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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF ALCOHOL USE DISORDERS

(57) Abstract: Compounds of formulas II and III (including nalfurafine and naltrexone) or their pharmaceutically acceptable salts administered in combination at low dosages may be used to reduce alcohol consumption in a person in need thereof. Additionally, compounds of formula II combined with one of compounds of formula III or compounds of formula IV (including nalmefene) may treat or reduce alcohol consumption and the likelihood of alcohol relapse. Methods of modulating the activity of an opioid receptor and methods of reducing alcohol consumption by administration of the combination are claimed. The composition of the compounds of formula II and one of formula III or formula IV and a pharmaceutically acceptable carrier is also claimed.



METHODS AND COMPOSITIONS FOR THE TREATMENT OF ALCOHOL USE DISORDERS

This invention was made with government support under grant number AA021970 awarded by the National Institutes of Health. The United States government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 62/803,922, filed on February 11, 2019 and U.S. Provisional Application Serial No. 62/880,415, filed on July 30, 2019, the contents of which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

Many people drink casually in social settings or in the evenings after a long day of work. However, for some, the line between casual, social drinking and alcohol abuse becomes blurred. When alcohol consumption begins to adversely affect a person's life and the lives of those around them on a regular basis, they may be suffering from an alcohol use disorder. Those with the disorder often become hyper-focused on attaining their next drink to the extent that relationships, jobs, and responsibilities suffer. Additionally, excessive drinking may cause short term physical effects like memory loss, hangovers, and blackouts or long term physical effects like cirrhosis of the liver, permanent memory loss, pancreatitis, high blood pressure, cancer, heart problems, and brain damage. Excessive drinking may even lead to death. A Centers from

Disease Control and Prevention (CDC) study estimates that excessive alcohol consumption causes 88,000 deaths each year in the U.S.

The economic losses due to excessive drinking are also staggering. The CDC estimates that in 2006, excessive drinking cost the U.S. \$223.5 billion and individual states a median of \$2.9 billion. The economic losses include losses in workplace productivity, health care expenses for treating problems caused by excessive drinking, law enforcement and other criminal justice expenses, and losses from motor vehicle crashes related to excessive alcohol use.

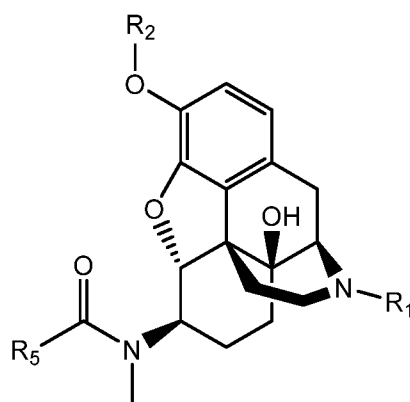
Unfortunately, an estimated 16 million people or more in the U.S. suffer from an alcohol use disorder. For many, recovering from an alcohol use disorder is a lifelong journey as alcohol abuse is a chronic disorder affecting the brain chemistry. The struggle to maintain sobriety and prevent alcohol relapse is on-going for people in recovery.

Excessive alcohol consumption is well known to affect the endogenous opioid systems. Specifically, activation of the kappa opioid receptor (KOP-r) system has been implicated in the negative reinforcing aspects of alcohol addiction. In rodents, KOP-r agonists attenuate alcohol drinking and decreases alcohol-induced reward. However, most “classic” KOP-r agonists such as U69,593 and U50,488H produce significant sedation and dysphoria, and those side effects limit their clinical use potential.

Accordingly, there is a need for a new drug or combination of drugs that is highly effective in reducing alcohol consumption in a person in need thereof without causing debilitating side effects and without the associated toxicity. There is also a need for a new drug or combination of drugs that will assist in treating or preventing the likelihood of alcohol relapse.

SUMMARY OF THE INVENTION

One aspect of the invention relates to a method of modulating the activity of an opioid receptor, including contacting the opioid receptor of a person in need thereof with (a) a compound of formula II



II

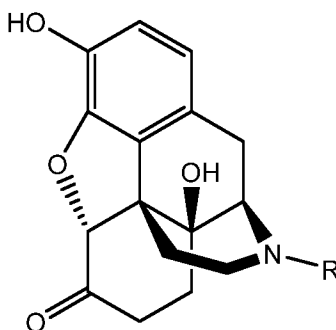
or a pharmaceutically acceptable salt thereof, wherein:

R₁ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R₂ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted optionally substituted alkynyl, and optionally substituted akanoyl;

R₅ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

and (b) a compound of formula III



III

or a pharmaceutically acceptable salt thereof, wherein R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl.

The opioid receptor is preferably selected from the μ -opioid receptor, δ -opioid receptor, κ -opioid receptor, or combinations thereof. The contacting preferably includes administering (a) and (b) to the person. Most preferably, (a) and (b) are administered simultaneously.

Another aspect of the invention relates to a method of reducing alcohol consumption in a person in need thereof, including administering a composition including (a) and (b) as defined above.

In a preferred embodiment,

R₁ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, and 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof;

R₂ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof; and C₂-C₆ optionally substituted alkanoyl; and

R₅ is optionally substituted heteroaryl.

R₁ is preferably an alkyl substituted with a cyclopropyl group. R₂ is preferably H. R₅ is preferably an alkyl substituted with furanyl. R is preferably cyclopropylmethyl.

In a preferred embodiment, (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof. The nalfurafine or a pharmaceutically acceptable salt thereof may be administered in a dosage of about 0.04 µg/kg to about 0.12 µg/kg per day. The naltrexone or a pharmaceutically acceptable salt thereof may be administered in a dosage of about 0.024 mg/kg to about 0.081 mg/kg per day.

The pharmaceutically acceptable salt of nalfurafine is preferably nalfurafine hydrochloride. The pharmaceutically acceptable salt of naltrexone is preferably naltrexone hydrochloride.

With the methods described above, it is contemplated that a person in need thereof is a person who suffers from alcohol addiction. Preferably (a) and (b) are administered to the person in need thereof prior to the person consuming alcohol. The administering may occur at least 30 min, at least 60 min, at least 90 min, at least 120 min, or at least 240 min prior to consumption of alcohol.

In one embodiment, the nalfurafine or a pharmaceutically acceptable salt thereof is administered in a low-dose, wherein low-dose is 5 µg or less nalfurafine or a pharmaceutically acceptable salt thereof per day.

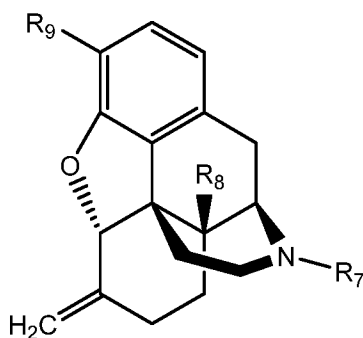
In another preferred embodiment, if (a) is nalfurafine or a pharmaceutically acceptable salt thereof and if (b) is naltrexone or a pharmaceutically acceptable salt thereof, then the dosage ratio of (a) : (b) is 0.1-10:500-2,000 or 0.5-2:500-2,000. The dosage ratio of (a) : (b) is 1:1,000-10,000 in another preferred embodiment.

The dosage of naltrexone or a pharmaceutically acceptable salt thereof is preferably about 1 mg to about 50 mg per day.

Preferably, alcohol consumption is reduced for at least 8 hours after administration of (a) and (b). The consumption of drugs of abuse is preferably also reduced. Drugs of abuse include cocaine, heroin, opiates, marijuana, or combinations thereof.

Another aspect of the invention relates to a composition including (a), (b), and a pharmaceutically acceptable carrier. Preferably (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof. In another embodiment, the active ingredients are limited to only nalfurafine or a pharmaceutically acceptable salt thereof and naltrexone or a pharmaceutically acceptable salt thereof.

Another aspect of the invention relates to a method of modulating the activity of an opioid receptor, including contacting the opioid receptor of a person in need thereof with (a) a compound of formula II or a pharmaceutically acceptable salt thereof, and one of (b) a compound of formula III or a pharmaceutically acceptable salt thereof, or (c) a compound of formula IV



IV

or a pharmaceutically acceptable salt thereof, wherein:

R₇ is selected from the group consisting of -CH₂-HC=CH₂ and cyclopropylmethyl;

R₈ is selected from the group consisting of hydrogen and hydroxyl; and

R₉ is selected from the group consisting of hydroxyl and methoxy.

Preferably, the opioid receptor is selected from the group consisting of μ -opioid receptor, δ -opioid receptor, κ -opioid receptor, and combinations thereof. The contacting preferably includes administering (a) and one of (b) or (c) to the person. Most preferably, (a) and one of (b) or (c) are administered simultaneously.

Another aspect of the invention relates to a method of treating or reducing likelihood of alcohol relapse in a person in need thereof, including administering a composition including (a) a compound of formula II or a pharmaceutically acceptable salt thereof, and one of (b) a compound of formula III or a pharmaceutically acceptable salt thereof, or (c) a compound of formula IV or a pharmaceutically acceptable salt thereof, wherein:

R₇ is selected from the group consisting of -CH₂-HC=CH₂ and cyclopropylmethyl;

R₈ is selected from the group consisting of hydrogen and hydroxyl; and

R₉ is selected from the group consisting of hydroxyl and methoxy.

In a preferred embodiment, R₁ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, and 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof;

R₂ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof; and C₂-C₆ optionally substituted alkanoyl; and

R₅ is optionally substituted heteroaryl.

R₁ is preferably an alkyl substituted with a cyclopropyl group. R₂ is preferably H. R₅ is preferably an alkyl substituted with furanyl. R is preferably cyclopropylmethyl. R₇ is preferably cyclopropylmethyl, and R₈ and R₉ are preferably hydroxyl.

Most preferably, (a) is nalfurafine or a pharmaceutically acceptable salt thereof, (b) is naltrexone or a pharmaceutically acceptable salt thereof, and (c) is nalmefene or a pharmaceutically acceptable salt thereof.

The nalfurafine or a pharmaceutically acceptable salt thereof is preferably administered in a dosage of about 0.04 µg/kg to about 0.12 µg/kg per day. The naltrexone or a pharmaceutically acceptable salt thereof is preferably administered in a dosage of about 0.024 mg/kg to about 0.80 mg/kg per day. The nalmefene or a pharmaceutically acceptable salt thereof is preferably administered in a dosage of about 0.05 mg/kg to about 1.0 mg/kg per day.

The pharmaceutically acceptable salt of nalfurafine is preferably nalfurafine hydrochloride. The pharmaceutically acceptable salt of naltrexone is preferably naltrexone hydrochloride. The pharmaceutically acceptable salt of nalmefene is preferably nalmefene hydrochloride.

With the methods described above, it is contemplated that the person suffers from alcohol addiction and may have been sober for a period of time. Preferably, (a) and one of (b) or (c) are administered to a person in need thereof prior to consumption of alcohol.

The nalfurafine or a pharmaceutically acceptable salt thereof is preferably administered in a low-dose, wherein low-dose comprises 5 µg or less nalfurafine or a pharmaceutically acceptable salt thereof per day. Preferably, (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (b) is 0.1-10:500-2,000 or 0.5-2:500-2,000. In another preferred embodiment, (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (b) is 1:1,000-10,000.

Preferably, (b) is naltrexone or a pharmaceutically acceptable salt thereof is administered in a dose of about 1 mg to about 50 mg per day. In another preferred embodiment, (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (c) is nalmefene or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (c) is 1:1,000-10,000. Preferably, (c) is nalmefene or a pharmaceutically acceptable salt thereof is administered in a dose of about 1 mg to about 50 mg per day.

It is contemplated that alcohol consumption is reduced for at least 8 hours after administration of the composition. It is also contemplated that consumption of drugs of abuse is also reduced. Drugs of abuse may include cocaine, heroin, opiates, marijuana, or combinations thereof. It is further contemplated that person's pain is reduced.

Another aspect of the invention relates to a composition including (a) a compound of formula II or a pharmaceutically acceptable salt thereof, and one of (b) a compound of formula

III or a pharmaceutically acceptable salt thereof, or (c) a compound of formula IV or a pharmaceutically acceptable salt thereof. Preferably, (a) is nalfurafine or a pharmaceutically acceptable salt thereof, (b) is naltrexone or a pharmaceutically acceptable salt thereof, and (c) is nalmeferone or a pharmaceutically acceptable salt thereof. In another preferred embodiment, the active ingredients consist of (1) nalfurafine or a pharmaceutically acceptable salt thereof and naltrexone or a pharmaceutically acceptable salt thereof; or (2) nalfurafine or a pharmaceutically acceptable salt thereof and nalmeferone or a pharmaceutically acceptable salt thereof.

It was surprisingly found that the administration of a compound of formula II in combination with a compound of formula III has a greater effect than either drug along in reducing alcohol consumption, even at low doses, e.g. 5 μ g or less of a compound of formula II and 25 mg or less of a compound of formula III. The compounds were found to be effective even at doses low enough such that each had no effect on alcohol consumption with either drug alone, thereby minimizing side effects and potential tolerance after regulated administration. Without being bound by theory, it is believed that by targeting both neurotransmitter pathways implicated in both KOP-r and MOP-r components of addiction to alcohol or drugs of abuse, the combination of the two compounds (formulas II and III) has an enhanced effect over traditional methods.

It was also surprisingly found that the administration of a compound of formula II in combination with a low-dose of either a compound of formula III or a compound of formula IV reduced both alcohol consumption and the alcohol deprivation effect (ADE) at doses lower than those individually effective, suggesting a synergistic effect between the compound of formula II and the compound of formula III or the compound of formula IV.

DESCRIPTION OF THE DRAWINGS

Figure 1 Effects of single administration of nalfurafine (NFF, 10 μ g/kg) on alcohol intake (A), water intake (B), and preference ratio (C) in male and female mice after 3-week chronic intermittent access drinking. (1) Control groups: males (n=7) and females (n=14) received saline injection (i.p.); and (2) NFF groups: males (n=8) and females (n=14) received one NFF injection (i.p.) 30 min before the drinking test. Alcohol and water intake values were recorded after 4, 8 and 24 hours. * $p < 0.05$ vs. control at the same time point.

Figure 2 Dose response of single administration of nalfurafine (NFF, 0, 0.3, 1, 3 or 10 μ g/kg) alone or combined with naltrexone (NTN, 0, 0.3 or 1 mg/kg) on decreasing alcohol intake (A) and alcohol preference ratio (B) in male and female mice after 3-week chronic intermittent access drinking. Data were collected at the first 4-hour recording time on the baseline and the testing day (24 hours later) and are expressed as a percentage of baseline intake to account for the differences in baseline that contribute to variation between experiments. * $p < 0.05$ or ** $p < 0.01$ vs. control (both NFF and NTN at 0 mg/kg); # $p < 0.05$ between treatment groups.

Figure 3 Effects of single nalfurafine (NFF) administration at 1 μ g/kg combined with naltrexone (NTN, 1mg/kg) on alcohol intake (A), water intake (B) and preference ratio (C) in male (left) and female (right) mice (n=6-8) after 3-week chronic intermittent access drinking. Control groups: mice received one saline (i.p.) followed by saline. Test groups: mice received one NFF injection (1 μ g/kg, i.p.) followed by one NTN injection (1mg/kg, i.p.) before the alcohol drinking test. Alcohol and water intake were recorded after 4, 8 and 24 hours. * $p < 0.05$ vs. control at the same time point.

Figure 4 Effects of repeated administrations of nalfurafine (10 μ g/kg) on alcohol intake (A) and preference ratio (B) after 3-week intermittent access drinking in male mice (n=6).

*p<0.05 vs. the baseline.

Figure 5 Effects of single administration of naltrexone (NTN, 3mg/kg) on alcohol intake (A), water intake (B), and preference ratio (C) in male and female mice after 3-week chronic intermittent access (IA) drinking. (1) Control groups (n=6-8): males and females received one vehicle injection (saline, i.p.); and (2) NTN group (n=6-8): males and females received one NTN injection (3mg/kg in saline, i.p.) 10 min before the drinking test. Alcohol and water intake were recorded after 4, 8 and 24 hours. * p<0.05 vs. control at the same time point.

Figure 6 Effects of single nalfurafine (NFF) at 3 μ g/kg combined with naltrexone (NTN) at 0.3mg/kg (left) or 1mg/kg (right) on alcohol intake (A), water intake (B) and preference ratio (C) in female mice (n=6-8) after 3-week chronic intermittent access drinking. Control groups: mice received one vehicle (saline, i.p.) followed by saline; Test groups: mice received one NFF injection (3 μ g/kg in saline, i.p.) followed by one NTN injection (0.3 or 1mg/kg, i.p.) before the drinking test. Alcohol and water intake were recorded after 4, 8 and 24 hours. * p<0.05 vs. control at the same time point.

Figure 7 Effects of nalfurafine (NFF, 3 and 10 μ g/kg) on alcohol intake in an alcohol deprivation effect (ADE) model at 4 hours in males (A) and females (B) after 1 week of abstinence from 3-week intermittent access alcohol drinking. * p<0.05 vs. control baseline, and + p<0.05 vs. control ADE.

Figure 8 Effects of nalfurafine (NFF, 1 μ g/kg) combined with naltrexone (NTX, 1 mg/kg) on alcohol intake in an alcohol deprivation effect (ADE) model at 4 hours in males (A,

n=10) and females (B, n=8-10) after 1 week of abstinence from 3-week intermittent access alcohol drinking. * $p<0.05$ vs. control baseline, and + $p<0.05$ vs. control ADE.

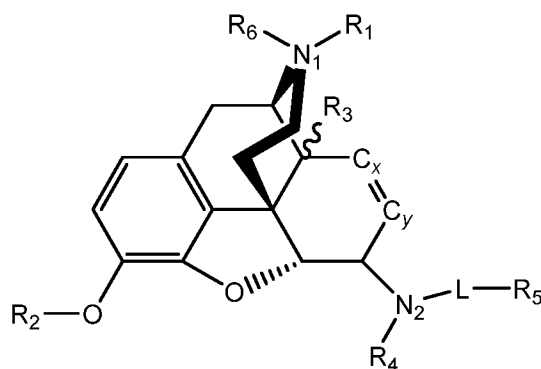
Figure 9 Effects of nalmefene (NMF, 0.125, 0.25 or 0.5mg/kg) on alcohol intake in an alcohol deprivation effect (ADE) model at 4 hours in males (A) and females (B) after 1 week of abstinence from 3-week chronic intermittent access alcohol drinking. * $p<0.05$ vs. control baseline, and ++ $p<0.01$ vs. control ADE.

Figure 10 Effects of nalfurafine (NFF, 1 μ g/kg) combined with nalmefene (NMF, 0.125mg/kg) on alcohol intake in an alcohol deprivation effect (ADE) model at 4 hours in males (A, n=8-12) and females (B, n=10) after 1 week of abstinence from 3-week intermittent access alcohol drinking. * $p<0.05$ vs. control baseline, and + $p<0.05$ vs. control ADE.

DETAILED DESCRIPTION

One aspect of the invention relates to a method of modulating the activity of an opioid receptor, including contacting the opioid receptor of a person in need thereof with (a) a compound of formula II or a pharmaceutically acceptable salt thereof; and (b) a compound of formula III or a pharmaceutically acceptable salt thereof. Formulas II and III are as described below.

The compound nalfurafine is described generically below in formula (I)



Formula (I)

wherein:

R_1 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R_2 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted optionally substituted alkynyl, and optionally substituted alkanoyl;

R₃ is selected from the group consisting of hydrogen, OH, and optionally substituted alkoxy;

R₄ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

L is a group linking N₂ and R₅ consisting of a bond, CH₂, C=O, S(=O)₂, (C=O)—NH—, and (C=O)—O—;

R₅ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R₆ is selected from the group consisting of hydrogen, O⁻, CH₃, and optionally substituted alkoxy, or R₆ is absent;

N₁ is a nitrogen atom, which is neutral when R₆ is absent, or is charged when R₆ is present, to satisfy the normal valence of a tertiary or quaternary nitrogen;

N₂ is a nitrogen atom, which is bound to the opiate nucleus in α or β stereochemistry or a mixture thereof; and

C_x and C_y together form an alkylidene group (—CH₂—CH₂—) or alkenylidene group (—CH=CH—); any of the attached hydrogens may be replaced to form a substituted alkenylidene group or substituted alkylidene of any possible stereochemistry.

“Substituted” or “optionally substituted” is intended to indicate that one or more hydrogens on the atom indicated in the expression using “substituted” is replaced with a selection from the indicated group(s), provided that the indicated atom's normal valence is not exceeded, and that the substitution results in a stable compound. Suitable indicated groups

include, e.g., alkyl, alkenyl, alkylidenyl, alkenylidenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and/or COOR_x , wherein each R_x and R_y are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxy, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring. When a substituent is keto (i.e., $=\text{O}$) or thioxo (i.e., $=\text{S}$) group, then 2 hydrogens on the atom are replaced.

