drug maintained lever press responding was demonstrated. During the syringe drive system setup and both maintenance and/or training sessions, an initial bolus priming injection of the syringe formulation was optionally given to the animal to insure that the animals' catheter line had been adequately 'primed' for the session and to signal or cue the animal to the initiation of a session.

[00360] The volumes were approximately 35 to 100 μL per infusion for cocaine and 100 μL per infusion for the test articles. The volumes were limited to less than 1 ml per infusion.

Table 14.

Treatment	**Dose mg/kg/infusion**	**Concentration mg/mL**	**Infusion Duration (seconds)**	**Volume (μL)**	**Maximum Number of Injections**
Cocaine	0	0	3 to 14	35 to 100	none
	0.032	0.224	3 to 14	35 to 100	none
	0.1	0.7	3 to 14	35 to 100	none
	0.32	2.24	3 to 14	35 to 100	none
	0.56	3.92	3 to 14	35 to 100	none
	1.0	7.0	3 to 14	35 to 100	none
Test Articles	see above for doses	See above for doses			
			3 to 14	35 to 100	none
			3 to 14	35 to 100	none
			3 to 14	35 to 100	none
			3 to 14	35 to 100	none

-Standard convention is used: 0 mg/kg cocaine or test article dose refers to vehicle

-For all test articles, infusion duration was 3-14 seconds, volume was 35-100 μL, and there was no number of maximum injection.

[00361] In the substitution studies carried out, no reinforcing behavior was observed in rats administered α -6-mPEG$_6$-O-hydroxycodone (Fig. 23B), even at the highest dose tested (1.0 mg/kg/injection), in contrast to rats administered the positive control compound, cocaine (Fig. 23A). The negative control, saline, shows extinction, i.e., is not reinforcing.

[00362] In the progressive ratio studies performed to compare quantitatively the abuse potential of various test articles, no reinforcing behavior was observed for α -6-mPEG$_6$-O-hydroxycodone at any of the doses tested. See Figs. 24A (saline control and other opioids) versus Fig. 24B (α -6-mPEG$_6$-O-hydroxycodone), where dose in mg/kg/injection is shown on the x-axis. In the progressive ratio studies, break points of 114 and 79 were observed for hydrocodone and oxycodone at unit doses of 0.18 and 0.03 mg/kg/injection, respectively, while a unit dose of 1.0 mg/kg/injection for α -6-mPEG$_6$-O-hydroxycodone produced a break point of 21, which is comparable to the level observed in saline treated rats (i.e., no reinforcing behavior observed).

EXAMPLE 25

Assessment of Side Effects in Mice Upon Administration of Various Opioids and their Oligomeric Conjugates

[00363] The objective of this study was to provide an approximation of the side effect profile of various oligomeric conjugates for comparison to the parent compounds by measuring various gross behavioral and physiological signs following administration of compound.

[00364] Comparative central nervous system activity was evaluated for oxycodone, morphine and α -6-mPEG$_6$-O-hydroxycodone along with additional oligomeric hydroxycodone and morphine conjugates using the straub tail response, which is known to reflect only CNS-mediated mu-opioid activity. (Nath, C., et al., Eur J Pharmacol. 1994, 263(1-2), p. 203-5). Additional evaluations included muscle rigidity and pinna reflex.

[00365] Male CD-1 mice from Charles River Laboratories, Raleigh, NC, weighing from 16-18 grams were maintained on a regular light/dark cycle (lights on 0600-1800) with ad libitum food and water for 1 week before commencement of testing. N =2 per treatment condition, i.e., separate mice for each dose of each test article. Injection was performed via a 25-gauge ⅝-inch needle on a 1-mL tuberculin syringe for the s.c. route and a 22-gauge stainless steel mouse feeding tube for the p.o. route (Becton, Dickinson & Co., Franklin Lakes, NJ). Each treatment condition was divided about evenly between two observers. The subcutaneous and oral doses of morphine, oxycodone, mPEG$_5$-O-hydroxycodone, mPEG$_6$-O-hydroxycodone, α -6-mPEG$_7$-O-hydroxycodone, and mPEG$_7$-O-morphine administered were as follows: 1 mg/kg, 3 mg/kg, 10 mg/kg, 30 mg/kg, and 100 mg/kg. Test article was administered (s.c. or p.o., and the mouse was placed immediately in the observation chamber). One-half, 1, 2, and 3 hours later, the animal was observed undisturbed for gross signs such as locomotor ataxia, tremor, hyperactivity, hypoactivity, convulsions, hindlimb splay, and Straub tail, and then removed and assessed for muscle tone (normal, rigid, flaccid), pinna reflex (presence or absence), righting reflex (intact or lost), and placing (whether or not the forepaws are extended when the mouse is placed near a surface).

[00366] The results provided in Tables 15, 16, and 17 are for oral administration, and provide comparative results for two known opioid compounds, oxycodone and morphine, along with mPEG$_6$-O-hydroxycodone.

Table 15. Straub Tail Effect

Dose (mg/kg)	Oxycodone	Morphine	mPEG$_6$-O-hydroxycodone
1	1	0	0
3	2	2	0
10	2	2	0
30	2	2	0
100	2	1	0
300	2	1	1

[00367] Each of the tables show the number of animals responding at each dose, where N=2. An entry of "2" indicates that 100% of animals responded; an entry of one indicates that 50% of animals responded. A bolded value indicates the lowest dose at which a response was detected. The lowest response at which α -6-mPEG$_6$-O-hydroxycodone caused a detectable response in the straub test was the highest dose tested, 300 mg/kg. At oral doses up to 100 mg/kg, where maximal analgesia was obtained with oral doses of 14 mg/kg for oxycodone, 20 mg/kg for morphine, and 100 mg/kg for α -6-mPEG$_6$-O-hydroxycodone, the straub tail response was observed in 100 percent of mice treated with morphine and oxycodone, but in none of the mice treated with α -6-mPEG$_6$-O-hydroxycodone.

Table 16. Muscle Rigidity

Dose (mg/kg)	**Oxycodone**	**Morphine**	**mPEG$_6$-O-hydroxycodone**
1	0	0	0
3	0	0	0
10	**2**	**1**	0
30	2	2	0
100	2	2	**1**
300	2	2	1

Table 17. Loss Pinna

Dose (mg/kg)	Oxycodone	Morphine	mPEG$_6$-O-hydroxycodone
1	0	0	0
3	0	2	0
10	1	1	0
30	1	2	0
100	2	2	0
300	2	2	0

[00368] The results above demonstrate a 10-100 fold decrease in CNS activity for α -6-mPEG$_6$-O-hydroxycodone.

[00369] Table 18 provides a summary of CNS responses for each of the compounds evaluated, where the value in the table for columns 4, 5, 6, and 7 represents the fold difference (i.e., reduction in CNS activity) relative to the corresponding parent compound, i.e., oxycodone or morphine.

Table 18. Summary of CNS Responses

Test	Mode	# / 2 responding	PEG-5-Oxy	PEG-6-Oxy	PEG-7-Oxy	PEG-7-Mor
Straub Tail	SC	2/2	100	100	10	100
		1/2	10	100	10	>100
	PO	2/2	>100	>100	100	>100
		1/2	100	100	30	>100
Muscle Rigidity	SC	2/2	33	100	30	3.3
		1/2	3	100	>300	>30
	PO	2/2	10	>30	30	>10
		1/2		>100		3
Loss Pinna	SC	2/2	10	33	100	100
		1/2	>10	>30		>30
	PO	2/2	1	>>	30	>100
		1/2	1	>>		>30

[00370] As can be seen from the above results, CNS-side effects associated with administration of the above oligomeric opioid compounds in mice was significantly decreased, i.e., from 10 to over 100 times, when compared to the corresponding opioid absent oligomer.

EXAMPLE 26

Assessment of Motor Coordination in Male Sprague-Dawley Rats Using the Rotarod Treadmill Upon Administration of an Illustrative Opioid and Its Oligomeric Conjugate

[00371] The following studies were conducted to evaluate the effect of orally administered PEG-6-O-hydroxycodone on motor coordination (i.e., sedation) in rats using the rat rotarod treadmill. Motor coordination was evaluated at 0.5 h and 1 h post-dose.

[00372] Sprague-Dawley male rats were maintained 2-3 per cage on a regular light/dark cycle (lights on 0600-1800) with *ad libitum* food (Purina Rodent Chow 5002) and water. The rats weighed between 240 to 280 grams on the day of study. Rats were not fasted prior to dosing.

[00373] Animals were trained to run on the treadmill for 2 consecutive days prior to the day of study. Animals were trained at a constant speed of 10 RPM and were given as many trials as necessary until they were able to stay on the rotarod for 300 seconds. Rats that were able to stay on the rod for 300 seconds were considered trained. On the day of study, animals were placed on the rotarod treadmill at 0, 30, and 60 minutes post dose. The treadmill was set at a constant speed of 4 RPM for 15 seconds, at this point the timer was started (T=0). After this 15 second period, the rotarod was set to accelerate from 4-40 RPM over a five minute period (using the built in program of the rotarod). The time (in seconds) that each animal stopped running and tripped the plate was recorded as the animal's run time. Animals that ran for 300 seconds were taken off the treadmill and 300 seconds was recorded as the run time.

[00374] Sterile Injectable Saline was used as the vehicle/negative control (Abbott Labs, Abbott Park, IL, Cat# 07-8009416, Lot# 73-505KL).

[00375] The objective of this study was to evaluate the effect of 10, 30, 100, and 300 mg/kg of PEG-6-O-hydroxycodone on motor coordination in rats using the rotarod treadmill. All doses were administered orally and evaluated at 0.5 h and 1 h post-dose. Animals dosed with 30 mg/kg PEG-6-O-hydroxycodone showed a reduction in time spent on the rotarod at 0.5 h post-dose compared to the saline control group. Animals dosed with 10 mg/kg, 100 mg/kg, or 300 mg/kg PEG-6-O-hydroxycodone did not exhibit impaired rotarod performance, compared to the saline control group, at 0.5 h and 1 h post-dose.

[00376] Due to the results seen in the initial study, the study was repeated. When the study was repeated, animals dosed with 300 mg/kg PEG-6-O-hydroxycodone showed a reduction in time spent on rotarod at 0.5 h and 1 h post-dose, compared to the saline control group. Animals dosed with 10 mg/kg, 30 mg/kg, or 100 mg/kg PEG-6-O-hydroxycodone did not demonstrate impaired rotarod performance, compared to the saline control group, at 0.5 h and 1 h post-dose. See Fig. 25.

[00377] Overall, oral administration of 300 mg/kg PEG-6-O-hydroxycodone showed a slight motor impairment at 0.5 h and 1 h post-dose. All other doses (10, 30, 100 mg/kg) failed to impair rotarod performance. That is to say, the illustrative oligomeric PEG-opioid compound evaluated produces less sedation than the parent opioid, oxycodone, at an equianalgesic dose thereby providing an additional indication of the ability of the subject compounds to reduce CNS-side effects normally associated with administration of unmodified opioids.

Example 27

Assessment of Respiratory Depression in Mice Following Administration of an Illustrative Opioid Versus Its Oligomeric PEG Conjugate

[00378] A study was undertaken to evaluate the respiratory depression associated with the administration of PEG-6-O-hydroxycodone versus oxycodone in mice.

[00379] 24 male (CD1) mice (8 to 10-weeks old upon arrival) weighing 20-28g were housed for 1 week, ear tagged and then randomized into groups based on body weight prior to the study. The animals were housed in SPF conditions. The animal housing facilities were maintained at 72° +/- 2° F with a light cycle of 12:12 hours (light:dark). Autoclaved rodent chow and water are provided *ad libitum*. The following dosing protocol was followed:

Table 19. Treatment Groups and Procedure Timing:

Group No.	N	Drug	Dose (mg/kg)	Concentration (mg/mL)
1	8	Saline	0	*
2	8	PEG-6-O-hydroxycodone	100	10
3	8	Oxycodone	30	3

* All animals dosed at 10mL/kg, vehicle was volume matched

In Vivo Measurements:

[00380] **Ventilation:** Approximate measurements of minute ventilation were carried out using Buxco unrestrained whole body plethysmographs (WBP). Digital computer aided analysis of the analog signal was used to report measurements of tidal volume, frequency of breathing, minute ventilation, inspiratory and expiratory times and flows as well as other derived measurements.

[00381] **CO_2 Challenge:** To more clearly delineate the effect of these test articless on respiratory depression, a hypercapnic ventilatory stimulus (CO_2) was added to the animals' breathing air supply to stimulate ventilation.

[00382] **CO_2 Challenge Protocol:** Mice received the drug by gavage and then were placed in the WBP. After 20 minutes, the breathing gas mixture was switched from zero grade air (21% O_2, balance N2) to 8% CO_2 (in 21% O_2, balance N_2). The mouse remained in that atmosphere for 10 minutes, after which time the chamber was flushed with zero grade air for another 20 min to allow ventilation to return to baseline. The mouse was challenged again with 8% CO_2 for 10 minutes. This process (20 min room air followed by 10 min 8% CO_2) was repeated until the mouse had been in the chamber for a total of 4 hours post test article administration. The last 2 minutes of each of the two conditions (Air or 8% CO_2) was recorded and analyzed for the following respiratory parameters: minute ventilation, respiratory frequency, tidal volume, and time of inspiration/expiration for animals in each dose group.

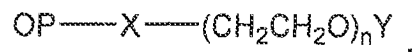
[00383] Analysis: Results are expressed as mean ± s.e.m. The appropriate statistical test used will be used. Significance will be accepted when $p<0.05$. 60 minutes of observation was determined to be sufficient to observe the effect on respiration.

[00384] As shown in Fig. 26, PEG-6-O-hydroxycodone produces less respiratory depression than oxycodone at equianalgesic doses, thereby further supporting the finding that the instant compounds advantageously reduce CNS-side effects upon administration at equi-efficaceous doses when compared to unmodified opiod.