“Alkyl” refers to a C1-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, $-\text{CH}_3$), ethyl (Et, $-\text{CH}_2\text{CH}_3$), 1-propyl (n-Pr, n-propyl, $-\text{CH}_2\text{CH}_2\text{CH}_3$), 2-propyl (i-Pr, i-propyl, $-\text{CH}(\text{CH}_3)_2$), 1-butyl (n-Bu, n-butyl, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-\text{CH}_2\text{CH}(\text{CH}_3)_2$), 2-butyl (s-Bu, s-butyl, $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-\text{C}(\text{CH}_3)_3$), pentyl (n-pentyl, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2-pentyl ($-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_3$), 3-pentyl ($-\text{CH}(\text{CH}_2\text{CH}_3)_2$), 2-methyl-2-butyl ($-\text{C}(\text{CH}_3)_2\text{CH}_2\text{CH}_3$), 3-methyl-2-butyl ($-\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)_2$), 3-methyl-1-butyl ($-\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$), 2-methyl-1-butyl ($-\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 1-hexyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2-hexyl ($-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3-hexyl ($-\text{CH}(\text{CH}_2\text{CH}_3)(\text{CH}_2\text{CH}_2\text{CH}_3)$), 2-methyl-2-pentyl ($-\text{C}(\text{CH}_3)_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3-methyl-2-pentyl ($-\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 4-methyl-2-pentyl ($-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}(\text{CH}_3)_2$), 3-methyl-3-pentyl ($-\text{C}(\text{CH}_3)(\text{CH}_2\text{CH}_3)_2$), 2-methyl-3-pentyl ($-\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}(\text{CH}_3)_2$), 2,3-dimethyl-2-butyl ($-\text{C}(\text{CH}_3)_2\text{CH}(\text{CH}_3)_2$), 3,3-dimethyl-2-butyl ($-\text{CH}(\text{CH}_3)\text{C}(\text{CH}_3)_3$).

The alkyl can optionally be substituted with one or more alkenyl, alkylidenyl, alkenylidenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle,

cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and/or COOR_x , wherein each R_x and R_y are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring. The alkyl can optionally be interrupted with one or more non-peroxide oxy (—O—), thio (—S—), carbonyl (—C(=O)—), carboxy (—C(=O)O—), sulfonyl (SO) or sulfoxide (SO_2). Additionally, the alkyl can optionally be at least partially unsaturated, thereby providing an alkenyl.

“Alkenyl” refers to a $\text{C}_2\text{—C}_{18}$ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e., a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl (—CH=CH_2), allyl ($\text{—CH}_2\text{CH=CH}_2$), cyclopentenyl ($\text{—C}_5\text{H}_7$), and 5-hexenyl ($\text{—CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH=CH}_2$).

The alkenyl can optionally be substituted with one or more alkyl, alkylidenyl, alkenylidenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and/or COOR_x , wherein each R_x and R_y are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring. Additionally, the alkenyl can optionally be interrupted with one or more peroxide oxy (—O—), thio (—S—), carbonyl (—C(=O)—), carboxy (—C(=O)O—), sulfonyl (SO) or sulfoxide (SO_2).

“Alkylidenyl” refers to a C₁-C₁₈ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methylenyl (=CH₂), ethylenyl (=CHCH₃), 1-propylenyl (=CHCH₂CH₃), 2-propylenyl (=C(CH₃)₂), 1-butylenyl (=CHCH₂CH₂CH₃), 2-methyl-1-propylenyl (=CHCH(CH₃)₂), 2-butylenyl (=C(CH₃)CH₂CH₃), 1-pentylenyl (=CHCH₂CH₂CH₂CH₃), 2-pentylenyl (=C(CH₃)CH₂CH₂CH₃), 3-pentylenyl (=C(CH₂CH₃)₂), 3-methyl-2-butylenyl (=C(CH₃)CH(CH₃)₂), 3-methyl-1-butylenyl (=CHCH₂CH(CH₃)₂), 2-methyl-1-butylenyl (=CHCH(CH₃)CH₂CH₃), 1-hexylenyl (=CHCH₂CH₂CH₂CH₂CH₃), 2-hexylenyl (=C(CH₃)CH₂CH₂CH₂CH₃), 3-hexylenyl (=C(CH₂CH₃)(CH₂CH₂CH₃)), 3-methyl-2-pentylenyl (=C(CH₃)CH(CH₃)CH₂CH₃), 4-methyl-2-pentylenyl (=C(CH₃)CH₂CH(CH₃)₂), 2-methyl-3-pentylenyl (=C(CH₂CH₃)CH(CH₃)₂), and 3,3-dimethyl-2-butylenyl (=C(CH₃)C(CH₃)₃).

The alkylidenyl can optionally be substituted with one or more alkyl, alkenyl, alkenylenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thio, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and/or COOR_x, wherein each R_x and R_y are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y, taken together along with the nitrogen atom to which they are attached form a heterocycle ring. Additionally, the alkylidenyl can optionally be interrupted with one or more non-peroxide oxy (—O—), thio (—S—), carbonyl (—C(=O)—), carboxy (—C(=O)O—), sulfonyl (SO) or sulfoxide (SO₂).

“Alkenylenyl” refers to a C₂-C₂₀ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e., a carbon-carbon, sp² double bond.

Examples include, but are not limited to: allylidenyl ($=\text{CHCH}=\text{CH}_2$), and 5-hexenylidenyl ($=\text{CHCH}_2\text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2$).

The alkenylidenyl can optionally be substituted with one or more alkyl, alkenyl, alkylidenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and/or COOR_x , wherein each R_x and R_y are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring. Additionally, the alkenylidenyl can optionally be interrupted with one or more non-peroxide oxy ($-\text{O}-$), thio ($-\text{S}-$), carbonyl ($-\text{C}(=\text{O})-$), carboxy ($-\text{C}(=\text{O})\text{O}-$), sulfonyl (SO) or sulfoxide (SO_2).

“Alkylene” refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene ($-\text{CH}_2-$), 1,2-ethyl ($-\text{CH}_2\text{CH}_2-$), 1,3-propyl ($-\text{CH}_2\text{CH}_2\text{CH}_2-$), 1,4-butyl ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$), and the like.

The alkylene can optionally be substituted with one or more alkyl, alkenyl, alkylidenyl, alkenylidenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and/or COOR_x , wherein each R_x and R_y are independently H, alkyl,

alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring. Additionally, the alkylene can optionally be interrupted with one or more nonperoxide oxy ($—O—$), thio ($—S—$), carbonyl ($—C(=O)—$), carboxy ($—C(=O)O—$), sulfonyl (SO) or sulfoxide (SO_2). Moreover, the alkylene can optionally be at least partially unsaturated, thereby providing an alkenylene.

“Alkenylene” refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene ($—CH=CH—$).

The alkenylene can optionally be substituted with one or more alkyl, alkenyl, alkylidenyl, alkenylidenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and/or $COOR_x$, wherein each R_x and R_y are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring. Additionally, The alkenylene can optionally be interrupted with one or more non-peroxide oxy ($—O—$), thio ($—S—$), carbonyl ($—C(=O)—$), carboxy ($—C(=O)O—$), sulfonyl (SO) or sulfoxide (SO_2).

The term “alkynyl” refers to unsaturated groups which contain at least one carbon-carbon triple bond and includes straight chain, branched chain, and cyclic groups, all of which may be

optionally substituted. Suitable alkynyl groups include ethynyl, propynyl, butynyl and the like which may be optionally substituted.

The term “alkoxy” refers to the groups alkyl-O—, where alkyl is defined herein. Preferred alkoxy groups include, e.g., methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

The alkoxy can optionally be substituted with one or more alkyl, alkylidenyl, alkenylidenyl, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and COOR_x , wherein each R_x and R_y are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring.

The term “aryl” refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings, wherein at least one ring is aromatic (e.g., naphthyl, dihydrophenanthrenyl, fluorenyl, or anthryl). Preferred aryls include phenyl, naphthyl and the like.

The aryl can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and COOR_x , wherein each R_x and R_y are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl or

hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring.

The term “cycloalkyl” refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The cycloalkyl can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and $COOR_x$, wherein each R_x and R_y are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring.

The cycloalkyl can optionally be at least partially unsaturated, thereby providing a cycloalkenyl.

The term “halo” refers to fluoro, chloro, bromo, and iodo. Similarly, the term “halogen” refers to fluorine, chlorine, bromine, and iodine.

“Haloalkyl” refers to alkyl as defined herein substituted by 1-4 halo groups as defined herein, which may be the same or different. Representative haloalkyl groups include, by way of example, trifluoromethyl, 3-fluorododecyl, 12,12,12-trifluorododecyl, 2-bromooctyl, 3-bromo-6-chloroheptyl, and the like.

The term “heteroaryl” is defined herein as a monocyclic, bicyclic, or tricyclic ring system containing one, two, or three aromatic rings and containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring, and which can be unsubstituted or substituted, for example, with one or more, and in particular one to three, substituents, like halo, alkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkylsulfinyl, and alkylsulfonyl. Examples of heteroaryl groups include, but are not limited to, 2H-pyrrolyl, 3H-indolyl, 4Hquinoliziny, 4nH-carbazolyl, acridinyl, benzo[b]thienyl, benzothiazolyl, β -carbolinyl, carbazolyl, chromenyl, cinnaoliny, dibenzo[b,d]furanyl, furazanyl, furyl, imidazolyl, imidizolyl, indazolyl, indolisiny, indolyl, isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthyridinyl, naphtho[2,3-b], oxazolyl, perimidinyl, phenanthridinyl, phenanthrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, thiadiazolyl, thianthrenyl, thiazolyl, thienyl, triazolyl, and xanthenyl. In one embodiment the term “heteroaryl” denotes a monocyclic aromatic ring containing five or six ring atoms containing carbon and 1, 2, 3, or 4 heteroatoms independently selected from the group non-peroxide oxygen, sulfur, and N(Z) wherein Z is absent or is H, O, alkyl, phenyl or benzyl. In another embodiment heteroaryl denotes an ortho-bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, or tetramethylene diradical thereto.

The heteroaryl can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and COOR_x ,

wherein each R_x and R_y are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring.

The term “heterocycle” refers to a saturated or partially unsaturated ring system, containing at least one heteroatom selected from the group oxygen, nitrogen, and sulfur, and optionally substituted with alkyl or $C(=O)OR_b$, wherein R_b is hydrogen or alkyl. Typically heterocycle is a monocyclic, bicyclic, or tricyclic group containing one or more heteroatoms selected from the group oxygen, nitrogen, and sulfur. A heterocycle group also can contain an oxo group ($=O$) attached to the ring. Non-limiting examples of heterocycle groups include 1,3-dihydrobenzofuran, 1,3-dioxolane, 1,4-dioxane, 1,4-dithiane, 2H-pyran, 2-pyrazoline, 4H-pyran, chromanyl, imidazolidinyl, imidazoliny, indoliny, isochromanyl, isoindoliny, morpholine, piperazinyl, piperidine, piperidyl, pyrazolidine, pyrazolidinyl, pyrazoliny, pyrrolidine, pyrroline, quinuclidine, and thiomorpholine.

The heterocycle can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, NR_xR_y and $COOR_x$, wherein each R_x and R_y are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl, or wherein R_x and R_y , taken together along with the nitrogen atom to which they are attached form a heterocycle ring.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole,

indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles. In one specific embodiment of the invention, the nitrogen heterocycle can be 3-methyl-5,6-dihydro-4H-pyrazino[3,2,1-jk]carbazol-3-ium iodide.

Another class of heterocyclics is known as “crown compounds” which refers to a specific class of heterocyclic compounds having one or more repeating units of the formula $[-(\text{CH}_2)_a\text{A}-]$ where a is equal to or greater than 2, and A at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only, $[-(\text{CH}_2)_3\text{NH}-]_3$, $[-((\text{CH}_2)_2\text{O})_4-((\text{CH}_2)_2\text{NH})_2]$ and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term “alkanoyl” refers to $\text{C}(=\text{O})\text{R}$, wherein R is an alkyl group as previously defined.

The term “substituted alkanoyl” refers to $\text{C}(=\text{O})\text{R}$, wherein R is a substituted alkyl group as previously defined.

The term “acyl” refers to $\text{C}(=\text{O})\text{R}$, wherein R is an optionally substituted alkyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted heterocyclyl group as previously defined. Examples of acyl groups include, but are not limited to acetyl, benzoyl, cyclohexanecarbonyl, nicotinoyl, and the like.

The term “acyloxy” refers to —O—C(=O)R , wherein R is an alkyl group as previously defined. Examples of acyloxy groups include, but are not limited to, acetoxy, propanoyloxy, butanoyloxy, and pentanoyloxy. Any alkyl group as defined above can be used to form an acyloxy group.

The term “alkoxycarbonyl” refers to C(=O)OR , wherein R is an alkyl group as previously defined.

The term “amino” refers to —NH_2 , and the term “alkylamino” refers to —NR_2 , wherein at least one R is alkyl and the second R is alkyl or hydrogen. The term “acylamino” refers to RC(=O)N , wherein R is alkyl, alkylidenyl, aryl, heteroaryl and the like.

The term “imino” refers to —C=N—[H or C—] .

The term “nitro” refers to —NO_2 .

The term “trifluoromethyl” refers to —CF_3 .

The term “trifluoromethoxy” refers to —OCF_3 .

The term “cyano” refers to —CN .

The term “hydroxy” or “hydroxyl” refers to —OH .

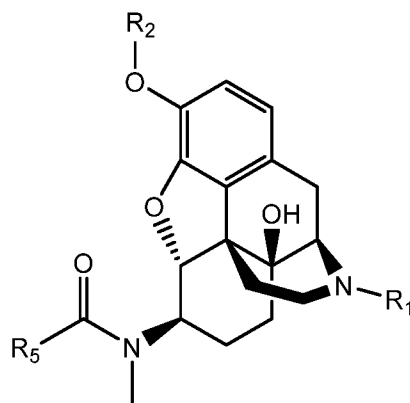
The term “oxy” refers to —O— .

The term “thio” refers to —S— .

The term “thioxo” refers to (=S) .

The term “keto” refers to ($=O$).

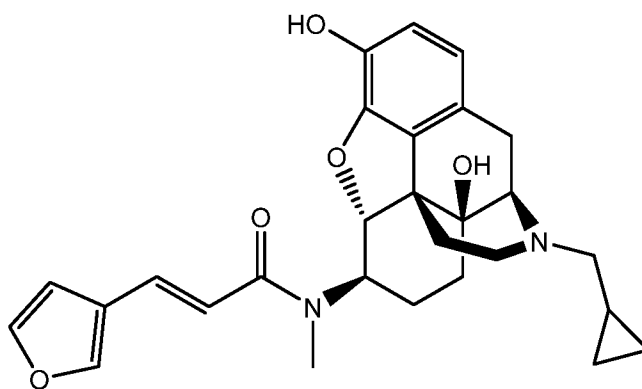
Nalfurafine is also described generically below in formula (II),



Formula (II)

wherein R_1 , R_2 , and R_5 are as described above.

The specific structure of nalfurafine is shown below:

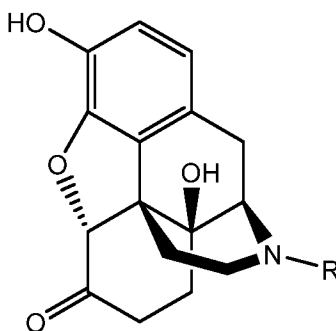


Nalfurafine

The structure of nalfurfine is formed from formula (I) when R_1 is an alkyl substituted with a cyclopropyl group; R_2 and R_3 are H; R_4 is methyl; L is $-C(O)-$; R_5 is an alkyl substituted with a heteroaryl (furanyl); and C_x and C_y form an alkyldiene group.

The structure of nalfurafine is formed from formula (II) when R_1 is an alkyl substituted with a cyclopropyl group; R_2 is H; and R_5 is an alkyl substituted with a heteroaryl (furanyl).

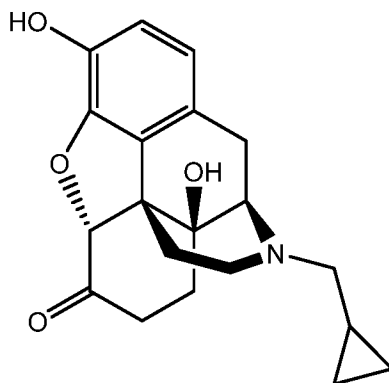
The compound naltrexone is described generically below in formula (III).



Formula (III)

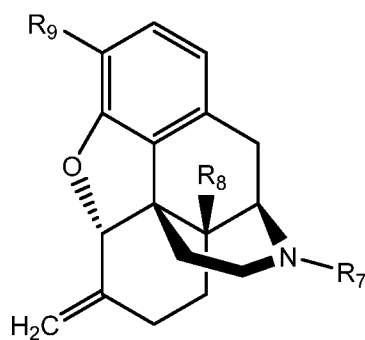
wherein R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl.

When R is cyclopropylmethyl, naltrexone is formed.



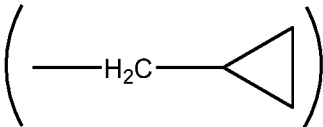
Naltrexone

The compound nalmefene is described generically below in formula (IV).

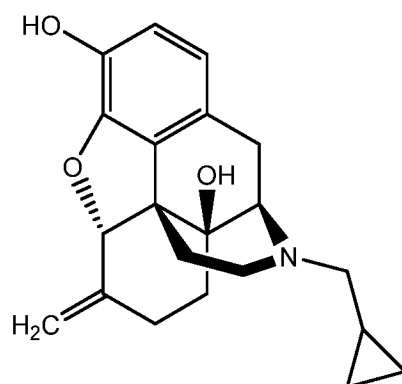


Formula (IV)

wherein R_7 is selected from the group consisting of allyl ($-\text{CH}_2\text{-HC=CH}_2$) and

cyclopropylmethyl ; R_8 is selected from the group consisting of hydrogen and hydroxyl; and R_9 is selected from the group consisting of hydroxyl and methoxy.

When R_7 is cyclopropylmethyl, R_8 is hydroxyl, and R_9 is hydroxyl, nalmefene is formed.



Nalmefene

Preferably, the opioid receptor is selected from the group consisting of μ -opioid receptor, δ -opioid receptor, κ -opioid receptor, and combinations thereof.

Pharmaceutically acceptable salts are well known in the art. Pharmaceutically acceptable salt means those salts of the compounds of formula I, II, III, and IV that are safe and effective for administration in mammals and that possess the desired biological activity. Pharmaceutically acceptable salts include salts of acidic or basic groups present in compounds of the invention. Pharmaceutically acceptable acid addition salts include, but are not limited to, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzensulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Certain compounds of the invention can form pharmaceutically acceptable salts with various amino acids. Suitable base salts include, but are not limited to, aluminum, calcium, lithium, magnesium, potassium, sodium, zinc, and diethanolamine salts. Pharmaceutically acceptable salts are discussed in *BERGE ET AL.*, 66 J. PHARM. SCI. 1-19 (1977).

For example, the most preferred pharmaceutically acceptable salt of nalfurafine is nalfurafine hydrochloride. The most preferred pharmaceutically acceptable salt of naltrexone is naltrexone hydrochloride. Likewise, the most preferred pharmaceutically acceptable salt of nalmeferene is nalmeferene hydrochloride.

Preferably, contacting includes administering (a) and (b), or (a) and (c) to the person in need thereof. In a preferred embodiment, compounds (a) and (b), or (a) and (c) are administered simultaneously. Simultaneous administration includes compounds (a) and (b), or (a) and (c) being within one formulation such as a tablet or capsule or compounds (a) and (b), or (a) and (c) being in two formulations and being taken at the same time.

For formula II, R₁ is preferably selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, and 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof. R₂ is preferably selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof; and C₂-C₆ optionally substituted alkanoyl. R₅ is preferably an optionally substituted heteroaryl.

In a preferred embodiment, R₁ is an alkyl substituted with a cyclopropyl group. R₂ is preferably H. R₅ is preferably an alkyl substituted with furanyl.

For formula II, R is preferably cyclopropylmethyl.

In a most preferred embodiment, (a) is nalfurafine or a pharmaceutically acceptable salt thereof (b) is naltrexone or a pharmaceutically acceptable salt thereof, and (c) is nalmefene or a pharmaceutically acceptable salt thereof.

Dosages and dosing frequency will be determined by a trained medical professional depending on the activity of the compounds of the invention, the characteristics of the particular formulation, the general physical condition of the person being treated, and whether the person has previously been administered any of the compounds of the invention. The dosages listed below are amounts to be administered per day. However, frequency of administration of the

dosage may be determined by a trained medical profession and includes, but is not limited to, daily administration for a certain period of time, administration every other day, administration of varying amount on weekdays versus weekends, and administration on an as needed basis.

Typically, the compound of formula I or II may be administered in a dosage of about 0.04 $\mu\text{g/kg}$ to about 0.12 $\mu\text{g/kg}$ per day. Depending upon the weight of the person, daily dosages may include, but are not limited to, the following amounts, or the following amounts may be a minimum dosage amount, a maximum dosage amount, or be used to form a range of dosages: 0.1 μg , 0.2 μg , 0.3 μg , 0.4 μg , 0.5 μg , 0.6 μg , 0.7 μg , 0.8 μg , 0.9 μg , 1.0 μg , 1.5 μg , 2.0 μg , 2.5 μg , 3.0 μg , 3.5 μg , 4.0 μg , 4.5 μg , 5.0 μg , 5.5 μg , 6.0 μg , 6.5 μg , 7.0 μg , 7.5 μg , 8.0 μg , 8.5 μg , 9.0 μg , 9.5 μg , 10.0 μg , 11.0 μg , 11.5 μg , and 12.0 μg .

For example, if the compound of formula I or II is nalfurafine or a pharmaceutically acceptable salt thereof, it may be administered in a dosage of less than 10 μg once a day. In another example, a person may take a dose of 2.5 μg or 5 μg nalfurafine or a pharmaceutically acceptable salt thereof daily. In yet another example, nalfurafine or a pharmaceutically acceptable salt thereof may be administered to a person in amounts from about 2 μg to 10 μg daily.

A low-dose of the compound of formula I or II or pharmaceutically acceptable salt thereof is defined as a dosage of 5 μg or less. For example, when the compound is nalfurafine or a pharmaceutically acceptable salt thereof, the low-dose would be 5 μg or less of the compound.

Typically, the compound of formula III may be administered in the same formulation or composition as the compound of formula I or II, or it may be administered at the same time in a different formulation.

The compound of formula III may be administered in a daily dosage of about 0.024 mg/kg to about 0.081 mg/kg, about 0.024 mg/kg to about 0.35 mg/kg, or even about 0.024 mg/kg to about 0.80 mg/kg. Depending upon the weight of the person and whether they have already taken the compound by itself, daily dosages may include, but are not limited to, the following amounts, or the following amounts may be a minimum dosage amount, a maximum dosage amount, or be used to form a range of dosages: 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg, 3 mg, 3.5 mg, 4 mg, 4.5 mg, 5 mg, 5.5 mg, 6 mg, 6.5 mg, 7 mg, 7.5 mg, 8 mg, 8.5 mg, 9 mg, 9.5 mg, 10 mg, 10.5 mg, 11 mg, 11.5 mg, 12 mg, 12.5 mg, 13 mg, 13.5 mg, 14 mg, 14.5 mg, 15 mg, 15.5 mg, 16 mg, 16.5 mg, 17 mg, 17.5 mg, 18 mg, 18.5 mg, 19 mg, 19.5 mg, 20 mg, 20.5 mg, 21 mg, 21.5 mg, 22 mg, 22.5 mg, 23 mg, 23.5 mg, 24 mg, 24.5 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, and 50 mg.

For example, if the compound is naltrexone or a pharmaceutically acceptable salt thereof the dosage may be 10 mg once or twice daily. In another example, naltrexone or a pharmaceutically acceptable salt thereof may be administered in a range from about 1 mg to about 50 mg daily or more preferably from about 1.5 mg to about 25 mg daily.

In a third example, a person may already be on a regimen of naltrexone for an alcohol use disorder and may have developed a tolerance to naltrexone after continued use. In these situations where a tolerance to one of the compounds of the invention has developed from prior continued exposure to that compound, the dosage of that compound will have to be adjusted accordingly. For example, a person who is already taking 50 mg naltrexone by itself daily may have to administered at least 50 mg naltrexone in combination with a dose of nalfurafine.

Low-doses of the compound of formula III may only be viable in a person who has either never taken the compound of formula III before or who has never developed a tolerance to the compound of formula III. A low-dose of the compound of formula III or pharmaceutically acceptable salt thereof is defined as a dosage of 25 mg or less; 20 mg or less; 15 mg or less, or 12.5 mg or less. For example, when the compound is naltrexone or a pharmaceutically acceptable salt thereof, the low-dose may be 25 mg or less of the compound.

Typically, the compound of formula IV may be administered in the same formulation or composition as the compound of formula I or II, or it may be administered at the same time in a different formulation.

The compound of formula IV may be administered in a daily dosage of about 0.024 mg/kg to about 0.081 mg/kg, about 0.024 mg/kg to about 0.35 mg/kg, or even about 0.024 mg/kg to about 0.80 mg/kg. Depending upon the weight of the person and whether they have already taken the compound by itself, daily dosages may include, but are not limited to, the following amounts, or the following amounts may be a minimum dosage amount, a maximum dosage amount, or be used to form a range of dosages: 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg, 3 mg, 3.5 mg, 4 mg, 4.5 mg, 5 mg, 5.5 mg, 6 mg, 6.5 mg, 7 mg, 7.5 mg, 8 mg, 8.5 mg, 9 mg, 9.5 mg, 10 mg, 10.5 mg, 11 mg, 11.5 mg, 12 mg, 12.5 mg, 13 mg, 13.5 mg, 14 mg, 14.5 mg, 15 mg, 15.5 mg, 16 mg, 16.5 mg, 17 mg, 17.5 mg, 18 mg, 18.5 mg, 19 mg, 19.5 mg, 20 mg, 20.5 mg, 21 mg, 21.5 mg, 22 mg, 22.5 mg, 23 mg, 23.5 mg, 24 mg, 24.5 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, and 50 mg.

For example, if the compound is nalmefene or a pharmaceutically acceptable salt thereof the dosage may be 10 mg once or twice daily. In another example, nalmefene or a pharmaceutically acceptable salt thereof may be administered in a range from about 1 mg to about 50 mg daily or more preferably from about 1.5 mg to about 25 mg daily.

In a third example, a person may already be on a regimen of nalmefene for an alcohol use disorder and may have developed a tolerance to nalmefene after continued use. In these situations where a tolerance to one of the compounds of the invention has developed from prior continued exposure to that compound, the dosage of that compound will have to be adjusted accordingly. For example, a person who is already taking 50 mg nalmefene by itself daily may have to administered at least 50 mg nalmefene in combination with a dose of nalfurafine.

Low-doses of the compound of formula IV may only be viable in a person who has either never taken the compound of formula IV before or who has never developed a tolerance to the compound of formula IV. A low-dose of the compound of formula IV or pharmaceutically acceptable salt thereof is defined as a dosage of 25 mg or less; 20 mg or less; 15 mg or less, or 12.5 mg or less. For example, when the compound is nalmefene or a pharmaceutically acceptable salt thereof, the low-dose may be 25 mg or less of the compound.

The dosage ratio of (a) a compound of formula II or a pharmaceutically acceptable salt thereof to (b) a compound of formula III or a pharmaceutically acceptable salt thereof ((a):(b)) may be about 0.1-10 : 500-2,000 or 0.5-2 : 500- 2,000. The dosage ratio of (a):(b) may also be about 1:1,000-10,000.

The dosage ratio of (a) a compound of formula II or a pharmaceutically acceptable salt thereof to (c) a compound of formula IV or a pharmaceutically acceptable salt thereof ((a):(c)) may be about 0.1-10 : 500-2,000 or 0.5-2 : 500- 2,000. The dosage ratio of (a):(c) may also be about 1:1,000-10,000.

The person using the method of invention, *i.e.*, a person in need thereof, is typically a person suffering from alcohol addiction and seeking a treatment method to reduce alcohol consumption. In addition to reducing alcohol consumption, the person may also be seeking treatment to reduce consumption of drugs of abuse such as cocaine, heroin, opiates, and marijuana. An additional benefit of the combination of compounds of the invention is an analgesic effect that provides pain relief.

It is contemplated that a person will take compounds (a) and (b) prior to consumption of alcohol. In a preferred embodiment, compounds (a) and (b) are taken at least 30 minutes, 45 minutes, 60 minutes, 75 minutes, 90 minutes, 120 minutes, 150 minutes, 180 minutes, 210 minutes, or 240 minutes prior to consumption of alcohol.

For people seeking reduction of drug use, it is contemplated that a person will take the compounds (a) and (b) prior to consumption of drugs of abuse. In a preferred embodiment, compounds (a) and (b) are taken at least 30 minutes, 45 minutes, 60 minutes, 75 minutes, 90 minutes, 120 minutes, 150 minutes, 180 minutes, 210 minutes, or 240 minutes prior to consumption of drugs of abuse.

It is contemplated that administration of the compounds of the invention will reduce alcohol consumption in a person in need thereof for at least 8 hours after administration. It is also contemplated that administration of the compounds will reduce consumption of drugs of

abuse for at least 8 hours after administration. It is further contemplated that administration of the compounds will relieve a person's pain for at least 8 hours after administration.

Another aspect of the invention relates to a method of reducing alcohol consumption in a person in need thereof, comprising administering a composition including (a) a compound of formula II or a pharmaceutically acceptable salt thereof and (b) a compound of formula III or a pharmaceutically acceptable salt thereof to the person in need thereof. Formulas II and III are as described above.

Another aspect of the invention relates to a method of treating or reducing likelihood of alcohol relapse in a person in need thereof, comprising administering a composition comprising (a) a compound of formula II or a pharmaceutically acceptable salt thereof, and one of (b) a compound of formula III or a pharmaceutically acceptable salt thereof, or (c) a compound of formula IV or a pharmaceutically acceptable salt thereof to the person in need thereof. Formulas II, III, and IV are as described above.

It is also contemplated that the person using the method of invention, *i.e.*, a person in need thereof, is a person recovering from alcohol abuse. A person recovering from alcohol abuse is a person who may have been previously treated for the alcohol abuse and has maintained sobriety for a period of time. In these cases, a composition of the invention may assist in preventing alcohol relapse.

Reducing the likelihood of alcohol relapse involves modulating the activity of an opioid receptor to reduce the desire to consume alcohol in a person recovering from alcohol abuse. Preventing alcohol relapse and assisting a person in maintaining sobriety is the ultimate goal of treatment.

Another aspect of the invention relates to a composition including (a) a compound of formula II or a pharmaceutically acceptable salt thereof; (b) a compound of formula III or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier. In a preferred embodiment, the active ingredients are only (a) and (b). In a preferred embodiment, compound (a) is nalfurafine or a pharmaceutically acceptable salt thereof and compound (b) is naltrexone or a pharmaceutically acceptable salt thereof. The dosages of the compounds in the composition are as described above.

The pharmaceutically acceptable carrier can be selected from pharmaceutically acceptable excipients and auxiliaries based on the route of administration. Pharmaceutical excipients are well known in the art.

Pharmaceutical excipients for a pharmaceutical composition may vary, the choice of excipients being guided by the intended route of administration, but excipients are well known to those skilled in the art, see e.g. Remington, The Science and Practice of Pharmacy, 21st Ed., 2005, University of the Sciences in Philadelphia, Publ. Lippincott Williams & Wilkins, incorporated by reference. For example, oral formulations may be protected from acidity in the stomach.

Another aspect of the invention relates to a composition including (a), one of (b) or (c), and a pharmaceutically acceptable carrier. In a preferred embodiment, the active ingredients are only (a) and one of (b) or (c). In a preferred embodiment, compound (a) is nalfurafine or a pharmaceutically acceptable salt thereof, compound (b) is naltrexone or a pharmaceutically acceptable salt thereof, and compound (c) is nalmefene or a pharmaceutically acceptable salt thereof. The dosages of the compounds in the composition are as described above.

The methods of the present invention can be carried out by administration of the compounds useful in practicing the invention, or pharmaceutical composition useful in practicing the invention, via any effective route of administration. The choice of route of administration will vary depending upon the circumstances of the particular subject, and taking into account such factors as age, gender, health, and weight of the recipient, condition or disorder to be treated, type of concurrent treatment (if any), the frequency of treatment, and the nature and extent of the desired effect.

In one embodiment, a pharmaceutical composition useful in practicing the invention can be administered orally and is formulated into tablets, dragees, capsules or an oral liquid preparation. A tablet is the preferred pharmaceutically acceptable carrier. In one embodiment, the oral formulation comprises extruded multiparticulates comprising the compound of the invention. In another embodiment, a pharmaceutical composition of the present invention is formulated to be administered by injection, such as intravenously, intramuscularly, subcutaneously or intrathecally.

In one embodiment, the pharmaceutical composition consists essentially of (a) and (b) or (a) and (c). Consisting essentially of excludes the presence of other active ingredients, but includes pharmaceutical excipients such as those well known in the art and described in Remington, The Science and Practice of Pharmacy, 21st Ed. The included pharmaceutical excipients do not change the function of the active ingredients (a) and (b) or (a) and (c).

In this specification, groups of various parameters containing multiple members are described. Within a group of parameters, each member may be combined with any one or more

of the other members to make additional sub-groups. For example, if the members of a group are a, b, c, d, and e, additional sub-groups specifically contemplated include any two, three, or four of the members, e.g., a and c; a, d, and e; b, c, d, and e; etc.

In some cases, the members of a first group of parameters, e.g., a, b, c, d, and e, may be combined with the members of a second group of parameters, e.g., A, B, C, D, and E. Any member of the first group or of a sub-group thereof may be combined with any member of the second group or of a sub-group thereof to form additional groups, i.e., b with C; a and c with B, D, and E, etc.

For example, in the present invention, groups of various parameters are defined (e.g. R₁, R₂, R₃, R₄, R₅, R₆, N₁, and N₂). Each group contains multiple members. For example, R₁ is represents hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, or optionally substituted heteroaryl. Each member may be combined with each other member to form additional sub-groups, e.g., hydrogen and optionally substituted heteroaryl; hydrogen and optionally substituted alkyl; and optionally substituted alkyl and optionally substituted alkynyl.

The instant invention further contemplates embodiments in which each element listed under one group may be combined with each and every element listed under any other group. For example, R₁ is represents hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, or optionally substituted heteroaryl. R₂ represents hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted

optionally substituted alkynyl, or optionally substituted alkanoyl. Each element of R_1 can be combined with each and every element of R_2 . For example, in one embodiment, R_1 may be hydrogen and R_2 may be optionally substituted alkyl. Alternatively, R_1 may be optionally substituted cycloalkyl and R_2 may be optionally substituted alkyl, etc. Similarly, a third group is R_3 , in which the elements are defined as hydrogen, OH, or optionally substituted alkoxy. Each of the above embodiments may be combined with each and every element of R_3 . For example, in the embodiment wherein R_1 is hydrogen, R_2 is optionally substituted alkyl, R_3 may be hydrogen (or any other chemical moiety within the element of R_3).

With each group, it is specifically contemplated that any one of more members can be excluded. For example, if R_3 is defined as hydrogen, OH, or optionally substituted alkoxy, it is also contemplated that R_3 is defined as hydrogen or OH.

The compounds of this invention are limited to those that are chemically feasible and stable. Therefore, a combination of substituents or variables in the compounds described above is permissible only if such a combination results in a stable or chemically feasible compound. A stable compound or chemically feasible compound is one in which the chemical structure is not substantially altered when kept at a temperature of 40°C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

A list following the word “comprising” is inclusive or open-ended, *i.e.*, the list may or may not include additional unrecited elements. A list following the words “consisting of” is exclusive or closed ended, *i.e.*, the list excludes any element not specified in the list.

Examples have been set forth below for the purpose of illustration. The scope of the invention is not to be in any way limited by the examples set forth herein.

EXAMPLES

EXAMPLE 1. Reducing Alcohol Intake

The researchers investigated whether nalfurafine alone or in combination with naltrexone changed excessive alcohol drinking in male and female C57BL/6J (B6) mice subjected to a chronic intermittent access drinking paradigm (two-bottle choice, 24-h access every other day) for 3 weeks. Neuronal proopiomelanocortin enhancer (nPE) knockout mice with brain-specific deficiency of beta-endorphin (endogenous ligand of MOP-r) were used as a genetic control for the naltrexone effects.

Animals.

C57BL/6J (B6) mice. Both male and female adult B6 mice purchased from Jackson lab were studied.

Pomc neuronal enhancers' knockout mice. Both male and female adult mice with targeted deletion of the two POMC neuronal enhancers (nPE^{-/-}) and the wildtype littermates (nPE^{+/+}) were studied.

Procedures.

1. Three-week Intermittent Access (IA) Drinking. This model of alcohol consumption in B6 mice has been widely studied in many laboratories [See, for example, Hwa LS, Chu A, Levinson SA, Kayyali TM, DeBold JF, Miczek KA (2011) Persistent escalation of alcohol drinking in C57BL/6J mice with intermittent access to 20% alcohol. Alcohol Clin Exp Res 35:1938-1947; and Zhou Y, Crowley RS, Ben K, Prisinzano TE, Kreek MJ (2017) Synergistic blockade of alcohol escalation drinking in mice by a combination of novel kappa opioid receptor agonist

Mesyl Salvinorin B and naltrexone. Brain Res 1662:75-86]. In their home cages, mice had access to alcohol when food and water were always available. In this 2-bottle free-choice model, mice exposed to chronic alcohol drinking every other day for 3 weeks. At 10:00 am (3 hours after lights off), both the alcohol (15%) solution and water sipper tubes were put on the home cages. The left/right location of the tubes was randomly set to avoid any development of side preference. The researchers recorded alcohol and water intake values at 3 time points (4, 8 and 24 hours after alcohol access) during the drinking days. Using these data, the researchers calculated alcohol intake (i.e., g/kg) and relative preference for alcohol (i.e., alcohol intake/total fluid intake). In each sex, the mice were assigned randomly to the vehicle-treated and drug-treated groups, with similar alcohol intake 1 day before the test day.

1.1. Single administration of nalfurafine, naltrexone or their combination in the IA model. On the drinking test day, alcohol was given 30 min after an injection of nalfurafine (0.3-10 μ g/kg in saline, i.p.) or vehicle (saline), and water and alcohol intake values were recorded in male and female B6 mice. Or alcohol was given 10 min after an injection of naltrexone (0.3-3mg/kg in saline, i.p.) or vehicle (saline).

When the combined effects of nalfurafine and naltrexone were studied, B6 mice received the first injection of nalfurafine (0.3-3 μ g/kg) or saline followed by the second injection of naltrexone (0.3 or 1mg/kg) or saline 20 min later. Then alcohol was given 10 min after the second injection.

Then, the researchers tested nor-BNI, a KOP-r antagonist, to pharmacologically block the KOP-r to confirm that the nalfurafine effects were mediated via KOP-r. Male B6 mice were pretreated with nor-BNI (2.5mg/kg) in saline (i.p.) 1 day before the drinking test, followed by one nalfurafine (10 μ g/kg) or vehicle injection 30 min before the drinking test.

Lastly, alcohol was given 30 min after an injection of nalfurafine (1µg/kg) or saline, and then alcohol and water intake values were recorded as described above in both nPE+/+ and nPE-/- mice of each sex.

1.2. Repeated administrations of nalfurafine in the IA model. After 3-week IA, male B6 mice received 5 consecutive injections of nalfurafine (10µg/kg) or saline every other day before each drinking session (total 5 sessions). In each session, alcohol was given 30 min after one nalfurafine or vehicle, and then intake values were recorded.

2. Three-week drinking-in-the-dark (DID). This model of alcohol consumption in B6 mice has been widely studied [See, for example, Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC (2005) Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiol Behav* 84:53-63; and Zhou Y, Crowley RS, Ben K, Prisinzano TE, Kreek MJ (2017) Synergistic blockade of alcohol escalation drinking in mice by a combination of novel kappa opioid receptor agonist Mesyl Salvinorin B and naltrexone. *Brain Res* 1662:75-86]. At 10:00 am (3 hours after lights off), the water bottle was switched to an alcohol (15%) bottle and kept for 4 hours before being switched back to the water bottle. The researchers recorded alcohol intake values after 4 hours of alcohol access. On the drinking test day, alcohol was given 30 min after an injection of nalfurafine (3 or 10µg/kg) or vehicle.

3. Sucrose (caloric reinforcer) or saccharin (non-caloric reinforcer) drinking in B6 mice. After 3-week IA, alcohol was replaced with sucrose or saccharin for 3 sessions to establish stable intakes. On the drinking test day, we recorded sucrose (4-16%) or saccharin (0.1-0.4%) and water intakes after 3 time points (4, 8 and 24 hours after single nalfurafine at 10µg/kg) in B6 mice of each sex.

The researchers also tested the nalfurafine effect in alcohol-naïve B6 mice. The procedures were identical to the above experiment, except the mice were only exposed to sucrose or saccharin.

4. Single administration of nalfurafine on locomotor activity in B6 mice. Both male and female alcohol-naïve mice were injected with nalfurafine (10µg/kg, i.p.) or vehicle. Thirty min after the injection, mice were located into the appropriate chamber in a place conditioning apparatus for 30 min, and locomotor activity was assessed as the number of “crossings”. The behavioral experiment was run between 10:00 and 11:00 am.

5. Single administration of nalfurafine on “anxiety” in B6 mice. Both male and female alcohol-naïve mice were injected with nalfurafine (10µg/kg, i.p.) or vehicle. Thirty mins after the injection, mice were placed on the central platform of the elevated plus maze facing an open arm and allowed to explore the maze for 5 min. The number of open arm entries and time spent in open arms were recorded manually as described in Maiya R, Zhou Y, Norris EH, Kreek MJ, Strickland S (2009) Tissue plasminogen activator modulates the cellular and behavioral response to cocaine. Proc Natl Acad Sci U S A. 106:1983-1988. The behavioral experiment was run between 10:00 and 11:00 am.