IT IS CLAIMED:

1. A method for reducing the addiction potential and reducing one or more central nervous system (CNS) side-effects related to administration of an opioid analgesic drug (OP), the method comprising:

administering to a mammalian subject suffering from pain a therapeutically effective amount of an opioid compound having the formula:

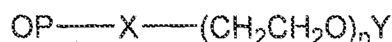


$$OP—X—(CH_2CH_2O)_nY\ ,$$

or a pharmaceutically acceptable salt form thereof,

wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group, whereby as a result of the administering, a degree of pain relief is experienced by the subject, and when evaluated in a suitable animal model, the opioid compound exhibits (i) a measurable reduction in addiction potential over the opioid analgesic drug in unconjugated form, and (ii) a ten-fold or greater reduction of at least one CNS-related side effect when compared to administration of the opioid analgesic drug in unconjugated form.

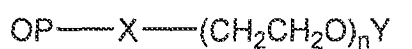
2. A method for reducing one or more central-nervous system side-effects related to administration of an opioid analgesic drug (OP) by administering the opioid analgesic drug to a mammalian subject in the following form:



$$OP—X—(CH_2CH_2O)_nY$$

wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group.

3. Use of an opioid compound having the formula:



$$OP—X—(CH_2CH_2O)_nY\ ,$$

wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group, for simultaneously reducing the addiction potential and one or more central nervous system (CNS) side-effects related to administration of the opioid analgesic drug (OP) in unconjugated form.

4. The use of an opioid compound having the formula:

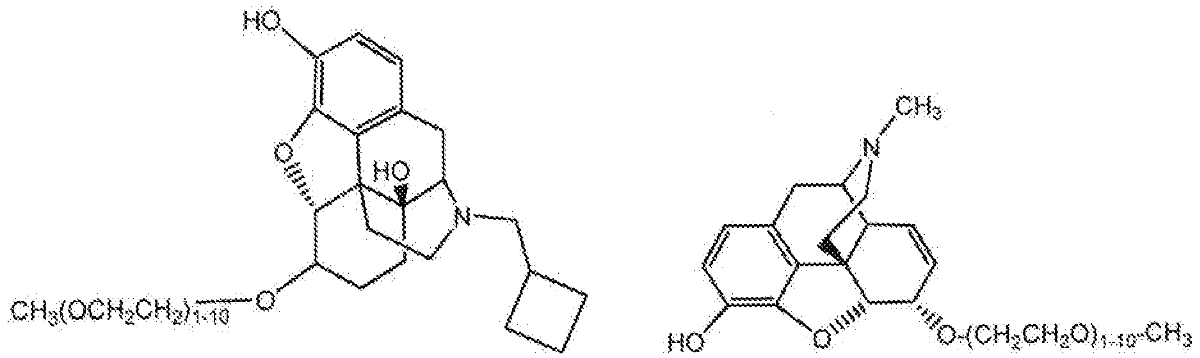
wherein OP is an opioid analgesic drug, X is a physiologically stable linker, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and Y is selected from a capping group, H, and a protecting group, for the manufacture of a medicament for reducing the addiction potential and reducing one or more central nervous system (CNS) side-effects related to administration of an opioid analgesic drug (OP).

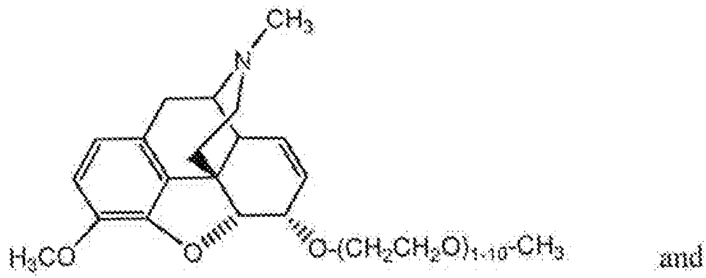
5. The method or use of any one of claims 1-4, wherein the opioid analgesic drug is a mu-opioid analgesic.

6. The method or use of any one of claims 1-5, wherein the opioid analgesic drug is selected from fentanyl, nalbuphine, hydromorphone, methadone, morphine, codeine, oxycodone, and oxymorphone.

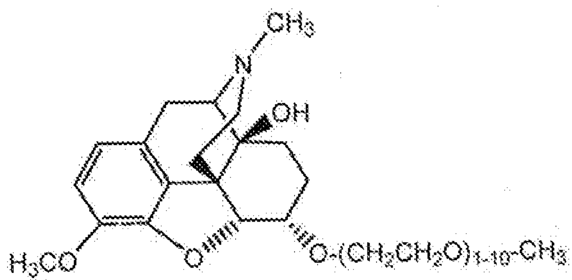
7. The method or use of claim 6, wherein X is oxygen.

8. The method or use of claim 7, wherein the opioid compound has a structure selected from:

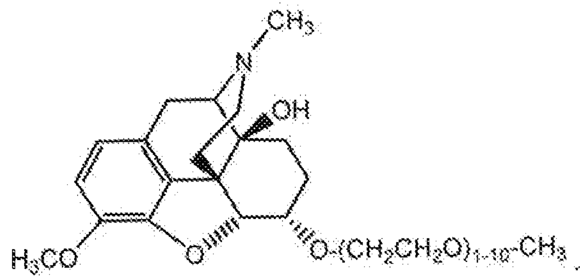




and



9. The method or use of claim 8, wherein the opioid compound has the structure:



.

10. The method of claim 1 or claim 2, wherein the administering comprises orally administering the opioid compound.

11. The method of claim 1 or claim 2, wherein the administering comprises parenterally administering the opioid compound.

12. The method of claim 1, wherein the opioid compound exhibits a measurable reduction in addiction potential over the opioid analgesic drug in unconjugated form when evaluated in an in-vivo self-administration model in rodents or primates.

13. The method of claim 1, wherein the opioid compound exhibits a ten-fold or greater reduction in at least one CNS-related side effect associated with administration of the opioid analgesic drug in unconjugated form when evaluated in a mouse model, wherein the one or more CNS-related side effects is selected from straub tail response, locomotor ataxia, tremor, hyperactivity, hypoactivity, convulsions, hindlimb splay, muscle rigidity, pinna reflex, righting reflex and placing.

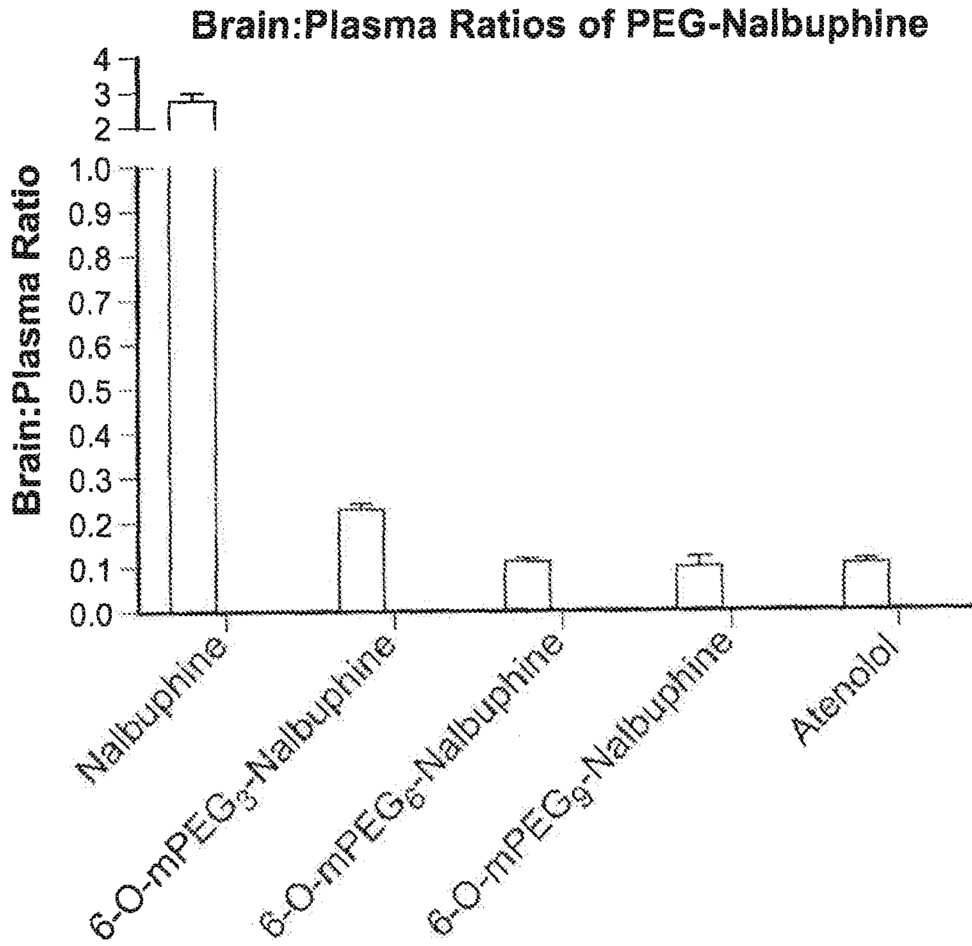
14. The method of claim 13, wherein the opioid compound exhibits a ten-fold or greater reduction in at least one CNS-related side effect associated with administration of the opioid analgesic drug in unconjugated form when evaluated in a mouse model, wherein the one or more CNS-related side effects is selected from straub tail response, muscle rigidity, and pinna reflex.

15. The method or use of any one of the foregoing claims, effective to reduce one or more central nervous system side-effects associated with administration of the opioid analgesic drug in unconjugated form in a mammalian subject selected from respiratory depression, sedation, myoclonus, and delirium.

16. The method or use of any one of the foregoing claims, wherein the amount of opioid compound provided results in both an analgesic effect and a reduction of one or more central nervous system side effects associated with administration of the opioid analgesic drug in unconjugated form in a mammalian subject.

17. The method or use of any one of the foregoing claims further comprising monitoring the patient over the course of treatment for abuse/addiction potential and/or the existence of one or more CNS-side effects associated with administration of the opioid analgesic.

18. The method or use of claim 17, wherein, in the event abuse/addiction potential and/or the existence of one or more CNS-side effects is observed, the monitoring further comprises an assessment of the degree of such abuse/addiction potential and/or CNS-side effect.



Molecule	Brain:Plasma Ratios
Nalbuphine	2.86
6-O-mPEG$_3$-Nalbuphine	0.23
6-O-mPEG$_6$-Nalbuphine	0.11
6-O-mPEG$_9$-Nalbuphine	0.10
Atenolol	0.11