6. Nalfurafine on “conditioned place aversion (CPA)” in B6 mice. The behavioral experiment in male and female alcohol-naïve mice was run between 10:00 and 11:00 am using a place conditioning apparatus. See Maiya R, Zhou Y, Norris EH, Kreek MJ, Strickland S (2009) Tissue plasminogen activator modulates the cellular and behavioral response to cocaine. Proc Natl Acad Sci U S A. 106:1983-1988. A dose of 10µg/kg (i.p.) nalfurafine was chosen because it was found to reduce alcohol drinking in mice in the above experiment. The protocol had 3 phases: (1) preconditioning: On day 1, the compartment doors were open, and mice could freely explore the

entire apparatus. Their behavior was monitored for 30 minutes; (2) CPA training: During days 2 through 8 of CPA, mice were first injected with nalfurafine (10 μ g/kg, i.p.) and placed in a compartment for 30 minutes. On the alternative days, the mice were injected with saline and placed in the compartment opposite to where they received nalfurafine; and (3) CPA test: On day 9 (1 day after the last injection), the mice received no injections and were placed in the neutral, central gray compartment of the apparatus, and allowed to freely explore the entire apparatus. Their behavior was monitored for 30 minutes.

Data analysis.

Power analyses were performed to determine the number of mice required to provide statistically significant results, based on the levels of differences reported before. See Zhou Y, Crowley RS, Prinszano TE, Kreek MJ (2018) Effects of Mesyl Salvinorin B alone and in combination with naltrexone on alcohol deprivation effect in male and female mice. *Neurosci Lett* 673:19-23. In the experiments with single nalfurafine, naltrexone, their combinations or nor-BNI, the group differences in alcohol (or sucrose, saccharin) intake, water intake, total fluid and preference ratio in each sex were analyzed using 2-way ANOVA with repeated measures for treatment (vehicle vs drug) and for time interval (0-4, 5-8 vs. 9-24). For dose response analysis on nalfurafine alone and nalfurafine + naltrexone combinations, the group differences at the first 4-hour time point for alcohol intake and preference ratios were analyzed using 2-way ANOVA for treatments with different doses and for sex (male vs. female). In experiments with repeated administrations of nalfurafine, the group differences were analyzed using 1-way ANOVA. In nPE mice, the group differences in each sex were analyzed using 2-way ANOVA for genotype (nPE+/+ vs. nPE-/-) and treatments (vehicle vs. drug). All the ANOVAs were followed by

Newman-Keuls *post-hoc* tests. The accepted level of significance was $p < 0.05$ (*Statistica* version 5.5, StatSoft Inc, Tulsa, OK).

RESULTS

1. Single administration of nalfurafine alone decreased alcohol intake and preference in a dose-dependent manner after chronic IA in male and female B6 mice. After two low doses (1 and 3 $\mu\text{g/kg}$) of nalfurafine, there was no effect on alcohol intake, water intake or alcohol preference ratio in either males or females. At a higher dose 10 $\mu\text{g/kg}$, nalfurafine significantly decreased alcohol intake in males [2-way ANOVA, $F(1,13)=5.7$, $p < 0.05$] at 4 hours [*post-hoc* test $p < 0.05$] (**Figure 1A**, left) and in females [2-way ANOVA, $F(1,26)=8.2$, $p < 0.01$] at 4 hours [*post-hoc* test $p < 0.05$] (**Figure 1A**, right). There was a compensatory increase in water intake in males [$F(1,13)=6.1$, $p < 0.05$] at 4 hours [$p < 0.05$] (**Figure 1B**, left) and in females [$F(1,26)=5.4$, $p < 0.05$] at 4 hours [$p < 0.05$] (**Figure 1B**, right), resulting in unaltered total fluid intake in both males and females (**Table 1A**). At 10 $\mu\text{g/kg}$ dose, there was also a significantly reduction of preference ratio in males [$F(1,13)=6.8$, $p < 0.05$] at 4 hours [$p < 0.05$] (**Figure 1C**, left) and in females [$F(1,26)=6.2$, $p < 0.05$] at 4 hours [$p < 0.05$] (**Figure 1C**, right).

Figure 2 presents the dose response of single nalfurafine administration (0, 0.3-10 $\mu\text{g/kg}$) in alcohol intake and preference at the first 4-hour recording time. For alcohol intake (**Figure 2A**), there was a significant effect of nalfurafine [2-way ANOVA, $F(10,136)=17$, $p < 0.0001$], and *post hoc* analysis revealed that (1) compared with the control group, the nalfurafine-treated mice had less intake at 10 $\mu\text{g/kg}$ in both males and females [$p < 0.05$ for both]; and (2) the decrease at 10 $\mu\text{g/kg}$ was greater than that at 1 $\mu\text{g/kg}$ [$p < 0.05$ for both sexes]. For preference ratio (**Figure 2B**), there was a significant effect of nalfurafine [2-way ANOVA, $F(10,136)=14$, $p < 0.0001$], and *post hoc* analysis revealed that (1) compared with the control, the nalfurafine-treated mice had

less preference at 10 μ g/kg in both sexes [$p<0.05$ for both]; and (2) the decrease at 10 μ g/kg was greater than that at 1 μ g/kg [$p<0.05$ for both sexes].

2. Single nalfurafine had no effect on sucrose or saccharin intake or preference in male or female B6 mice.

In these experiments, we verified the specificity of the nalfurafine effect on alcohol intake by testing the effects of 10 μ g/kg nalfurafine on sucrose (caloric reinforcer) or saccharin (non-caloric reinforcer) consumption in mice. As shown in **Table 2**, no significant effect of 10 μ g/kg nalfurafine on 4% sucrose (**Table 2A**) or 0.1% saccharin (**Table 2C**) intake or preference was found in either males or females at the first 4-hour recording time. There was no effect observed after 8 or 24 hours neither (data not shown). We also tested the effects of nalfurafine on other concentrations of sucrose (8% or 16%) or saccharin (0.2% or 0.4%) in both males and females (n=4-5) and did not observe any significant difference. Similarly, no effect of acute nalfurafine at 10 μ g/kg on sucrose or saccharin drinking was found in alcohol-naïve males and females (data not shown).

3. nor-BNI pretreatment blocked the effect of nalfurafine on reducing alcohol drinking in male B6 mice.

For intake (**Table 3**), 2-way ANOVA showed significant effects of nalfurafine [$F(1,24)=7.7$, $p<0.05$], nor-BNI pretreatment [$F(1,24)=6.9$, $p<0.05$] and interaction between the nor-BNI pretreatment and nalfurafine [$F(1,24)=7.0$, $p<0.05$]. For preference ratio (**Table 3**), 2-way ANOVA showed significant effects of nalfurafine [$F(1,24)=17$, $p<0.05$], nor-BNI pretreatment [$F(1,24)=11$, $p<0.05$] and interaction between the nor-BNI pretreatment and nalfurafine [$F(1,24)=7.9$, $p<0.05$]. At 10 μ g/kg, nalfurafine significantly decreased alcohol intake and preference ratio [$p<0.05$ for both], and pretreatment with nor-BNI at 2.5mg/kg blunted the nalfurafine effect.

4. Single administration of naltrexone decreased alcohol consumption in a dose-dependent manner after chronic IA in male and female B6 mice. At two lower doses of naltrexone (0.3 or 1mg/kg), there was no effect on alcohol drinking in either sex. At a higher dose 3mg/kg, naltrexone significantly decreased alcohol intake in males [2-way ANOVA, $F(1,10)=7.7$, $p<0.05$] after 4 hours [$p<0.05$] (**Figure 5A**, left) and in females [2-way ANOVA, $F(1,14)=8.2$, $p<0.05$] after 4 hours [$p<0.05$] (**Figure 5A**, right). The naltrexone-treated mice showed a compensatory increase in water intake in males [$F(1,10)=6.1$, $p<0.05$] after 4 hours [$p<0.05$] and in females [$F(1,14)=5.9$, $p<0.05$] after 4 hours [$p<0.05$] (**Figure 5B**), resulting in unaltered total fluid intake (**Table 1B**). For preference ratio, two-way ANOVA showed a significant effect of naltrexone in males [$F(1,10)=9.3$, $p<0.01$] at 4 hours [$p<0.05$] (**Figure 5C**, left) and in females [$F(1,14)=10$, $p<0.01$] at 4 hours [$p<0.05$] (**Figure 5C**, right).

Like the previous reports [See Zhou et al. 2017 and 2018 cited above], naltrexone at this dose range (0.3-3mg/kg) had no effect on sucrose (4-16%) or saccharin (0.1-0.4%) consumption in either males or females (data not shown).

5. Single administration of low-dose nalfurafine combined with low-dose naltrexone decreased alcohol consumption in a dose-dependent manner after chronic IA in male and female B6 mice, with no effect on sucrose or saccharin drinking.

5.1. Nalfurafine combined with naltrexone on alcohol drinking. As shown in **Figure 2**, single administration of nalfurafine (0, 0.3 or 1 μ g/kg) combined with naltrexone (0, 0.3 or 1mg/kg) decreased alcohol intake and preference in a dose-dependent manner in both sexes (data at the first 4-hour time point are analyzed together). There was no effect of nalfurafine at 0.3 μ g/kg dose combined with naltrexone at 0.3mg/kg in either sex. Combined with a higher dose of 1mg/kg naltrexone, however, nalfurafine at 0.3 μ g/kg significantly decreased both alcohol

intake [$p<0.05$] (**Figure 2A**) and preference ratio [$p<0.05$] (**Figure 2B**) in both sexes, when compared with the vehicle control. Also, $1\mu\text{g/kg}$ nalfurafine with either 0.3 or 1mg/kg naltrexone significantly decreased alcohol intake [$p<0.05$ and $p<0.01$, respectively] (**Figure 2A**) and preference ratio [$p<0.05$ for both] (**Figure 2B**) in both sexes. The reductions at $1\mu\text{g/kg}$ nalfurafine with 0.3 or 1mg/kg naltrexone were greater than those at $1\mu\text{g/kg}$ nalfurafine alone for both sexes [$p<0.05$ and $p<0.01$, respectively].

Of note, combined with 0.1mg/kg naltrexone, other experiments also tested in both male and female mice using low doses of nalfurafine (0.3 or $1\mu\text{g/kg}$), and no significance was found (data not shown).

Figure 3 presents alcohol and water intake values at 4, 8 and 24 hours following the combination dose ($1\mu\text{g/kg}$ nalfurafine + 1mg/kg naltrexone) in males and females. In males, the combination significantly decreased alcohol intake [$F(1,10)=5.2$, $p<0.05$] between 0-4 and 5-8 hour intervals [$p<0.05$ for both] (**Figure 3A1**) and preference ratio [$F(1,10)=5.5$, $p<0.05$] after 4 hours [$p<0.01$] (**Figure 3C1**). In females, the combination decreased alcohol intake [$F(1,14)=9.8$, $p<0.01$] between 0-4 and 5-8 hour intervals [$p<0.05$ for both] (**Figure 3A2**) and preference ratio [$F(1,14)=8.7$, $p<0.01$] between 0-4 and 5-8 hour intervals [$p<0.05$ for both] (**Figure 3C2**). The combination did not have any effect on total fluid intake (**Table 1C**).

At $3\mu\text{g/kg}$ nalfurafine with 0.3 or 1mg/kg naltrexone, alcohol and water intake in females are presented in **Figure 6**. Combined with 0.3mg/kg naltrexone, $3\mu\text{g/kg}$ nalfurafine significantly decreased alcohol intake [2-way ANOVA, $F(1,10)=5.6$, $p<0.05$] between 0-4 hour interval [$p<0.05$] (**Figure 6A1**). This combination significantly decreased preference ratio [$F(1,10)=5.5$, $p<0.05$] between 0-4 interval [$p<0.05$] (**Figure 6C1**). With 1mg/kg naltrexone, $3\mu\text{g/kg}$ nalfurafine decreased alcohol intake [2-way NOVA, $F(1,14)=13.7$, $p<0.01$] between 0-4 and 5-8

hour intervals [$p < 0.05$ for both] (**Figure 6A2**), coupled with a compensatory increase in water intake between 0-4 hour interval [$p < 0.05$] (**Figure 6B2**). The combination also significantly decreased preference ratio [2-way ANOVA, $F(1,14)=11.2$, $p < 0.01$] between 0-4 and 5-8 hour intervals [$p < 0.05$ for both] (**Figure 6C2**). Neither combination dose had any effect on total fluid intake (data not shown).

5.2. No effect of single administration of nalfurafine with naltrexone on sucrose or saccharin drinking. We tested the specific effect of $1\mu\text{g/kg}$ nalfurafine + 1mg/kg naltrexone combination on sucrose and saccharin drinking after chronic IA. After 4 hours, no effect of the combination (the most effective combination for reducing alcohol) on 4% sucrose or 0.1% saccharin drinking was observed in either sex (**Table 2B, 2D**). Similarly, there was no effect of the combination on other concentrations of sucrose (8% or 16%) or saccharin (0.2% or 0.4%) intake in either males or females ($n=4-5$). Finally, no effect of the combination on sucrose or saccharin in alcohol-naïve males or females was observed (data not shown).

6. Repeated administrations of nalfurafine did not developed tolerance on decreasing

alcohol drinking. To evaluate the effect of repeated nalfurafine on alcohol intake after 3-week IA, one group of males received 5 repeated administrations of $10\mu\text{g/kg}$ nalfurafine and the effects were compared among the 5 sessions (**Figure 4**). For intake, 1-way ANOVA showed a significant effect of nalfurafine (**Figure 4A**) [$F(5,24)=4.1$, $p < 0.01$], and nalfurafine significantly decreased alcohol intake in all the sessions [$p < 0.05$ for all], with no tolerance development. For preference ratio, 1-way ANOVA showed a significant effect of nalfurafine (**Figure 4B**) [$F(5,24)=3.7$, $p < 0.05$], and nalfurafine significantly decreased preference ratio in all the sessions [$p < 0.05$ for all], without showing any tolerance.

7. Acute administration of nalfurafine at a sub-effective dose (1 μ g/kg) decreased alcohol intake after chronic IA in male and female nPE mice. In nPE males after 4 hours (**Table 4A**), 2-way ANOVA showed significant effects of genotype [$F(1,20)=61$, $p<0.005$] and nalfurafine [$F(1,20)=5.6$, $p<0.05$] on alcohol intake. *Post hoc* analysis showed that: (1) between genotypes, nPE-/- had less intake than nPE+/+ [$p<0.05$]; and (2) nalfurafine decreased intake in nPE-/- [$p<0.05$], but not nPE+/+. For water intake, 2-way ANOVA showed a significant effect of genotype [$F(1,20)=5.4$, $p<0.05$]. For alcohol preference, 2-way ANOVA showed a significant effect of genotype [$F(1,20)=37$, $p<0.01$], and nPE-/- had less preference than nPE+/+ [$p<0.05$]. Between 5-8 hours (**Table 9A**), 2-way ANOVA showed significant effects of genotype [$F(1,20)=33$, $p<0.01$], nalfurafine [$F(1,20)=5.0$, $p<0.05$] and interaction between genotype and nalfurafine [$F(2,20)=4.7$, $p<0.05$] on alcohol intake: between genotypes, nPE-/- had less intake than nPE+/+ [$p<0.05$] and nalfurafine decreased intake in nPE-/- [$p<0.05$]. There was no effect of nalfurafine between 9-24 hours (**Table 9C**).

In nPE females after 4 hours (**Table 4B**), 2-way ANOVA showed significant effects of genotype [$F(1,20)=49$, $p<0.005$] and nalfurafine [$F(1,20)=5.6$, $p<0.05$] on alcohol intake. *Post hoc* analysis found that: (1) nPE-/- had less intake than nPE+/+ [$p<0.05$] on alcohol intake; and (2) nalfurafine decreased intake in nPE-/- [$p<0.05$]. For water intake, 2-way ANOVA showed a significant effect of genotype [$F(1,20)=5.5$, $p<0.05$]. For alcohol preference, 2-way ANOVA showed a significant effect of genotype [$F(1,20)=50$, $p<0.01$], and nPE-/- had less preference than nPE+/+ [$p<0.05$]. Between 5-8 hours (**Table 9B**), 2-way ANOVA showed significant effects of genotype [$F(1,20)=29$, $p<0.01$], nalfurafine [$F(1,20)=5.1$, $p<0.05$] and interaction between genotype and nalfurafine [$F(2,20)=4.6$, $p<0.05$] on alcohol intake: nPE-/- had less

intake than nPE+/+ [$p < 0.05$], and nalfurafine further decreased intake in nPE-/- [$p < 0.05$]. There was no effect of nalfurafine between 9-24 hours (**Table 9D**).

8. Single administration of nalfurafine had no effect on alcohol intake after chronic 3-week DID in male or female B6 mice. We also determined the effect of nalfurafine using the DID model (a short-access “binge” alcohol drinking model) and found no effect on alcohol intake in either male or female mice after nalfurafine at 3 or 10 $\mu\text{g/kg}$ (**Table 5**).

9. Single administration of nalfurafine had no effect on locomotor activity in male or female alcohol-naïve B6 mice. We further tested whether nalfurafine could induce sedation in mice at the dose required to attenuate alcohol drinking. The dose of 10 $\mu\text{g/kg}$ and injection schedule (30 min before the test) were based on the above alcohol drinking experiments. There was no effect of 10 $\mu\text{g/kg}$ nalfurafine on locomotor activity in either sex (**Table 6**).

10. Single administration of nalfurafine had no effect on anxiety-like activity in male or female alcohol-naïve B6 mice. Male and female mice displayed relatively similar basal levels of anxiety, as measured by both open arm entries and time spent in the open arms after vehicle injection in an elevated plus maze test (**Table 7**). In both male and female mice, the number of open arm entries and time spent in open arms were scored 30 min after nalfurafine at 10 $\mu\text{g/kg}$, and there was no significant effect of nalfurafine.

11. Single administration of nalfurafine did not induce conditioned place aversion in male or female alcohol-naïve B6 mice. There was no significant effect of nalfurafine at 10 $\mu\text{g/kg}$ on the time spent in either the nalfurafine-paired compartment or the saline-paired compartment (**Table 8**).

To summarize, the researchers found that single administration of nalfurafine decreased alcohol intake and preference in both male and female B6 mice in a dose-dependent manner. Pretreatment with nor-BNI (a selective KOP-r antagonist) blocked the nalfurafine effect on alcohol drinking, indicating a KOP-r mediated mechanism. Pharmacological effects of a five-dosing nalfurafine regimen were further evaluated: the repeated nalfurafine administrations decreased alcohol consumption without showing any blunted effects, suggesting nalfurafine did not develop a tolerance after the multi-dosing regimen tested. Nalfurafine did not produce any “anhedonia” (sucrose preference test), “anxiety” (elevated plus maze test), “dysphoria” (conditioned place aversion test) or sedation (spontaneous locomotor activity), suggesting that nalfurafine had few side effects. To investigate synergistic effects between low-dose naltrexone and nalfurafine, the researchers found that single combinations of nalfurafine and naltrexone, at doses lower than individual effective dose, profoundly decreased excessive alcohol intake in both sexes. The effect of nalfurafine on decreasing alcohol consumption was confirmed in nPE -/- mice, suggesting independent mechanisms by which nalfurafine and naltrexone reduced alcohol drinking.

Table 1. No effects of single administration of nalfurafine (NFF, 10µg/kg) (**A**), naltrexone (NTN, 3mg/kg) (**B**), or their combination (1µg/kg NFF+1mg/kg NTN) (**C**) on total fluid intake (ml) in male and female mice after 3-week intermittent access drinking. Data in table are presented as mean ± SEM.

A.	male (n=7-8)		female (n=14)	
time	Vehicle	10 μ g/kg NFF	Vehicle	10 μ g/kg NFF
0-4h	1.5 $\pm$ 0.21	1.6 $\pm$ 0.15	1.5 $\pm$ 0.20	1.6 $\pm$ 0.17
5-8h	1.1 $\pm$ 0.16	1.1 $\pm$ 0.21	1.6 $\pm$ 0.21	1.4 $\pm$ 0.22
9-24h	2.9 $\pm$ 0.24	2.9 $\pm$ 0.31	3.2 $\pm$ 0.30	3.3 $\pm$ 0.40

B.	male (n=6-8)		female (n=6-8)	
time	Vehicle	3mg/kg NTN	Vehicle	3mg/kg NTN
0-4h	1.7 $\pm$ 0.32	1.6 $\pm$ 0.31	1.4 $\pm$ 0.25	1.5 $\pm$ 0.30
5-8h	1.3 $\pm$ 0.30	1.4 $\pm$ 0.26	1.7 $\pm$ 0.41	1.4 $\pm$ 0.33
9-24h	3.1 $\pm$ 0.41	3.0 $\pm$ 0.40	3.3 $\pm$ 0.40	3.3 $\pm$ 0.35

C.	male (n=6-8)		female (n=6-8)	
time	Vehicle	1 μ g/kg NFF+ 1mg/kg NTN	Vehicle	1 μ g/kg NFF+ 1mg/kg NTN
0-4h	1.3 $\pm$ 0.28	1.3 $\pm$ 0.33	1.7 $\pm$ 0.37	1.4 $\pm$ 0.29
5-8h	1.3 $\pm$ 0.30	1.8 $\pm$ 0.40	1.5 $\pm$ 0.31	1.6 $\pm$ 0.33
9-24h	3.1 $\pm$ 0.44	3.2 $\pm$ 0.59	3.4 $\pm$ 0.40	3.5 $\pm$ 0.47

Table 2. No effect of single administration of nalfurafine (NFF, 10 μ g/kg) alone or the combination of NFF (1 μ g/kg) and naltrexone (NTN, 1 mg/kg) on 4% sucrose (A and B) or 0.1% saccharin (C and D) intake, water intake and their preference ratio in male or female mice (n=6-7) at 4-hour time point. Data in table are presented as mean $\pm$ SEM.