FIG. 1

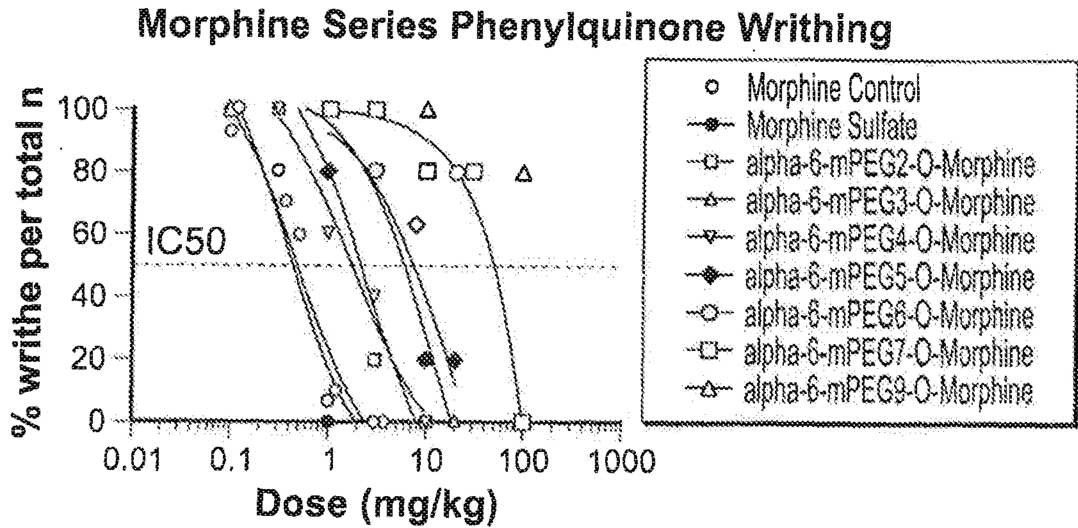


FIG. 2

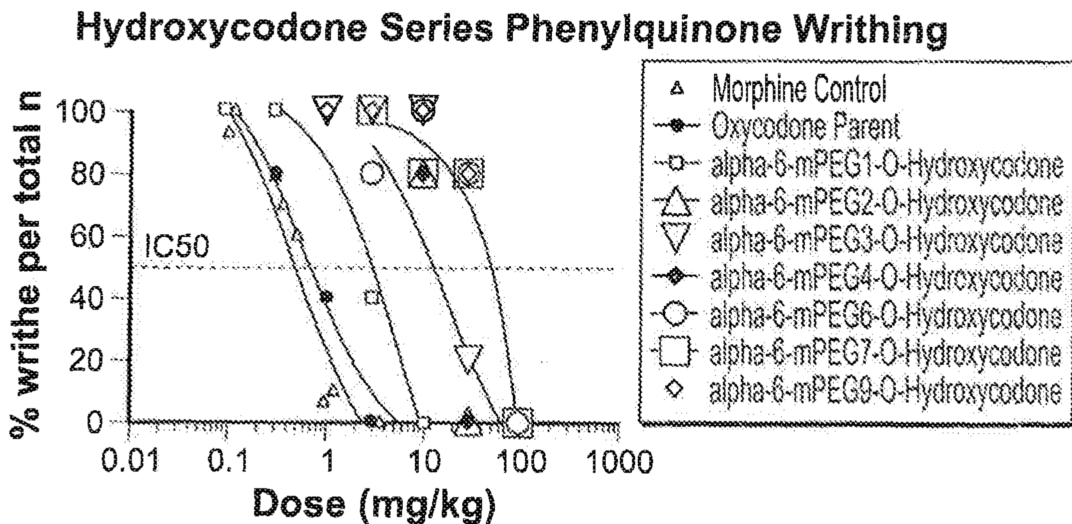


FIG. 3

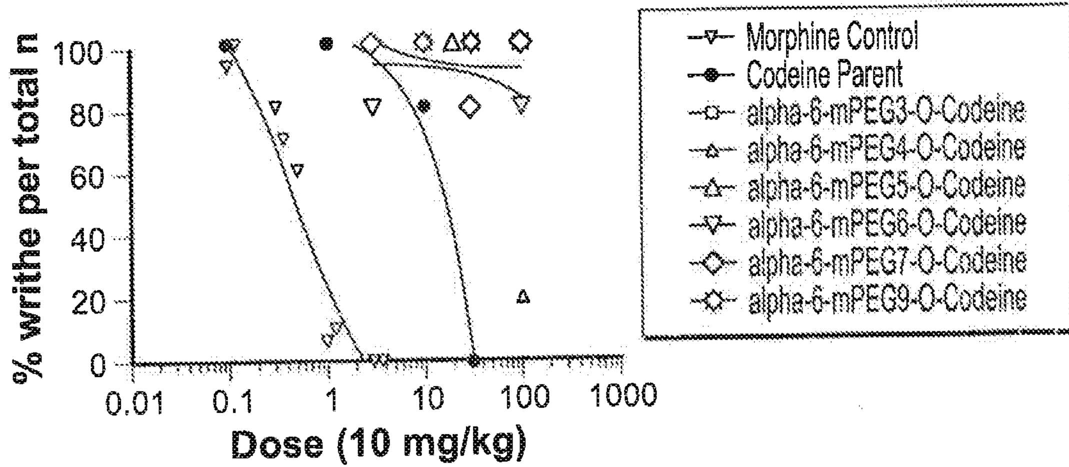
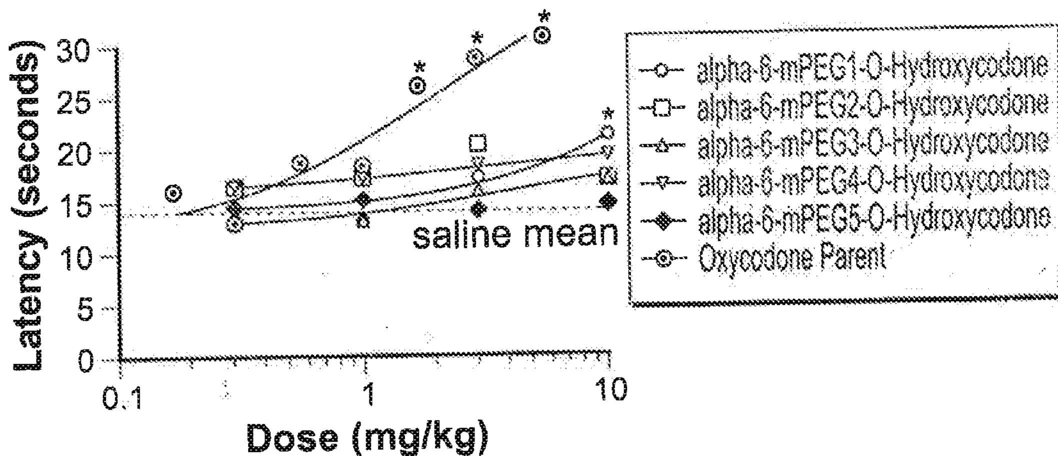


FIG. 4



* indicates $p < .05$ vs. Saline by ANOVA/Dunnett's.

FIG. 5

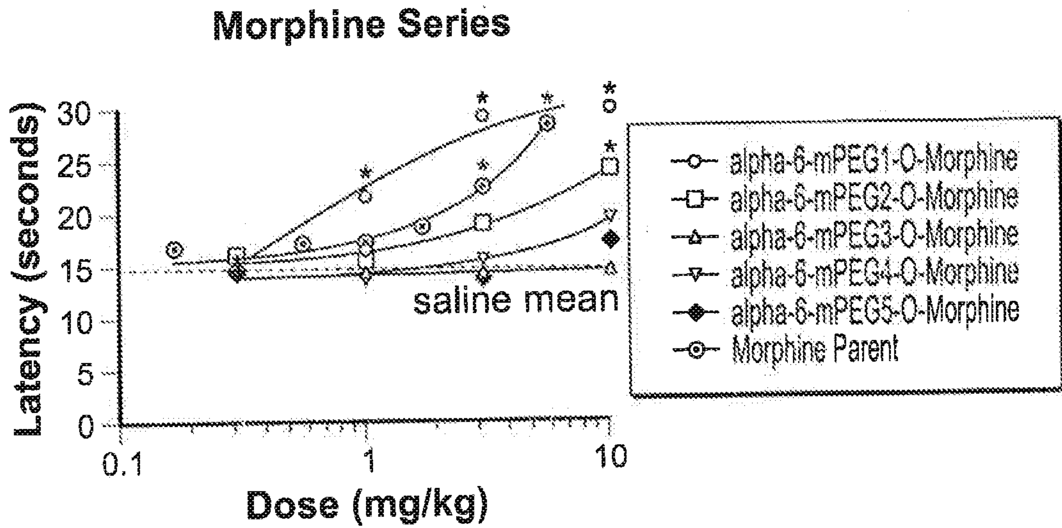


FIG. 6

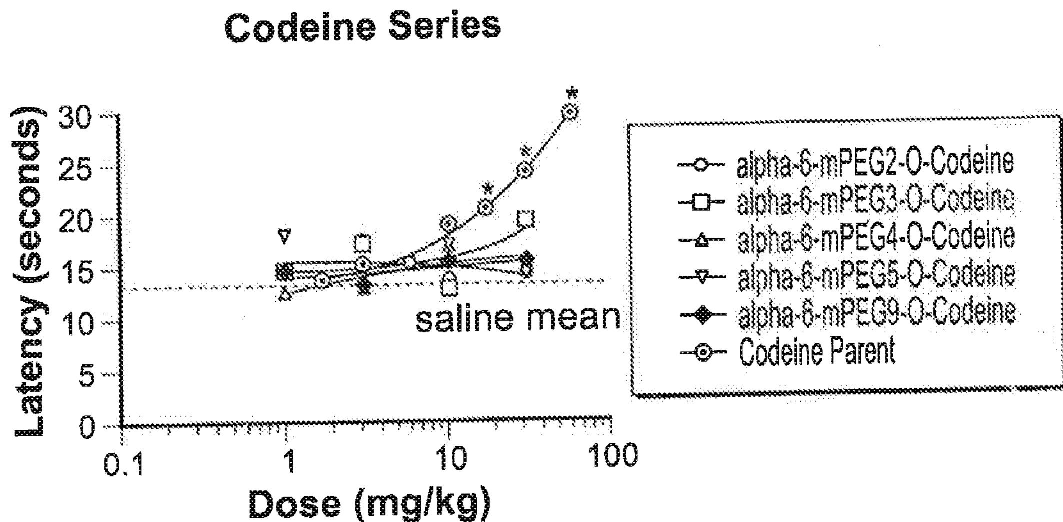


FIG. 7

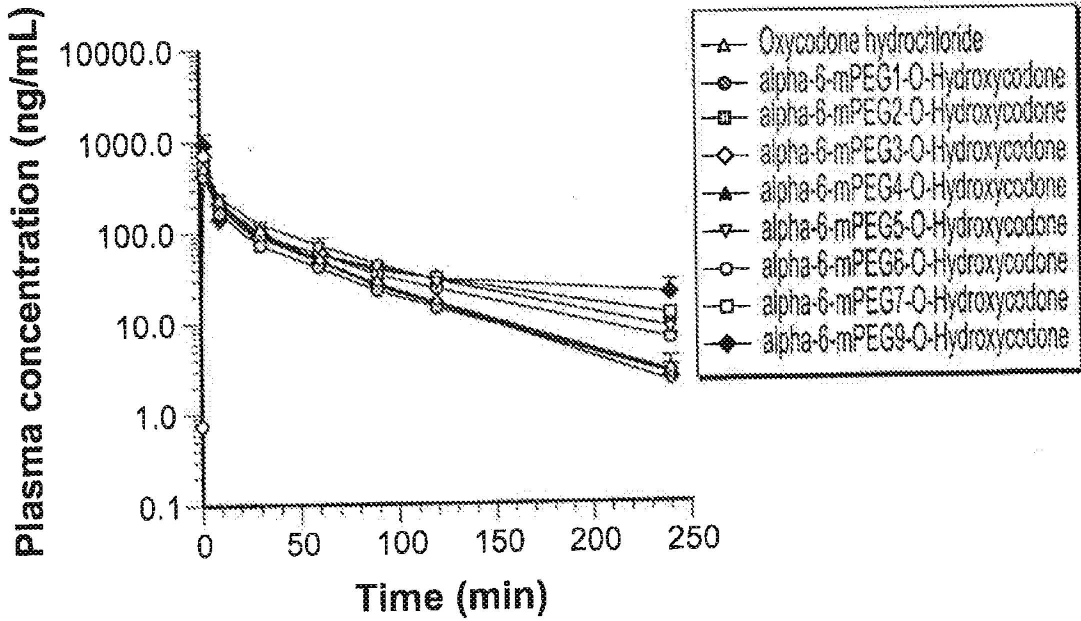


FIG. 8

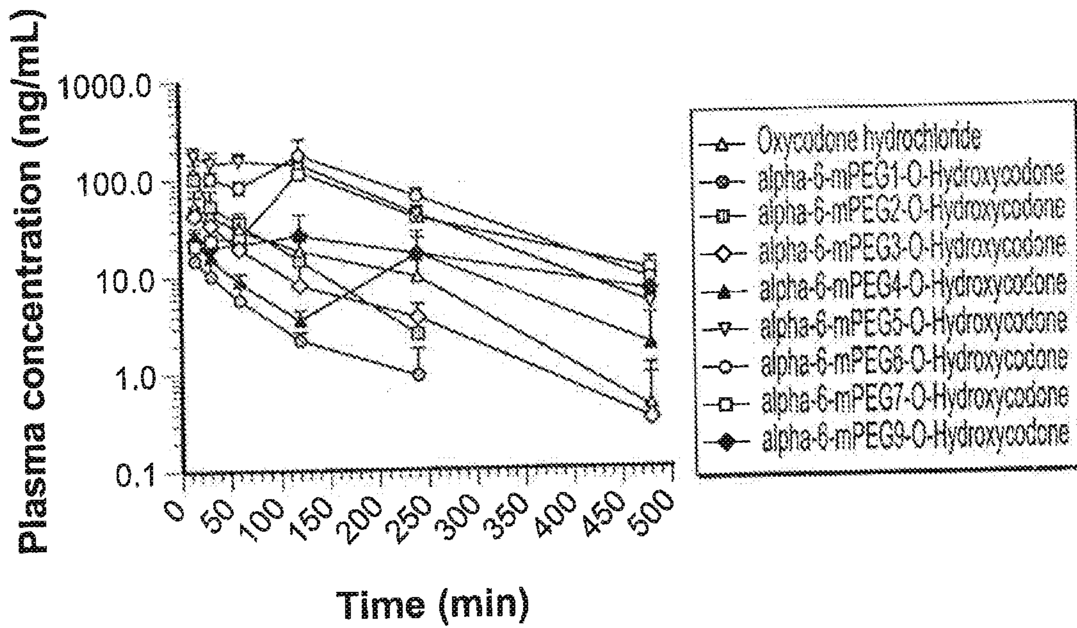


FIG. 9

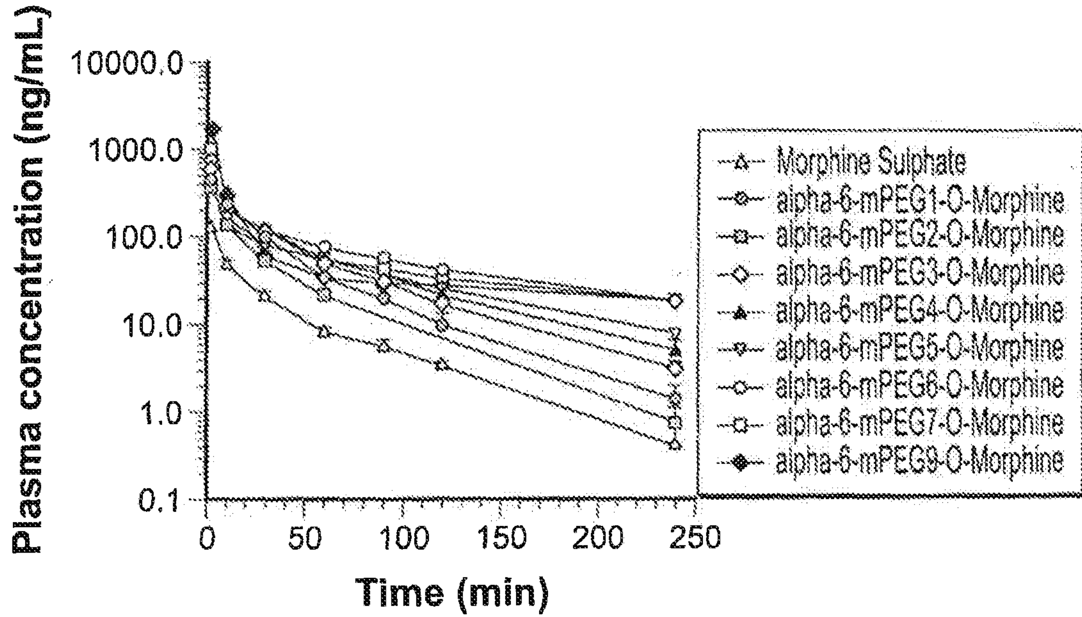


FIG. 10

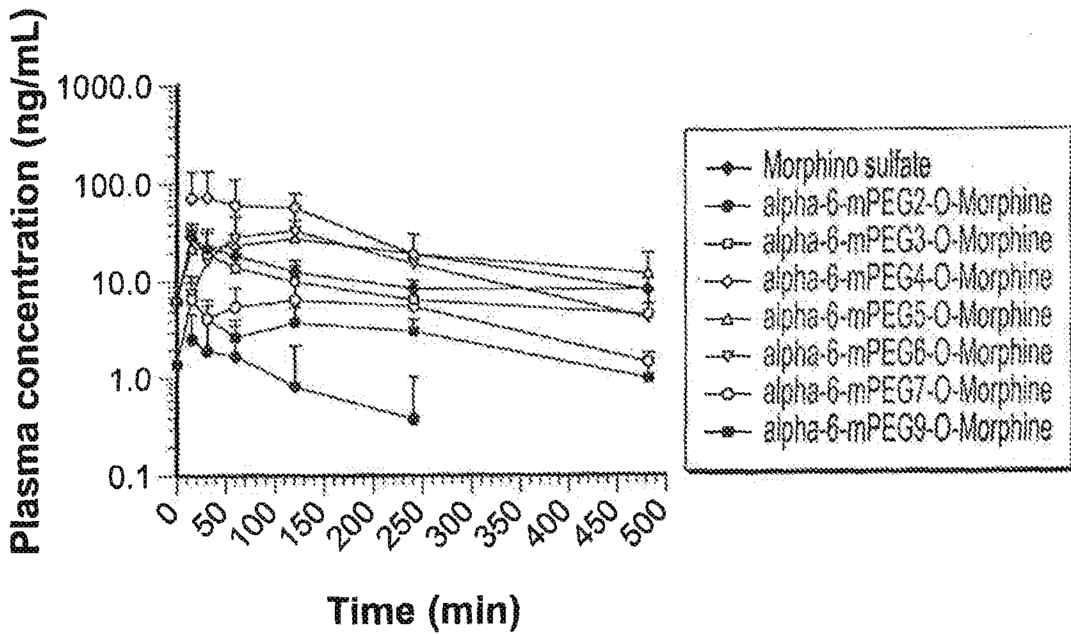


FIG. 11

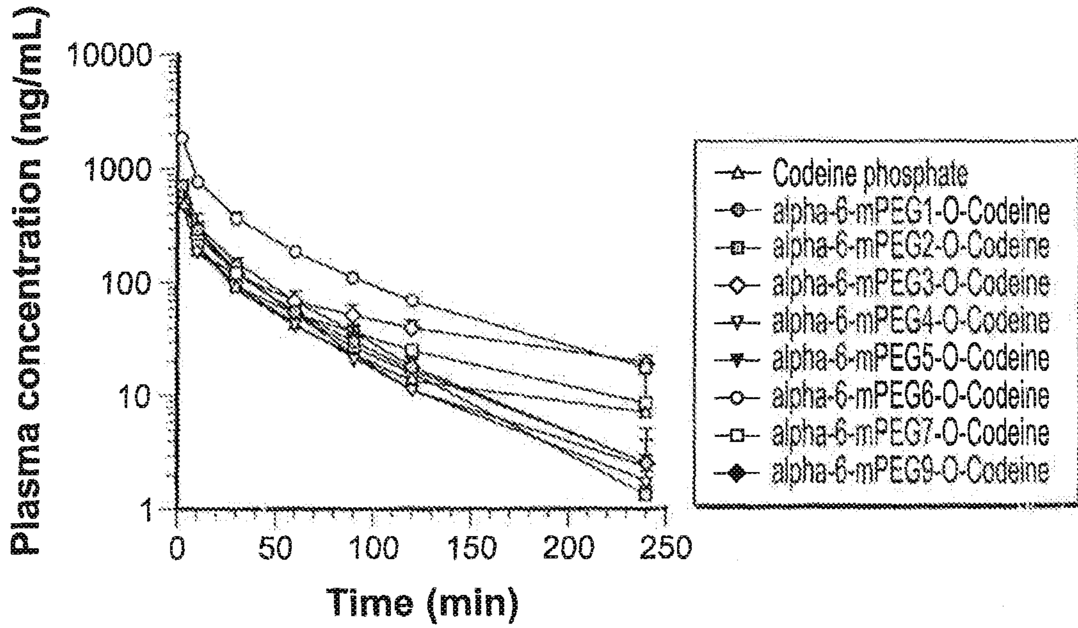


FIG. 12

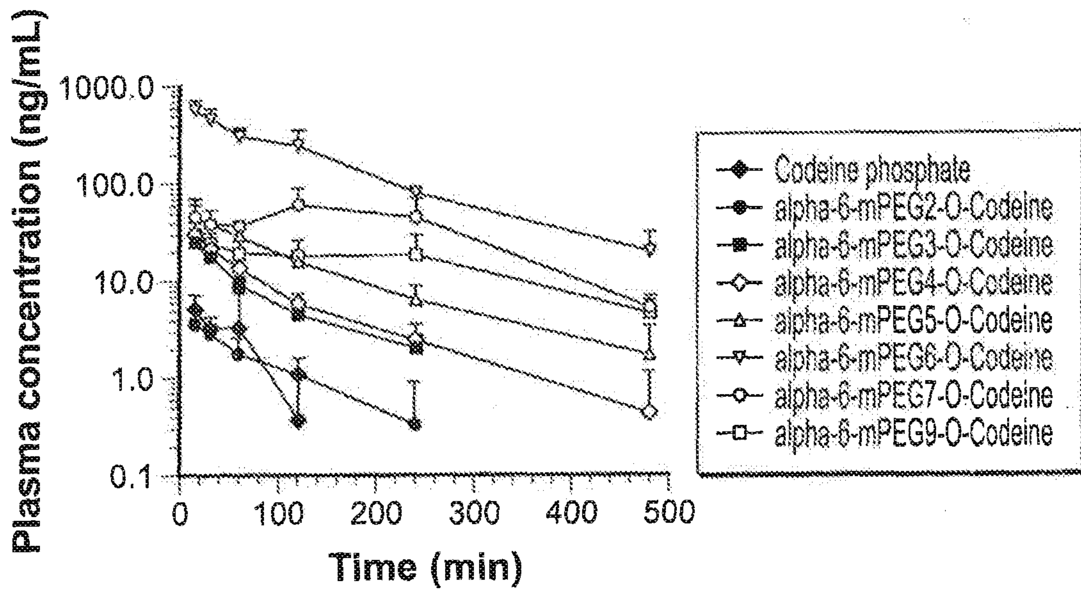
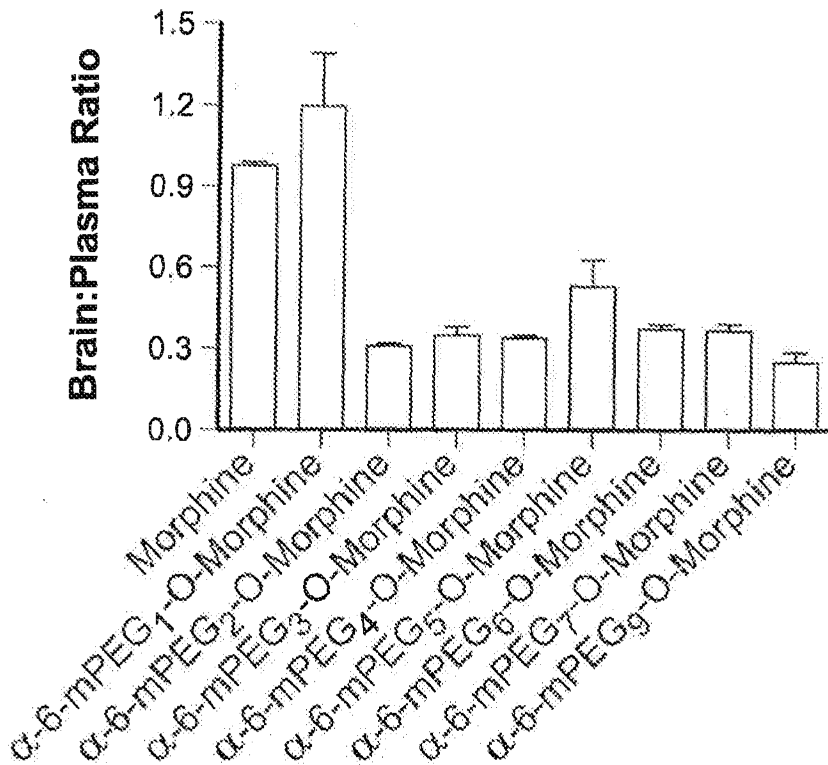
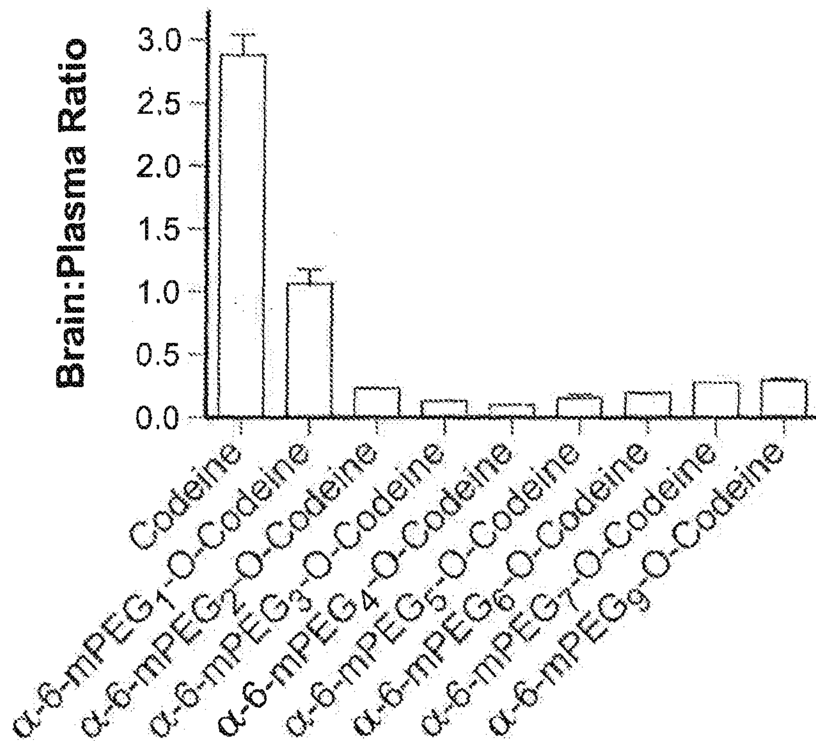


FIG. 13



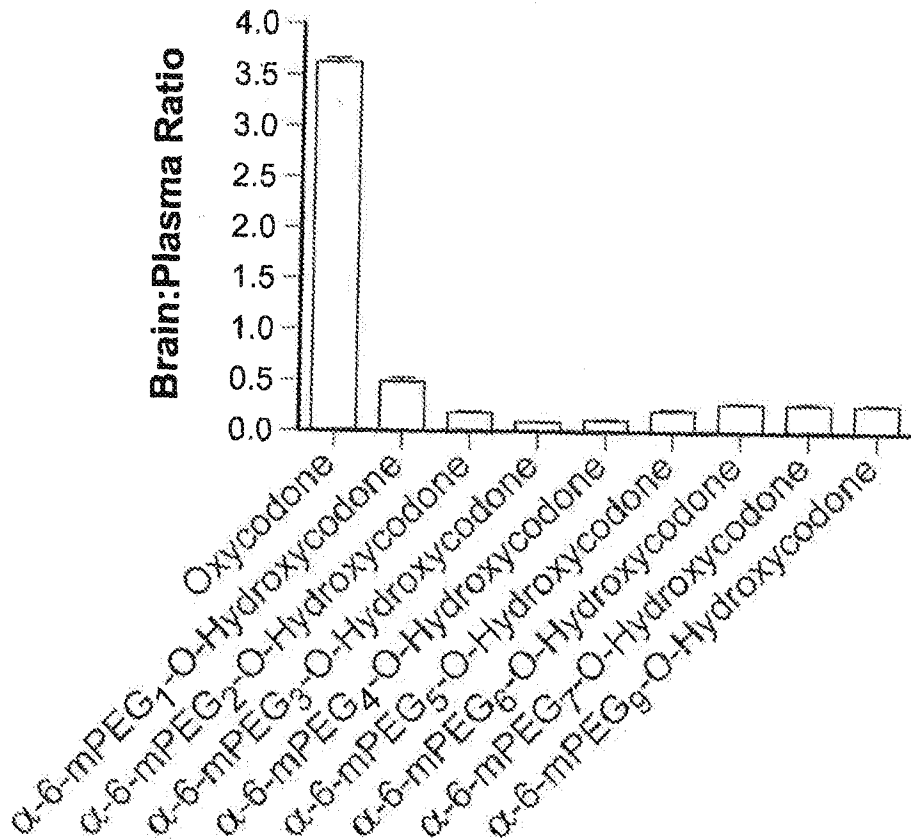
	A / B			C / D		
	Mean	SD	N	Mean	SD	N
Morphine	0.97	0.019	3			
α-6-mPEG$_1$-O-Morphine	1.19	0.341	3			
α-6-mPEG$_2$-O-Morphine	0.31	0.019	3			