A	male		female	
Treatment	Vehicle	10 μ g/kg NFF	Vehicle	10 μ g/kg NFF
Sucrose (4%) intake (g/kg)	10.9 $\pm$ 0.72	10.5 $\pm$ 0.93	12.3 $\pm$ 0.95	13.1 $\pm$ 1.2
Water intake (ml)	0.15 $\pm$ 0.08	0.13 $\pm$ 0.03	0.15 $\pm$ 0.05	0.18 $\pm$ 0.11
Preference ratio	0.97 $\pm$ 0.01	0.96 $\pm$ 0.02	0.98 $\pm$ 0.02	0.97 $\pm$ 0.01

B	male		female	
Treatment	Vehicle + Saline	1 μ g/kg NFF + 1mg/kg NTN	Vehicle + Saline	1 μ g/kg NFF + 1mg/kg NTN
Sucrose (4%) intake (g/kg)	9.8 $\pm$ 0.98	9.9 $\pm$ 0.92	11.9 $\pm$ 0.91	10.8 $\pm$ 1.0
Water intake (ml)	0.17 $\pm$ 0.06	0.20 $\pm$ 0.09	0.16 $\pm$ 0.07	0.19 $\pm$ 0.10
Preference ratio	0.97 $\pm$ 0.02	0.98 $\pm$ 0.01	0.97 $\pm$ 0.01	0.98 $\pm$ 0.01

C	male		female	
Treatment	Vehicle	10 μ g/kg NFF	Vehicle	10 μ g/kg NFF
Saccharin (0.1%) intake (g/kg)	0.13 $\pm$ 0.02	0.14 $\pm$ 0.03	0.18 $\pm$ 0.01	0.17 $\pm$ 0.02
Water intake (ml)	0.18 $\pm$ 0.10	0.21 $\pm$ 0.04	0.14 $\pm$ 0.05	0.15 $\pm$ 0.03
Preference ratio	0.96 $\pm$ 0.04	0.97 $\pm$ 0.02	0.98 $\pm$ 0.01	0.97 $\pm$ 0.02

D	male		female	
Treatment	Vehicle + Saline	1 μ g/kg NFF + 1mg/kg NTN	Vehicle + Saline	1 μ g/kg NFF + 1mg/kg NTN
Saccharin (0.1%) intake (g/kg)	0.19 $\pm$ 0.04	0.17 $\pm$ 0.05	0.20 $\pm$ 0.04	0.18 $\pm$ 0.03
Water intake (ml/4h)	0.16 $\pm$ 0.05	0.19 $\pm$ 0.08	0.16 $\pm$ 0.05	0.20 $\pm$ 0.05
Preference ratio	0.97 $\pm$ 0.03	0.98 $\pm$ 0.02	0.98 $\pm$ 0.02	0.97 $\pm$ 0.03

Table 3. Pretreatment with selective KOP-r antagonist nor-BNI (2.5mg/kg) blocks the effect of single nalfurafine (NFF, 10µg/kg) on decreasing alcohol drinking in male mice (n=6-8). *p<0.05 vs. vehicle control with the same pretreatment. Data in table are presented as mean ± SEM.

Pretreatment	Saline	Saline	Nor-BNI	Nor-BNI
Treatment	Vehicle	NFF	Vehicle	NFF
Alcohol intake (g/kg/4h)	4.8 ± 0.39	3.2 ± 0.42 *	4.9 ± 0.52	4.7 ± 0.61
Water intake (ml/4h)	0.25 ± 0.07	0.50 ± 0.22	0.22 ± 0.09	0.27 ± 0.08
alcohol preference	0.74 ± 0.04	0.54 ± 0.12 *	0.73 ± 0.05	0.70 ± 0.05

Table 4. Genotype differences in the effects of acute nalfurafine (1µg/kg) on alcohol intake and preference ratio after 3-week intermittent access drinking at 4 hours in male (A) and female (B) nPE mice. In each sex, mice were assigned to one of four groups: (1) nPE+/+ with vehicle; (2) nPE+/+ with 1µg/kg nalfurafine; (3) nPE-/- with vehicle; and (4) nPE-/- with 1µg/kg nalfurafine. Genotype difference: *p<0.05 vs. nPE+/+ mice after the same treatment; Nalfurafine effect: #p<0.05 vs. vehicle control in the same genotype. Data in table are presented as mean ± SEM.

A. Males (n=6)

Genotype	nPE +/+		nPE -/-	
Treatment	vehicle	1µg/kg nalfurafine	vehicle	1µg/kg nalfurafine
Intake, g/kg	5.2 ± 0.50	5.0 ± 0.44	2.9 ± 0.20 *	1.5 ± 0.23 * #
Water, ml	0.32 ± 0.10	0.30 ± 0.11	0.50 ± 0.15	0.61 ± 0.14
Preference	0.75 ± 0.05	0.70 ± 0.08	0.54 ± 0.06 *	0.44 ± 0.07 *

B. Females (n=6)

Genotype	nPE +/+		nPE -/-	
Treatment	vehicle	1µg/kg nalfurafine	vehicle	1µg/kg nalfurafine
Intake	6.4 ± 0.49	6.3 ± 0.38	3.1 ± 0.17 *	1.5 ± 0.22 * #
Water, ml	0.29 ± 0.09	0.27 ± 0.07	0.50 ± 0.12	0.49 ± 0.10
Preference	0.84 ± 0.07	0.83 ± 0.06	0.58 ± 0.05 *	0.45 ± 0.09 *

Table 5. No Effect of single administration of nalfurafine (3 or 10µg/kg) on alcohol intake after 3-week drinking-in-the-dark (DID) in male or female mice. Data in table are presented as mean ± SEM.

Sex	Male (n = 8)			Female (n = 8)		
Treatment	vehicle	3µg/kg	10µg/kg	vehicle	3µg/kg	10µg/kg
Alcohol intake (g/kg/4h)	5.0 ± 0.59	4.8 ± 0.51	4.7 ± 0.57	5.8 ± 0.55	5.7 ± 0.75	5.6 ± 0.82

Table 6. No effect of single administration of nalfurafine (10µg/kg) on locomotor activity in alcohol-naïve male and female mice. Locomotor activity is assessed as the number of “crossings,” defined as breaking the light beams at either end of the conditioning chamber. Data in table are presented as mean ± SEM.

Sex	Male (n = 6)		Female (n = 6)	
Treatment	vehicle	10 µg/kg	vehicle	10 µg/kg
Crossings (30 min)	228 ± 60	199 ± 48	268 ± 55	251 ± 42

Table 7. No effect of single administration of nalfurafine (10 μ g/kg) on anxiety-like activity in alcohol-naïve male and female mice. “Anxiety” levels were measured for 5 min by elevated plus maze 30 min after nalfurafine. Data in table are presented as mean $\pm$ SEM.

Sex	Male (n = 7-8)		Female (n = 8)	
Treatment	vehicle	10 μ g/kg	vehicle	10 μ g/kg
Number of open arm entries	11 $\pm$ 1.0	8.9 $\pm$ 1.2	10 $\pm$ 1.5	9.2 $\pm$ 1.1
Time spent in open arms (sec)	38 $\pm$ 4.1	40 $\pm$ 4.9	42 $\pm$ 4.5	39 $\pm$ 7.1

Table 8. No effect of single nalfurafine (10 μ g/kg) on conditioned place aversion (CPA) in alcohol-naïve male and female mice. The time spent in either the nalfurafine-paired compartment or the vehicle (saline)-paired compartment was recorded for 30 min in both the preconditioning session and CPA test for 30 min. Data in table are presented as mean $\pm$ SEM.

Sex	Male (n = 6)		Female (n = 6)	
Treatment	vehicle	10 μ g/kg	vehicle	10 μ g/kg
Preconditioning: time spent in chambers (sec)	462 $\pm$ 49	477 $\pm$ 60	431 $\pm$ 55	429 $\pm$ 61
CPA test: time spent in chambers (sec)	438 $\pm$ 52	420 $\pm$ 46	452 $\pm$ 45	449 $\pm$ 69

Table 9. Genotype differences in the effects of single nalfurafine (1 μ g/kg) on alcohol intake and preference ratio after 3-week intermittent access drinking between 5-8 hours in male (**A**) and female (**B**) nPE mice or between 9-24 hours in male (**C**) and female (**D**) nPE mice. Mice were assigned to one of four treatment groups: (1) nPE+/+ with vehicle; (2) nPE+/+ with 1 μ g/kg nalfurafine; (3) nPE-/- with vehicle; and (4) nPE-/- with 1 μ g/kg nalfurafine. Alcohol was presented 30 min after single i.p. injection of nalfurafine or vehicle, and then alcohol and water

intake were recorded after 4, 8 or 24 hours. Genotype difference: * $p < 0.05$ vs. nPE+/+ mice after the same treatment; Nalfurafine treatment effect: # $p < 0.05$ vs. vehicle control in the same genotype.

A. Males between 5-8 h (n=6)

Genotype	nPE +/+		nPE -/-	
Treatment	vehicle	1 μ g/kg nalfurafine	vehicle	1 μ g/kg nalfurafine
Intake, g/kg	4.4 $\pm$ 0.50	4.0 $\pm$ 0.59	2.6 $\pm$ 0.30 *	1.2 $\pm$ 0.15 * #
Preference	0.68 $\pm$ 0.09	0.66 $\pm$ 0.09	0.49 $\pm$ 0.14	0.39 $\pm$ 0.12

B. Females between 5-8 h (n=6)

Genotype	nPE +/+		nPE -/-	
Treatment	vehicle	1 μ g/kg nalfurafine	vehicle	1 μ g/kg nalfurafine
Intake, g/kg	6.1 $\pm$ 1.0	6.0 $\pm$ 0.77	3.9 $\pm$ 0.37 *	2.5 $\pm$ 0.13 * #
Preference	0.72 $\pm$ 0.08	0.69 $\pm$ 0.07	0.51 $\pm$ 0.10	0.38 $\pm$ 0.14

C. Males between 9-24 h (n=6)

Genotype	nPE +/+		nPE -/-	
Treatment	vehicle	1 μ g/kg nalfurafine	vehicle	1 μ g/kg nalfurafine
Intake, g/kg	8.9 $\pm$ 2.9	10.5 $\pm$ 1.6	3.7 $\pm$ 1.1 *	3.8 $\pm$ 1.0 *
Preference	0.55 $\pm$ 0.07	0.52 $\pm$ 0.09	0.49 $\pm$ 0.12	0.51 $\pm$ 0.14

D. Females between 9-24 h (n=6)

Genotype	nPE +/+		nPE -/-	
Treatment	vehicle	1 μ g/kg nalfurafine	vehicle	1 μ g/kg nalfurafine
Intake, g/kg	10.3 $\pm$ 1.4	10.2 $\pm$ 0.85	5.4 $\pm$ 0.7 *	5.8 $\pm$ 0.9 *
Preference	0.64 $\pm$ 0.04	0.61 $\pm$ 0.05	0.49 $\pm$ 0.10	0.50 $\pm$ 0.08

EXAMPLE 2. Reducing Likelihood of Alcohol Relapse

After certain time period of abstinence, there is a transient increase in alcohol intake observed in both humans and rodents, which is termed as the alcohol deprivation effect (ADE). See, *e.g.*, Burish TG, Maisto SA, Cooper AM, Sobell MB. Effects of voluntary short-term abstinence from alcohol on subsequent drinking patterns of college students. *J Stud Alcohol* 42 (1981) 1013-1020 and Vengeliene V, Bilbao A, Spanagel R. The alcohol deprivation effect model for studying relapse behavior: a comparison between rats and mice. *Alcohol* 48 (2014) 313-320. It has been demonstrated that ADE, as an appropriate animal model for alcohol relapse, has been widely studied in rats and mice. See, *e.g.*, Holter SM, Henniger MS, Lipkowski AW, Spanagel R. Kappa-opioid receptors and relapse-like drinking in long-term ethanol-experienced rats. *Psychopharmacology* 153 (2000) 93-102.; Heyser CJ, Moc K, Koob GF. Effects of naltrexone alone and in combination with acamprosate on alcohol deprivation effect in rats. *Neuropsychopharmacology* 28 (2003) 1463-1471.; Zhou Y, Schwartz B, Giza J, Gross S, Lee F, Kreek M.J. Blockade of alcohol escalation and "relapse" drinking by pharmacological FAAH inhibition in male and female C57BL/6J mice. *Psychopharmacology* 234 (2017) 2955-2970.; and Zhou Y, Crowley RS, Prisinzano TE, Kreek MJ. Effects of Mesyl Salvinorin B alone and in combination with naltrexone on alcohol deprivation effect in male and female mice. *Neurosci Lett* 673 (2018) 19-23.

Specifically, in the mouse model used, after chronic (1-week) abstinence from chronic (3-week) intermittent-access alcohol drinking with excessive alcohol consumption, both males and females show a significant increase in alcohol intake after 4 hours of alcohol access when alcohol is provided again.

Animals.

C57BL/6J mice (8-week-old) in both sexes from The Jackson Laboratory (Bar Harbor, ME, USA) were purchased and housed in a temperature-controlled room (21 °C). After arrival, mice were maintained on a 12-hour reverse light-dark cycle (lights off at 7:00 am) and acclimated for at least one week prior to the experiments. Mice were individually housed and given *ad libitum* access to food and water. Animal care and experimental procedures were conducted according to Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources Commission on Life Sciences 1996) and were approved by the IACUC (Institutional Animal Care and Use Committee) of the Rockefeller University.

Materials.

Ethanol solution was prepared from 190 proof absolute ethyl alcohol (Pharmco-AAPER, Brookfield, CT, USA) and dissolved in tap water. Naltrexone hydrochloride (NTX) was purchased from Sigma-Aldrich, nalmefene hydrochloride (NMF) from Baker Norton Pharmaceuticals, and both dissolved in physiological saline. Nalfurafine (NFF) and nor-BNI was obtained from the NIDA Division of Drug Supply and Analytical Services and dissolved in saline.

Procedures.

Alcohol deprivation effect (ADE) after chronic intermittent-access alcohol drinking

Table 10. The alcohol deprivation effect (ADE) model after 1-week abstinence from a 3-week chronic intermittent-access (IA) drinking (alcohol vs. water).

Weeks 1-2	Week 3	Week 4	Week 5
24-h alcohol vs water	24-h alcohol vs water Baseline	Alcohol abstinence	24-h alcohol vs water ADE

Chronic intermittent-access alcohol drinking model is a two-bottle choice paradigm with alcohol drinking every other day for 3 weeks. Three hours after lights off, both the water and alcohol (15%) solution sipper tubes were provided, and the sides (left or right one of the cage) of the bottles were randomly positioned on their home cages to avoid the development of side preference. The alcohol bottle was filled with fresh 15% alcohol and kept for 24 h before being replaced by the water bottle. Both alcohol and water intake values were recorded after 4, 8 and 24 hours of alcohol access in the drinking days and calculated consumed alcohol intake (g/kg) and relative preference for alcohol (alcohol intake/total fluid intake).

At the end of the 3-week intermittent-access alcohol drinking, 30% alcohol and water bottles were provided on day 21 (week 3) and their intake values at 4, 8 and 24 hours were recorded in the Baseline session. Then, alcohol bottle was not provided for the following 7 days. On day 28 (week 5), after the 1-week abstinence, we provided alcohol (30%) bottles again 3 h after lights off and recorded the alcohol and water intakes at 4, 8 and 24 h in the ADE session.

Administration of NFF, NTX or NFF+NTX in ADE

Mice in each sex were randomly assigned into the drug-treated and vehicle groups with similar alcohol consumption in the Baseline session on day 21. On day 28, the experimenter who was blinded to the treatment codes injected the vehicle and drugs before the ADE test. The mice in the vehicle control groups received one or two vehicle injections; and the mice in the drug groups received one drug (NFF, NTX, or NMF) or two drug (NFF+NTX) injections. Then, the alcohol bottles were presented and alcohol and water intakes were recorded. [a] The range of NFF doses was based on the inventor's publication. See Zhou Y, Kreek MJ. Combination of human kappa opioid receptor agonist nalfurafine with low-dose naltrexone reduces excessive

alcohol drinking in male and female mice. Alcohol Clin Exp Res 43 (2019) 1077-1090. Mice received NFF (1, 3 or 10 μ g/kg, i.p.) or vehicle (saline) 30 min before the ADE test; [b] The range of NTX doses was based on the inventor's study See Zhou Y, Crowley RS, Prisinzano TE, Kreek MJ. Effects of Mesyl Salvinorin B alone and in combination with naltrexone on alcohol deprivation effect in male and female mice. Neurosci Lett 673 (2018) 19-23. Mice received NTX (0.3, 1 or 3 mg/kg, i.p.) or vehicle (saline) 10 min before the ADE test; and [c] The NFF+NTX dose chosen was also based on the above NFF and NTX experiments: mice received the first injection of NFF at low-dose 1 μ g/kg 30 min before the ADE test, followed by the second one of NTX (0.3 or 1 mg/kg) in saline vehicle 10 min before the ADE test.

Administration of NMF or NFF+NMF in ADE

The range of NMF doses (0.125, 0.25, 0.5 or 1 mg/kg, i.p.) was based on pilot studies and a previous publication. Using the same paradigm for the above drugs, mice received one NMF injection or one saline 30 min before the ADE test. And the NFF+NMF dose chosen was also based on the above NFF and NMF experiments: mice received the first injection of NFF at low-dose 1 μ g/kg 30 min before the ADE test, immediately followed by the second one of NMF (0.125 mg/kg) in saline vehicle before the ADE test.

Administration of nor-BNI or nor-BNI+NTN in ADE

Selective KOP-r antagonist nor-BNI was tested on ADE, using the same paradigm for the above drugs with an exception: mice were pretreated with nor-BNI (5, 10 or 20 mg/kg, i.p.) or saline on day 27 (1 day before the ADE test). The nor-BNI dose was based on our pilot studies testing in a drinking-in-the-dark (DID) model and on an early publication in the intermittent-access drinking model. And the nor-BNI+NTX dose chosen was based on the above experiments. Using the same paradigm, mice were pretreated with nor-BNI (20 mg/kg, i.p.) or

saline on day 27 (1 day before the ADE test), followed by the second one of NTX (1 mg/kg) in saline 10 min before the ADE test.

Data analysis.

Based on the levels of differences in the previous experiments [Zhou et al 2017, 2018], power analyses were performed to determine the number of animals required to provide statistical significances. It was predicted that 8-12 mice per group in each sex were required in the present studies. If similar effects on the ADE with no significant sex differences were seen after each compound or their combination, data of each sex were analyzed and presented separately. Alcohol intake differences across the different groups were analyzed using two-way ANOVA for treatment (vehicle vs drug doses) and for sessions (Baseline vs ADE) in each sex, with testing our *a priori* hypothesis that there were effects of ADE or drug treatment, based on the published findings and the new hypothesis. Two-way ANOVA was followed by Newman-Keuls *post-hoc* tests, and the accepted level of significance was $p < 0.05$. All statistical analyses were performed using *Statistica* (version 5.5, StatSoft Inc, Tulsa, OK).

Effect of NFF on ADE. In the 1st experiment, the effect of NFF at 1 µg/kg dose was tested in both sexes, and there was no difference between saline control and NFF treatment in either males or females at 4, 8 or 24 hours (data not shown). In the 2nd experiment, both 3 and 10 µg/kg doses were tested, and the results on alcohol intake at 4 hours are shown in **Figure 7**.

In males (**Figure 7A**), two-way ANOVA revealed a significant effect of NFF [$F(1,48)=4.6$, $p < 0.05$], Session [$F(1,48)=5.6$, $p < 0.05$], and a significant interaction between Session and NFF [$F(1,48)=6.4$, $p < 0.01$]. *Post hoc* analysis showed that: (1) males had more intake in the ADE session than that in the Baseline [$p < 0.05$]; and (2) the 10 µg/kg NFF-treated

males had less intake than the vehicle controls in the ADE session [$p < 0.01$]. NFF at a low dose ($3\mu\text{g/kg}$), however, did not significantly reduce ADE at 4 hours in males.