α-6-mPEG$_3$-O-Morphine	0.35	0.057	3			
α-6-mPEG$_4$-O-Morphine	0.34	0.023	3			
α-6-mPEG$_5$-O-Morphine	0.53	0.179	3			
α-6-mPEG$_6$-O-Morphine	0.37	0.016	3			
α-6-mPEG$_7$-O-Morphine	0.36	0.050	3			
α-6-mPEG$_9$-O-Morphine	0.25	0.067	3			
Atenolol				0.060	0.008	3

FIG. 14A



Molecule	Brain:Plasma Ratios					
	A / B			C / D		
	Mean	SD	N	Mean	SD	N
Codeine	2.88	0.276	3			
α-6-mPEG$_1$-O-Codeine	1.06	0.214	3			
α-6-mPEG$_2$-O-Codeine	0.24	0.014	3			
α-6-mPEG$_3$-O-Codeine	0.14	0.011	3			
α-6-mPEG$_4$-O-Codeine	0.12	0.009	3			
α-6-mPEG$_5$-O-Codeine	0.16	0.049	3			
α-6-mPEG$_6$-O-Codeine	0.21	0.006	3			
α-6-mPEG$_7$-O-Codeine	0.30	0.017	3			
α-6-mPEG$_9$-O-Codeine	0.31	0.047	3			
Atenolol				0.060	0.013	3

FIG. 14B



Molecule	Brain:Plasma Ratios					
	A / B			C / D		
	Mean	SD	N	Mean	SD	N
Oxycodone	3.63	0.073	3			
α-6-mPEG$_1$-O-Hydroxycodone	0.47	0.108	3			
α-6-mPEG$_2$-O-Hydroxycodone	0.18	0.008	3			
α-6-mPEG$_3$-O-Hydroxycodone	0.10	0.006	3			
α-6-mPEG$_4$-O-Hydroxycodone	0.12	0.028	3			
α-6-mPEG$_5$-O-Hydroxycodone	0.21	0.029	3			
α-6-mPEG$_6$-O-Hydroxycodone	0.29	0.026	3			
α-6-mPEG$_7$-O-Hydroxycodone	0.29	0.020	3			
α-6-mPEG$_9$-O-Hydroxycodone	0.27	0.039	3			
Atenolol				0.059	0.015	3

FIG. 14C

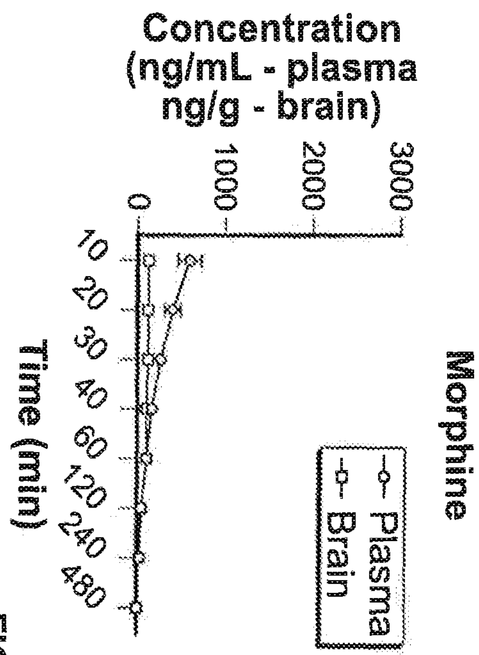


FIG. 15A

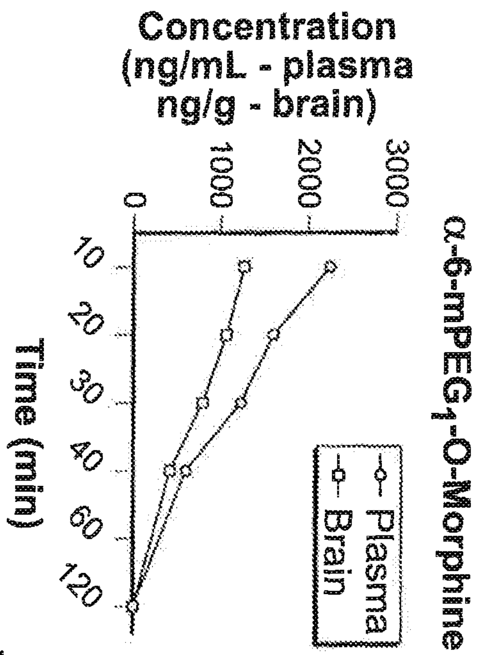


FIG. 15B

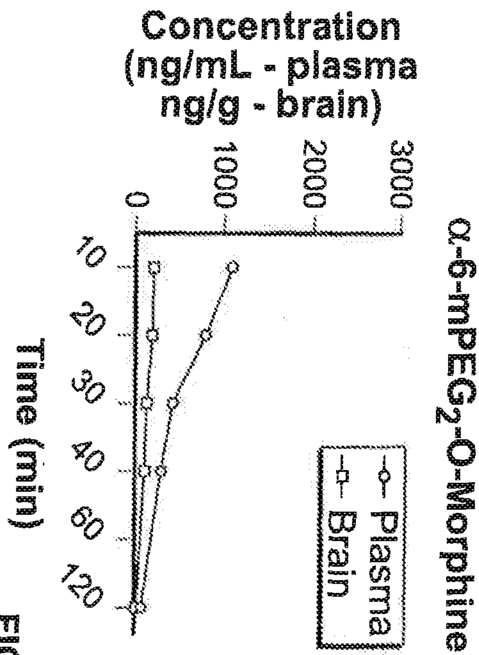


FIG. 15C

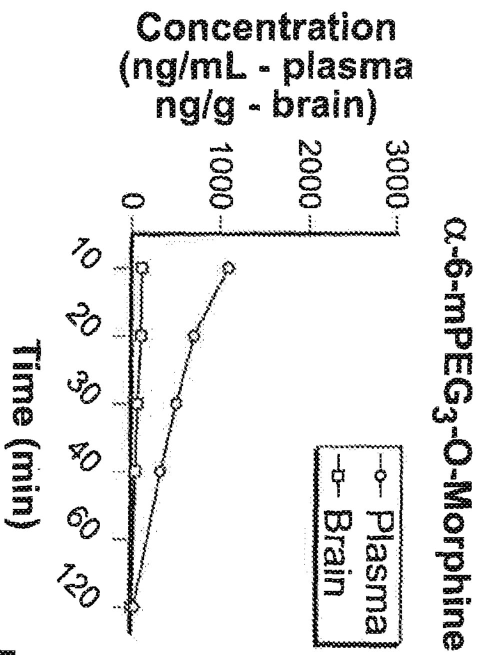


FIG. 15D

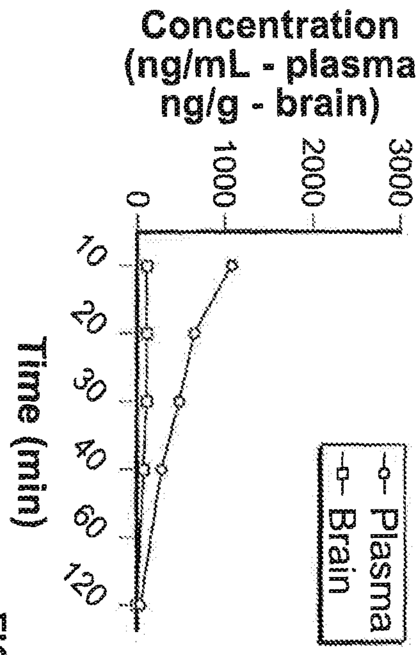


FIG. 15E

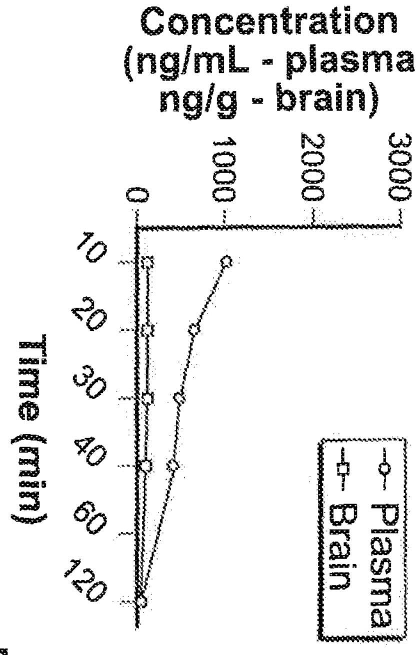


FIG. 15F

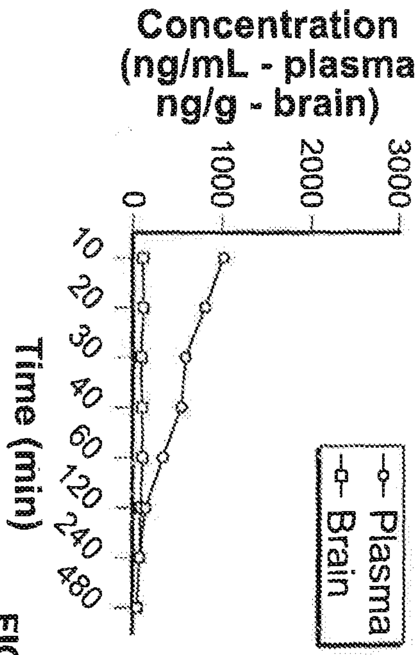


FIG. 15G

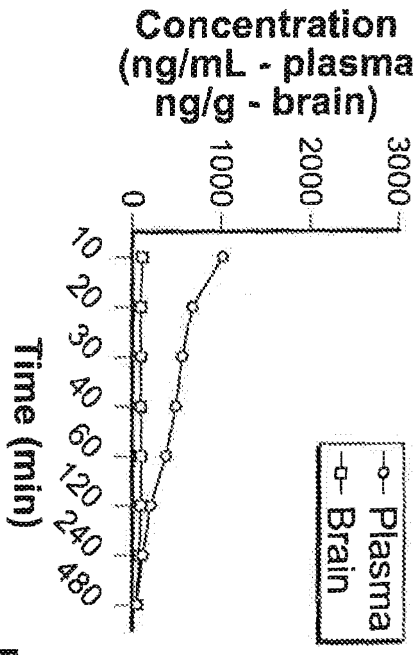


FIG. 15H

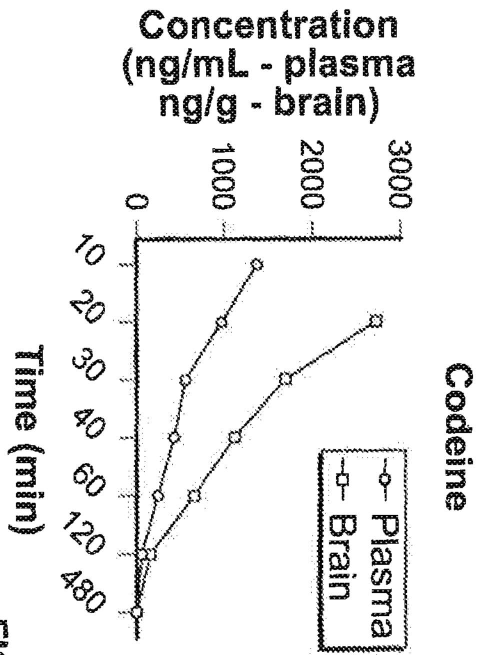


FIG. 16A

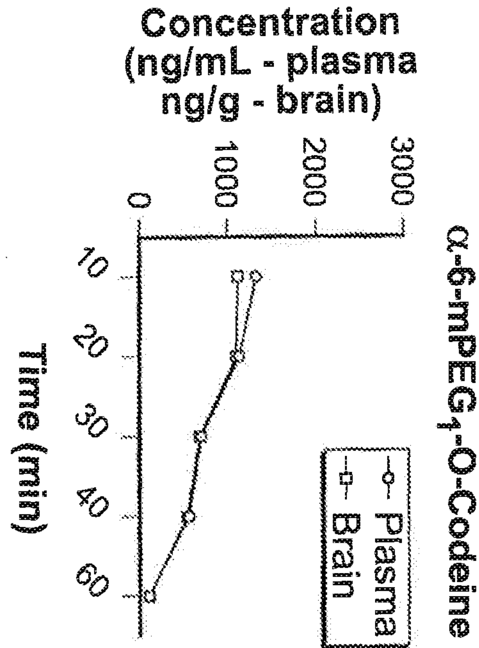


FIG. 16B

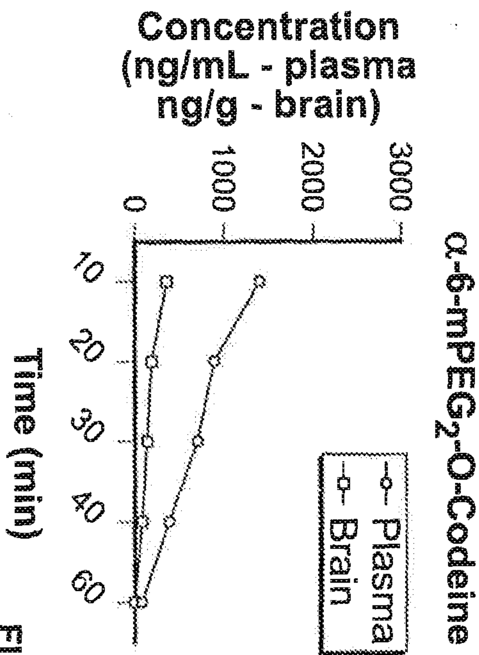


FIG. 16C

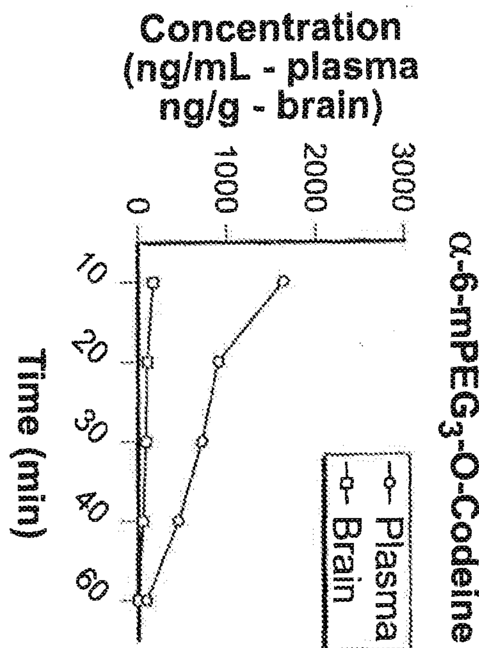


FIG. 16D