In females (**Figure 7B**), two-way ANOVA showed a significant effect of Session [$F(1,48)=4.1$, $p < 0.05$] and a marginally significant effect of NFF [$F(1,48)=2.9$, $p=0.07$]. To test our *a priori* hypothesis that NFF would reduce ADE, we included the Newman-Keuls *post-hoc* results: (1) females had more intake in the ADE session than that in the Baseline [$p < 0.05$]; and (2) the $10\mu\text{g/kg}$ NFF-treated females had less intake than the vehicle-treated controls in the ADE session [$p < 0.05$], though 2-way ANOVA did not show a significant effect of NFF. Similar to males, NFF at $3\mu\text{g/kg}$ did not reduce ADE at 4 hours in females.

After 8 or 24 hours, there were no significant effects of NFF on alcohol intake at these 3 doses in either sex.

For alcohol preference, there was no significant effect of ADE or NFF on preference ratio in either sex at any doses tested. NFF at these doses did not change water intake after 4, 8 or 24 hours.

Effect of NTX on ADE. In a pilot study, the researchers tested the effect of 1mg/kg NTX, and found the NTX had no significant effect on ADE at 4 hours in either sex.

In males with NTX at 3mg/kg (**Table 11A**), two-way ANOVA revealed significant effects of Session [$F(1,24)=5.1$, $p < 0.05$], NTX [$F(1,24)=4.6$, $p < 0.05$] and a significant interaction between Session and NTX [$F(1,24)=4.5$, $p < 0.05$]. The *post-hoc* results showed that: (1) males had more intake in the ADE session than that in the Baseline [$p < 0.05$]; and (2) the NTX-treated males had less intake than the vehicle controls in the ADE session [$p < 0.05$]. After 8 or 24 hours, there were no significant effects of NTX on alcohol intake in males (data not shown).

In females with NTX at 3mg/kg (**Table 11B**), two-way ANOVA revealed significant effects of Session [$F(1,26)=5.3$, $p<0.05$], NTX [$F(1,26)=5.4$, $p<0.05$], and a significant interaction between Session and NTX [$F(1,26)=4.8$, $p<0.05$]. The *Post-hoc* analysis showed that: (1) females had more intake in the ADE session than that in the Baseline [$p<0.05$]; and (2) the NTX-treated females had less intake than the controls in the ADE session [$p<0.05$]. After 8 or 24 hours, there were no significant effects of NTX on alcohol intake in females (data not shown).

For alcohol preference, there was no significant effect of ADE or NTX in either sex at any time points (data not shown).

Effect of NFF combined with NTX on ADE. In a pilot study, the researchers tested the effect of 1 μ g/kg NFF combined with NTX at 0.3mg/kg, and found the combination had no significant effect on ADE at 4 hours in either sex, though a slight reduction in male. With a higher dose of NTX at 1mg/kg combined with 1 μ g/kg NFF, the ADE intake at 4 hour are shown in **Figure 8**.

In males (**Figure 8A**), two-way ANOVA revealed significant effects of Session [$F(1,36)=5.9$, $p<0.05$], Combination [$F(1,36)=4.7$, $p<0.05$] and a marginally significant interaction between Session and Combination [$F(1,36)=3.0$, $p=0.06$]. To test our *a priori* hypothesis that there was an effect of the combination, we included the *post-hoc* results showing that: (1) males had more intake in the ADE session than that in the Baseline [$p<0.05$]; and (2) the NFF+NTX-treated males had less intake than the vehicle controls in the ADE session [$p<0.05$]. After 8 or 24 hours, there were no significant effects of the combination on alcohol intake in males (data not shown).

In females (**Figure 8B**), two-way ANOVA revealed significant effects of Session [$F(1,32)=10.6$, $p<0.01$] and Combination [$F(1,32)=4.4$, $p<0.05$], and a significant interaction

between Session and Combination [$F(1,32)=4.3$, $p<0.05$]. *Post-hoc* analysis showed that: (1) females had more intake in the ADE session than that in the Baseline [$p<0.05$]; and (2) the NFF+NTX-treated females had less intake than the vehicle controls in the ADE session [$p<0.05$]. After 8 or 24 hours, there were no significant effects of the combination on alcohol intake in females (data not shown).

For alcohol preference, there was no significant effect of ADE or NFF+NTX in either sex at any time points (data not shown).

Effect of NMF on ADE. In this experiment, the effect of NMF at 0.125, 0.25 or 0.5mg/kg on alcohol intake was tested and **Figure 9** presents the data at 4 hours.

In males (**Figure 9A**), two-way ANOVA showed a significant effect of NMF [$F(1,72)=2.8$, $p<0.05$], Session [$F(1,72)=7.1$, $p<0.01$] and a significant interaction between Session and NMF [$F(1,72)=3.7$, $p<0.05$]. *Post hoc* analysis showed that: (1) the males had more intake in the ADE session than that in the Baseline [$p<0.05$]; and (2) the 0.5 mg/kg NMF-treated males had less intake than the vehicle control in the ADE session [$p<0.01$]. NMF at low doses (0.125 or 0.25 mg/kg), however, did not significantly reduce ADE at 4 hours in males.

In females (**Figure 9B**), two-way ANOVA revealed a significant effect of NMF [$F(1,84)=3.5$, $p<0.05$], Session [$F(1,84)=11$, $p<0.01$] and a significant interaction between Session and NMF [$F(1,84)=2.8$, $p<0.05$]. *Post hoc* analysis showed that: (1) the females had more intake in the ADE session than that in the Baseline [$p<0.05$]; and (2) the 0.5mg/kg NMF-treated females had less intake than the vehicle-treated controls in the ADE session [$p<0.01$]. Similar to males, NMF at two lower doses (0.125 and 0.25 mg/kg) did not significantly reduce ADE in females.

After 8 or 24 hours, there were no significant effects of NMF on either alcohol intake in either sex (data not shown). For alcohol preference, there was no significant effect of ADE or NMF on preference ratio in either sex at any time points (data not shown).

Effect of NFF combined with NMF on ADE. With NMF at 0.125mg/kg combined with 1µg/kg NFF, the ADE intake at 4 hour is shown in **Figure 10**.

In males (**Figure 10A**), two-way ANOVA showed significant effects of Session [$F(1,36)=6.0$, $p<0.05$], Combination [$F(1,36)=4.8$, $p<0.05$] and a significant interaction between Session and Combination [$F(1,36)=4.5$, $p<0.05$]. *Post-hoc* analysis showed that: (1) males had more intake in the ADE session than that in the Baseline [$p<0.05$]; and (2) the NFF+NMF-treated males had less intake than the controls in the ADE session [$p<0.05$]. After 8 or 24 hours, there were no significant effects of the combination on alcohol intake (data not shown).

In females (**Figure 10B**), two-way ANOVA revealed significant effects of Session [$F(1,36)=7.6$, $p<0.01$], Combination [$F(1,36)=4.9$, $p<0.05$] and a significant interaction between Session and Combination [$F(1,36)=4.7$, $p<0.05$]. *Post-hoc* analysis revealed that: (1) females had more intake in the ADE session than that in the Baseline [$p<0.05$]; and (2) the NFF+NMF-treated females had less intake than the controls in the ADE session [$p<0.05$]. After 8 or 24 hours, there were no significant effects of the combination on alcohol intake (data not shown).

There was no significant effect of ADE or NFF+NMF on alcohol preferences in either sex at any time points (data not shown).

2.6. Effect of nor-BNI on ADE. At two low doses tested: 5 or 10 mg/kg, there were no significant effects of nor-BNI on alcohol intake in either sex (data not shown). The results at 20 mg/kg are shown in **Table 12**.

In males at 4 hours (**Table 12A**), two-way ANOVA revealed a significant effect of Session [$F(1,48)=8.5$, $p<0.01$]. *Post hoc* analysis showed that males had more intake in the ADE session than that in the Baseline [$p<0.05$]. In females at 4 hours (**Table 12B**), two-way ANOVA revealed a significant effect of Session [$F(1,48)=9.6$, $p<0.01$]. *Post hoc* analysis showed that females had more intake in the ADE session than that in the Baseline [$p<0.05$]. After 4 hours, there were no significant effects of nor-BNI on alcohol intake in either sex at 20 mg/kg.

2.7. Effect of nor-BNI plus NTN on ADE. After 4, 8 or 24 hours, there were no significant effects of nor-BNI+NTN on ADE in either sex (data not shown).

Table 11. Effects of NTX (3 mg/kg) on alcohol intake in an alcohol deprivation effect (ADE) model at 4 hours in males (A, $n=6$) and females (B, $n=7-9$) after 1 week of abstinence from 3-week intermittent-access alcohol drinking. * $p<0.05$ vs. control Baseline, and + $p<0.05$ vs. control ADE.

A. Male

	Time point	Vehicle		20 mg/kg Nor-BNI	
		Baseline	ADE	Baseline	ADE
Intake, g/kg	0-4 hour	5.3 ± 0.3	8.6 ± 1.1 *	5.5 ± 0.5	6.1 ± 0.9 * +

B. Female

	Time point	Vehicle		20 mg/kg Nor-BNI	
		Baseline	ADE	Baseline	ADE
Intake, g/kg	0-4 hour	6.5 ± 0.6	11.9 ± 1.2 *	6.4 ± 0.9	7.8 ± 0.9 * +

Table 12. No effects of nor-BNI (20 mg/kg) on alcohol intake in an alcohol deprivation effect (ADE) model at 4 hours in males (A, $n=9$) and females (B, $n=9$) after 1 week of abstinence from 3-week intermittent-access alcohol drinking. * $p<0.05$ vs. control Baseline.

A. Male

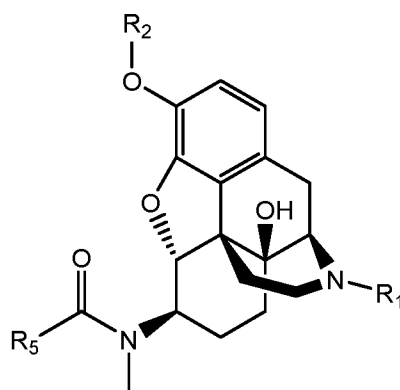
	Time point	Vehicle		20 mg/kg Nor-BNI	
		Baseline	ADE	Baseline	ADE
Intake, g/kg	0-4 hour	5.2 ± 0.5	7.6 ± 0.9 *	5.3 ± 0.7	7.1 ± 1.0 *

B. Female

	Time point	Vehicle		20 mg/kg Nor-BNI	
		Baseline	ADE	Baseline	ADE
Intake, g/kg	0-4 hour	6.7 ± 0.7	12.3 ± 1.1 *	6.6 ± 0.5	12.0 ± 1.2 *

Claims

1. A method of modulating the activity of an opioid receptor, comprising contacting the opioid receptor of a person in need thereof with (a) a compound of formula II



II

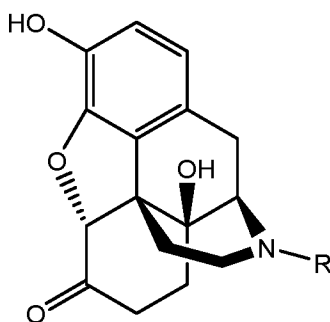
or a pharmaceutically acceptable salt thereof, wherein:

R_1 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R_2 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl;

R_5 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

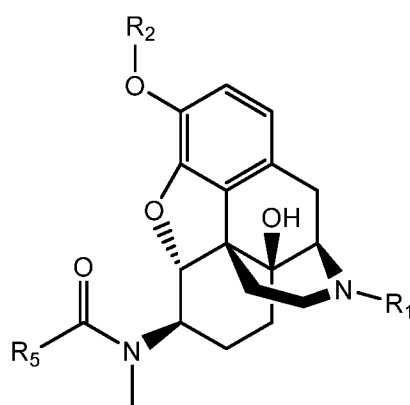
and (b) a compound of formula III



III

or a pharmaceutically acceptable salt thereof, wherein R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl.

2. The method according to any one of the preceding claims, wherein the opioid receptor is selected from the group consisting of μ -opioid receptor, δ -opioid receptor, κ -opioid receptor, and combinations thereof.
3. The method according to any one of the preceding claims, wherein the contacting comprises administering (a) and (b) to the person.
4. The method according to any one of the preceding claims, wherein (a) and (b) are administered simultaneously.
5. A method of reducing alcohol consumption in a person in need thereof, comprising administering a composition comprising (a) a compound of formula II



II

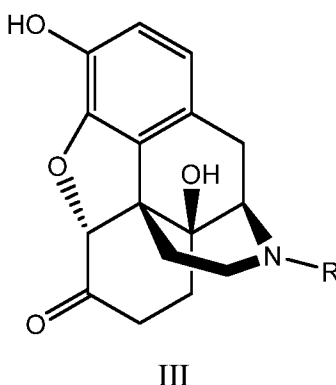
or a pharmaceutically acceptable salt thereof, wherein:

R_1 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R_2 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted optionally substituted alkynyl, and optionally substituted alkanoyl;

R₅ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

and (b) a compound of formula III



or a pharmaceutically acceptable salt thereof to the person in need thereof, wherein R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl.

6. The method according to any one of the preceding claims, wherein

R₁ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, and 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof;

R₂ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof; and C₂-C₆ optionally substituted alkanoyl; and

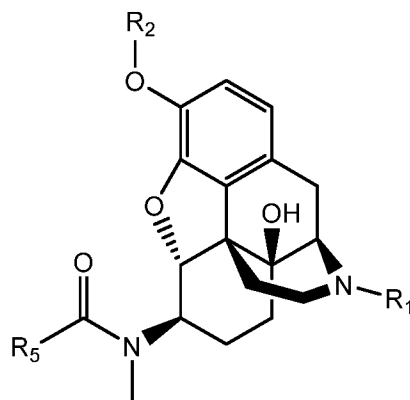
R₅ is optionally substituted heteroaryl.

7. The method according to any one of the preceding claims, wherein R₁ is an alkyl substituted with a cyclopropyl group.

8. The method according to any one of the preceding claims, wherein R₂ is H.
9. The method according to any one of the preceding claims, wherein R₅ is an alkyl substituted with furanyl.
10. The method according to any one of the preceding claims, wherein R is cyclopropylmethyl.
11. The method according to any one of the preceding claims, wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof.
12. The method according to any one of the preceding claims, wherein the nalfurafine or a pharmaceutically acceptable salt thereof is administered in a dosage of about 0.04 µg/kg to about 0.12 µg/kg per day.
13. The method according to any one of the preceding claims, wherein the naltrexone or a pharmaceutically acceptable salt thereof is administered in a dosage of about 0.024 mg/kg to about 0.80 mg/kg per day.
14. The method according to any one of the preceding claims, wherein the pharmaceutically acceptable salt of nalfurafine is nalfurafine hydrochloride.
15. The method according to any one of the preceding claims, wherein the pharmaceutically acceptable salt of naltrexone is naltrexone hydrochloride.
16. The method according to any one of the preceding claims, wherein the person suffers from alcohol addiction.
17. The method according to any one of the preceding claims, wherein (a) and (b) are administered to a person in need thereof prior to consumption of alcohol.

18. The method according to any one of the preceding claims, wherein the administering occurs at least 30 min, at least 60 min, at least 90 min, at least 120 min, or at least 240 min prior to consumption of alcohol.
19. The method according to any one of the preceding claims, wherein the nalfurafine or a pharmaceutically acceptable salt thereof is administered in a low-dose, wherein low-dose comprises 5 µg or less nalfurafine or a pharmaceutically acceptable salt thereof per day.
20. The method according to any one of the preceding claims, wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (b) is 0.1-10:500-2,000 or 0.5-2:500-2,000.
21. The method according to any one of the preceding claims, wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (b) is 1:1,000-10,000.
22. The method according to any one of the preceding claims, wherein (b) naltrexone or a pharmaceutically acceptable salt thereof is administered in a dose of about 1 mg to about 50 mg per day.
23. The method according to any one of the preceding claims, wherein alcohol consumption is reduced for at least 8 hours after administration of the composition.
24. The method according to any one of the preceding claims, wherein consumption of drugs of abuse is also reduced.
25. The method according to claim 24, wherein the drugs of abuse comprise cocaine, heroin, opiates, marijuana, or combinations thereof.
26. The method according to any one of the preceding claims, wherein the person's pain is reduced.

27. A composition comprising (a) a compound of formula II



II

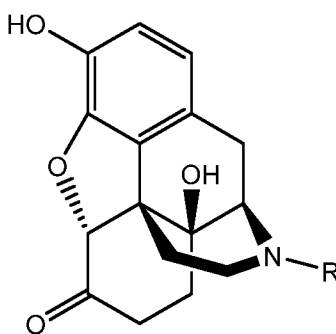
or a pharmaceutically acceptable salt thereof, wherein:

R_1 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R_2 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted acanoyl;

R_5 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

- (b) a compound of formula III



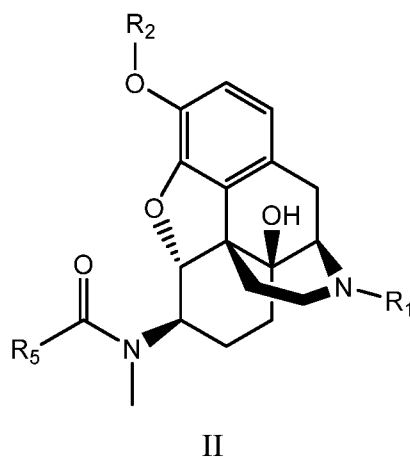
III

or a pharmaceutically acceptable salt thereof, wherein R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl; and a pharmaceutically acceptable carrier.

28. The composition according to claim 27 wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof.

29. The composition according to claim 27 or 28, wherein the active ingredients consist of nalfurafine or a pharmaceutically acceptable salt thereof and naltrexone or a pharmaceutically acceptable salt thereof.

30. A method of modulating the activity of an opioid receptor, comprising contacting the opioid receptor of a person in need thereof with (a) a compound of formula II



or a pharmaceutically acceptable salt thereof, wherein:

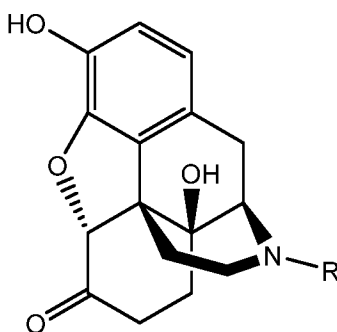
R₁ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R₂ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted optionally substituted alkynyl, and optionally substituted alkanoyl; and

R₅ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted

cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

and one of (b) a compound of formula III

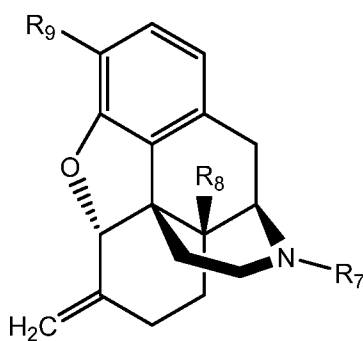


III

or a pharmaceutically acceptable salt thereof, wherein:

R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl; or

(c) a compound of formula IV



IV

or a pharmaceutically acceptable salt thereof, wherein:

R₇ is selected from the group consisting of -CH₂-HC=CH₂ and cyclopropylmethyl;

R₈ is selected from the group consisting of hydrogen and hydroxyl; and

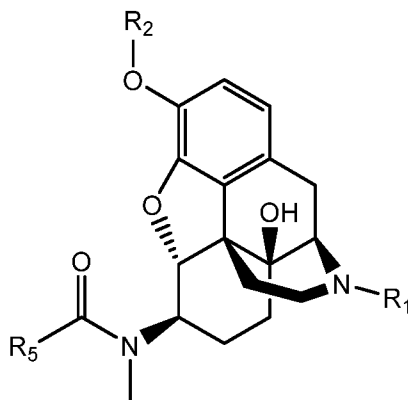
R₉ is selected from the group consisting of hydroxyl and methoxy.

31. The method according to claim 30, wherein the opioid receptor is selected from the group consisting of μ -opioid receptor, δ -opioid receptor, κ -opioid receptor, and combinations thereof.

32. The method according to claim 30 or 31, wherein the contacting comprises administering (a) and one of (b) or (c) to the person.

33. The method according to claims 30-32, wherein (a) and one of (b) or (c) are administered simultaneously.

34. A method of treating or reducing likelihood of alcohol relapse in a person in need thereof, comprising administering a composition comprising (a) a compound of formula II



II

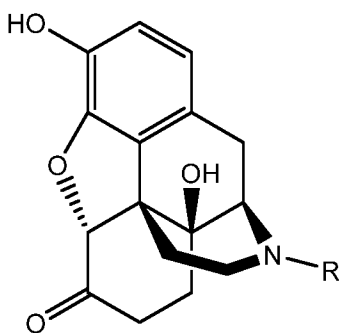
or a pharmaceutically acceptable salt thereof, wherein:

R₁ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R₂ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted optionally substituted alkynyl, and optionally substituted akanoyl; and

R₅ is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

and one of (b) a compound of formula III

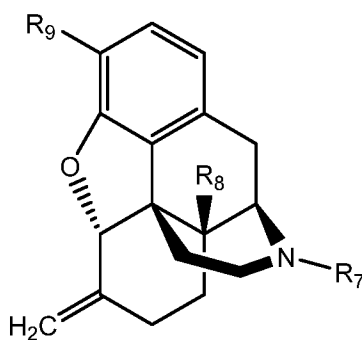


III

or a pharmaceutically acceptable salt thereof, wherein:

R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl; or

(c) a compound of formula IV



IV

or a pharmaceutically acceptable salt thereof, wherein:

R₇ is selected from the group consisting of -CH₂-HC=CH₂ and cyclopropylmethyl;

R₈ is selected from the group consisting of hydrogen and hydroxyl; and

R₉ is selected from the group consisting of hydroxyl and methoxy.