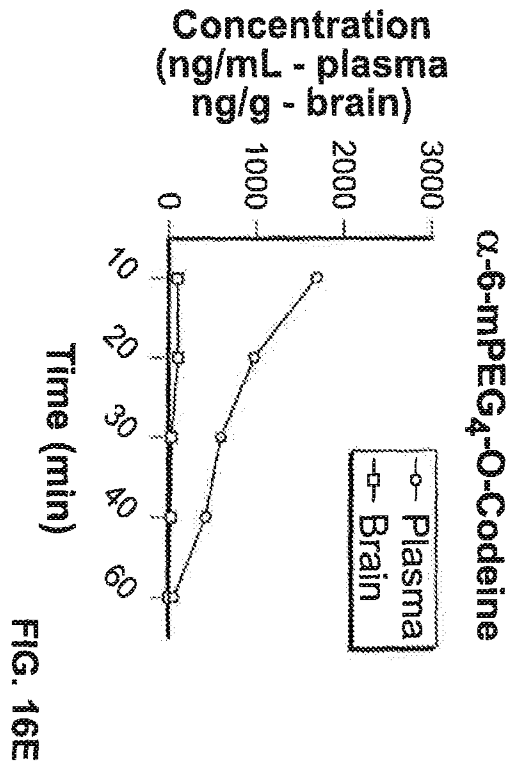


FIG. 16E

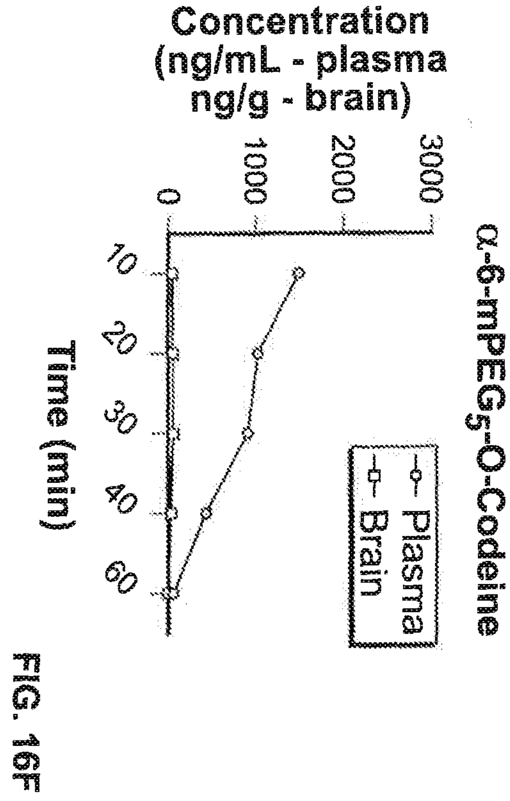


FIG. 16F

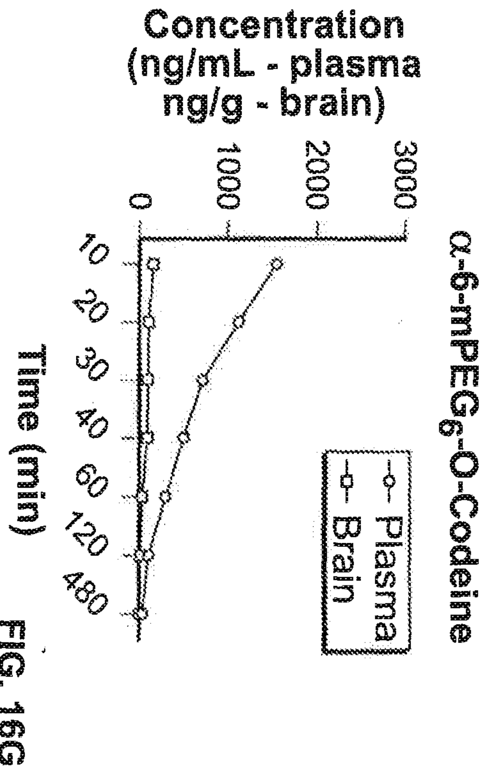


FIG. 16G

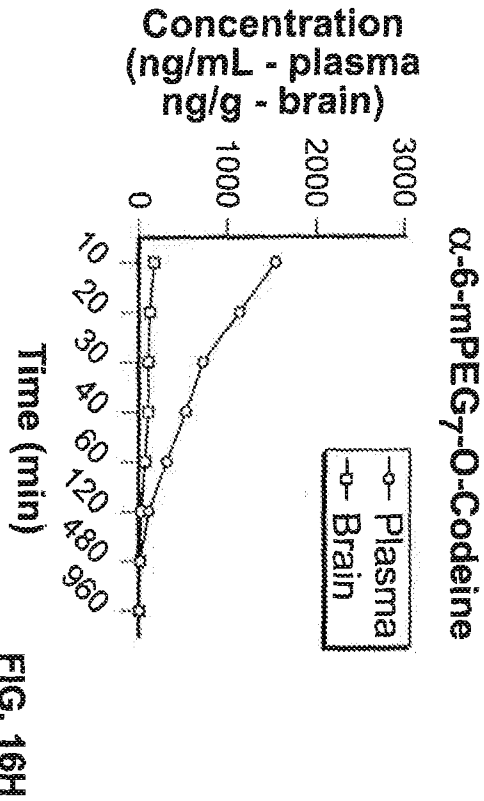


FIG. 16H

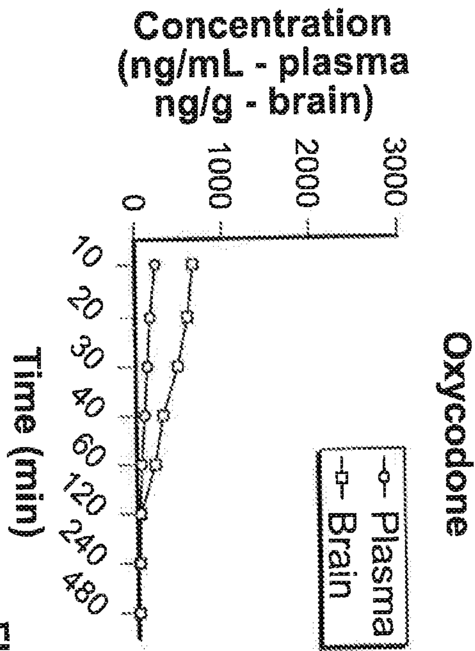


FIG. 17A

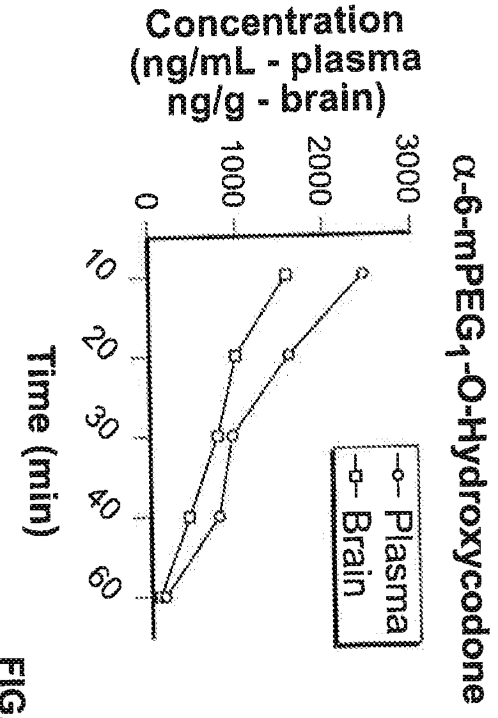


FIG. 17B

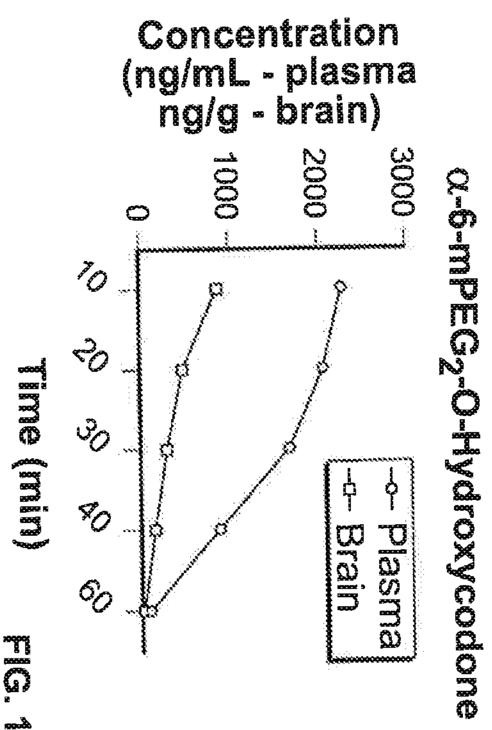


FIG. 17C

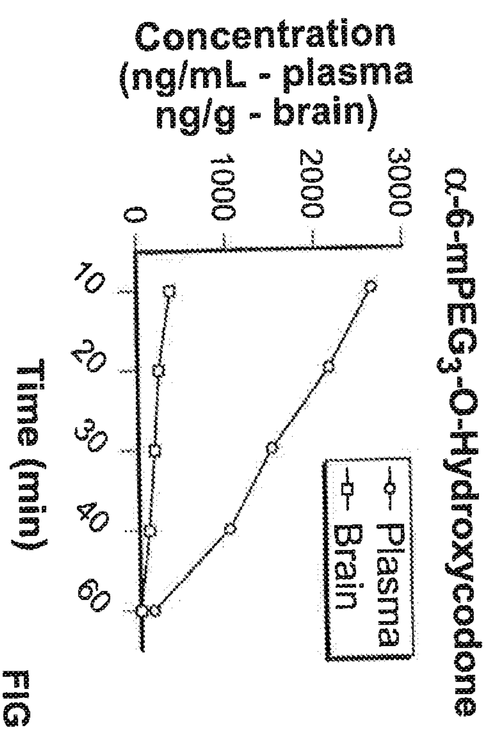


FIG. 17D

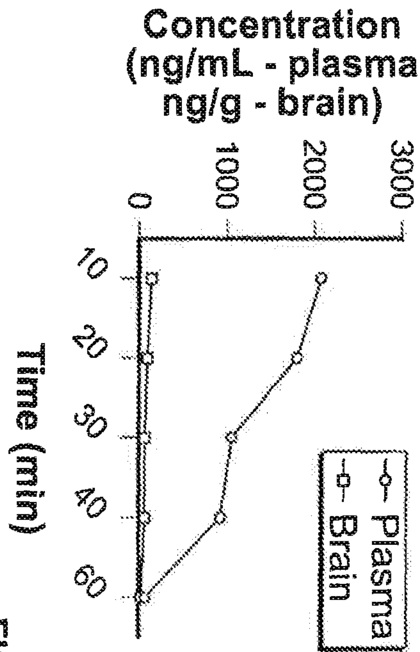


FIG. 17E

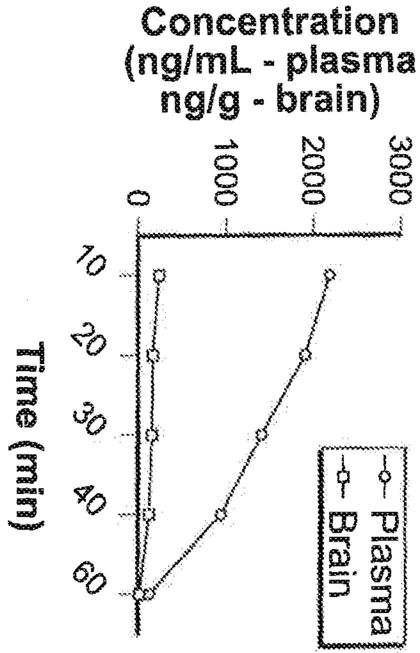


FIG. 17F

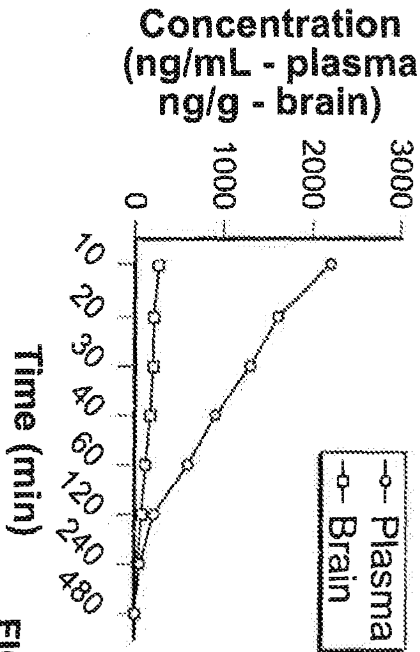


FIG. 17G

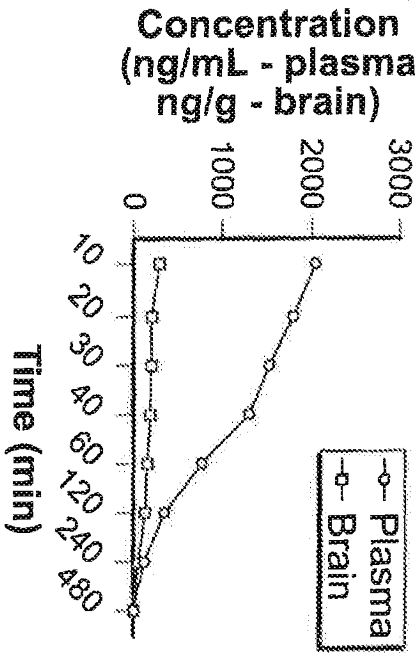


FIG. 17H

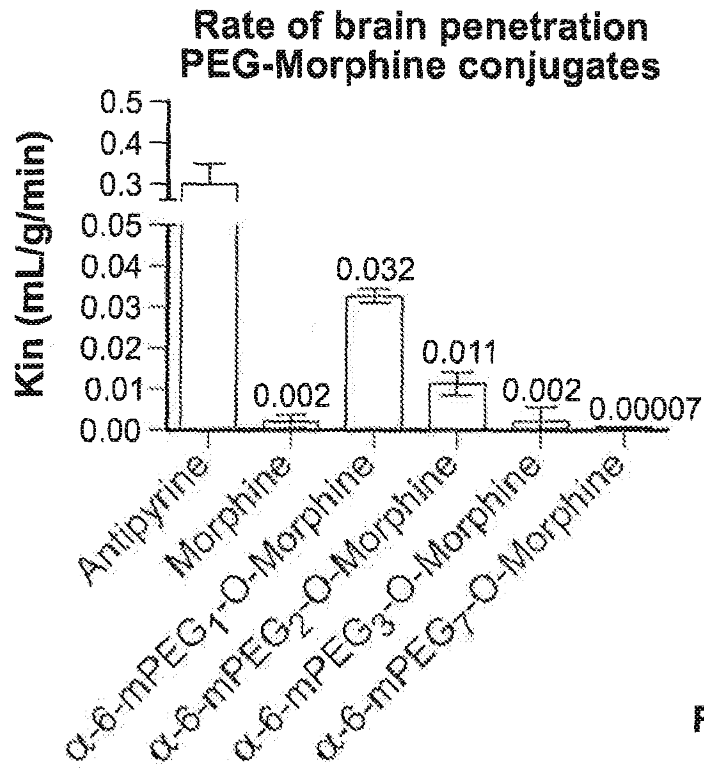


FIG. 18A

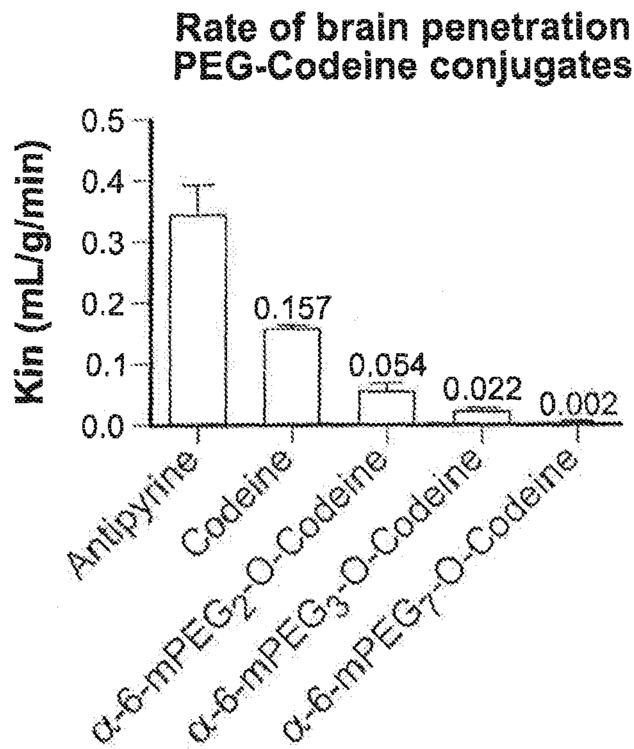


FIG. 18B

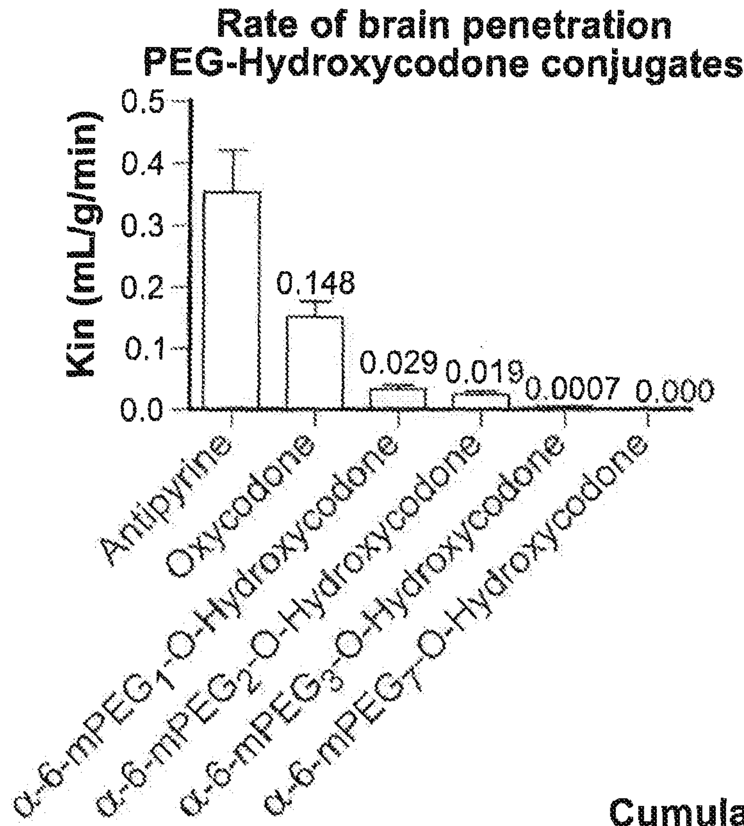
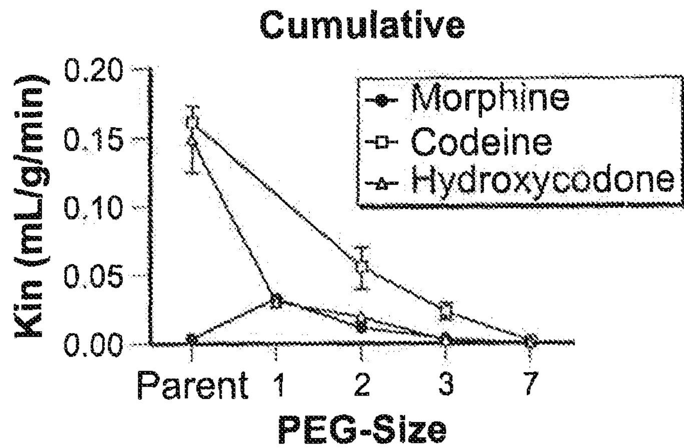


FIG. 18C



	Fold change in Kin versus parent					
	Morphine		**Codeine**		**Hydroxycodone**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Parent	1.000	0.707	1.000	0.029	1.000	0.130
1	15.833	7.929			0.199	0.022
2	5.500	2.854	0.343	0.055	0.129	0.018
3	1.000	1.118	0.142	0.021	0.005	0.005
7	0.033	0.037	0.011	0.011	0.000	

FIG. 19

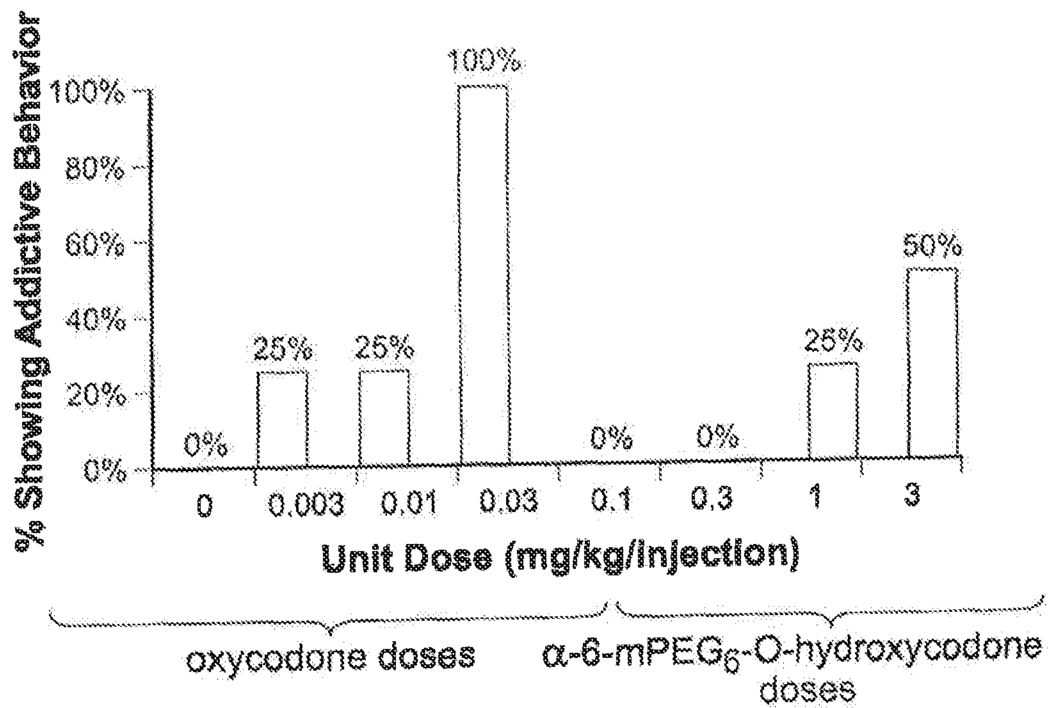


FIG. 20

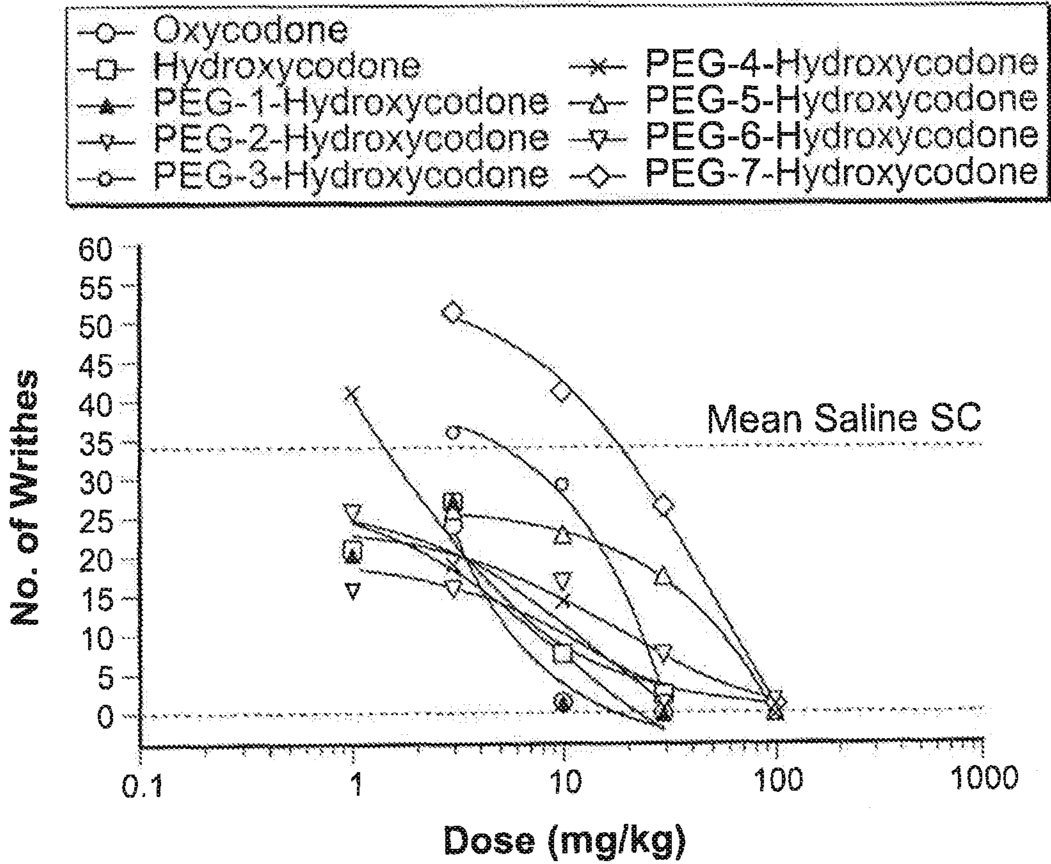


FIG. 21

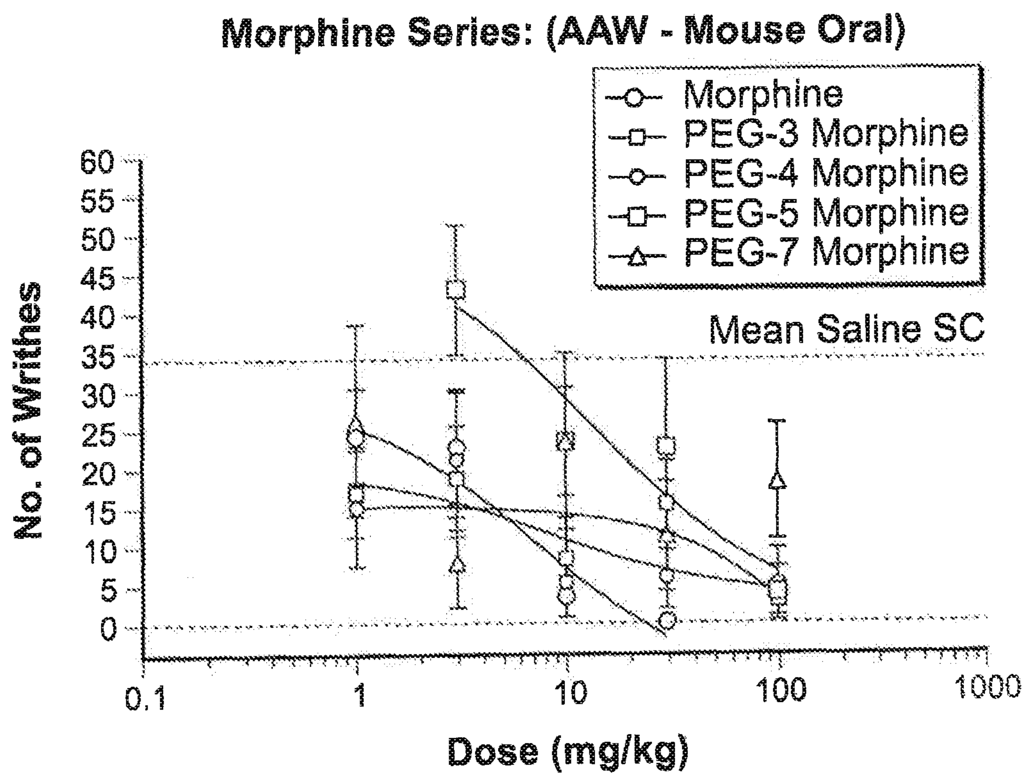


FIG. 22

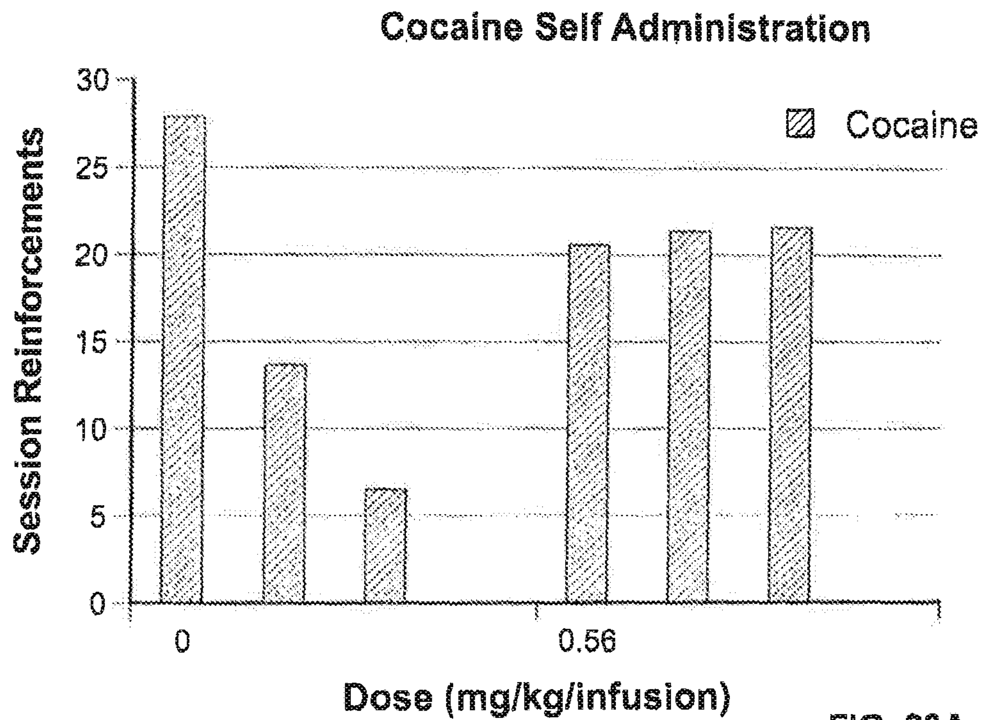


FIG. 23A

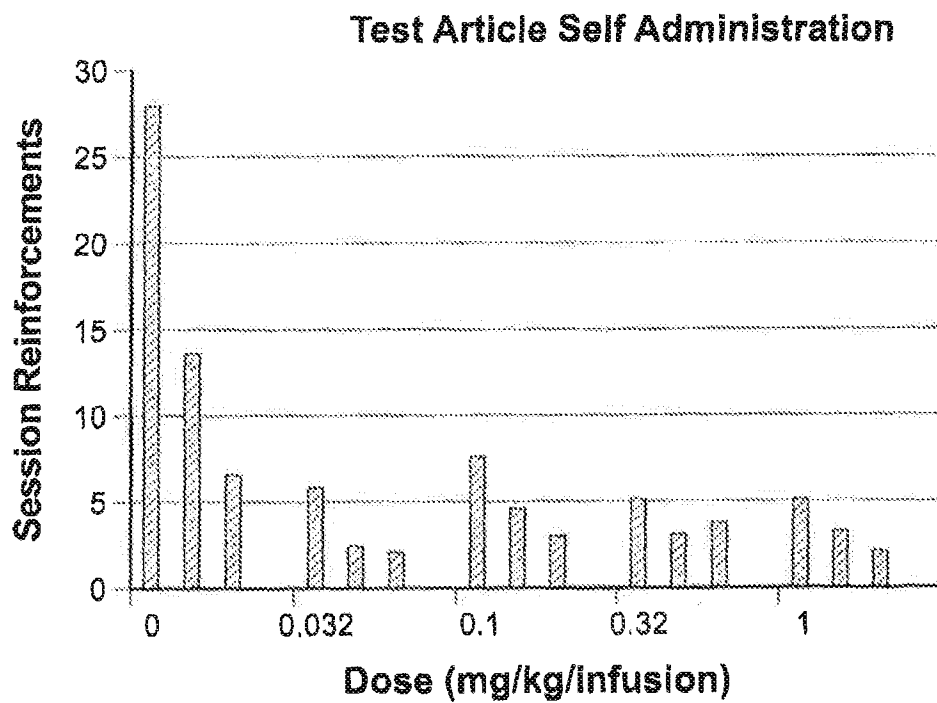


FIG. 23B

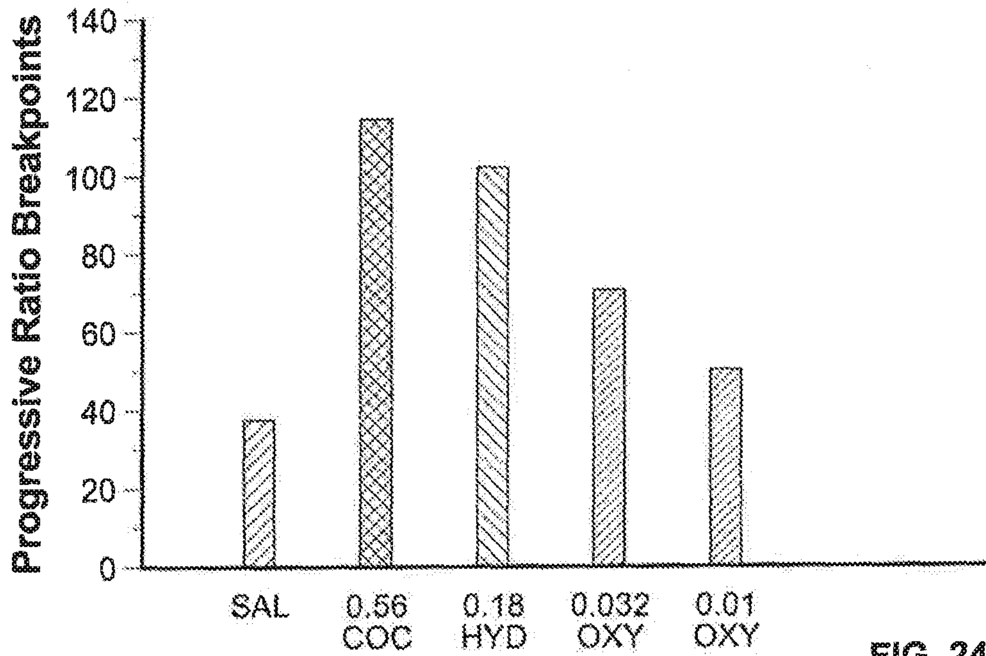


FIG. 24A

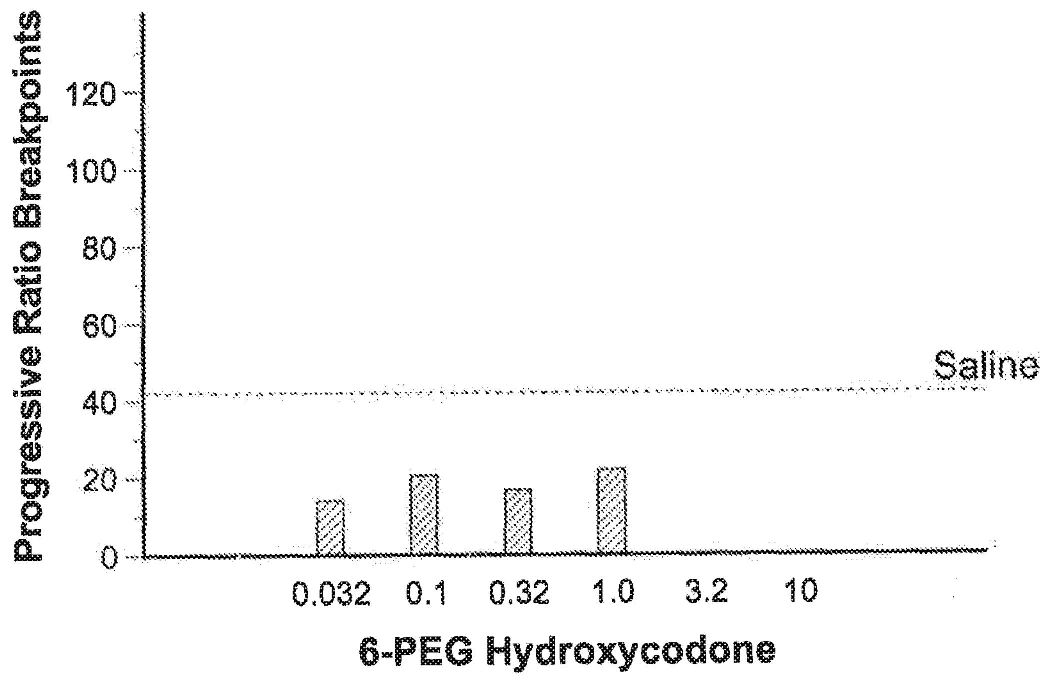


FIG. 24B

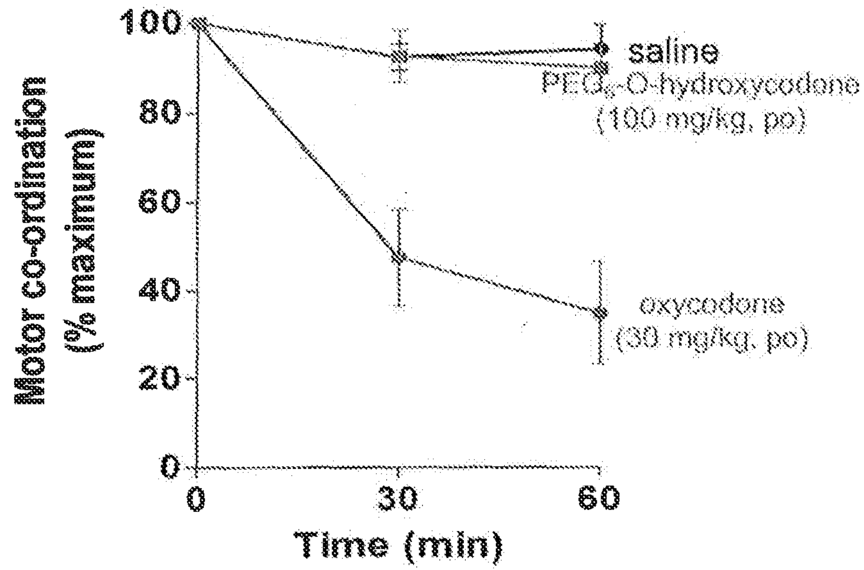


FIG. 25

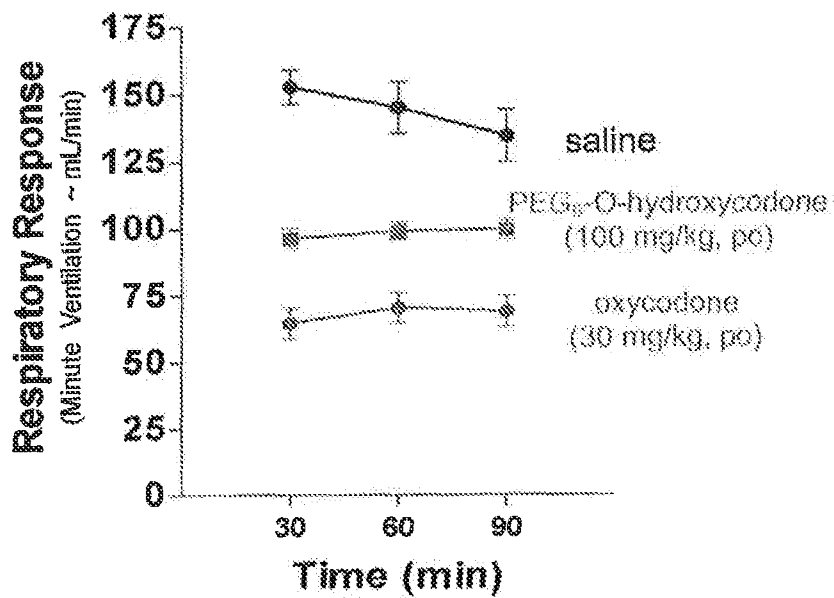


FIG. 26

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/021017

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/00 A61K31/075 A61K31/485
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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 practical, search terms used)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/036980 A1 (ALLTRANZ INC [US]; STINCHCOMB AUDRA LYNN [US]; GOLINSKI MIROSLAW JERZY) 27 March 2008 (2008-03-27)	1-18
Y	claims, in particular claims 8, 13; 0008-0009; 0044; 0029	1-18

X	WO 2008/112288 A2 (NEKTAR THERAPEUTICS AL CORP [US]; RIGGS-SAUTHIER JENNIFER [US]; DENG B) 18 September 2008 (2008-09-18)	1-18
Y	0060-0061; 0050; example 4, table 1	1-18

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document 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
15 March 2011	22/03/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dahse, Thomas

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANDERSON W R ET AL: "Structure-activity relationship assessment of conjugated enkephalins in centrally mediated analgesia", ABSTRACTS OF THE ANNUAL MEETING OF THE SOCIETY FOR NEUROSCIENCE, SOCIETY FOR NEUROSCIENCE, WASHINGTON, DC, US, vol. 25, no. 1/2, 1 January 1999 (1999-01-01), page 180, XP008118335, ISSN: 0190-5295	1-5, 11-18
Y	the whole document	1-18
Y	US 2005/266070 A1 (MICKLE TRAVIS [US] ET AL MICKLE TRAVIS [US] ET AL) 1 December 2005 (2005-12-01) 0114; 0117	1-5, 10-18
A	WITT K A ET AL: "PHARMACODYNAMIC AND PHARMACOKINETIC CHARACTERIZATION OF POLY(ETHYLENE GLYCOL) CONJUGATION TO MET-ENKEPHALIN ANALOG not D-PEN2,D-PEN5 3/4 ENKEPHALIN (DPDPE)", JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, US, vol. 298, no. 2, 1 August 2001 (2001-08-01), pages 848-856, XP001020459, ISSN: 0022-3565 * abstract	1-18
X	WO 2009/094209 A1 (NEKTAR THERAPEUTICS AL CORP [US]; REN ZHONGXU [US]; RIGGS-SAUTHIER JEN) 30 July 2009 (2009-07-30)	1-5, 10-18
Y	tables 1 and 2	1-18
X,P	WO 2010/033195 A1 (NEKTAR THERAPEUTICS [US]; JUDE-FISHBURN C SIMONE [US]; RIELY TIMOTHY A) 25 March 2010 (2010-03-25) 0016-0019	1-18
Y,P	WO 2011/002995 A1 (KEMPHARM INC [US]; MICKLE TRAVIS [US]; GUENTHER SVEN [US]; MICKLE CHRI) 6 January 2011 (2011-01-06) 0008; 0017-19	1-18

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2011/021017

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