35. The method according to claims 30-34, wherein

R₁ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, and 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof;

R₂ is selected from the group consisting of hydrogen, C₁-C₅ optionally substituted alkyl, C₂-C₅ optionally substituted alkenyl, C₂-C₅ optionally substituted alkynyl, C₃-C₆ optionally substituted cycloalkyl, C₃-C₆ optionally substituted cycloalkenyl, C₆-C₁₂ optionally substituted aryl, 5 or 6-membered optionally substituted heteroaryl containing 1-3 nitrogen, oxygen, or sulfur atoms, or a combination thereof; and C₂-C₆ optionally substituted alkanoyl; and

R₅ is optionally substituted heteroaryl.

36. The method according to claims 30-35, wherein R₁ is an alkyl substituted with a cyclopropyl group.

37. The method according to claims 30-36, wherein R₂ is H.

38. The method according to claims 30-37, wherein R₅ is an alkyl substituted with furanyl.

39. The method according to claims 30-38, wherein R is cyclopropylmethyl.

40. The method according to claims 30-39, wherein R₇ is cyclopropylmethyl, and R₈ and R₉ are hydroxyl.

41. The method according to claims 30-40, wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof, (b) is naltrexone or a pharmaceutically acceptable salt thereof, and (c) is nalmefene or a pharmaceutically acceptable salt thereof.

42. The method according to claims 30-41, wherein the nalfurafine or a pharmaceutically acceptable salt thereof is administered in a dosage of about 0.04 µg/kg to about 0.12 µg/kg per day.

43. The method according to claims 30-42, wherein the naltrexone or a pharmaceutically acceptable salt thereof is administered in a dosage of about 0.024 mg/kg to about 0.80 mg/kg per day.

44. The method according to claims 30-43, wherein the nalmefene or a pharmaceutically acceptable salt thereof is administered in a dosage of about 0.05 mg/kg to about 1.0 mg/kg per day.

45. The method according to claims 30-44, wherein the pharmaceutically acceptable salt of nalfurafine is nalfurafine hydrochloride.

46. The method according to claims 30-45, wherein the pharmaceutically acceptable salt of naltrexone is naltrexone hydrochloride.

47. The method according to claims 30-46, wherein the pharmaceutically acceptable salt of nalmefene is nalmefene hydrochloride.

48. The method according to claims 30-47, wherein the person suffers from alcohol addiction.

49. The method according to claims 30-48, wherein (a) and one of (b) or (c) are administered to a person in need thereof prior to consumption of alcohol.

50. The method according to claims 30-49, wherein the person suffers from alcohol addiction.

51. The method according to claims 30-50, wherein the nalfurafine or a pharmaceutically acceptable salt thereof is administered in a low-dose, wherein low-dose comprises 5 µg or less nalfurafine or a pharmaceutically acceptable salt thereof per day.

52. The method according to claims 30-51, wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (b) is 0.1-10:500-2,000 or 0.5-2:500-2,000.

53. The method according to claims 30-52, wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (b) is naltrexone or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (b) is 1:1,000-10,000.

54. The method according to claims 30-53, wherein (b) is naltrexone or a pharmaceutically acceptable salt thereof is administered in a dose of about 1 mg to about 50 mg per day.

55. The method according to claims 30-54, wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof and (c) is nalmefene or a pharmaceutically acceptable salt thereof and dosage ratio of (a) : (c) is 1:1,000-10,000.

56. The method according to claims 30-55, wherein (c) is nalmefene or a pharmaceutically acceptable salt thereof is administered in a dose of about 1 mg to about 50 mg per day.

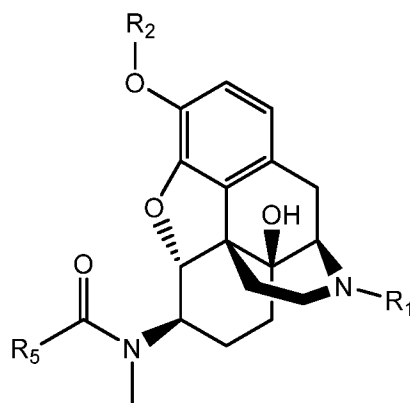
57. The method according to claims 30-56, wherein alcohol consumption is reduced for at least 8 hours after administration of the composition.

58. The method according to claims 30-57, wherein consumption of drugs of abuse is also reduced.

59. The method according to claim 58, wherein the drugs of abuse comprise cocaine, heroin, opiates, marijuana, or combinations thereof.

60. The method according to claims 30-59, wherein the person's pain is reduced.

61. A composition comprising (a) a compound of formula II



II

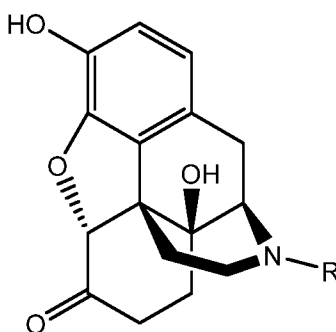
or a pharmaceutically acceptable salt thereof, wherein:

R_1 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

R_2 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted acanoyl; and

R_5 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, and optionally substituted heteroaryl;

and one of (b) a compound of formula III

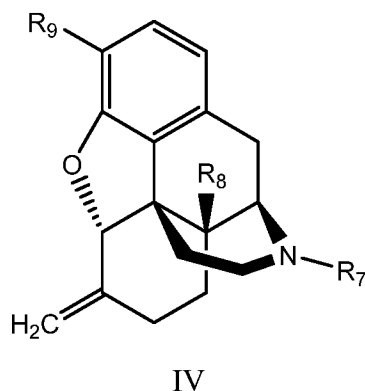


III

or a pharmaceutically acceptable salt thereof, wherein:

R is 3'-methyl-2-butenyl, cyclopropylmethyl, or cyclobutylmethyl; or

(c) a compound of formula IV



or a pharmaceutically acceptable salt thereof, wherein:

R_7 is selected from the group consisting of $-\text{CH}_2\text{-HC=CH}_2$ and cyclopropylmethyl;

R_8 is selected from the group consisting of hydrogen and hydroxyl; and

R_9 is selected from the group consisting of hydroxyl and methoxy.

62. The composition according to claim 61 wherein (a) is nalfurafine or a pharmaceutically acceptable salt thereof, (b) is naltrexone or a pharmaceutically acceptable salt thereof, and (c) is nalmefene or a pharmaceutically acceptable salt thereof.

63. The composition according to claim 61 or 62, wherein the active ingredients consist of (1) nalfurafine or a pharmaceutically acceptable salt thereof and naltrexone or a pharmaceutically acceptable salt thereof; or (2) nalfurafine or a pharmaceutically acceptable salt thereof and nalmefene or a pharmaceutically acceptable salt thereof.

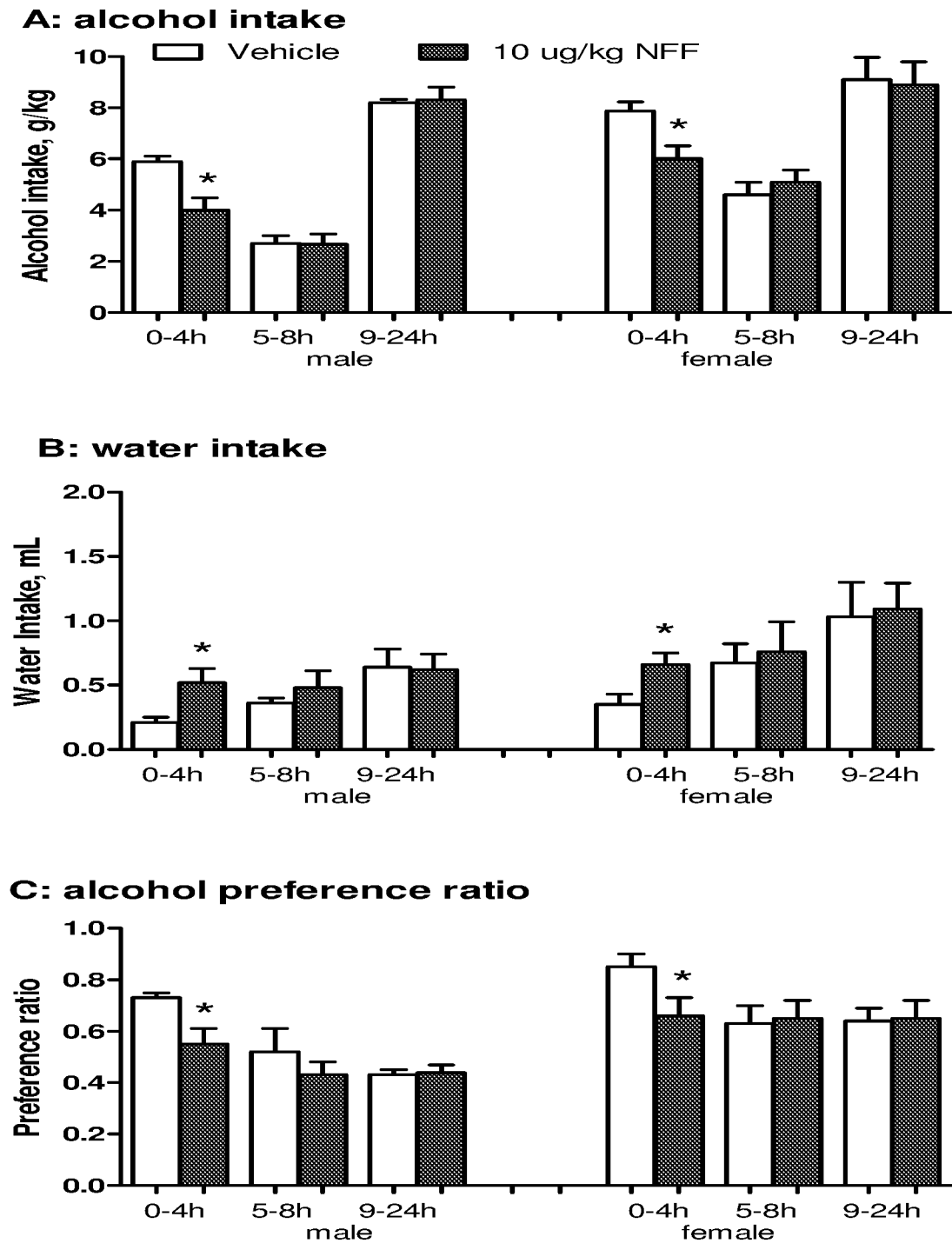


FIG. 1

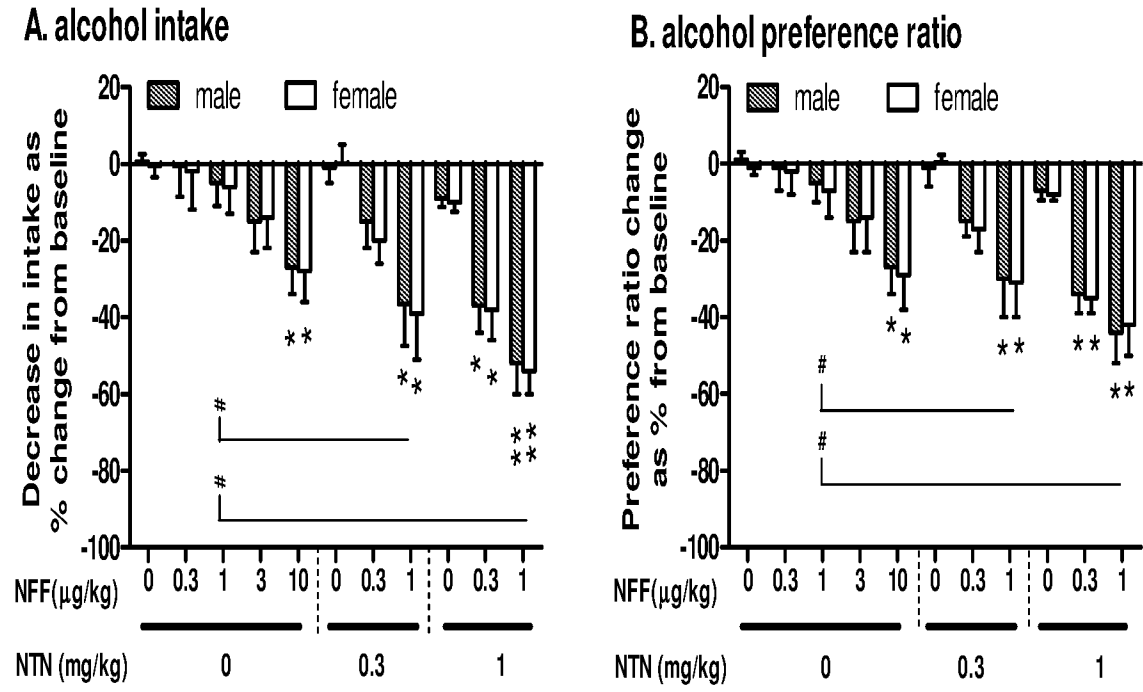


FIG. 2

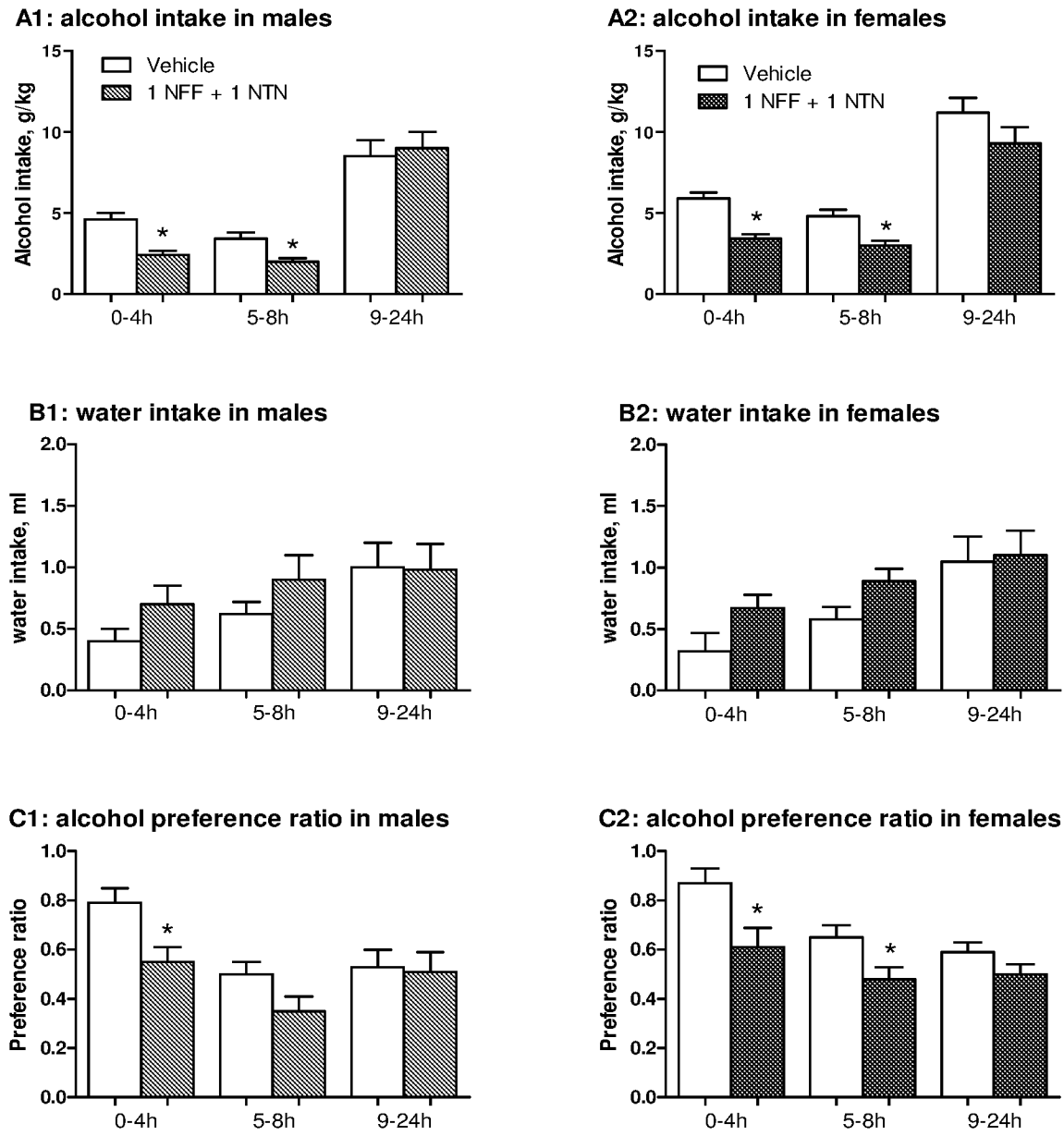


FIG. 3

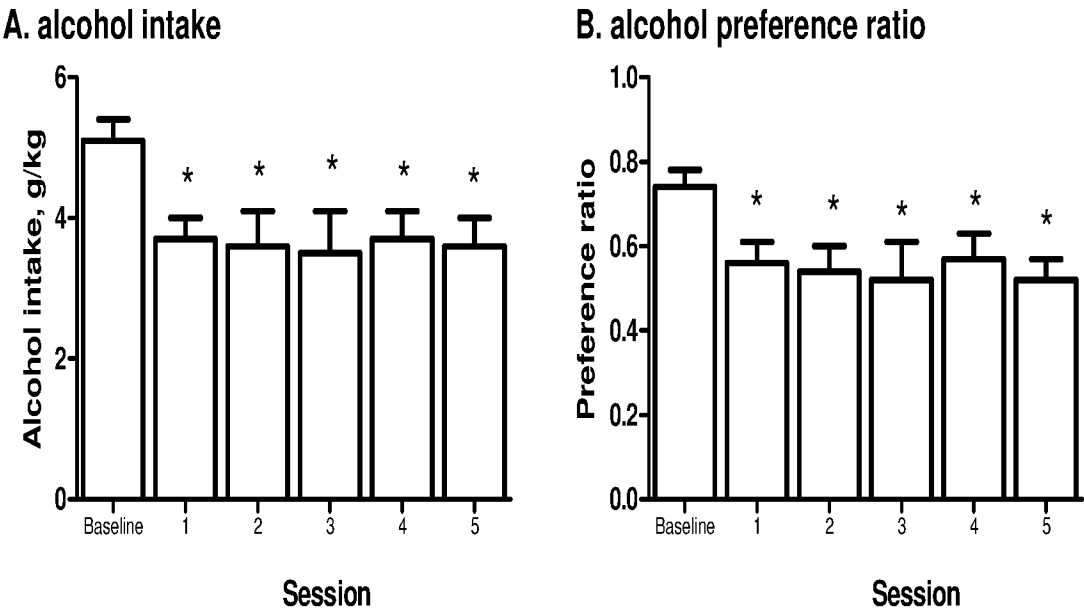


FIG. 4

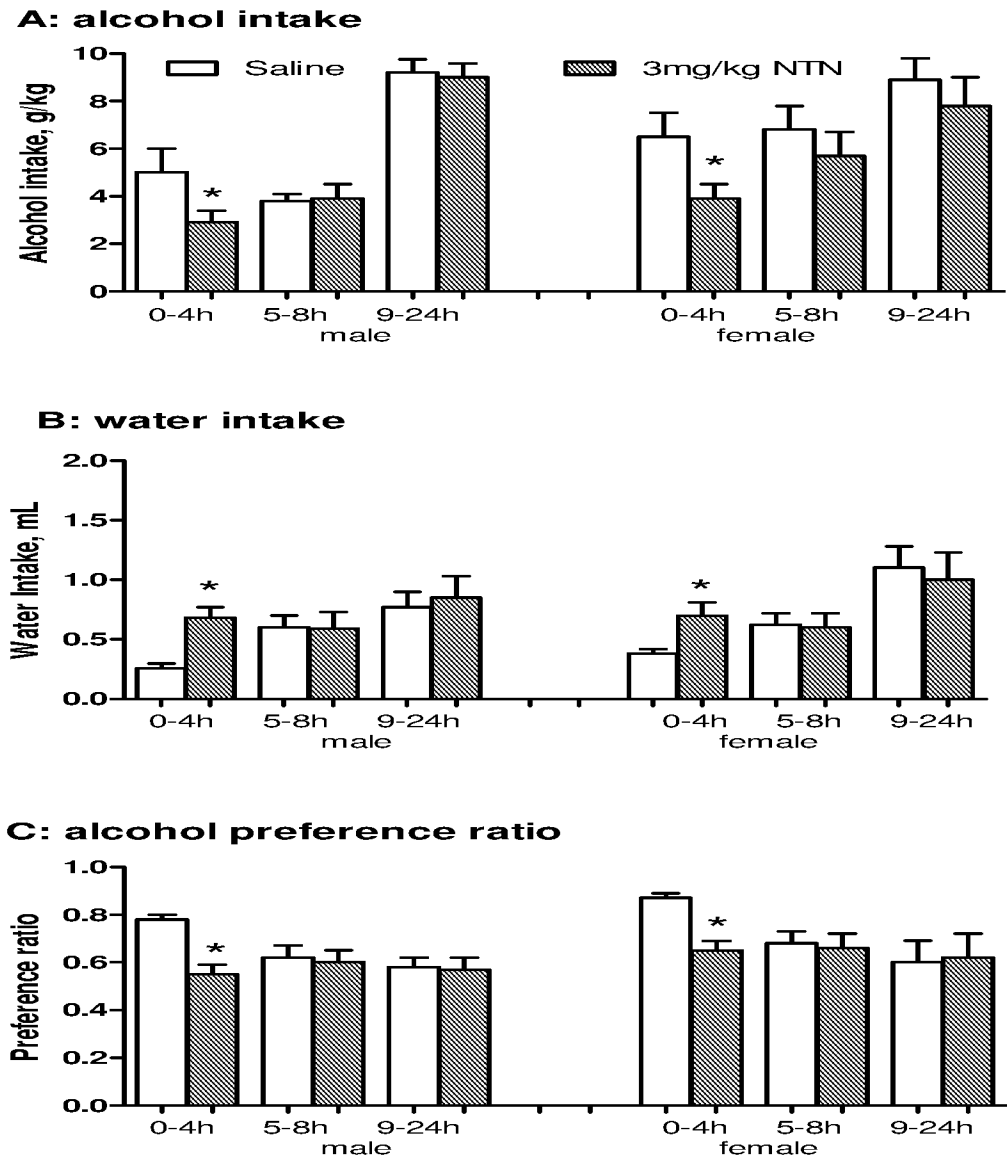


FIG. 5

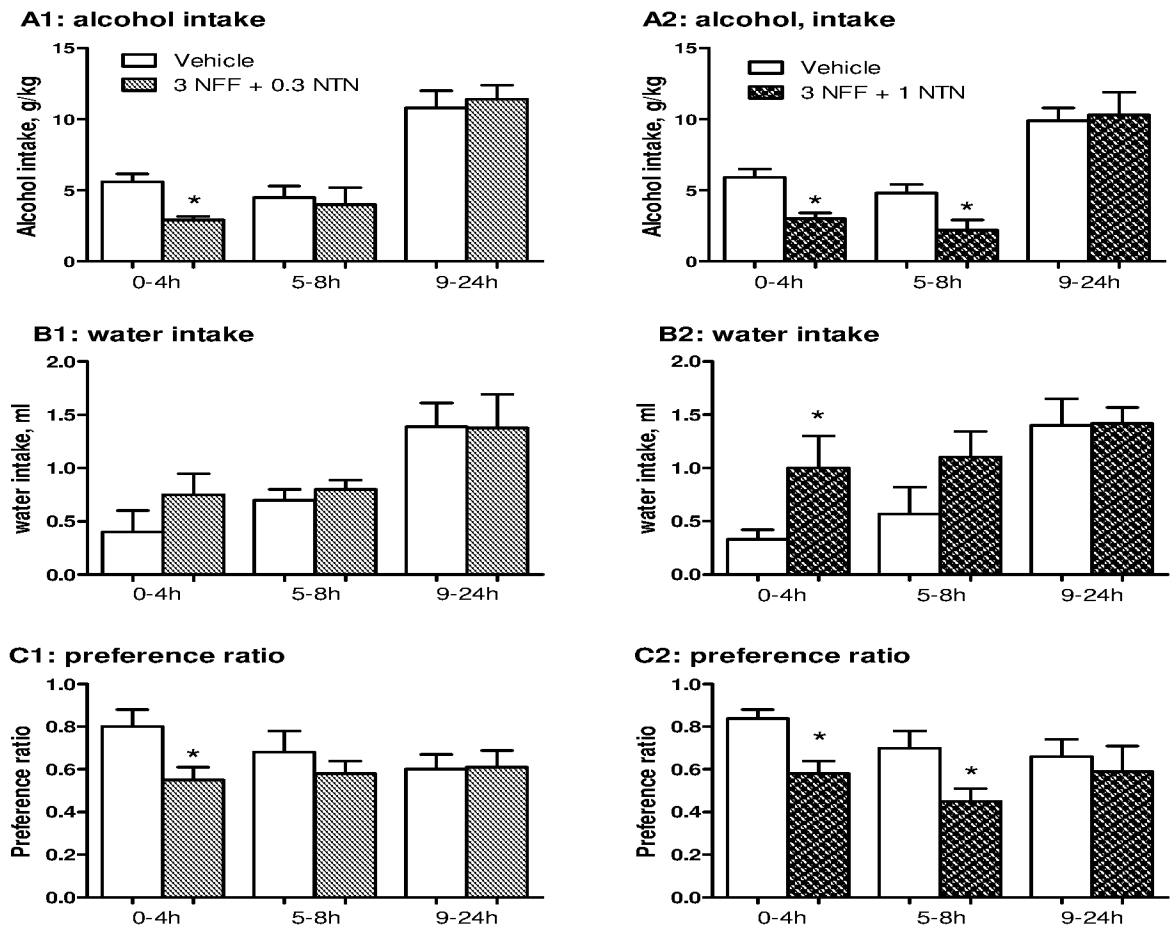


FIG. 6

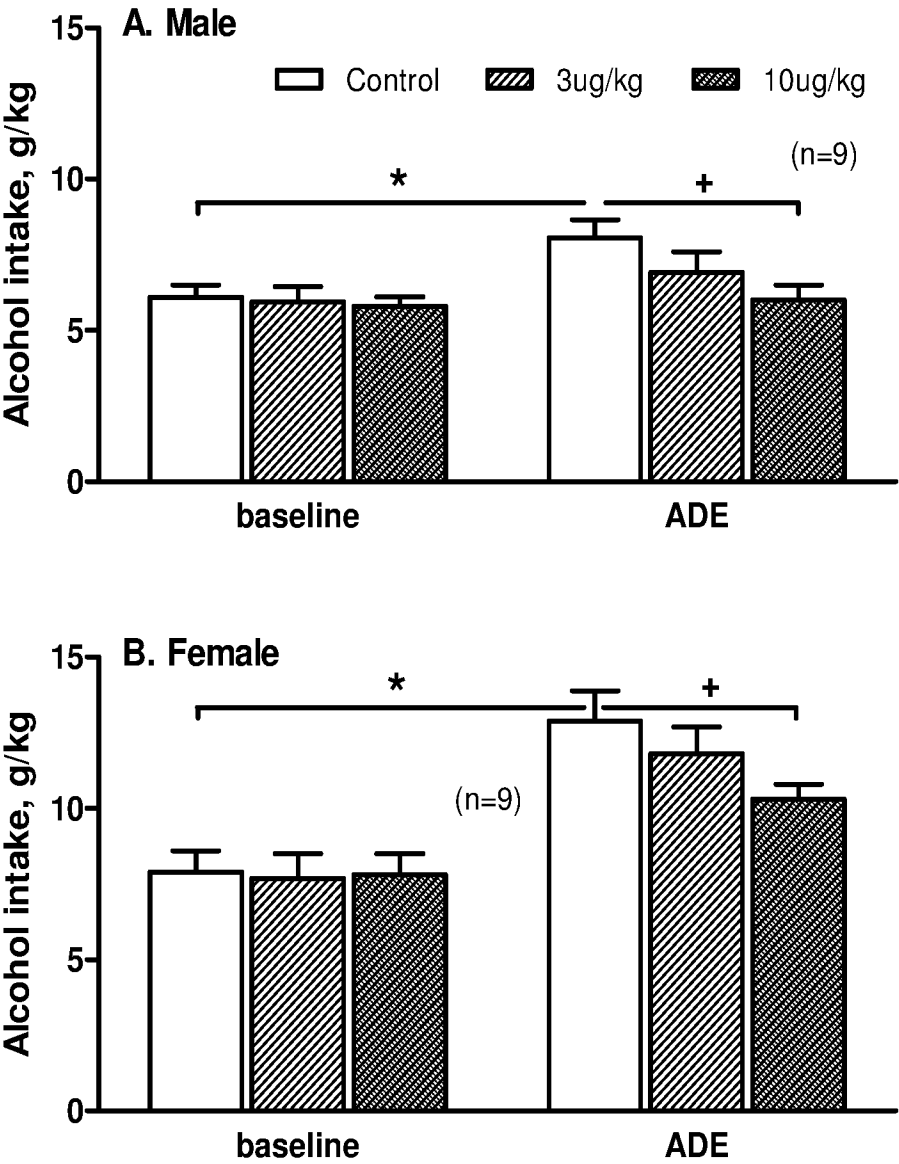


FIG. 7

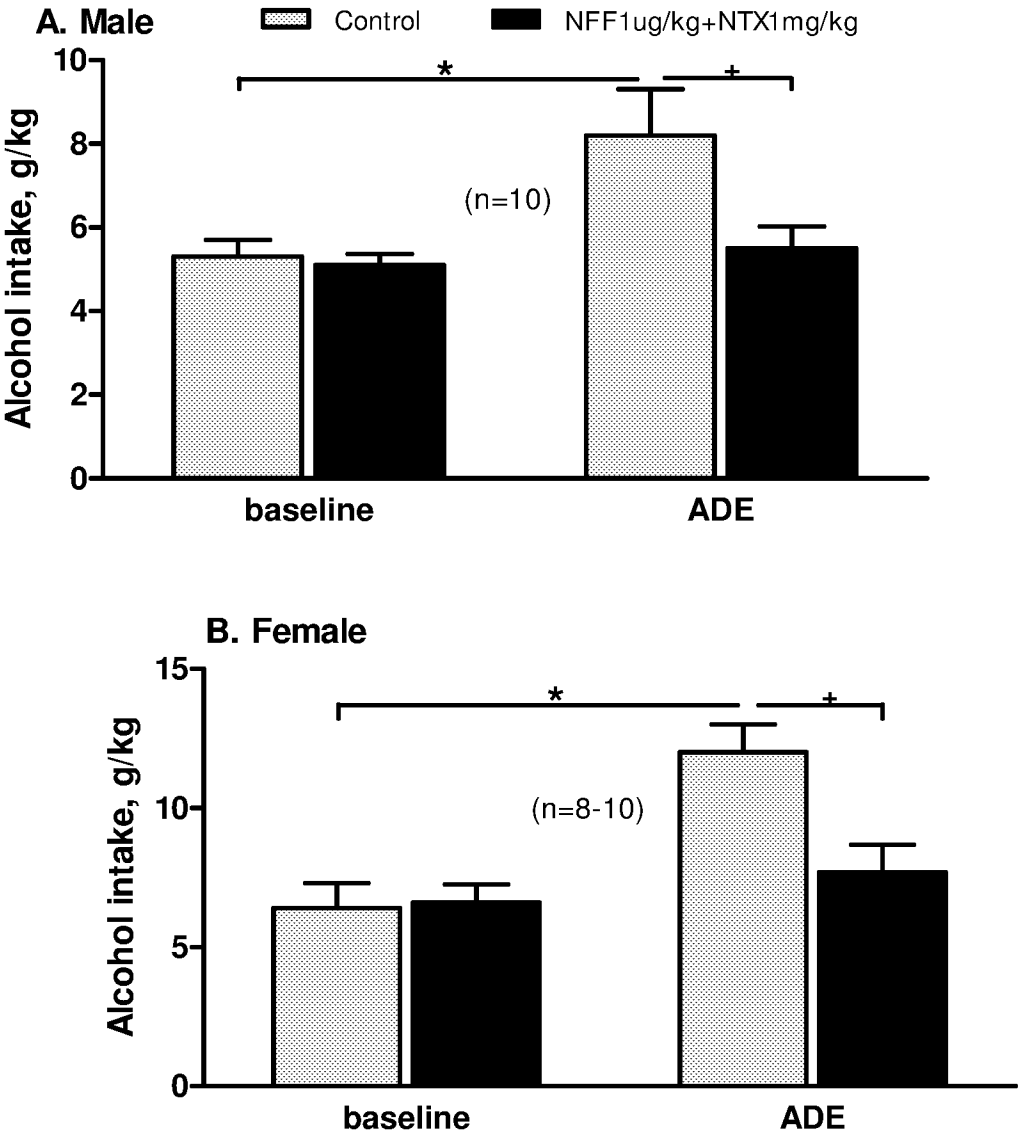


FIG. 8

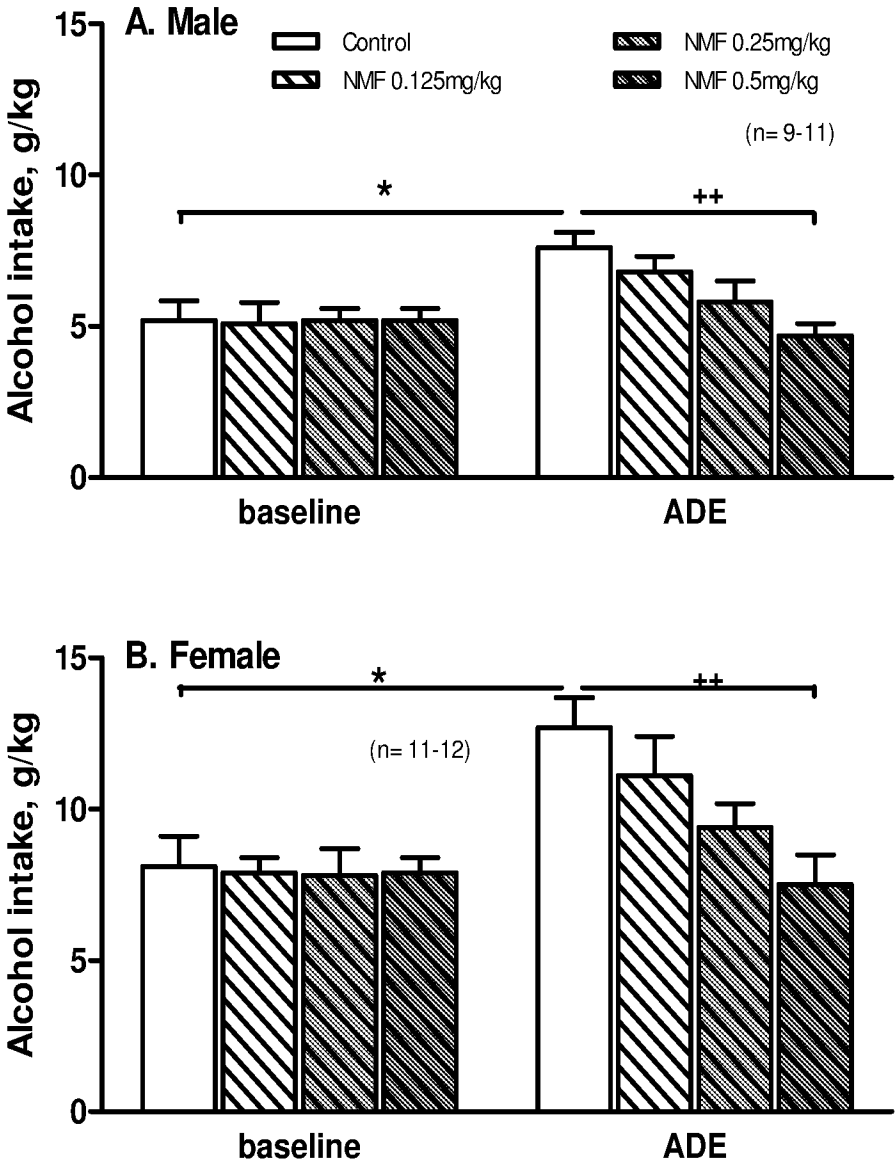


FIG. 9

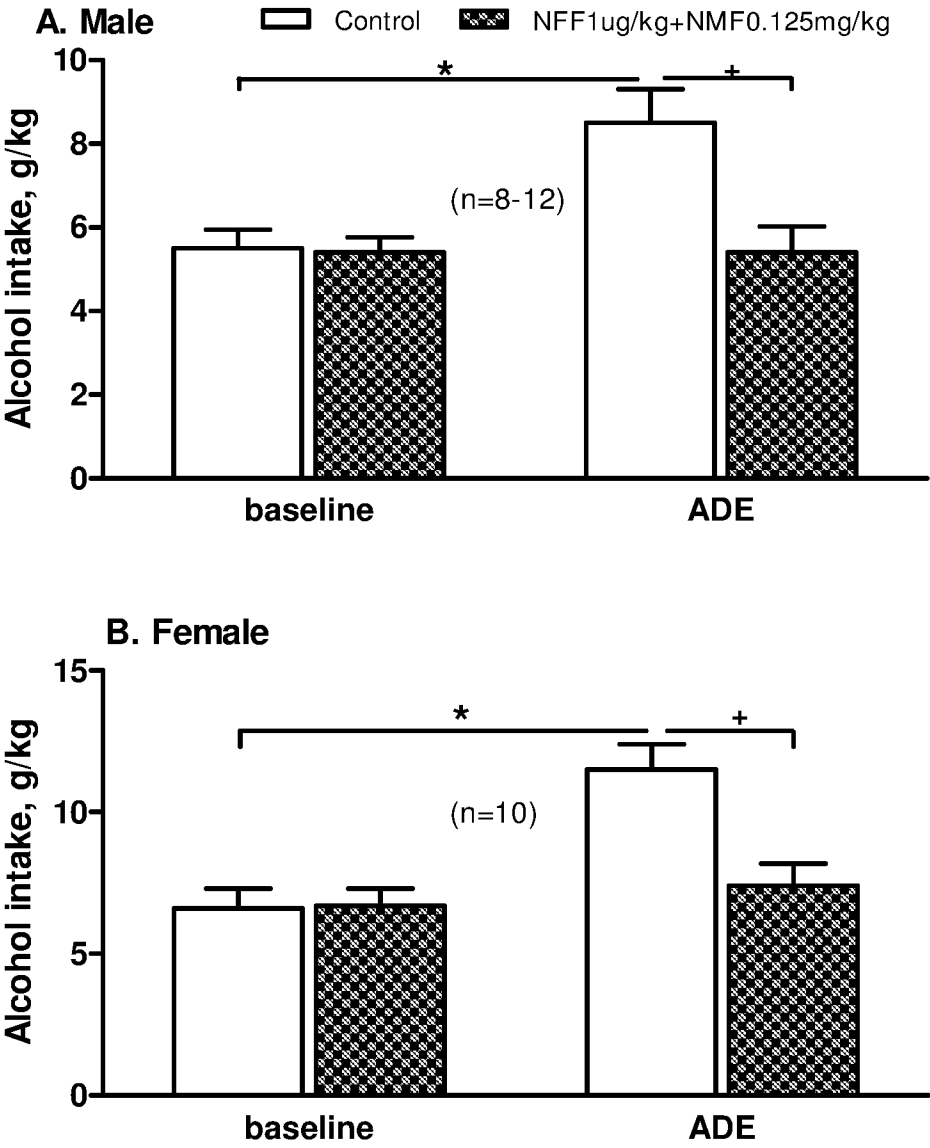


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US20/16930

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07D 489/02, 489/08 (2020.01)

CPC - C07D 489/02, 489/08

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0304529 A1 (THE UNIVERSITY OF BATH, et al.) 20 October 2016; paragraphs [0101]-[0103], [0105], [0164], [0169]	1-2, 3/1-2, 5, 27-28, 29/27-28, 30-31, 32/30-31, 34, 61-62, 63/61-62
Y	(TOWNSEND, EA et al.) 'Effects of nalfurafine on the reinforcing, thermal antinociceptive, and respiratory-depressant effects of oxycodone: Modeling an abuse-deterrent opioid analgesic in rats'; September 2017, Psychopharmacology; Volume 234, Issue 17, pages 2597-2605; abstract	1-2, 3/1-2, 5, 27-28, 29/27-28, 30-31, 32/30-31, 34, 61-62, 63/61-62
A	WO 2017/148919 A1 (G.L. PHARMA GMBH) 08 September 2017; entire document	1-2, 3/1-2, 5, 27-28, 29/27-28, 30-31, 32/30-31, 34, 61-62, 63/61-62
P, X	(ZHOU, Y et al.) 'Clinically utilized kappa-opioid receptor agonist nalfurafine combined with low-dose naltrexone prevents alcohol relapse-like drinking in male and female mice'; 01 December 2019, Brain Research; Volume 1724, page 146410; abstract only	1-2, 3/1-2, 5, 27-28, 29/27-28, 30-31, 32/30-31, 34, 61-62, 63/61-62

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

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

19 March 2020 (19.03.2020)

Date of mailing of the international search report

08 APR 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US20/16930

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

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4, 6-26, 33, 35-60
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.